Degradation of Cry1Ac Protein Within Transgenic *Bacillus thuringiensis* Rice Tissues Under Field and Laboratory Conditions

YUNHE LI,[1,2,3] KONGMING WU,[1,4] YONGJUN ZHANG,[1] AND GUOHUI YUAN[2]

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ABSTRACT To clarify the environmental fate of the Cry1Ac protein from *Bacillus thuringiensis* subsp. *kurstaki* (*Bt*) contained in transgenic rice plant stubble after harvest, degradation was monitored under field conditions using an enzyme-linked immunosorbent assay. In stalks, Cry1Ac protein concentration decreased rapidly to 50% of the initial amount during the first month after harvest; subsequently, the degradation decreased gradually reaching 21.3% when the experiment was terminated after 7 mo. A similar degradation pattern of the Cry1Ac protein was observed in rice roots. However, when the temperature increased in April of the following spring, protein degradation resumed, and no protein could be detected by the end of the experiment. In addition, a laboratory experiment was conducted to study the persistence of Cry1Ac protein released from rice tissue in water and paddy soil. The protein released from leaves degraded rapidly in paddy soil under flooded conditions during the first 20 d and plateaued until the termination of this trial at 135 d, when 15.3% of the initial amount was still detectable. In water, the Cry1Ac protein degraded more slowly than in soil but never entered a relatively stable phase as in soil. The degradation rate of Cry1Ac protein was significantly faster in nonsterile water than in sterile water. These results indicate that the soil environment can increase the degradation of *Bt* protein contained in plant residues. Therefore, plowing a field immediately after harvest could be an effective method for decreasing the persistence of *Bt* protein in transgenic rice fields.

KEY WORDS *Bacillus thuringiensis*, rice plant, Cry1Ac protein, soil, degradation

Many crop plants, such as cotton, corn, potato, and rice, have been genetically modified to express *cry* genes derived from various subspecies of *Bacillus thuringiensis* (*Bt*) Berliner encoding insecticidal proteins (Lewellyn et al. 1994, Jouanin et al. 1998, Shelton et al. 2002). Since the commercialization of the first *Bt* crops in the United States in 1996, the area planted with transgenic *Bt* crops is steadily increasing worldwide (James 2006). A major advantage of *Bt* crops is the reduction in the use of chemical insecticides (Pray et al. 2001, Shelton et al. 2002), which has brought benefits to farmers and the environment. However, concerns have been raised that genetically modified crops may pose risks to agricultural and natural ecosystems (Williamson 1992, Rissler and Mellon 1996, Hails 2000, Dale et al. 2002). One important aspect is the possible accumulation and persistence of plant-produced *Bt* proteins in agricultural ecosystems, especially in soils where *Bt* crops are grown and residues of the crop plants are incorporated by tillage or as litter, because this could affect sensitive nontarget organisms or interfere with biological processes (Head et al. 2002, Giovannetti et al. 2005).

There are several ways by which *Bt* proteins expressed by transgenic crops can enter soil: (1) direct release of *Bt* protein into soil through root exudates and/or leachates from plant injuries (Saxena et al. 1999, 2002a, b, 2004, Saxena and Stotzky 2000); (2) input through pollen during tasseling (Losey et al. 1999, Obrycki et al. 2001); and (3) degradation of residues of *Bt* plants after harvest (Tapp and Stotzky 1998, Flores et al. 2005). A number of laboratory studies have shown that insecticidal *Cry* proteins from *B. thuringiensis* subsp. *kurstaki* and subsp. *tenebrionis* are readily adsorbed and bound to clay minerals and humic acids, and that their insecticidal activity is maintained or enhanced in the soil–protein complexes (Venkateswerlu and Stotzky 1992, Tapp et al. 1994, Tapp and Stotzky 1995, Crecchio and Stotzky 1998, 2001, Stotzky 2000, Zhou et al. 2005). The binding of the toxins on these surface-active particles reduced their availability to microbial decomposers, which reveals that the *Bt* protein released into soil from transgenic plants may accumulate and persist in soil for relatively long time (Crecchio and Stotzky 1998, 2001, Stotzky 2000, Flores et al. 2005). Palm et al. (1996), Koskella and Stotzky (1997), and Tapp and Stotzky (1998) reported that purified *Bt* protein persisted in

[1] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China.
[2] College of Plant Protection, Henan Agricultural University, Zhengzhou 450002, China.
[3] Present address: Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstr. 191, 8046 Zurich, Switzerland.
[4] Corresponding author, e-mail: wkm@caascose.net.cn.

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soil for up to 234 d, at which time the tests were terminated. Crecchio and Stotzky (1998) reported that Cry1Ab protein that is bound on soil humic acids degrades more slowly than free protein. Saxena and Stotzky (2001) and Saxena et al. (2002) showed that *Bt* protein from root exudates of transgenic corn and from degradation of *Bt* corn biomass could persist in soil for up to 350 d. However, Sims and Holden (1996), who studied the degradation of Cry1Ab protein in transgenic *Bt* corn tissue in soil or without any soil contact, concluded that Cry1Ab protein in corn plant tissue would be unstable under field conditions and would likely degrade rapidly under cultivation practices. Zwahlen et al. (2003) reported that the Cry1Ab protein of *Bt* corn leaves in soil or on the top of soil could be detected after 200 and 240 d, respectively. A study of degradation of Cry1Ab protein from *Bt* transgenic rice under both aerobic and flooded conditions in paddy soil by Wang et al. (2007) showed that Cry1Ab protein degraded significantly more rapidly under aerobic conditions, with half-lives ranging from 19.6 to 41.3 d, compared with flooded conditions, with half-lives extended to 45.9–141.0 d.

After harvest, transgenic crop plant residues, which still contain *Bt* protein, may be immediately tilled into the soil or remain on the soil surface until the following sowing season. *Bt* protein would be released into the soil ecosystem continually and slowly with the decomposition of *Bt* plant residues, which possibly results in different exposures of nontarget organisms, such as the soil microbiota and beneficial insects (Giovannetti et al. 2005, Romeis et al. 2006) to the *Bt* protein. It is, therefore, essential to understand the dynamics of the *Bt* protein in decomposing plant residues (Sims and Holden 1996, Zwahlen et al. 2003).

To control the major rice pest, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), which is widely distributed in all the rice-growing areas of China, a series of transgenic rice lines have been developed expressing Cry1Ab or Cry1Ac protein (Han et al. 2006, Wang et al. 2007). These transgenic rice lines have been released into the environment for field evaluation and may be approved for commercialization in the near future. Therefore, it is urgent to evaluate the risk that *Bt* rice may pose to the soil ecosystem before commercialization. Wang et al. (2007) studied the influence of aerobic and flooded conditions on the biodegradation of purified Cry1Ab protein from *Bt* transgenic rice in paddy soil. However, there have been no comparative studies on the degradation of Cry1Ac toxin within *Bt* rice residues, especially rice plant stubble, which are normally left in the field untouched after harvest in autumn until the following spring sowing season in northern China.

In this study, we monitored the dynamics of the decomposition of Cry1Ac insecticidal protein in *Bt* rice stubble under field conditions after harvest. To predict the environmental fate of Cry1Ac, we conducted a laboratory trial to study the persistence of the insecticidal protein released from rice leaves in water and paddy soil under flooded conditions.

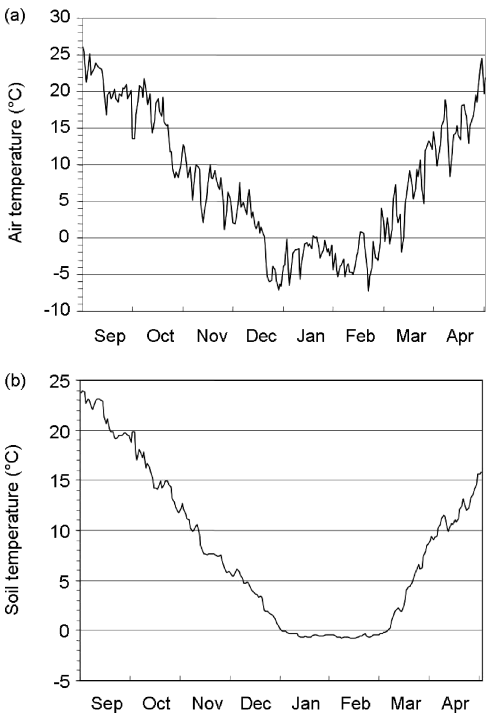

Fig. 1. Average daily air (a) and soil (b) temperatures (°C) from September 2004 to April 2005.

Materials and Methods

Plants. Two rice hybrids were used: one was a genetically modified hybrid (MSB) containing a truncated, synthetic version of a gene from *B. thuringiensis* subsp. *kurstaki* coding for Cry1Ac and a gene encoding the cowpea trypsin inhibitor (*CpTI*), which exhibited high control efficacy against *C. suppressalis* (Han et al. 2006), and the second was the corresponding nontransformed near-isoline (Minghui86). Both rice lines were obtained from the Key Laboratory of Agriculture Genetic Engineering, Fujian Academy of Agricultural Sciences (Fuzhou, China). All experimental plants were grown in the same field at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, located in the northeast of Beijing (39.90° N, 116.30° E). The field was divided into two plots; each plot was ≈40 m^2, with a 1-m buffer area of blank soil between the plots. The plots were managed according to the common growing practices in the Beijing region but without insecticide application.

Air and Soil Temperature. During the field experiment, soil temperature was measured at a depth of 10 cm, and air temperature was measured at 1.5 m above ground level (Fig. 1, a and b) at the Agricultural Weather Station, Chinese Academy of Agricultural Sciences (Beijing, China), which is 6 km from the field site.

Field Experiments. The experiment was conducted from October 2004 to April 2005. After the *Bt* and non-*Bt* rice plants were harvested at the end of September 2004, the field was not plowed, the roots and

rice stalks were left intact in the field, and no further agricultural activity was applied during the sampling period until the next seeding. At half-monthly intervals, from 1 October 2004 to 1 May 2005, rice stubble (roots and above-ground stalks) was collected randomly on 15 sampling dates. Five replicate stubble samples from non-*Bt* or *Bt* plants were collected on each sampling date, resulting in a total of 75 samples for each rice line. The field-collected materials were immediately brought to the laboratory, cleaned from soil particles with distilled water, and surface dried with filter paper. For each stubble sample, stalks with leaf sheaths at 10 cm above the roots and the corresponding roots were cut into small pieces (<2 mm) by scissors, separately. Approximately 5 g of plant material (stalk or root pieces) was weighed and stored in a minigrip plastic bag (9 by 13.5 cm) at −70°C until analysis by enzyme-linked immunosorbent assay (ELISA).

Laboratory Experiments. Leaf tissue (excluding sheathes) of *Bt* and non-*Bt* rice lines was collected at the booting stage in the second half of July 2004, when the content of *Bt* proteins expressed in MSB reached its highest level (based on ELISA, unpublished data) and immediately placed on dry ice before storage at −20°C. Frozen leaf material was lyophilized, ground using a mortar and pestle, and passed through a 2-mm sieve. ELISA analysis indicated that the level of Cry1Ac protein was ≈8 μg/g dry weight of the lyophilized transgenic rice leaf powder of MSB, and no Cry1Ac protein was detected in the corresponding non-*Bt* rice leaves. *Bt* leaf powder was stored at −70°C before use.

Six treatments were compared: *Bt* rice tissue plus soil; *Bt* rice tissue plus nonsterile water; *Bt* rice tissue plus sterile water; and three corresponding non-*Bt* rice tissue treatments. Soil samples (paddy soil: 3.91% organic matter, 0.136% total nitrogen, 0.84% total potassium, 0.23% total phosphorus, pH 7.48, and 20.6% particle content [<0.001 mm]) were collected from the surface layer (0–15 cm) in rice fields in Beijing, where transgenic crops had never been planted, and air-dried for 48 h in darkness at room temperature before passing though a 1.4-mm sieve (U.S. Standard Sieve No. 14) to remove extraneous debris and larger plant residues.

Lyophilized rice tissue powder was mixed with air-dried soil at a ratio of 1:5 (plant tissue:soil), and distilled water was added into the mixtures until the mixtures were completely soaked and a 1-cm layer of water was present on top. In water treatments, rice tissue powder was mixed with water taken directly from a rice field (nonsterile) or boiled for 30 min (sterile water) at a ratio of 1:5. Approximately 10 g of the mixtures was added to a 50-ml polypropylene centrifuge tube with two 1-mm-diameter holes punched into the cap for ventilation. A total of 36 incubation tubes were prepared per treatment and maintained in a climate chamber at 25 ± 1°C, 80 ± 5% RH, and continuous darkness. Water lost from incubation tubes, as determined by mass loss, was restored weekly during the incubation. For each treatment, triplicate tubes were chosen randomly after different intervals of incubation (0, 5, 10, 20, 30, 40, 50, 60, 75, 95, 115, and 135 d) and frozen at –70°C to be analyzed at the end of the experiment. Cry1Ac protein concentration for each sample was determined.

ELISA Analysis. Quantification of the Cry1Ac protein was conducted with ELISA kits (Envirologix Cry1Ab/Cry1Ac plate Kit; Envirologix, Portland, ME) with a limit of detection of 0.14 ppb in sample extracts. Cry1Ac standards at concentrations of 0, 5, 10, and 25 ppb were used for calibration. Spectrophotometric measurements were taken at 450 nm with a microtiter plate reader (model 680; Bio-Rad, Hercules, CA).

To extract and effectively determine the level of Cry1Ac protein in the samples, different extraction methods were adopted for different test materials. For rice tissue without soil, 0.2 g of lyophilized plant material powder was mixed with 5 ml of extraction buffer (PBST, provided in ELISA kit) on a shaker for 2 h at 4°C, followed by centrifugation at 10,000 rpm for 5 min. The supernatant was collected for ELISA quantitative detection according to the manufacturer's protocol. For the soil samples, the water covering the top of the tissue-soil mixture was decanted, and the soil mixture was again mixed thoroughly. Two replicates of 1 g soil mixture from each sample were weighed: one was for ELISA analysis and the other was dried at 40°C for 48 h to determine the dry weight of the sample. Each of the subsamples of soil was mixed with 2 ml of extraction buffer (50 mM sodium borate, 0.75 M potassium chloride, 0.075% Tween 20, and 10 mM ascorbic acid, pH = 10.5) as described by Palm et al. (1994) and ground with a mortar and pestle. The soil solutions were shaken on a platform shaker (200 rpm) at 4°C overnight and centrifuged at 10,000 rpm for 8 min. A preliminary test with this method showed that the extraction recovery of Cry1Ac toxin from the soil ranged from 41 to 54%, and the limit of detection of the ELISA test was ≈3 ng of extractable Cry1Ac per g dry weight of soil.

Data Analysis. The data are presented as mean values with SE. Cry1Ac concentration data were analyzed using repeated-measures analysis of variance (ANOVA). Percentage data (protein concentration in rice stubble) were transformed by arcsine [square root (x)] before analysis. All analyses were conducted with STATISTICA (version 6; StatSoft, Tulsa, OK).

Results

Field Experiment. ELISA analysis indicated that the initial mean (±SE) concentration of Cry1Ac protein was 1501.3 ± 200.5 ng/g dry weight in *Bt* rice stalks and 516.1 ± 86.4 ng/g dry weight of *Bt* rice roots. No *Bt* protein was detected in stalks or roots from the nontransgenic rice hybrid on any sampling date. Two nonlinear degradation rate curves of the Cry1Ac protein in *Bt* rice stalks and roots with time were observed (Fig. 2). Repeated-measures ANOVA revealed no difference in the degradation rate between rice stalks and roots ($F_{1,4} = 0.43$, $P = 0.55$). However, date × treatment interactions were significant ($F_{13,52} = 2.79$,

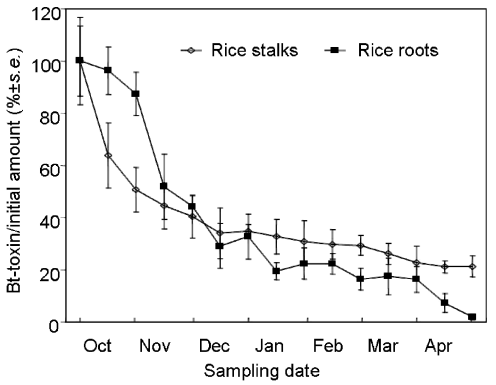

Fig. 2. Degradations (% ± SE) of the Cry1Ac protein in stalks and roots of *Bt* transgenic rice plants.

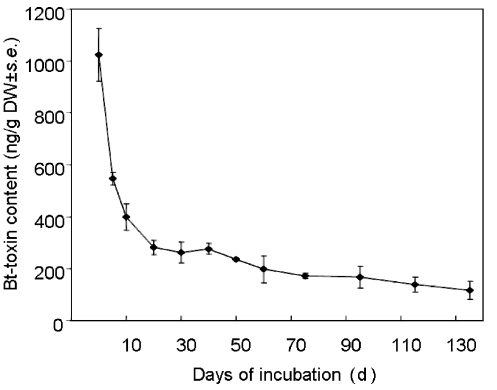

Fig. 3. Mean (±SE) of the Cry1Ac protein concentration (ng/g dry weight) in transgenic rice leaf tissue incorporated into soil.

$P = 0.0043$). In rice stalks, the concentration of *Bt* protein decreased from 1501.3 to 762.0 ng/g dry weight of stalks, i.e., to 50% of the initial amount, in the first month of the experiment (Fig. 2). During this period, the mean air temperature decreased from 20 to 10°C (Fig. 1a). Subsequently, the rate of degradation of the Cry1Ac protein decreased. From early-November to mid-December, the daily mean air temperature ranged from 1.2 to 12.7°C (Fig. 1a), and 16% of the initial amount of protein was degraded, i.e., the Cry1Ac concentration decreased from 762.0 to 510.8 ng/g dry weight of stalks. After mid-December, little Cry1Ac protein was degraded, even though the air temperature reached >15°C in late March and April. Consequently, rice stalks sampled at the end of the experiment in April still contained 319.8 ± 59.8 ng Cry1Ac protein/g dry weight of stalks representing 21.3% of the initial protein amount.

The Cry1Ac protein contained in rice roots degraded at a slower rate than in stalks during the first month (Fig. 2). From the beginning of the experiment to the end of October when the daily mean soil temperatures were >10°C (Fig. 1b), the concentration of Cry1Ac protein declined from 516.1 to 451.0 ng/g dry weight of roots, i.e., to 74.1% of the initial amount of protein (Fig. 2). From November to January, the largest portion of the protein was degraded, reaching 100.4 ± 16.5 ng Cry1Ac/g dry weight of roots, ≈20% of the initial amount of protein, by the end of January. During this period, the daily mean soil temperatures declined from 12.6 to −0.6°C (Fig. 1b). Subsequently, no further degradation was observed until mid-March, a period during which mean soil temperatures remained below 5°C (Fig. 1b). From April onward, the soil temperature increased gradually to >10°C, and the concentration of the Cry1Ac protein declined again. By the end of the experiment in late April, only 0.02% of the initial amount of *Bt* protein in rice roots could be detected.

Laboratory Experiments. The Cry1Ac protein, contained in powder of *Bt* rice leaf tissue, degraded quickly when leaf powder was mixed and incubated with field soil (Fig. 3). During the first 20 d, the concentration of Cry1Ac protein decreased from an initial mean (±SE) of 1,023.5 ± 101.0 ng/g dry weight of soil-leaf mixture to 282.1 ± 27.8 ng/g, i.e., a 72.4% decrease. After this initial decline, the rate of degradation slowed and gradually entered a relatively stable state until the end of incubation at 135 d. At the end of the experiment, 15.3% of the initial amount of Cry1Ac protein was still detectable in the soil.

In general, the Cry1Ac protein also degraded quickly in water (Fig. 4). However, compared with the soil experiment, the degradation rate of the Cry1Ac protein during the first 20 d was significantly slower in water and never plateaued but continued to degrade (Fig. 4). The initial mean (±SE) concentrations of the Cry1Ac protein in nonsterile and sterile water were 1,318.9 ± 105.1 and 1,369.6 ± 71.1 ng/g dry weight of leaf powder, respectively. During the first 40 d of incubation, the concentration of *Bt* protein decreased to 270.1 ± 52.8 ng/g dry weight of leaf powder (i.e., 79.5% of the initial amount) in nonsterile water, and to 658.8 ± 34.7 ng/g dry weight of leaf power (i.e., 51.9% of the initial amount) in sterile water (Fig. 4). After 115 d of incubation, no Cry1Ac protein was detected

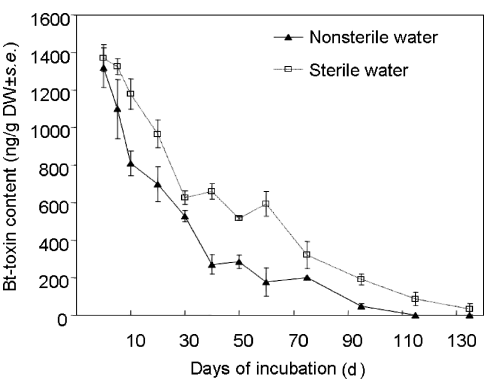

Fig. 4. Mean (±SE) Cry1Ac protein concentration (ng/g dry weight) in transgenic rice leaf powder mixed in nonsterile water and sterile water.

in nonsterile water; however, in sterile water, 87.2 ± 36.2 ng of Cry1Ab protein/g dry weight of leaf powder, i.e., 6.4% of the initial amount, was detected, and 2.4% of the protein was still detectable after 135 d when the experiment was terminated (Fig. 4). Repeated-measures ANOVA analysis showed a significantly faster rate of degradation of the *Bt* protein in nonsterile water ($F_{1,4} = 225.18$, $P = 0.0001$). Furthermore, the time × treatment interaction also was significant ($F_{11,44} = 3.62$; $P = 0.0011$).

Discussion

Bt protein contained in the stubble may be an important source contributing to the input of *Bt* protein in soil and to the exposure of nontarget species to the toxins (Zwahlen et al. 2003, Flores et al. 2005). Therefore, the degradation of the insecticidal proteins contained in transgenic *Bt* plant stubble should be taken into account when evaluating the potential risks associated with the release of *Bt* protein to the environment from transgenic plants. To our knowledge, this is the first study investigating the dynamics of the degradation of the Cry1Ac in *Bt* rice stubble between harvest of the mature crop and the next seeding.

The degradation of Cry1Ac protein in *Bt* rice stubble in this study followed, in general, the degradation curves described in previous studies (Palm et al. 1994, Sims and Ream 1997, Zwahlen et al. 2003) of protein in transgenic *Bt* plants, which showed a two-phase degradation: a rapid decline during the first stage of the experiments followed by a slow decline (Fig. 2). In rice stalks above ground, Cry1Ac protein concentration declined quickly, with ≈66% of the initial protein already degraded by the end of the first 2 mo. This is consistent with the observation in the second field experiment of Zwahlen et al. (2003), where corn leaf residues were left on the soil surface. In contrast to rice stalks, the Cry1Ac protein contained in rice roots degraded at a much lower rate. Approximately 25% of the initial amount of protein was degraded during the first month, with rapid degradation occurring in the second month of incubation (Fig. 2). This is similar to the finding in the first field experiment of Zwahlen et al. (2003), where corn leaf residues were incorporated into the soil. There are several possible reasons for these results: (1) rice stalks rapidly dried when exposed to the sunlight and decayed in rain water, which thereby provided a relatively favorable access for some decomposer organisms; (2) Cry1Ac may be degraded under high temperatures (Chen et al. 2005) and also may have been inactivated by sunlight (Koskella and Stotzky 1997); and (3) rice roots were still alive and protected against microbial decomposition by the plant's defenses for some days after the rice plants were harvested. However, with the root tissues dying gradually, the Cry1Ac protein became available for degradation by microorganisms (Zwahlen et al. 2003, Saxena et al. 1999). During the winter months (from December until March) when soil and air temperature were both <0°C, the concentrations of the Cry1Ac protein declined very slowly in both stalks and roots, probably because of reduced microbial activity (Palm et al. 1996, Zwahlen et al. 2003). When temperatures increased and the ice and snow thawed in early spring, there was no apparent decrease of protein in the stalks above ground until ≈210 d, but the concentration of Cry1Ac protein decreased again when the soil temperature increased to >10°C in April. This probably can be attributed to the dry air environment in the Beijing region, to which the stalks exposed were completely dry after a whole winter season, and the hardy character of dry rice stalks protected the Cry1Ac protein from microbial or enzymatic attack (Hopkins et al. 2001, Hopkins and Gregorich 2003). However, the subsequent wet and warm soil environment provided a good environment for decomposition of rice roots by soil microbes. In general, these studies imply that the soil environment is propitious to decomposition of *Bt* insecticidal proteins within plant residues (Sims and Holden 1996, Zwahlen et al. 2003).

These laboratory experiments studied the degradation dynamics of Cry1Ac protein released from rice leaf tissues in water and paddy soil under flooded conditions, which can be useful for predicting the environmental fate of Cry1Ac in the field (Sims and Ream 1997, Palm et al. 1996, Wang et al. 2006). The Cry1Ac protein concentration in the soil also decline rapidly during the first 20 d ,followed by a slow decline (120 d) until termination of incubation, when 15% of the protein was still detected (Fig. 2). The reduced rate of degradation in the final 120 d of the experiment may have been a consequence of lowered microbial activity after depletion of available nutrients in the plant tissues (Sims and Ream 1997). In addition, free *Bt* protein can bind tightly to surface-active particles, such as humic acids and clay minerals (Venkateswerlu and Stotzky 1992, Tapp et al. 1994, Crecchio and Stotzky 1998, 2001, Zhou et al. 2005), where they are partially protected against microbial degradation (Koskella and Stotzky 1997).

This degradation pattern is consistent with recent studies (Wang et al. 2006, 2007), where Cry1Ab toxin purified from rice was used. Wang et al. (2006) amended Cry1Ab protein exacted from rice plants in five different soils, and ELISA tests indicated that the half-lives of Cry1Ab protein varied from 11.5 to 34.3 d, with an initial rapid decline during the first 20 d of the 90-d incubation period, followed by a slower decline. Additionally, an initial rapid decline in the extractable Cry1Ab protein concentration also was observed for all tested soil under aerobic conditions during the first 20 d of incubation and was followed by a slower decline (Wang et al. 2007). Conversely, Wang et al. (2007) found that Cry1Ab protein degraded considerably more slowly in soil under anaerobic conditions compared with our results, with half-lives of 45.9–141 d in different types of soil. This probably can be attributed to differences in the *Bt* protein, soil, and/or the microbial composition of the soil (Sims and Holden 1996). Cry1Ac protein was not purified in this study, but amended into the soil together

with leaf material. Thus, the Cry1Ac protein contained in the plant tissue may be more accessible to soil microorganisms because of the degradative enzymes and compounds released when the plant decays and/or the increase in microbial populations with the addition of plant material to the soil (Palm et al. 1996).

Some earlier bioassay studies also have indicated that *Bt* insecticidal proteins expressed by cotton or corn plants as components of plant tissue lose bioactivity rapidly during the initial stage of experiments than more slowly when incorporated into soils. Ream et al. (1992), using a bioassay with *Heliothis virescens* to study the degradation in soil of Cry1Ac protein in transgenic cotton tissue, reported a DT_{50} (50% dissipation time) of 41 (31–62) days and a DT_{90} of 136 (101–205) days. Sims and Holden (1996) reported that Cry1Ab protein, added to soil with transgenic corn tissue, had an estimated DT_{50} of 1.6 d and a DT_{90} of 15 d. Sims and Ream (1997) also used larvae of *H. virescens* to estimate DT_{50} values of the Cry2A protein in transgenic cotton plant tissue, which was incubated in soil for 120 d in the laboratory and field. Results indicated that the dissipation rate of Cry2A protein from *Bt* cotton was relatively rapid during the first 40 d of incubation but slower during the next 80 d, and DT_{50} values were 15.5 and 31.7 d for the laboratory and field treatments, respectively. In both environments, <25% of the initial bioactivity remained after 120 d. The rates of decay of *Bt* protein varied greatly between experiments because of different experimental and environmental conditions, such as the nature of the soil type, microbial composition, and climate, as well as different transgenic plant species and *Bt* proteins tested (Sims and Ream 1997). Therefore, it was suggested that the degradation rates should be compared within each experiment and not between experiments. Nevertheless, all of the studies described above support the general conclusion that *Bt* insecticidal proteins, as components of plant tissue, degrade rapidly after incorporation into soils, and only small amounts of protein may persist in the soil for a relatively long time by binding surface-active soil particles.

Possible accumulation of a small amount of Cry1Ac protein in the soil ecosystem and whether it retains its insecticidal proprieties for a long period has potential implications for the activity and survival of root-feeding invertebrates and decomposer organisms. In a field study, Saxena and Stotzky (2000) found that the toxin released from root exudates in soil was still detectable and insecticidally active several months after the occurrence of frost and the death of the plants. However, Head et al. (2002) showed that Cry1Ac protein was not detectable by ELISA tests or insect bioassays in soil after multiple years of transgenic *Bt* cotton use. A recent study indicated no evidence of persistence or accumulation of Cry1Ab protein in soil from fields planted for at least three consecutive growing seasons with *Bt* corn hybrids (Dubelman et al. 2005). In addition, Wang et al. (2006) found that *Bt* rice (KMD) grown in the field did not result in any serious accumulation of the Cry1Ab protein in the rhizosphere soil. Differences in the findings might be caused by the different crops, toxins, soil types, and/or detection methods used. Hence, further studies using more sensitive detection methods will be needed to establish the fate of *Bt* proteins released from transgenic crops.

No Cry1Ac protein was detected in nonsterile water after 120 d. However, 2.4% of the initial amount of protein added was still detected in sterile water at the end of the laboratory experiment (135 d). Similarly, a study by Palm et al. (1996) indicated that the *Bt* var. Kurstaki toxin decreased much more slowly in sterile than nonsterile soil, where the microbial numbers were much greater in the latter (10^5 CFUs/g soil) than in the former (<100 CFUs/g soil). In addition, recent research showed that the degradation of Cry1Ab protein from *Bt* transgenic rice is significantly prolonged under flooded conditions compared with aerobic conditions (Wang et al. 2007). These results all confirm that microbial decomposers play an important role in the decomposition of *Bt* protein (Koskella and Stotzky 1997, Crecchio and Stotzky 2001).

In summary, these field and laboratory experiments suggest that degradation of the *Bt* protein is greatly influenced by microbial organisms in the soil ecosystem, and protein concentration decreases rapidly after the *Bt* plant material enters the soil. Therefore, an effective method for decreasing the persistence of the *Bt* protein in a *Bt* rice field would be to plow the fields after harvest, so that the stubble is destroyed and the unprotected *Bt* protein can be degraded.

Acknowledgments

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