JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fate of the Cry1Ab Protein from Bt-maize MON810 Silage in Biogas Production Facilities

STEFAN RAUSCHEN* AND INGOLF SCHUPHAN

Institute of Environmental Research (Biologie V), Chair of Ecology, Ecotoxicology, Ecochemistry, RWTH Aachen University, Worringerweg 1, D-52074 Aachen, Germany

Biogas plants fuelled with renewable sources of energy are a sustainable means for power generation. In areas with high infestation levels with the European corn borer, *Ostrinia nubilalis* (Hbn.), it is likely that transgenic Bt-maize will be fed into agricultural biogas plants. The fate of the entomotoxic protein Cry1Ab from MON810 maize was therefore investigated in silage and biogas production-related materials in the utilization chains of two farm-scale biogas plants. The Cry1Ab content in silage exhibited no clear-cut pattern of decrease over the experimental time of 4 months. Mean content for silage was 1878 ± 713 ng Cry1Ab g^{-1}. After fermentation in the biogas plants, the Cry1Ab content declined to trace amounts of around 3.5 ng g^{-1} in the effluents. The limit of detection of the employed ELISA test corresponded to 0.75 ng Cry1Ab g^{-1} sample material. Assays with larvae of *O. nubilalis* showed no bioactivity of the reactor effluents. The utilization of this residual material as fertilizer in agriculture is therefore deemed to be ecotoxicologically harmless.

KEYWORDS: Bt-maize; MON810; genetically modified plants; *Ostrinia nubilalis*; biogas plants; ELISA.

INTRODUCTION

In the last ten to twenty years, biogas plants have become a major point in the effort to use renewable sources of energy to generate electricity. The international Kyoto protocol that came into force on 16 February 2005, and even more so the revised Renewable Energy Sources Act EEG from 2004, encourage German energy producers to generate electricity from organic materials and wastes (*1*, *2*). These circumstances have led to a rapid increase in the number of biogas plants in Germany (*3*), a development which is also expected in other parts of the world (*4*). On a farm scale, biogas plants can have very different technical implementations with respect to capacity, sophistication, process control, and power output, allowing for an adaptation to the individual needs and resources of the farmer (*5*). This makes biogas plants very attractive as a source of income for farmers. Additionally, the residual materials from the biomethanation process can be utilized as fertilizer, either for their personal use or as a marketed product.

Maize has recently been established as an energy-rich and technically advantageous substrate for the production of electricity from renewable sources of energy in biogas plants (*6*). In areas of high infestation levels with the European corn borer, *Ostrinia nubilalis* (Hbn.), it is likely that Bt-transgenic maize expressing the protein Cry1Ab will be fed into agricultural biogas plants.

Two important questions arise from this instance: First, is the amount of protein in the residual material, which can be subsequently deployed onto fields or sold as fertilizer, substantially reduced or retained? This is important because these materials would have to be labeled when marketed and their application could constitute an input of Cry1Ab into the environment. Second, is there any indication of toxicity of the residual protein to organisms that may come into contact with it?

To address these questions, we studied the fate of the transgenic expression product in the utilization chains of two farm-scale biogas plants. The two plants under investigation exemplified two different fermentation strategies and quite different technological implementations. Sample materials that were subjected to commercially available enzyme-linked immunosorbent assay (ELISA) tests encompassed MON810 maize silage of different age stages and reactor effluents and solid residual wastes derived from Bt-maize silage after fermentation in the biogas plant reactors. Silage and reactor effluent samples were also tested in bioassays for their toxicity against larvae of the pest *Ostrinia nubilalis* (Hbn.) (Lepidoptera), the European corn borer, used here as a highly sensitive model for Bt-toxin-susceptible organisms, to assess their possible residual ecotoxicological relevance to a susceptible target organism.

MATERIALS AND METHODS

Bt-maize Cultivation and Silage Preparation. Bt-maize MON810 (Monsanto, St. Louis, MO) was cropped in 2004 at two farms, one situated near Rheinbach (North Rhine-Westphalia, Germany), the other in Schwarzenau near Wuerzburg (Bavaria, Germany). All stages of maize cultivation, harvest, and silage preparation were carried out according to the local common agricultural practice. No additives were used in silage preparation.

* Corresponding author. E-mail: rauschen@bio5.rwth-aachen.de. Telephone: +49 (0) 241 80 25237. Fax: +49 (0) 241 80 22182..

10.1021/jf0525380 CCC: $33.50
Published on Web 01/12/2006

Technical Aspects of the Biogas Plants and Silage Feed-in. The plant in Rheinbach featured a reactor of 1500 m^3 and was equipped with a process water recycling and complete pasteurization unit with which the material fed into the plant was heated to the temperature ambient in the reactor. Additionally, the plant material was incubated after the pasteurization step with bacterial inoculums from the digester. The co-generator was a gas engine of 500 kW h^{-1} power output. The plant had been erected in 2003 by Krieg & Fischer Ingenieure GmbH (Göttingen, Germany, http://www.kriegfischer.de) and was representative of a large-scale, modern, high-technology plant. Fermentation of organic substrates was under thermophilic conditions. During the period of investigation, only maize silage was being fed into the digester. Varying amounts of Bt-maize and non-Bt-maize silage (with Bt-maize silage constituting at least 50% of the feed-in) were fed into the digester at different intervals, depending on the performance of the biomethanation process. The biogas plant in Schwarzenau had a volumetric capacity of 850 m^3, an integrated hydrolysis and a downstream digester for further fermentation, and two pilot injection power units of 50 kW h^{-1} each. It had been erected in 2000 by the engineering company Rückert Biogasanlagen (Neukirchen, Germany, http://www.rueckert-naturgas.de/frames.html) and represented a more basic technological implementation, adapted to typical agricultural substrates. Fermentation conditions were mesophilic. The Bt-maize silage was co-fermented with manure from cattle and pigs and liquid wastes from a nearby potato processing facility.

Silage and Reactor Effluent Sampling. Silage sampling began 3 months after ensilation. Bulk samples of approximately 200 g were transferred to small plastic bags, sealed tight, immediately frozen, and stored at −20 °C until analysis. Samples were taken on a monthly basis during the whole period of silage storage. During the time of silage feed-in into the digesters, sampling was carried out every second to third day. Reactor effluent samples were taken daily to every third day during a period of 3−4 weeks, but not until 3 weeks after the first feed-in of Bt-maize-derived material into the digester. This period was deemed necessary to ensure a level of Cry1Ab high enough for detection. Additionally, reactor effluent samples were taken in Schwarzenau up to 8 weeks after the last input of Bt-maize into the reactor and of effluent that had been stored for the same length of time in separate containers. Bulk samples of 250 mL were taken at the bleeder or downpipe of the plant, transferred to a plastic flask, immediately frozen, and stored at −20°C until analysis.

Cry1Ab Protein Measurement (DAS-ELISA). Determination of the Cry1Ab content of different sample materials was carried out with a commercially available ELISA kit (Agdia Inc., Elkhart, IN, USA; purchased via Linaris GmbH, Wertheim-Bettingen, Germany). Silage samples were prepared as follows: 10 g of the frozen bulk samples were preground in a mortar with a pestle. An amount of 200 mg of this material was transferred to an Eppendorf tube and further ground under liquid nitrogen until completely pulverized. Reactor effluent samples were thawed, and 200 μL of each bulk sample were pipetted into Eppendorf tubes. Sample materials were extracted with 2 mL of the sample extraction buffer provided with the kit for duration of up to 1 h. Three parallel extractions were prepared from each bulk sample. The extracts were then centrifuged, and in the case of silage samples, diluted with the extraction buffer to a ratio of 1:400. All other steps were carried out according to the manufacturer's instructions. After addition of 50 μL of 3 M sulfuric stop solution (Agdia, sold separately) to each testwell, samples were measured in duplicates at 450 nm by using a MRX plate reader (Dynatech). To allow quantitation of the Cry1Ab content, the positive control provided with the kit was used to create a standard curve with concentrations of 0.025, 0.05, 0.075, 0.1, 0.3, 0.5, 0.75, 1, 2, and 3 ng mL^{-1}.

Bioassays with *Ostrinia nubilalis* (Hbn.). Bioassays, as described by Broadbent et al. (*7*), were carried out, slightly modified, in 128-well trays (Bio-Ba-128, Color-Dec, Italy) to test the bioavailability and potential toxicity of Cry1Ab residues and presumed breakdown products in Bt-maize silage and reactor effluents from the biogas plants to neonate larvae of the European corn borer. Bt-maize silage (200 mg) was transferred to each testwell of the bioassay tray and wetted with approximately 500 μL of distilled water. For tests with reactor effluents, 1 mL of nutrient solution medium (*8*) was dispensed into each testwell and treated with 100 μL of the appropriate reactor effluent after solidification of the medium. Silage of isogenic maize and reactor effluents from 3 months after the last feed-in of Bt-maize into the biogas plants were used as controls. After the introduction of one neonate larva taken from a laboratory colony maintained at the Institute of Environmental Research (RWTH Aachen University) to each well, the trays were sealed with vented covers provided with the bioassay trays (Bio-Cv-16, Color-Dec, Italy). After seven days, weights and developmental stages of the surviving larvae and the percentage of dead larvae were recorded. For data on the origin of the *O. nubilalis* strains and the rearing conditions see Saeglitz (*9*).

Table 1. Cry1Ab Contents (ng g^{-1} Fresh Sample) in Silage, Reactor Effluent, and Residual Solid Waste Samples from the Two Biogas Plants Fed with MON810 Maize Silage

		Cry1Ab content statistics			
sample origin	sample material	N	mean	SEM[a]	range
both locations[b]	silage	14	1878	713	10 196
Rheinbach	reactor effluent	15	0.74	0.28	2.76
Schwarzenau	solid residual waste	13	3.52	0.75	9.37
	reactor effluent	18	2.43	0.36	5.05

[a] Standard error of the mean. [b] Silage samples were pooled over both locations for statistical analysis.

Statistical Data Analysis. The nonparametric Kruskal−Wallis H-test was used to check the sample materials for significant differences in Cry1Ab contents. Subsequently, the Mann−Whitney U-test was used to check all pairs of sample materials individually for significant differences. The data for larval weights were subjected to Mann−Whitney U-tests to elucidate differences between the treatment groups and their respective control groups. All statistical analyses were performed by using SPSS 12.0 for Windows (SPSS Inc.).

RESULTS

Cry1Ab Protein Measurement (DAS-ELISA). Silage had a mean content of Cry1Ab of 1878 ng g^{-1} (fresh sample) (n = 14) (**Table 1**). Note that silage samples exhibited a considerable range of 10 196 ng g^{-1} and a standard error of the mean of 713 ng g^{-1}. The limit of detection (LOD) for the ELISA test was between 0.05 and 0.075 ng purified Cry1Ab mL^{-1}, corresponding to 0.5 and 0.75 ng Cry1Ab g^{-1} sample material, respectively. The limit of quantitation (LOQ) was at 0.3 ng mL^{-1}, equivalent to 3 ng g^{-1} material. Reactor effluent and solid residual waste samples contained only trace amounts of immunoreactive protein: 3.52 ng g^{-1} (fresh sample) for residual solid waste (from Schwarzenau) (n = 13), 2.43 ng g^{-1} for reactor effluent from Schwarzenau (n = 18), and 0.74 ng g^{-1} for reactor effluent from Rheinbach (n = 15). Note that the latter two values fall within the range between the LOD and the LOQ of the test. Of the reactor effluent bulk samples taken at Rheinbach, only 5 out of 15 tested were positive, and 2 out of 3 parallel extractions prepared from each of these samples were negative in all cases. Taking into consideration only the positive results, the mean content of immunoreactive protein in the effluent from Rheinbach was 1.86 ng g^{-1} of fresh sample. At Schwarzenau, all reactor effluent samples taken during the period of feed-in of Bt-maize into the digester contained trace amounts ranging from 0.99 to 6.04 ng g^{-1}. After storage of such effluents for 2 months before field application, they were tested negative, as were effluent samples taken 2 months after the last input of Bt-maize material into the reactor.

The differences in Cry1Ab content between the silage samples (values from both locations) and the solid waste samples from Schwarzenau and the reactor effluent samples from both biogas reactors were significant (**Table 2**). While reactor effluent

Table 2. Comparisons of Mean Cry1Ab Protein Contents between Silage, Reactor Effluent, and Solid Residual Waste Samples from Both Biogas Plants with Mann–Whitney U-Tests

comparisons	Cry1Ab ng/g (mean ± SEM)	*U*	*p*
silage (pooled) vs	1878 ± 713		
solid waste (Schwarzenau)	3.52 ± 0.75	0.000	<0.001
silage (pooled) vs	1878 ± 713		
reactor effluent Schwarzenau	2.43 ± 0.36	0.000	<0.001
silage (pooled) vs	1878 ± 713		
reactor effluent Rheinbach	0.74 ± 0.28	0.000	<0.001
solid waste vs	3.52 ± 0.75		
reactor effluent Schwarzenau	2.43 ± 0.36	72.500	0.075
reactor effluent Schwarzenau vs	2.43 ± 0.36		
reactor effluent Rheinbach	0.74 ± 0.28	47.000	0.001

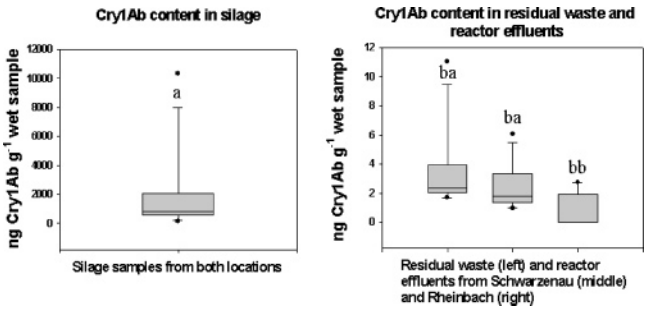

Figure 1. 1. Cry1Ab contents in different sample materials. The limit of detection of the employed ELISA test corresponded to 0.75 ng Cry1Ab g^{-1} sample material. The horizontal lines in the middle of the box plots indicate the median, and the boxes and dashes represent the range of 75% and 90% of all values, respectively. The filled circles mark the outliers. Significantly different groups are marked with different letters (a: difference between silage samples and all pooled residual waste and reactor effluent samples, $p < 0.001$; ba: no difference between residual solid waste and reactor effluent from Schwarzenau, $p = 0.075$; bb: difference between all pooled reactor effluent samples form the Schwarzenau and from the Rheinbach plant, $p = 0.001$)

samples also differed significantly between both plants, there was no significant difference between solid residual waste and reactor effluent from the Schwarzenau plant (**Figure 1**). Taking into account the contents of ELISA-active compounds of only the five Bt-positive reactor effluent samples from the total of 15 from Rheinbach, there were no significant differences between effluent from the two plants under investigation ($U = 47.0$, $p = 0.673$).

Bioassays with *Ostrinia nubilalis* (Hbn.). Toxicity tests with silage exhibited a high mortality for first instar larvae of the European corn borer for both Bt-maize silage and silage prepared from isogenic maize. While only 2 out of 96 larvae exposed to Bt-maize silage were alive after 7 days (mortality = 97.92%), the number of larvae that survived on silage derived from isogenic maize was 28 out of 160 (mortality = 82.5%). Mean weight of larvae was 0.13 mg for those kept on Bt-silage and 0.23 mg for larvae on isogenic maize silage, respectively. None of the larvae reached the second instar stage. Data were not statistically analyzed due to the high mortality in the control. Three different reactor effluent samples were tested: a reactor effluent from Rheinbach (RH) with a content of ELISA-active compounds of 0.92 ng g^{-1} (sample RH 24.02.), another effluent sample from Rheinbach with 2.67 ng g^{-1} (sample RH 03.03.), and a reactor effluent sample from Schwarzenau (SW) with a concentration of 2.40 ng g^{-1} (sample SW 11.01.) (**Table 3**). Effluent samples used as control treatments derived from an ELISA-negative sample taken from the Schwarzenau plant 3 months after the last input of Bt-maize silage (sample SW 10.05.) and a sample from the Rheinbach plant that had been established to be Bt-toxin negative (sample RH 06.03). The calculated resulting Cry1Ab concentrations per cm^2 of the test wells were 0.05 ng (RH 24.02.), 0.14 ng (RH 03.03.), and 0.15 ng (SW 11.01.). Mortality was low in all treatment groups, 3.13% in the group exposed to sample SW 11.01., 4.84% in the group exposed to sample RH 03.03., 6.25% in the control group with sample RH (06.03.), 6.67% in the test group with effluent RH 24.02., and 9.38% in the control group with sample SW 10.05. No statistically significant differences in mean larval weights between the test and their respective control groups or between the three test groups could be found by applying Mann−Whitney U-tests. All larvae reached the second instar stage during the testing period.

Table 3. Toxicity Tests with ECB Larvae Exposed to Reactor Effluents from Both Biogas Plants (For Sample Origins, See Text)[b]

	Control SW 10.05. -	Test sample SW 11.01. (0.15 ng Cry1Ab cm^{-2})
Exposed (n)	32	96
Alive after 7d	29	93
Mortality (%)	9.38	3.13
Mean weight (mg)	0.66	0.72
	p=0.249[a]	

	Control RH 06.03. -	Test sample RH 24.02. (0.05 ng Cry1Ab cm^{-2})	Test sample RH 03.03. (0.14 ng Cry1Ab cm^{-2})
Exposed (n)	32	15	62
Alive after 7d	30	14	59
Mortality (%)	6.25	6.67	4.84
Mean weight (mg)	0.85	0.82	0.77
	p=0.562[a]		
	p=0.167[a]		

[a] Significance of differences between control and test group tested with Mann–Whitney U-test. [b] RH: plant at Rheinbach. SW: plant at Schwarzenau.

DISCUSSION

Cry1Ab Content Change during Ensilation and Biomethanation. Statements about the degradation of the Cry1Ab protein during silage preparation and storage are not easily made: silage samples had a mean Cry1Ab content of 1878 ng Cry1Ab g^{-1} fresh weight ranging between 139 and 10 335 ng g^{-1} (fresh weight). The vast differences in toxin content can be attributed to basically three reasons: First, plant tissues differ considerably in Cry1Ab expression levels (*10*). MON810 maize that was grown in the greenhouse and served as a basis for comparison revealed contents of 3325 ± 180 ng g^{-1} fresh sample in leaves ($n = 4$), 820 ± 67 ng g^{-1} fresh sample in the stalks ($n = 3$), and 1238 ± 378 ng g^{-1} fresh sample in the developing cobs (including husks and silks) ($n = 3$) during the early stage of flowering. Similar differences between different tissues are frequently observed in the field (*11*). During silage preparation, the whole plant is chopped into small particles. The actual size distribution of particles tracing back to different plant tissues can lead to an inhomogeneity within the sample material that may not be compensated for by laboratory sample preparation. A given test sample may thus have a disproportionate composition not representative of the whole plant. Second, the material dries up differently before being ensiled and during

storage, explaining the high variability of values. Third, silage fragments may be subjected to microbial activity with different intensities; depending on the structural damage caused by chopping and differences in the packing density and the insulation against oxygen, some particles may be more vulnerable to attack by bacteria than others, leading to very different contents of Cry1Ab in the course of degradation. In summary, the Cry1Ab content varied greatly, but in mean did not consistently decline. This is in stark contrast to the reports of other researchers, who describe a complete breakdown of Cry1Ab in Bt11 and Bt176-maize (*12*, *13*, *14*). The work on hand shows, however, that the decomposition of the transgenic Cry1Ab protein in silage prepared from MON810 Bt-maize is by far not as pronounced, as demonstrated in works with silages from both Bt11 and Bt176 maize varieties.

After the fermentation of the maize silage in the two biogas plants, detection of the transgenic protein was still possible, with values between those corresponding to the limit of detection (LOD) and the limit of quantitation (LOQ). This means that, while a qualitative statement about the presence of immunologically active compounds was possible, a quantitation of the actual content was unwarranted. While the solid residual waste held more detectable ELISA-active compounds than the liquid reactor effluents, their content was still smaller in order of magnitudes (factor 500−1000) than that of the original plant material, and in many cases, the values fell between the LOD and the LOQ of the test. It is important to note that, in works with Bt176 maize silage, fragmentation of the protein structure appeared to be from extensive to near complete. Lutz et al. (*15*) showed that, in the gastrointestinal tract of cattle, Cry1Ab was present only as fragments of 34 and 17 kDa sizes. These particles could still be detected in the ELISA kit used, the same kit employed in the work on hand. This breakdown pattern could also be observed in silage already after a very short period of time (*14*). It can be concluded that fragmentation of the same nature, as described in the works of Lutz et al. (*14*, *15*), also occurred in samples analyzed in this work. With application rates of approximately 15 000 l of reactor effluent as fertilizer distributed over an area of 10 000−20 000 m^2, an amount of estimated 4.5 μg Cry1Ab residues would be applied per m^2. This is a negligible addition when compared to what can be measured in soils of Bt-maize fields (*16*, *17*).

Toxicity of Residual Wastes toward *Ostrinia nubilalis* (Hbn.). The reasons for the apparent negative impact of non-Bt-maize silage to larvae of *O. nubilalis* are unknown. The low pH of the silage of around 4.2 can be ruled out because the artificial diet used in the assays and for rearing has itself a pH of less than 5. The fact that silage was very dry seemed to have played a more important role.

A Bt toxicity of the reactor effluent samples from the two biogas plants against first instar larvae of the European corn borer could not be demonstrated. The bioassays showed no statistically significant differences in mean weight of the larvae and no considerably higher mortality in the treatment groups compared to the control groups. There was neither a direct toxic nor any sublethal inhibitory or stunting effect. This can be attributed to two reasons: First, the concentration of toxic protein in the samples was to low for any effects to be observable and/or second, the proteinaceous compounds or break down products had lost their entomotoxic capacity, although they could still be detected in ELISA. The very low concentration of immunologically active compounds is demonstrated by the results from the ELISA test. A decomposition of the Cry1Ab to fragments of smaller size that probably are no longer toxic to target organisms is likely. It seems safe to conclude that the reactor effluent samples are not toxic to the target organism used here as a sensitive model organism. The possibility that other susceptible or nontarget organisms would be affected by this material can be assumed to be very low.

After biomethanation, there is no evidence of any toxicity of the residual material derived from this process; the concentration of Cry1Ab in sample materials was extensively reduced to trace amounts. The assessment of the ecotoxicological implications of using Bt-maize MON810 in agricultural biogas plants and the use of the derived residual materials as fertilizer in agriculture exhibited no reason for concern.

LITERATURE CITED

(1) Weiland, P. Anaerobic waste digestion in Germany: Status and recent developments. *Biodegradation* **2000**, *11*, 415−421.

(2) Erneuerbare-Energien-Gesetz 2004. Gesetz für den Vorrang Erneuerbarer Energien vom 21. Juli 2004. *Bundesgesetzblatt Teil I, Nummer 40*; Federal Republic of Germany: Berlin, 2004; pp 1918−1930.

(3) Fischer, T.; Krieg, A. Operating biogas plants for solid waste digestion in Germany. *Biocycle* **2002**, *43*, 34−36.

(4) Fischer, T.; Krieg, A. Agricultural Biogas Plants Worldwide. International Congress, Renewable Energy Sources in the Verge of XXI Century, Warschau, 10−11 December, 2001.

(5) Fischer, T.; Krieg, A.; Chae, K. J.; Yim, S. K.; Choi, K. H.; Park, W. K.; Eom, K. C. Farm-Scale Biogas Plants. *J. Korean Org. Waste Recycl. Counc.* **2002**, *9*, 136−144.

(6) Deutsches Maiskomitee e.V. 2005. *Mais Sonderheft: Mais zur Biogaserzeugung*; Verlag Th. Mann: Gelsenkirchen, 2005; http://www.maiskomitee.de/dmk_download/dcenter/infodocs/Mais_Sonderheft_Druckfassung.pdf.

(7) Broadbent, B.; McNeil, J.; Hilton, S.; Whistlecraft, J. Final Report to Monsanto Canada, Pioneer Hi-Bred Seeds, Syngenta Canada: Baseline Monitoring of Bt-Resistance in the European Corn Borer in Ontario and Quebec (2000−2003). 2005, Available online under http://www.cornpest.ca/documents/baselinemonitoring.pdf.

(8) Draft Protocol for the Monitoring of European Corn Borer Resistance to Bt-Maize (Document XI/157/98) submitted by DGXI 29/04/98 (SCP/GMO/014), 1998.

(9) Saeglitz, C. Untersuchungen der genetischen Diversität von Maiszünsler-Populationen (*Ostrinia nubilalis* (Hbn.)) und ihrer Suszeptibilität gegenüber dem *Bacillus thuringiensis* (Bt)-Toxin als Grundlage für ein Resistenzmanagement in Bt-Maiskulturen. Ph.D. Thesis, RWTH Aachen, Aachen, 2004; English abstract at http://sylvester.bth.rwth-aachen.de/dissertationen/2004/110/04_110_enzu.htm and full German text at http://sylvester.bth.rwth-aachen.de/dissertationen/2004/110/04_110.pdf.

(10) U. S. EPA. Biopesticides Registration Action Document: *Bacillus thuringiensis* Plant-Incorporated Protectants, 2001; accessible at http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm.

(11) Nguyen Thu, H. Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais: Expression, Nachweis und Wirkung von rekombinantem Cry1Ab in heterologen Expressionssystemen. Ph.D. Thesis, Georg-August-Universität, Göttingen, 2004; accessible via http://webdoc.sub.gwdg.de/diss/2005/nguyen/.

(12) Fearing, P. L.; Brown, D.; Vlachos, D.; Meghji, M.; Privalle, L. Quantitative analysis of CryIA(b) expression in Bt-maize plants, tissues, and silage and stability of expression over successive generations. *Mol. Breed.* **1997**, *3*, 169−176.

(13) Folmer, J. D.; Grant, R. J.; Milton, C. T.; Beck, J. Utilization of Bt-corn residues by grazing beef steers and Bt-corn silage and grain by growing beef cattle and lactating dairy cows. *J. Anim. Sci.* **2002**, *80*, 1352−1361.

(14) Lutz, B.; Wiedemann, S.; Einspanier, R.; Mayer, J.; Albrecht, C. Degradation of transgenic Cry1Ab DNA and protein in Bt176

maize during the ensiling process. *J. Anim. Physiol. Anim. Nutr*. **2005**, in press (DOI: 10.1111/j.1439−0396.2005.00571.x).

(15) Lutz, B.; Wiedemann, S.; Einspanier, R.; Mayer, J.; Albrecht, C. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. *J. Agric. Food Chem.* **2005**, *53*, 1453−1456.

(16) Hopkins, D. W.; Gregorich, E. G. Detection and decay of the *Bt* endotoxin in soil from a field trial with genetically modified maize. *Eur. J. Soil Sci.* **2003**, *54*, 793−800.

(17) Baumgarte, S.; Tebbe, C. C. Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. *Mol. Ecol.* **2005**, *14*, 2539−2551.

Received for review October 13, 2005. Revised manuscript received December 8, 2005. Accepted December 13, 2005.

JF0525380