Appendix 5.2. MON 810 Literature Review – Environment

MON 810 literature review (July 2013)

Appendix 5.2 - Environment

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Review of peer-reviewed publications

Area of the environmental risk assessment: Environmental Safety – Non-Target Organisms (NTO)

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<tr><th>Publication</th><th>Summary of research and results</th><th>Conclusion</th><th>Protection Goal</th><th>Adverse effects</th></tr>
<tr><td rowspan="3">(Meissle et al., 2012)</td><td rowspan="3">Objective: To test whether the leaf beetle Oulema melanopus is susceptible to Cry3Bb1 or Cry1Ab proteins expressed in Bacillus thuringiensis (Bt) insect resistant maize (MON 88017 and MON 810).
Experimental Design: MON 88017 and MON 810 maize, the corresponding near-isolines and two conventional Swiss cultivars were grown in the glasshouse in 2006-2008 and used at the 6-8 leaf stage. Over the same period, O. melanopus adults were collected from wheat fields close to Zurich, Switzerland and used to produce laboratory-bred larvae. Feeding experiments were conducted by placing neonate larvae on the maize plants. On Days 4 and 7, mortality and consumed leaf area were noted. Larvae were then kept in individual cages on the same maize plant and checked daily until the prepupal stage. Bt protein concentrations were determined in all prepupae and in leaves where the larvae were feeding. O. melanopus insects (prepupae, larvae and beetles), feces and plant samples were lyophilized and dry weight was recorded. The sex of the beetles was determined. Fresh weights at test start and after up to 3 weeks were compared using ANOVA. Feeding experiments were also conducted with newly emerged adult beetles using MON 88017 maize only. Beetles were kept in plastic trays and offered Bt or control maize leaves as food. Mortality and bodyweight were recorded; the sex of the beetles was determined. Beetles that survived the 3 weeks of the experiment were processed for Cry3Bb1 analysis.
Results: Larval survival was reduced on Cry3Bb1-, but not on Cry1Ab-expressing maize compared with conventional varieties. Differences among conventional varieties were also present. Amount of eaten leaf material, developmental time to prepupal stage and prepupal weight did not differ between Bt maize and the near-isolines. In the study with newly emerged adults, survival and beetle weight did not differ between test and control groups when leaves of Cry3Bb1-expressing maize were offered as food over 3 weeks. Across all experiments, larvae feeding on Bt maize contained Cry3Bb1 or Cry1Ab levels in the same order of magnitude as the leaves. In contrast, concentrations in feces and prepupae were one and two orders of magnitude lower, respectively.</td><td rowspan="3">Feeding on Cry3Bb1-expressing Bt maize affected neonate larvae of O. melanopus but not older larvae and adults. Cry1Ab-expressing maize had no effect on larvae or adult beetles.
The experimental results suggests that, in the field, new O. melanopus adults which frequently feed on young maize plants are likely to be exposed to Bt proteins but will not be affected. Larvae do show sensitivity to CryBb1 protein but this developmental stage is not commonly found in maize crops because eggs are laid before maize seedlings emerge. In conclusion, neither MON 88017 nor MON 810 maize is likely to contribute to the reduction in O. melanopus leaf beetle damage in the field.</td><td>Environment</td><td>No adverse effects were determined in this study</td></tr>
<tr><td>Observed parameter</td><td>Feedback on initial environmental risk assessment</td></tr>
<tr><td>Interaction between the GM plant and NTO</td><td>There are no changes to the conclusions of the safety of the initial risk assessment.</td></tr>
</table>

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Wang *et al.*, 2012)	***Objective:*** To determine whether the termite *Coptotermes formosanus* is susceptible to Cry1Ab, Cry1A.105, Cry2Ab2, Cry 3Bb1, Cry1F and Cry34Ab1/Cry35Ab1 proteins. ***Experimental Design:*** Three insect resistant *Bacillus thuringiensis* (Bt) maize hybrids were included in the study: DKC 67-23 RR2 containing the YieldGard*Corn Borer Trait, DKC 67-88 expressing the Genuity*VT Triple Pro™ traits and DC 61-21 expressing SmartStax™ traits. Two non-Bt hybrids served as controls: DKC 61-21 and DKC 67-86. Leaves, stalks and roots of each maize hybrid were collected. The expression of Cry proteins was confirmed with an ELISA-based technique. A two-way completely random design was used in the study. For each maize hybrid, tests were conducted in five different ways as food sources for the termites: (1) wood block containing maize leaf extract, (2) filter paper containing maize leaf extract, (3) maize leaf tissue, (4) maize stalks and (5) maize roots. In addition, wood block and filter paper treated with distilled water only were included as blank controls. There were five replications in each treatment combination. Fifty termite workers and two nymphs were introduced into each experimental unit. The bioassays were kept at room temperature (23±1°C) for two weeks. Survival rate, food consumption and tunnel length were assessed. ***Results:*** Significant differences in survival rates, food consumption and tunnel length were found between the different food sources, but the effect of maize hybrid and the interaction of food source and maize hybrid was not significant.	No significant differences in survival rate, food consumption and length of tunnels between termites feeding on Bt and non-Bt maize materials were observed.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and NTO	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Grabowski and Dabrowski, 2012)	***Objective:*** Evaluation of the impact of Cry1Ab *Bacillus thuringiensis* (Bt) insecticidal protein expressed by genetically modified MON 810 maize on honey bee behaviour. ***Experimental Design:*** The insects used were part of a single honeybee hive including one queen bee and approximately 800 worker bees. The plant material consisted of transgenic maize plants of the variety DKC3421 Yield Gard (MON 810) and the corresponding near-isogenic hybrid (DKC 3420) grown in pots in a garden tunnel (6 m x 1.5 m) covered with white fabric. The pots with the plants were arranged in two rows, one for each of the two cultivars (DKC3421 and DKC3420). The hive with the bees was introduced when the plants reached BBCH stage 63 (flowering/anthesis). The number of bees visiting the maize flowers of each of the maize cultivars during BBCH stage 69 was counted. The observations were made at 8.00 a.m. and 15.00 p.m. The experiment was carried out in two vegetation seasons, 2010 and 2011. The statistical analysis was made using the One-Way Anova test with significant differences at $p \leq 0.05$. ***Results:*** No significant differences in the number of the honey bees visiting the two maize cultivars that would be caused by the potential negative impact of the genetically modified maize MON 810 were found.	No differences in behaviour were detected between honeybees feeding on MON 810 or near-isogenic maize plants.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and NTO	There are no changes to the conclusions of the safety of the initial risk assessment.

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<tr><td rowspan="3">(Alcantara, 2012)</td><td rowspan="3">Objective: To determine the long-term impacts of insect resistant Bacillus thuringiensis (Bt) maize on the arthropod communities of commercial farms and adjacent riparian areas in the Philippines.
Experimental Design: The maize varieties used were “Dekalb 818 YieldGard” and the near isoline “Dekalb 818”. Six farms were included in the study. The experiment was carried out as a randomized complete-block design by using the three pairs of commercial farms, with each pair of farms serving as a replicate. The farms of each pair were approximately 1 ha each and no more than 1 km from each other. The adjacent riparian areas were less than 25 m from the maize fields. The study was conducted over five cropping seasons. Arthropod counts in each farm were determined by visual sampling using a “W” pattern that covered the whole farm. In riparian areas, sweep net sampling was used. Arthropods were identified to species level where possible. The results were analysed using principal response curve (PRC) analysis.
Results: The PRC analyses of the five cropping seasons of data did not detect any adverse impacts on arthropod community either inside the maize crop or in adjacent riparian areas.</td><td rowspan="3">There were no long-term adverse effects on non-target organism communities in farms planted with Bt maize and adjacent riparian areas in the Philippines.</td><td>Environment</td><td>No adverse effects were determined in this study</td></tr>
<tr><td>Observed parameter</td><td>Feedback on initial environmental risk assessment</td></tr>
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Publication	Summary of research and results	Conclusion[1]	Protection Goal	Adverse effects
(Perez-Hedo *et al.*, 2012)	***Objective:*** To determine the fate of Cry1Ab *Bacillus thuringiensis* (Bt) protein when ingested in sub-lethal concentrations by two maize pests that are poorly susceptible to Bt, i.e. true armyworm *Mythimna unipuncta* and corn earworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). ***Experimental Design:*** The varieties used for the study were Bt maize PR33P67 containing the transformation event MON 810 and its corresponding non-Bt near-isogenic line PR33P66. Sixth instar larvae of *M. unipuncta* and *H. armigera* reared in laboratory cultures were used in the study. Larvae were fed on Bt, non-Bt or a mixed diet, two types of mixed diet were tested: (i) Bt for one day and then on non-Bt diet for four days; and (ii) Bt diet for three days and then on non-Bt for two days. The different treatments were compared at three time periods (L6d0–L6d1, L6d1–L6d3 and L6d3–L6d5). Larvae were weighed before and after treatment and some tissues were extracted (hemolymph, the peritrophic membrane and its contents, and the midgut epithelium) to measure Cry1Ab protein content by ELISA. ***Results:*** The results indicate that multiple mechanisms could be involved in the low susceptibility of *M. unipuncta* and *H. armigera* to Cry1Ab protein. In larvae of both species fed on a Bt diet, the low content of the toxin inside the peritrophic membrane and its contents 48 h after ingestion indicate a high rate of toxin degradation in this space, a fast toxin excretion or both. Larvae of both species fed on the Bt toxin recovered very quickly, showing overcompensation mechanisms. This recovery was detected not only in the weight gain but also in the midgut epithelium.	*M. unipuncta* and *H. armigera* fed on Bt maize showed changes in weight gain and the morphology of gut epithelium. However, once fed on non-Bt maize, larvae showed rapid recovery and also showed overcompensation mechanisms.	Environment **Observed parameter** Interaction between the GM plant and NTO	No adverse effects were determined in this study **Feedback on initial environmental risk assessment** There are no changes to the conclusions of the safety of the initial risk assessment.

[1] Because of the low susceptibility of these species to this particular Bt protein (Cry1Ab), it is not surprising to see the results of comparable growth between test groups fed non-Bt or Bt plant tissues. It is not clear from this study what the various weight differences mean (sometimes equal, less, or greater) between non-Bt and Bt tissue in the diet fed to the 6^{th} instar larvae fed non-Bt or Bt tissues in their diet. This study was conducted with just a single replicate. Also, diet renewal schedules were not provided, so it is not clear if all treatments were provided fresh diet when the diets of certain groups were changed from one type to the other. The authors speculate on potential mechanisms for the low susceptibility on these two insect species to Bt proteins, but they only tested these species against a single Bt protein (Cry1Ab).

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Kim *et al.*, 2012)	***Objective:*** To investigate the potential effects of insect resistant *Bacillus thuringiensis* (Bt) maize on the yellow mealworm *Tenebrio molitor* and potential accumulation of Cry proteins that could result in exposure of higher trophic levels. ***Experimental Design:*** Imported Bt maize grain for poultry was obtained from several feed stores (source not available). The type of protein expressed was determined using a PathoScreen Kit for Bt-Cry1Ab/Ac, Cry1F and Cry3A protein. Only Cry1Ab/Ac proteins were detected. Two different varieties of non-Bt control maize were purchased from a Korean domestic grain dealer. Seventeen nutritional components, including protein, carbohydrate, vitamins, water and minerals were analysed in the Bt and non-Bt maize to determine equivalence. The amounts of Cry1A protein were analysed by ELISA. The test organisms were *T. molitor* larvae. The study analysed host preference of three-month-old larvae, accumulation of Bt Cry proteins in the insect's body, survival and growth rate. ***Results:*** Slightly increased survival rate and head capsule width of Bt maize-fed *T. molitor* were observed, indicating that Bt maize has no sub-chronic adverse effects. Approximately 70% of *T. molitor* larvae were found in the Bt maize powder, indicating that the larvae had a strong preference for Bt versus non-Bt maize. An ELISA test revealed that concentrations of Cry1A proteins slowly increased in the body of *T. molitor* when the insects were fed Bt maize. The substantial amounts of Cry protein remaining in the digestve tract of the larvae indicated that Cry1A toxins can be transferred to the higher trophic level of predatory insects. However, no Cry proteins were detected in the hemolymph of the Bt maize-fed larvae, suggesting that there is little possibility of Cry toxin exposure via *T. molitor* to the higher endoparasitoids.	No adverse effects on the survival and growth of *T. molitor* larvae were observed following long-term exposure to Bt maize. However, *T. molitor* larvae fed with Bt maize retained Cry toxin in the body, which could act as an exposure route to predators of higher trophic levels.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and NTO	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects[1]
(Hansen *et al.*, 2013)	***Objective:*** To test the impact of insect resistant *Bacillus thuringiensis* (Bt) MON 810 maize on the weevil pest *Sitophilus zeamais* and its natural enemy *Lariophagus distinguendus*, and identify possible trophic effects of transgenic crops. ***Experimental Design:*** The study was conducted with the maize cv. Elgino (event MON 810, producing the lepidopteran-specific Bt protein Cry1Ab) and its isogenic line (cv. Cecilia P66). Both cultivars were grown in Lombardy, Italy, during 2005. Cry1Ab protein content in transgenic and isogenic maize kernels was determined by ELISA. Maize weevils, *S. zeamais*, were obtained from the Central Science Laboratory in the UK and *L. distinguendus* originated from a strain collected in Denmark in 2005. The trial was conducted in a controlled climate chamber. Adult *S. zeamais* were collected by sieving colonies that had been producing weevils for 7 d. These weevils were transferred to clean wheat kernels and incubated for 14 d to obtain 14-21 d old individuals. After an oviposition period of 2 d, the weevils were removed by sieving and the maize was returned to the climate chamber for incubation. Parasitoids (< 1 d old) were added to ten containers of each type, resulting in ten replicates of the following treatments: weevil-infested non-transgenic maize, no parasitoids; weevil-infested transgenic maize, no parasitoids; weevil-infested non-transgenic maize with parasitoids; weevil-infested transgenic maize with parasitoids. Parasitoids were then removed by sieving after 2 d. ***Results:*** Cry1Ab toxin levels in Bt maize kernels were in agreement with literature data. Bt maize did not affect emergence or development time of *S. zeamais*. However, weevil females developing on Bt maize had greater body mass than those on non-Bt maize. On the other hand, smaller weevils were found in units with parasitoids. Egg-laying parasitoids select larger hosts for oviposition, and, as these will be killed by the parasitoid larvae, on average smaller or young hosts will be left alive. The parasitoids were also affected: ca. 40% fewer females emerged in units with Bt maize. The parasitoids that successfully emerged showed no statistically significant differences in body mass, wing length or development time.	Bt maize did not affect measured parameters in *S. zeamais*. Emergence of female parasitoids was reduced in Bt maize and the authors stipulated that "*this could not be explained by the known lepidopteran-specific toxicity of Cry1Ab protein*". The parasitoids do not feed on maize, so the effect was assumed to be indirect via the hosts. According to the authors, "*the effect was unexpected, because hosts were larger in transgenic maize, and female parasitoids generally deposit eggs in larger hosts. The Bt toxin titre in maize kernels is lower than in the growing plant tissue. In spite of this, the parasitoids were affected, underlining that a simple toxicological approach cannot fully explain tritrophic effects. It seems that hosts developing in Bt maize constitute some developmental challenge to parasitoid larvae and the compatibility of biological control of stored-product pests and transgenic crop cultivation is not straightforward*."	Environment **Observed parameter** Interaction between the GM plant and NTO	The suggestion that *Bt* maize presents a developmental challenge to wasps is speculative and unsupported as no empirical data is provided that clearly establishes a cause and effect relationship for the observed difference in the number of female offspring and presence or absence of the *Bt* trait in the host food source. **Feedback on initial environmental risk assessment** There are no changes to the conclusions of the safety of the initial risk assessment.

[1] The authors acknowledge in their abstract that "*this effect cannot be explained by the known lepidopteran-specific toxicity of the Bt Cry1Ab toxin* " but offer no other plausible explanation for the decrease in female wasp offspring other than surmising that a toxicological approach therefore cannot explain the effect.
It is well known that several factors influence the sex ratio and clutch size of parasitic wasps such as *L. distinguendus* including: the number of hosts available for parasitizing; the age of the host; the amount of time that female wasps are allowed to locate and parasitize hosts; the number of males available for mating; the number of other females that compete for hosts; and artificial conditions present within a laboratory setting.
Additionally, parasitic wasps as used in the study design of Hansen *et al*. (2012). are known to exhibit a higher incidence of superparasitism (multiple females parasitizing the same host) when confined in the laboratory. Superparasitizing wasps generally lay fewer eggs and the sex ratio of the offspring is male-biased.
The fact that the both the Bt and non-Bt treatments in Hansen *et al*. (2012) showed a male bias in wasp sex ratio may be indicative of confinement induced effects regardless of treatment and provide alternate hypothesis for the observed effect on female wasps emergence.

Publication	**Summary of research and results**	**Conclusion**	**Protection Goal**	**Adverse effects[1]**
(Holst *et al.*, 2013)	***Objective:*** The objective of this study was to assess the potential effects of *Bacillus thuringiensis* (Bt) protein in pollen from insect resistant genetically modified (GM) maize on larvae from the herbivorous lepidopteran butterfly *Inachis io* under European farmland conditions, using a new model BtButTox. ***Experimental Design:*** Using climatic records, maize and butterfly phenology data, a simulation model (BtButTox[2]) of the butterfly's annual life cycle was built, overlaid with the phenology of maize pollen deposition on the leaves of the food plant *Urtica dioica* (nettle). These data were linked with the dose–response curve of *I. io* larvae to Bt maize pollen (event MON 810). Two scenarios were set up to mimic the climatic and agroecological conditions in Northern Europe (scenario 1) and Southern and Central Europe (scenario 2). Historical data on weather and maize phenology were obtained for Northern Germany (scenario 1) and Southern Germany (scenario 2). The phenology of *I. io* differs between the two regions where it is either univoltine or bivoltine. The model was not spatially explicit but simulated the worst case where nettles are growing in close proximity to a Bt maize field. *Results:* The simulations indicated that in Northern Europe, where *I. io* is univoltine, Bt-maize pollen would not be present on the food plant at the same time as the butterfly larvae. However, in Central and Southern Europe, where *I.io* is bivoltine, Bt maize pollen and the second generation larvae would coincide and an increased mortality of the larvae was predicted. This conclusion differs from earlier studies which predicted negligible effect of field-grown Bt maize on *I. io* larvae.	According to the authors, the BtButTox model indicated that "*in European farmland, the exposure of butterfly larvae to Bt maize protein constitutes a realistic risk. Specifically I. io is at risk in Central and Southern Europe where it is bivoltine. This suggests that a more comprehensive assessment is warranted of the risk implied to butterflies when and where Bt maize is grown*." The authors further contend that "*such an assessment is best carried out using empirical data, which invites scientific review and integration of knowledge, rather than on expert opinion, on which a qualified assessment is not possible*."	Environment	Holst (2013) have assembled their BtButTox model to simulate a risk for *I. io* rather than to provide a model for any predictive assessment of realistic potential risk.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and NTO	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] Importantly, the model used by Holst *et al*. (2013) was not spatially explicit and its application was directed only to nettles growing in worse case proximity to maize with assumed worse case inputs for pollen deposition, exposure, efficacy, and phenology. Meaningful models for practical risk assessment, such as that provided by Perry *et al*. (2010), apply worse case "potential mortality as measured in the laboratory or under controlled experimental conditions" to empirically realistic observations of physical and temporal associations in the field. The worse case scenarios of the Perry *et al.* (2010) model provided a robust overestimate of mortality and parametrized for the most susceptible life stage of *I. io* (first instar). Perry *et al.* (2010) determined that risk to *I. io* and other non-target lepidopteran larvae from potential hazards of pollen deposited on host plants from MON 810 fields was low.

Schuppener *et al*. (2012) and Rauschen Rauschen (2008-2011) also carried-out studies in Europe on maize pollen deposition on nettle, the level of *Bt* exposure to non-target caterpillars of *I.io* and the small tortoiseshell (*Aglais urticae)* from potential deposition of maize pollen on their food plant, laboratory feeding studies with, and Bt protein quantification, to assess the potential risk to the butterfly populations in the agricultural landscape; this extensive research determined the risk to *I. Io* and *A. urticae* to be negligible.

[2] The BtButTox model was implemented as an open-source C++ program using the Universal Simulator framework version 1.41. Model source code, an installation file to run the model on Microsoft Windows and a manual can be found on www.ecolmod.org.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects[1]
(van der Merwe *et al.*, 2012)	***Objective:*** The objective of this study was to assess the potential effects of Cry1Ab protein from insect resistant *Bacillus thuringiensis* (Bt) maize on biomass, reproduction and Neutral red retention time (NRRT, a cellular metal-stress biomarker) of the earthworm *Eisenia andrei* after long-term exposure. Additional objectives were to determine the usefulness of lysosomal membrane destabilisation as measured by NRRT as a biomarker for stress related to Cry1Ab exposure as well as exploring the use of Randomly Amplified Polymorphic DNA sequences (RAPDs) to identify genotoxic effects of this protein. ***Experimental Design:*** Maize plants from two isolines were used, the transgenic variety DKC 78-15B (event MON 810) expressing Cry1Ab protein and its closest non-transgenic isoline, CRN3505. Ten adult *E. andrei* were placed in potting soil amended with finely shredded Bt or non-Bt isoline maize leaves (dry weight soil:maize leaf ratio of 90:10). There were three replicates per group. Rearing containers were kept in the dark at 25°C for 28 days. *E. andrei* were individually weighed each week and NRTT (Nuetral Red Tetention Time; a cellular metal-stress biomarker) was measured for three earthworms per replicate. After 28 days, earthworms were removed and the number of cocoons was noted. Cocoons were then returned to the soil and the number of hatched earthworms was recorded after a further 56 days. Cry1Ab protein concentrations in soil were measured before earthworms were added and after hatchlings were counted. For the detection of potential genotoxic effects, Randomly Amplified Polymorphic DNA (RAPD) profiles generated from control and treated earthworm DNA were compared. ***Results:*** NRRT results indicated no differences between treatments. Also, no significant differences were found for cocoon production or hatching success. Conversely, biomass data indicated a significant difference from the second week onwards, with the Bt treatment losing significantly more weight than the isoline treatment. Possible confounding factors included nutritional value of the maize lines. From the RAPD profiles, there was no conclusive data linking observed genetic variation to exposure of *E. andrei* to Bt maize Cry1Ab proteins.	Long-term exposure to Cry1Ab-producing Bt maize did not affect coccon production, hatching success or NRRT of the earthworm *E. andrei*. Also, no link was observed between Cry1Ab exposure and genotoxic effects. Earthworms exposed to Bt maize lost significantly more weight than controls in the second week of exposure, however this may have been linked to confounding factors such as differences in the composition / nutritional value of the maize lines. The authors suggest that further studies should be conducted to investigate multi-generation effects on the sub-organismal and organismal level.	Environment	Multiple sensitive endpoints measured did not provide any data that could link Cry1Ab exposure to genontoxic effects in *E. andrei*. Biomass differences may have be due to nutritional differences between the test and the control maize plant material.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and NTO	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] As all other measured endpoints showed no significant differences between the test and control, and because multiple studies of earthworms exposed to Bt plant material show no affect on the biomass of earthworms, the authors believe it is important to acknowledge the presence of possible confounding factors such as nutritional difference of Bt maize and its isoline (e.g. amount of lignin, sugar, protein, and soluble carbohydrates). The authors also provide evidence (Svendsen et al., 2004) that the NRRT was a more sensitive endpoint of toxicity, and "if adverse effects were caused in earthworms by cry1Ab, the NRRT assay would provide the first indication of this."

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects[1]
(Bowers *et al.*, 2013)	***Objective:*** To examine the efficacy of hybrid maize expressing Cry1Ab and Vip3Aa insecticidal proteins for the control of mixed infestations of Lepidopteran pests, and measure levels of *Fusarium* infection and fumonisin occurrence in harvested grain compared to Cry1Ab-only maize and a non-*Bacillus thuringiensis* (Bt) isoline. ***Experimental Design:*** Two hybrids genetically engineered to express Bt genes (Cry1Ab or Cry1Ab x Vip3Aa) and one near-isogenic non-Bt maize were used in the study. Field trials were conducted in 2008-2009 and 2011 in Indiana, US. The experiments were organised as randomised complete blocks with four replicates of each hybrid x insect infestation treatment. In 2008, three treatments were included: ECB (European corn borer), CEW (corn earworm) and natural infestation. In 2009 and 2011, there were four treatments: ECB, CEW, WBC (Western bean cutworm) and natural infestation. In all experiments, ten primary ears from each plot were harvested and scored visually for *Fusarium* fungal infection and insect injury to kernels. Fumonisin concentrations in ground maize were determined using the AgraQuant Total Fumonisin Assay. Analysis of variance was conducted to evaluate insect feeding injury, severity of *Fusarium* ear rot symptoms and total fumonisin concentration in grain. Factorial analysis was used to examine simple effects of year, maize hybrid, insect treatment and hybrid x treatment interactions. ***Results:*** Results differed significantly between years. In all years, significant positive correlations were present between insect injury, *Fusarium ear* rot, insect injury and grain fumonisin levels, and *Fusarium ear* rot and grain fumonisin levels. Under all insect infestation treatments, Cry1Ab x Vip3Aa hybrids were the most resistant with regard to grain quality measurements. Averaged over all insect infestations and years, insect injury, *Fusarium* ear rot and grain fumonisin levels were lowest in Cry1Ab x Vip3Aa maize, the highest average levels being found in non-Bt hybrids. Only grain obtained from Cry1Ab x Vip3Aa hybrids consistently had acceptable fumonisin content according to US guidance and EU regulatory limits.	In the present study, the presence of transgenic insect protection (Cry1Ab x Vip3Aa or Cry1Ab) resulted in significant reductions in all grain quality measurements in maize compared to non-Bt hybrids. The results indicate that Cry1Ab x Vip3Aa maize hybrids are more likely to yield high quality and low-fumonisin grain compared to hybrids expressing only Cry1Ab or lacking insect resistance.	Environment	Results from this study are not unexpected. The dual toxin hybrid (Cry1Ab x Vip3Aa) has a broader spectrum of activity than either the single toxin or the non *Bt*, so as the study reported, there was generally less damage, ear rot, and fumonisins in the dual toxin treatment.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and fungi	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] The authors erroneously report that Cry1Ab hybrids had higher than acceptable fumonisins relative to FDA and EU guidelines. Figure 3 shows the fumonisins data, and because of the way the samples were handled, the best comparisons are between figures 3B (2009 results) and 3C (2011 results) whose ranges are somewhat similar. Crucially from those two figures, the mean levels of fumonisins in both the single and dual toxin are less than the FDA maximum of 4mg/kg, regardless of insect infestation. There is a more consistent response in the dual toxin but the single toxin, though slightly more variable (see different levels for single toxin in Figure 3B (2009 results) and 3C (2011 results)), is also efficient at keeping fumonisin levels well within the EU and FDA ranges.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Dutra *et al.*, 2012)	***Objective:*** To determine if *Harmonia axyridis* displays a preference between prey larvae fed genetically modified (GM) *Bacillus thuringiensis* (Bt) or conventional maize (non-Bt), which could in turn potentially impact resistance development. ***Experimental Design:*** Two maize hybrids, DKC-5048 RR2 (event MON 810, expressing Cry1Ab gene) and DKC-4840 (non-Bt), were used in the study. Leaf tissue was taken from plants between V3 and V6 growth stages to feed the prey species *Spodoptera frugiperda* larvae. The predators were third instar larvae and female adults of *H. axyridis*, starved for 24 h prior to test start. Individual predators were offered Bt or non-Bt fed prey larvae that had fed for 24, 48 or 72 h. Then, 15 larvae of each prey type were offered to third instar and adult predators, respectively. Observations of arenas were conducted at 1, 2, 3, 6, 15 and 24 h after test start to determine the number and type of prey eaten. To assess the potential for cannibalism between Bt- and non-Bt fed prey, experiments were conducted with only prey in the arena with the same time treatment than the choice test. Cry1Ab was quantified in leaf, *S. frugiperda* and *H. axyridis* using ELISA. ***Results:*** As expected, a sublethal effect of Cry1Ab on *S. frugiperda* larvae was apparent as larvae fed on non-Bt maize leaves were significantly larger than those fed Bt leaves. Despite sublethal effects of Bt maize on prey size and the presence of Bt toxins in Bt fed prey, *H. axyridis* showed no preference between Bt fed and non-Bt fed prey. No consistent numeric trends were found between the numbers of prey consumed. Both predator stages ate nearly all the prey by the end of the experiment. In all combinations of predator stage and prey age, the number of prey type consumed did not differ significantly. Further, overall rates of cannibalism were low and did not differ significantly between Bt fed and non-Bt fed prey. ELISA measurement confirmed the presence of Cry1Ab in leaf tissue (23-33 µg Cry1Ab/g dry weight) and *S. frugiperda* (2.1-2.2 µg Cry1Ab/g dry weight), while mean concentrations in *H. axyridis* were very low (0.01-0.2 µg Cry1Ab/g dry weight), confirming that Cry1Ab protein is highly diluted along the food chain.	The results confirm the predatory status of *H. axyridis* on *S. frugiperda* and that both *H. axyridis* adults and larvae show no preference between prey types. The lack of preference between Bt-fed and non Bt-fed prey should act in favour of insect resistance management strategies using mixtures of GM and non-GM maize seed.	Environment **Observed parameter** Trophic interactions	No adverse effects were determined in this study **Feedback on initial environmental risk assessment** There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Environmental Safety – Effects on soil organisms

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Barriuso *et al.*, 2012)	***Objective:*** To monitor the effect of Cry1Ab protein from insect protected *Bacillus thuringiensis* (Bt) maize on maize rhizobacterial communities over a period of four years. ***Experimental Design:*** Bt maize MON 810 and the isogenic line DKC6450 were grown in experimental maize fields located in Madrid, Spain. Current agricultural practices were maintained throughout the four years cultivation (2008, 2009, 2010 and 2011) and crop residues were removed after each vegetative cycle. Non-Bt and Bt maize plants were harvested at two different growth stages: about 90 days after seeding (first sampling time), when the plants had around 8 leaves, and just before crop harvesting at final growth (final sampling time). Rhizospheres from each collection time were pooled and the soil was subjected to three independent DNA extractions. Soil DNA from each of the three independent extractions was used as template for PCR amplification of the V6 hypervariable region of the 16S rRNA gene. The obtained sequences were subjected to taxonomic, phylogenetic and taxonomic-independent diversity studies. **Results:** Three predominant phyla were found in all rhizospheres: Proteobacteria, Acidobacteria and Actinobacteria. The overall distribution of these three phyla did not change between the non-Bt to the Bt maize at any sampling time. The structure of the rhizobacterial community changed at the final sampling time in the first year, probably due to a heavier period of rainfall. There were no consistent statistically significant differences in the number of groups of microorganisms, enzyme activities or pH between non-Bt and Bt maize rhizospheres, or in soil improved with Bt maize biomass. Overall, the effects of Bt maize on the bacterial community structure were minimal, and the growth stage of plant or environmental factors exerted a more noticeable effect on microbial community.	The cultivation of Bt maize during the four-year period did not change the maize rhizobacterial communities when compared to those of non-Bt maize.	Environment **Observed parameter** Interaction between the GM plant and bacteria	No adverse effects were determined in this study **Feedback on initial environmental risk assessment** There are no changes to the conclusions of the safety of the initial risk assessment.

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<tr><th>Publication</th><th>Summary of research and results</th><th>Conclusion</th><th>Protection Goal</th><th>Adverse effects</th></tr>
<tr><td rowspan="3">(Lupwayi and Blackshaw, 2013)</td><td rowspan="3">Objective: To evaluate the effects of insect tolerant Bt (Bacillus thuringiensis) maize producing Cry1Ab protein and deltamethrin insecticide application on soil microbial biomass C (MBC), β-glucosidase enzyme activity, bacterial functional diversity and bacterial community-level physiological profiles (CLPPs) in maize monoculture over five seasons. To determine the impact of growing Bt maize in rotation with other crops.
Experimental Design: The study was conducted on a dark brown Chernozem soil in Alberta, Canada. Bt (DKC26-82) and isogenic (DKC26-75) maize were grown in monoculture with or without insecticide application. In the last four treatments, Bt maize was grown in rotation with glufosinate ammonium resistant (GfR) and glyphosate resistant (GR) canola (Invigor 5020 and Pioneer 45H21, respectively), with equivalent rotations of conventional canola (Pioneer 46H02) and maize (DKC26-75) included as controls. From 2002 to 2007 (except 2005), soil samples were collected at the tasselling stage of maize. Soil MBC was measured using the substrate-induced respiration method. In 2007 samples, the activity of β-glucosidase was measured colorimetrically. Community-level physiological profiles (CLPP) of soil bacteria were evaluated using the Biolog® method. On the basis of CLPPs, functional diversity was determined.
Results: Statistical analysis of pooled data across seasons did not show any effects of Bt technology, insecticide application or crop rotation on soil MBC or diversity even though differences between seasons and between the rhizosphere and bulk soil were observed. Annual analyses of results also indicated that neither the Bt technology nor insecticide application affected soil MBC, enzyme activity or functional diversity of bacteria in the maize rhizosphere, but shifts in bacterial CLPPs due to Bt trait were observed in one year. Crop rotation effects on soil microbial properties were not observed in most cases. Where effects were observed, Bt maize grown in rotation resulted in greater MBC, enzyme activity and functional diversity than when grown in monoculture or conventional maize grown in rotation, and these effects were observed only in bulk soil.</td><td rowspan="3">The overall statistical analysis of results across years did not show any treatment (Bt technology, insecticide application or crop rotation) effects on soil microbial biomass or diversity even through differences between sampling location (rhizosphere vs. bulk soil) and years were observed. The authors concluded that ‘Therefore, the Bt technology is safe with respect to the non-target effects measured in this study. However, the effects of repeated use of Bt crops over many years on the soil environment should continue to be monitored.’</td><td>Environment</td><td>No adverse effects were determined in this study.</td></tr>
<tr><td>Observed parameter</td><td>Feedback on initial environmental risk assessment</td></tr>
<tr><td>Interaction between the GM plant and microbia</td><td>There are no changes to the conclusions of the safety of the initial risk assessment.</td></tr>
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Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Cotta *et al.*, 2013)	***Objective:*** effects of MON810 on structure and abundance of microbial communities in the rhizosphere. ***Experimental Design:*** Two events were included: MON810 and Herculex and their corresponding near-isogenic parental lines. The plants were grown in a completely randomized fourfold replicated 5x5m plots. Two different types of soil, representing typical agricultural soils in Brazil, were used. Samples were taken at three time points: growth (30 days), flowering (60 days) and during grain filling (90 days). Three sets of three plants were collected. The soil adhering to the roots of each set of three plants was pooled and homogenized. This was conducted during the traditional period for maize cultivation in Brazil "Safra" and during a non-traditional period of cultivation "Safrinha". Total microbial community DNA was extracted from the samples. PCR techniques were used. ***Results:*** no differences in the diversity of the microbial communities evaluated were observed between the MON810 line and the isogenic control. However, shifts in the composition of microbial communities due to soil type, plant age and period of cultivation were observed.	No differences between the microbial communities in the rhizosphere of MON810 maize and control maize were detected.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and microbia	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	**Summary of research and results**	**Conclusion**	**Protection Goal**	**Adverse effects**
(Verbruggen *et al.*, 2012)	***Objective:*** To test and compare the response of arbuscular mycorrhizal fungi (AMF) communities to two genetically modified (GM) *Bacillus thuringiensis* (Bt) and two non-Bt maize varieties. ***Experimental Design:*** Maize seeds were sown into pots that contained soil cores collected from a field in which the AM fungal community had been characterized for multiple years. In each pot (containing approximately 6 kg of soil), one of four different maize cultivars (two GM and two non-GM isolines) was grown: (i) Monumental MON 810; (ii) DKC3421YG (MON 810); (iii) non-GM Monumental; and (iv) non-GM DKC3420. Two maize seeds were planted in each pot using three replicates per treatment. Soil samples from the pots were taken after 47, 104 and 130 of plant growth. From each sample, 2 g of soil were used for simultaneous RNA and DNA isolation. The DNA from total RNA-enriched samples was removed by DNase I. The total RNA was measured and cDNA synthesized. Resulting DNAs and cDNAs served as the template in parallel analyses using 454 pyrosequencing and T-RFLP. RNA, owing to its fast degradation, is more suitable for analysis of active communities at a given time point, whereas DNA analyses provide a historical component. ***Results:*** Roots of all maize plants were colonized by AM fungi, with percentage root length colonization varying from 12 to 64%. This variation was not attributable to the GM trait, as colonization percentages did not differ significantly between plant varieties. No significant differences between AM fungal communities associated with GM and non-GM maize plants were found. Moreover, variation, including GM-induced variation, was much lower than the natural variation of AMF communities across a wide range of fields.	No significant differences between arbuscular mycorrhizal fungal communities associated with GM and non-GM maize plants were found.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Interaction between the GM plant and fungi	There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Environmental Safety – Effect on biochemical processes in soil

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<tr><th>Publication</th><th>Summary of research and results</th><th>Conclusion</th><th>Protection Goal</th><th>Adverse effects</th></tr>
<tr><td rowspan="3">(Londono et al., 2013)</td><td rowspan="3">Objective: To evaluate whether short-term decomposition dynamics, the decomposer micro-arthropod communities or the bacterial and fungal communities colonizing residue surfaces of two different hybrids of Bacillus thuringiensis (Bt) maize producing Cry1Ab protein differ from non-transgenic isolines.
Experimental Design: Field experiments were carried out in North Platte, USA. The soil was a Holdrege silt loam with an organic matter content of 1.8% and a pH of 6.5 in the top 20 cm. Litterbags were used to measure the decomposition rate of maize residues from 4 maize hybrids: Pioneer 34N44 Bt, Pioneer34N43 (non-Bt), NC+4990 Bt and NC+4880 (non-Bt) and three plant parts: cobs, stalks and leaves; with 2 depths of litterbag placement: soil surface and 10 cm. The four maize hybrid treatments were planted in the spring of 2003.
Results: After five months, no significant differences in either the rates of residue mass loss or in the bacterial, fungal or micro-arthropod communities colonizing the transgenic versus the non-transgenic residues were observed. Instead, both residue mass loss and detritivore colonizers were significantly affected by residue placement (surface vs. buried) and plant part. The study demonstrated that environmental factors and residue quality, not the presence of the Cry1Ab protein, were the key drivers of residue decomposition and detritivore colonization.</td><td rowspan="3">In conclusion, this study contributes to the current scientific data suggesting that cultivating Cry1Ab Bt maize is unlikely to result in any significant change in rates of residue turnover or cause significant changes in soil decomposer activity or community composition.</td><td>Environment</td><td>No adverse effects were determined in this study.</td></tr>
<tr><td>Observed parameter</td><td>Feedback on initial environmental risk assessment</td></tr>
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<tr><td rowspan="3">(Sander et al., 2012)</td><td rowspan="3">Objective: Study the mechanism adsorption of Cry1Ab protein to humic substances in soil.
Experimental Design: purified and lyophilized Cry1Ab protein was used in this study. The experiments were conducted at various pH (5, 6, 7 and 8). Measurements were taken using quartz crystal microbalance with dissipation monitoring (QCM-D), optical waveguide lightmode spectroscopy (OWLS), used OWLS, Leonardite humic acid (LHA) and polyacrylic acid (PAA) films.
Results: This study shows that there can be adsorption of Cry1Ab to humic substances at pH above 5. Therefore, Cry1Ab is expected to strongly adsorb to the surfaces of relatively apolar organic matter in agricultural soils, including apolar humic acids and humin. This work provides evidence that, in addition to PCEA, soil organic matter surface polarity is a key factor determining the kinetics, extent and reversibility of adsorption of Cry1Ab and likely of other (Cry) proteins.</td><td rowspan="3">Cry1Ab is expected to strongly adsorb to apolar organic matter in agricultural soils.</td><td>Environment</td><td>No adverse effects were determined in this study</td></tr>
<tr><td>Observed parameter</td><td>Feedback on initial environmental risk assessment</td></tr>
<tr><td>Effect on biochemical processes</td><td>There are no changes to the conclusions of the safety of the initial risk assessment.</td></tr>
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Area of the environmental risk assessment: Environmental Safety – Insect Resistance Management (IRM)

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Atsumi *et al.*, 2012)	***Objective:*** To confirm that mutation of the ATP-binding cassette (ABC) transporter gene *ABCC2* is causally related to *Bacillus thuringiensis* (Bt) Cry1Ab protein resistance in the silkworm (*Bombyx mori*) and to explore the function of this gene in the resistance mechanism. ***Experimental Design:*** Two *B. mori* strains, C2 (resistant to Cry1Ab toxin) and Rin (susceptible to Cry1Ab, were reared on mulberry leaves or artificial diet. Cry1Ab protein was expressed in *Escherichia coli*. An established silkworm GAL4[1]/UAS[2] system was used for transgenesis. The eye-color mutant strain w1-pnd, a non-diapausing mutant of diapausing strain w1-c, was used as recipient. Two EGFP[3]-positive UAS lines were established and maintained by crossing with w1-c. Females which expressed the GAL4 protein in the midgut and DsRed2[4] in the eyes were crossed with males of the UAS lines. DsRed- and EGFP-positive offspring were selected. To confirm expression of the exogenous transformed gene, the endogenous and exogenous genes were detected using primers that amplified each of the genes separately. Both genes in transgenic silkworms were quantified on a real-time thermal cycler. Midguts were dissected from fourth-instar larvae and total RNA extracted. cDNA was synthetized from the RNA. ***Results:*** Six genes were predicted in the candidate region by gene models in KAIKObase version 2.1.0. Gene *007792-93*, expressed only in the midgut of silkworm, was the most plausible candidate for Bt resistance. One sequence of three consecutive nucleotides encoding tyrosine was a fixed difference between resistant and susceptible strains. Introduction of the *Rin-007792-93* gene into Bt-resistant silkworm strains made them highly susceptible to Cry1Ab toxin. The gene *007792-93* showed high homology to human ABC transporter gene *ABCC4*, which is known to be involved in multidrug resistance.	Confirmation of the role of the ATP-binding cassette transporter gene in Bt resistance by converting a resistant silkworm strain into a susceptible one using germline transformation. This study represents a direct demonstration of Bt resistance gene function in insects with the use of transgenesis.	Environment **Observed parameter** Insect susceptibility	No adverse effects were determined in this study **Feedback on initial environmental risk assessment** There are no changes to the conclusions of the safety of the initial risk assessment.

[1] GAL4: positive regulator of gene expression for galactose-induced genes; [2] UAS: Upstream Activating Sequence; [3] EGFP: Enhanced Green Fluorescent Protein; [4] DsRed2: red fluorescent protein.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Rios-Diez *et al.*, 2012)	***Objective:*** To compare the susceptibility of two strains of fall armyworm (*Spodoptera frugiperda)* to Cry1Ab and Cry1Ac protein. ***Experimental Design:*** About 180 fall armyworm (*Spodoptera frugiperda*) larvae were collected at two sites, one on maize and one on rice. The larvae were genotyped and reared in the laboratory under controlled conditions and maintained as two separate populations. Once the populations were established they were separated into three groups: the check group of larvae of both strains fed only artificial diet, the second group fed with artificial diet treated with Cry1Ab and the third group with artificial diet treated with Cry1Ac. Purified Cry1Ab and Cry1Ac were provided by the University of Nebraska. The bioassays consisted of first instar neonates fed artificial diets. The purified endotoxins were applied to the surface of the diet at five concentrations (62.5, 250, 500, 700 and 1000 ng/cm^2). Fifty to 75 larvae were assayed per toxin concentration, with three replicates per concentration. Mortality was assessed three days after exposure. Four generations were analysed to detect changes in tolerance. A t-test was used to compare differences in mortality to the endotoxins between the fall armyworm maize and rice strains. ***Results:*** Both fall armyworm strains tolerated greater concentrations of Cry1Ac than Cry1Ab. The rice strain was more susceptible to Cry1Ac than the maize strain. Values for mortality rate showed that the rice strain was significantly more susceptible than the maize strain to Cry1Ab for the parental, F_1 and F_2 generations. The strains did not significantly differ in mortality rate for the Cry1Ac endotoxin. The authors conclude that in central Colombia, integrated pest management of fall armyworm should be different between maize and rice.	Maize and rice strains of *S. frugiperda* showed different susceptibility to Cry1Ab and Cry1Ac. In central Colombia, integrated pest management of fall armyworm should be different between maize and rice.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Insect susceptibility	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Gryspeirt and Gregoire, 2012)	***Objective:*** To assess the impact of *Bacillus thuringiensis* (Bt) maize Cry1Ab toxin on mortality and life cycle of the pest *Plodia interpunctella*. ***Experimental Design:*** Two different varieties of Bt maize were used in the study: MON 810 and TC1507. Only results for MON 810 are presented below. A standard non-Bt maize from biological agriculture ("Les 4 saisons", Andrimont-Linea verde Bio maize) was chosen as control. Diets were prepared using 100 g of cracked maize (containing different mixture levels of control and Bt-maize) mixed with 20 g of glycerol. Cry1Ab concentrations in the diet ranged from 0.05 to 0.23 µg/g fresh weight grain. Fifty newly laid eggs of *P. interpunctella* were placed in each box. Three replicates per concentration and per maize variety were used. Three times a week, the number of surviving adults emerging from each treatment and the days required from egg to adult emergence were counted. At emergence, adults were isolated in individual empty cups to determine life duration. Mortality induced by the toxin was based upon comparison of adult emergence between the different diets. Larval preference between the diets was also tested. The percentage of adults emerging simultaneously from the non-Bt and the Bt diets was calculated. This was considered as the proportion of insects available for random mating. ***Results:*** The susceptibility of larvae to Cry1Ab was demonstrated as mortality increased with the presence of the toxin in the diet. In diets containing 50% Cry1Ab, mortality was 88.1%. However, mortality in the control group was also high (56.0 ± 7.6%). Larval distribution was uniform between treatments as larvae did not avoid the diet containing Cry1Ab. A significant positive linear regression was observed between Cry1Ab concentration in the diet and female adult weight. The protein increased the development time from egg to adult regardless of sex and had no impact on male adult lifespan. However, Cry1Ab increased female lifespan and the effect was correlated with dose. A time lag of adult emergence between the non-Bt and the Bt diets was observed, resulting in asynchrony in adult emergence between control and Bt diets.	Asynchronous emergence of adults of *P. interpunctella* in Bt and non-Bt maize could result in faster development of resistance due to failure in insect resistance management (IRM) strategies based on the high dose-refuge strategy. These experiments increase the knowledge base of the impact of Cry toxins of the biology of *Plodia interpunctella*	Environment	The high control mortality in these laboratory tests, the diet used (ground maize with non-uniform particle sizes) and the methodology used (potential shortcomings discussed by the authors) make the results from this study questionable and their relevance in field situations unclear, especially when Bt maize is not targeted for control of stored grain pests such as *Plodia interpunctella* and therefore an IRM plan (e.g. including refugia) is not required.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Insect susceptibility	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Kruger *et al.*, 2012)	***Objective:*** To compare life history characteristics as well as fecundity and longevity of *Busseola fusca* moths of field-collected *Bacillus thuringiensis* (Bt) resistant and susceptible populations. ***Experimental Design:*** The life history parameters of a *B. fusca* population known to be resistant to Bt and a susceptible population were compared in a laboratory study using 1st generation spring moths and 2nd generation summer populations from maize field in South-Africa. **Spring larvae** were collected in August 2008 in the Vaarlharts area. The Bt-resistant population (designated R-VH1gen) was taken after harvest from late-planted four to seven week-old plants that exhibited symptoms of borer damage. The Bt-susceptible population was collected from non-Bt maize in August 2008 at Fochville (approx. 400 km from the Vaalharts irrigation scheme). **Summer populations** were collected in January 2009. The Bt-susceptible population was from a locality in the Viljoenskroon area. Larvae (2nd-3rd instar) were collected from maize ears and stems of non-Bt maize plants. A bioassay was conducted to determine reproductive fitness (fecundity and fertility) of moths. Pupae from all populations were weighted and sexed to determine the sex ratio. An attempt was made to have at least 30 breeding pairs from each population. The number of eggs per batch was counted and records of the eggs laid by each female were kept. After removal, each egg batch was placed separately in a test tube plugged with cotton wool to prevent desiccation and incubated at 26 ± 1°C. Moth longevity was determined as the number of days from eclosion until death. The total number of eggs and egg batches were determined for each female. ***Results:*** Sex ratio, pupal mass, fecundity and longevity of moths of field-collected Bt-resistant and susceptible *B. fusca* populations were compared. Slight adverse effects of Bt maize on fitness of the resistant summer-population were observed. The sex ratio was biased towards males in some resistant populations and towards females in susceptible populations. The resistant population had a lower mean pupal mass, shorter longevity of moth and reduced fecundity.	The study indicated that the general fitness of a Bt-resistant summer generation (pupae and moths) on Bt maize was poorer compared to that of a susceptible summer generation on non Bt maize.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Insect susceptibility	There are no changes to the conclusions of the safety of the initial risk assessment.

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Burkness and Hutchison, 2012)	***Objective:*** The objective of this study was to assess the degree to which conventional maize hybrids in refuge areas are cross-pollinated by nearby insect resistant *Bacillus thuringiensis* (Bt) maize hybrids. The implications for the success of a 'refuge in a bag' insect resistance management (IRM) strategy is discussed. ***Experimental Design:*** Trials were conducted in 2009 and 2010 in Rosemount, MN (USA). Fields of Bt maize expressing Cry1Ab protein (DKC 50-48, event MON 810) were planted in a block within 4.0 - 8.1 ha fields with 12 - 36 rows of a non-Bt hybrid (DKC 48-40) planted adjacent to the Bt hybrid on all four sides. Measurements of pollen movement and cross pollination were made by collecting non-Bt ears just before harvest and testing the kernels for the presence of Cry1Ab protein. Green leaf tissue was also tested on each plant to verify that it was true to type. Data were obtained for wind speed and direction during pollen shed. ***Results:*** During both years, Cry1Ab protein was found in kernel samples taken from all sides of the refuge. The highest percentages of positive samples were found within the first four rows (3 m) and generally declined as the distance from Bt maize increased. In both years, each of the first four rows of non-Bt refuge maize, in downwind directions (north and east), had ≥25% of the ears containing kernels that expressed Cry1Ab. Highly variable rates of pollen movement in the maize fields were observed throughout the study.	Cross pollination between Bt and refuge maize occurs at a high percentage in the first rows of refuge maize, decreasing with distance. The authors state that cross pollination rates can be reduced by temporal isolation or by using hybrids of different maturity in the Bt and refuge areas. The conclusion is that, based on the nature of pollen dispersal and cross pollination in maize, there is a high probability that non-Bt ears of maize will be cross pollinated by Bt pollen under a' refuge in a bag' scenario thus reducing the effectiveness of the refuge plants in producing susceptible adults. More research is needed to fully measure the impact of Bt pollen on lepidopteran pests for sustainable IRM practices in Bt maize.	Environment	Cross pollination of refuge ears (relatively close and/or downwind to the traited ears, which would especially occur with seed blends or "refuge in a bag)) resulting in kernels expressing Bt toxin would reduce the effectiveness of refuge ears in producing susceptible adults of target insect species that primarily feed on corn ears such as corn earworm (Helicoverpa zea). The actual impact (from a refuge standpoint) on a target corn ear pest is yet unknown and would most likely be dose and trait-dependent. Currently, there are no commercially available Bt seed blends (refuge in a bag) that target primarily a corn ear pest.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Cross-pollination	There are no changes to the conclusions of the safety of the initial risk assessment.

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