Appendix 5.2. MON 810 Literature Review – Environment

MON 810 literature review (July 2014)

Appendix 5.2 - Environment

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Review of peer-reviewed publications

Area of the environmental risk assessment: Environmental Safety – Non Target Organisms

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Habustova *et al.*, 2014)	***Objective:*** To determine the effect of *Bacillus thuringiensis* (Bt) insect resistant maize (MON 810) on the diversity and abundance of plant-dwelling insects. ***Experimental Design:*** The study was carried out from 2003 to 2005 on 10 plots of 0.5 ha each, that were set in two rows in a field of 14 ha in Southern Bohemia. The rows were separated by a 10 m wide strip of bare land and the plots within each row by 2 m wide unseeded walkways. Bt maize and non-Bt parental cultivar were each planted on five alternating plots. The area surrounding the plots (30–70 m wide) was seeded with the non-transgenic cultivar. Temperature and precipitation were recorded monthly by the Hydrometeorological Institute. The field was treated yearly with Guardian EC herbicide (3 L/ha), fertilized with DAM (225 L/ha) before sowing and with AMOFOS (88 kg/ha) simultaneously with maize sowing. The maize was cut in the waxy stage of kernel ripening. Each plot was planted with the same cultivar in all 3 years. Ten randomly selected plants were taken from each plot during the vegetation period (between stages BBCH14-16 and BBCH 89) in about 2 week intervals. One plant for each plot was analyzed to determine the amount of Bt protein (Cry1Ab) by using a commercial ELISA. All the collected plants were thoroughly examined in the laboratory for the presence of arthropods. The insects were identified to the level of genus and species. The insect counts were statistically analyzed with CANOCO with respect to the Bt toxin, developmental stage of maize and the year of cultivation. ***Results:*** The content of Cry1 Ab protein in different plant tissues changed in the course of season in a similar manner every year, with the highest concentration being in the leaves at the flowering stage. No significant effect of Bt maize on plant-dwelling non-target insects was detected. Correlation between the number of plants and detected insect diversity revealed that the analysis of 20 plants (four per each of five plots) provided data reliable at 95% probability level; six plants per plot were sufficient for the analysis of aphids, thrips and *Orius* bugs.	The authors concluded that "*our study confirms and extends results of similar field trials (Candolfi et al. 2004; Novillo et al. 2004; Dively 2005) demonstrating that Cry1Ab expression in maize does not affect plant-dwelling non-target insects.*"	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Diversity and abundance of plant-dwelling insects	There are no changes to the conclusions of the safety of the initial risk assessment.

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Kocourek *et al.*, 2013)	***Objective:*** To compare three control strategies against European maize borer (*Ostrinia nubilalis* Hubner) in maize with respect to carabid beetles, beneficial epigeal arthropods. ***Experimental Design:*** The study was conducted at two sites in the Czech Republic over three year (2002-2004). Insect resistant *Bacillus thuringiensis* (Bt) maize MON 810 was planted on one plot. The remaining plots were planted with varieties susceptible to *O. nubilalis*: an isogenic hybrid (Monumental in 2002, 2003 and DKC 3420 in 2004) and a local hybrid (Raissa). At each locality, one non-transgenic plot was treated with *Trichogramma* wasps as a biological control method. Epigeal arthropods were sampled using pitfall traps. Observed species richness was calculated for each plot, sampling date and year. Because richness is a function of sample size, the Chao 1 index was used to estimate true species richness, a predicted value taking into account the number of unrecorded but present species based on the number of species found in one or two specimens. The composition of carabid assemblages was compared between fields using the estimated abundance-based Chao-Jaccard similarity index, which takes into account the contribution made per species estimated to be present at both sites. ***Results:*** In total, 48 carabid species and 9,782 individuals were collected over the three seasons. The rarefaction curves revealed that the type of control strategy against *O. nubilalis* did not significantly affect carabid species richness. Also, the Chao-Jaccard similarly indices were high for all pairs of plots within each year and locality. The use of different control strategies against *O. nubilalis* did not affect populations of carabid beetles in the study plots between the years. Pooled across treatments, the species richness remained unchanged at both localities, so did the composition of carabid assemblages. In conclusion, no differences were found in species richness or species composition between treatments, seasons or sites, suggesting no effect of planting MON 810 maize on the assemblage of carabid beetles in the fields.	The authors concluded that: *"No differences were found in species richness or species composition between treatments, seasons or sites, suggesting no effect of planting transgenic insect resistant Bt maize MON 810 on the assemblages of carabid beetles in the study fields".*	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Diversity and abundance of carabid beetles	There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Environmental Safety – Non Target Organisms

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Twardowski *et al.*, 2014)	***Objective:*** To determine the impact of genetically modified (GM) MON 810 maize expressing the *Bacillus thuringiensis* (Bt) Cry 1Ab insecticidal protein on the population density of rove beetles (Coleoptera: Staphylinidae). ***Experimental Design:*** The study was carried out from 2008 to 2010 in two locations in southern Poland where infestation by the European corn borer is a substantial problem. The following five treatments were used for the experiment: Bt transgenic maize MON 810 (DKC 3421 Yield Gard®), the corresponding non-GM isogenic counterpart (DK 3420) without insecticide application, DK 3420 with insecticide application and 2 non-Bt conventional cultivars (Bosman and Wigo). The experimental design consisted of randomized complete blocks with five treatments and four replicates. The experimental field covered an area of 1,600 m^2 divided into 20 plots (40 x 40 m) with an alley distance of 4.5 m. A total of 80 circular plastic pitfall traps were used in each location to collect the epigeal arthropods (four for each plot). In each of the three growing seasons, 14 - 16 sample sets were collected. Data were analyzed using the Generalized Linear Model. To avoid the influence of seasonal trends, statistical analyses were calculated separately for each date. ***Results:*** The average number of rove beetle populations in the plots cultivated with Bt-maize did not differ significantly from the number of beetles in the plots cultivated with conventional maize. Significant differences in the number of beetles occurred on individual dates and this was probably due to environmental factors rather than the cultivar effect.	The authors concluded that '*also in our trials no significant differences were recorded in the abundance of the total rove beetle assemblages in Bt-maize with Cry1Ab endotoxin in comparison to conventional cultivars in both research areas. Cases when DKC 3421YG differ significantly from all other objects were confirmed in single date measurements only. They related most often to a considerably smaller number of beetles. Our findings suggest that environmental conditions had the greatest impact on staphylinid assemblages, rather than the crop itself (Bt or isoline)'.*	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Rove beetle population	There are no changes to the conclusions of the safety of the initial risk assessment.

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Cheeke *et al.*, 2013)	***Objective:*** To evaluate arbuscular mycorrhizal fungi (AMF) colonization and growth response for seven different lines of genetically modified *Bacillus thuringiensis* (Bt) maize and five corresponding non-Bt parental isolines, and assess plant growth responses at three different physiological time points. **Experimental Design:** The field experiment was conducted from May to November 2009 near Corvallis, USA. The soil at the field site had a clay loam texture (22% sand, 50% silt and 27% clay), pH 5.7 to 6.1, medium levels of nitrogen and potassium and high levels of available phosphorus. The Bt maize lines used in this study differed in type (sweet maize or field maize), Bt protein expressed (Cry1Ab, Cry34/ Cry35Ab1, Cry1F plus Cry34/35Ab1, Cry1F or Cry3Bb1) and background genetics (P1 to P5), representing a cross-section of the broad range of Bt maize lines commercially available. Five soil samples were collected and pooled to determine initial spore abundance and diversity in each plot prior to planting. Spores were counted and assigned to five different morphological categories based on colour and size. Plants were harvested at 60, 90 and 130 days after sowing when they were in an active growth stage, at tasseling and at maturity, respectively. Plant height and leaf number were recorded 45 days after sowing and at each harvest to determine whether plants with higher levels of AMF colonization exhibited any growth benefits as a result of the symbiosis. Root samples were stained with a trypan blue solution to visualize fungal structures and scored for mycorrhizal fungus colonization using the slide-intersect method. **Results:** Plant growth and AMF colonization did not differ between Bt and non-Bt maize at any harvest period, but AMF colonization was positively correlated with leaf chlorophyll content at the 130 day harvest. Cultivation of Bt maize had no effect on spore abundance and diversity in Bt versus non-Bt plots over one field season. Plot had the most significant effect on total spore counts, indicating spatial heterogeneity in the field.	The authors concluded that: "*although previous greenhouse studies demonstrated that AMF colonization was lower in some Bt maize lines, our field study did not yield the same results, suggesting that the cultivation of Bt maize may not have an impact on AMF in the soil ecosystem under field conditions"*.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Root colonization and growth of arbuscular mycorrhizal fungi	There are no changes to the conclusions of the safety of the initial risk assessment.

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Velasco *et al.*, 2013)	***Objective:*** To evaluate the potential impact of two lines of MON 810 insect resistant *Bacillus thuringiensis* (Bt) maize expressing the Cry 1Ab protein on rhizosphere microorganisms. ***Experimental Design:*** A time-course field experiment was conducted over two years in two fields in Spain, with monthly sampling from April to September. The transgenic (TG) lines were PR33P67 and DKc6575, derived from the unmodified (WT) lines PR33P66 and Tietar, respectively. Rhizosphere soil was collected from TG and WT plants at different sampling times for the analysis of functional and structural soil quality parameters. Soil organic carbon and pH were determined for all replicates and repetitions at each sampling time. Free nitrogen fixation, denitrification and soil respiration were evaluated under laboratory conditions. Total microbial activity, as determined by methyl-[^{3}H]-thymidine and L-[^{14}C]-leucine incorporation in the rhizosphere of the maize lines, was measured. Bacterial and fungal communities were characterized by their metabolic fingerprints. Finally, total DNA was extracted from 0.25 g of rhizosphere soil from the first and last sampling times of each year. Four 16S rRNA gene libraries were generated from TG and WT samples. Amplifications of 16S rRNA genes fragments were carried out using the primers 16sF and 16sR. **Results:** Significant differences between TG and WT were only found in percentage of organic carbon in the first year and in pH in the second year. Total microbial activity was higher in the rhizosphere of the TG plants. Similarly, differences in potential ammonification and nitrification were observed in the second year. In contrast, bacterial and fungal microbial catabolic abilities, were more influenced by sampling time than the transgenic nature of the plants. Microbial community structure was also studied by bacterial and phylum-specific PCR-DGGE and PCR cloning approaches. In general, differences were again more pronounced between sampling times, as opposed to between TG versus WT plants, although marked differences were observed within Betaproteobacteria between plant lines. For the first time, the presence of the *Iamiaceae* family was described in soil.	The authors concluded that: "*the study showed that some important properties of rhizosphere microbes may be impacted by Bt maize cultivation and highlighted the fact that such potential effects need to be viewed within the context of seasonal and spatial variability*".	Environmental	No adverse effects were determined in this study[1]
			Observed parameter	**Feedback on initial environmental risk assessment**
			Soil microbe performance characteristics	There are no changes to the conclusions of the safety of the initial risk assessment.

[1] The authors do not make any conclusions regarding adverse effects of MON 810 on soil microorganisms and note that season and spatial variability, not the presence of the GM trait, were the most important factors for determining the catabolic profile and community

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Ondreickova *et al.*, 2014)	***Objective:*** 1) To compare bacterial communities in the rhizosphere of *Bacillus thuringiensis* (Bt) insect-resistant MON 810 maize and non-genetically modified (GM) hybrids, and 2) to characterize bacteria by creating a 16S rDNA clone library from the rhizosphere of MON 810 maize. ***Experimental Design:*** Experimental fields were located at Borovce in Slovakia. Rhizosphere samples were collected from Bt-maize (DK-C4442YG, MEB483BT and TPA422-H) and conventional (DKC3511) hybrids at four sampling dates (July and September 2008/2009). Each sample was homogenized and metagenomic DNA was extracted by the PowerSoil DNA Isolation Kit. Bacterial 16S rRNA genes were amplified and analysed by Terminal Restriction Fragment Length Polymorphism (T-RFLP). Purified PCR products were digested separately with restriction enzymes *CfoI*, *MspI* and *RsaI*. The rhizosphere sample from Bt maize MEB483BT collected in September 2009 was elected for constructing the 16S rDNA clone library. Twenty clones with specific T-RFLP profiles were sequenced and compared with 16S rDNA sequences available in GenBank database. The clones were digested *in silico* using *CfoI*, *MspI* or *RsaI* to determine the expected sizes of the digested fragments. ***Results:*** T-RFLP analysis yielded between 22 and 65 T-RFs in individual community profiles. Nevertheless, significant differences between samples were not statistically confirmed. The intensities of the fluorescent signals of these T-RFs were different for each sample. Genetic variation evaluated by the Principal Component Analysis indicated that there were no significant differences between bacterial rhizosphere of GM and non-GM plants. Soil bacteria diversity in rhizosphere was influenced by seasonal changes caused by external environmental conditions. The 16S rDNA clone library creation from rhizosphere samples of MON 810 maize followed by DNA sequencing revealed that Proteobacteria were in majority; Actinobacteria, Firmicutes and Chloroflexi were less represented.	The authors concluded that: *"this study did not confirm any changes in the soil ecosystem, which would have been larger than normal variations caused by external conditions"*.	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Soil bacteria diversity	There are no changes to the conclusions of the safety of the initial risk assessment.

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Perez-Hedo *et al.*, 2013)	***Objective:*** To resolve the mechanism underlying the low susceptibility of sixth instar (L6) Armyworm moth (*Mythimna unipuncta*) larvae to *Bacillus thuringiensis* (Bt) Cry1Ab protein present in insect resistant genetically modified maize (MON 810) and determine whether the response depends on amount of protein ingested. ***Experimental Design:*** L6 caterpillars of *M. unipuncta* were exposed to lyophilized leaves of Bt maize, variety PR33P67, containing the transformation event MON 810 and its corresponding near-isogenic line PR33P66. Before reaching the L6 stage, larvae were fed on a semi-artificial diet with lyophilized non-Bt maize leaves. The larvae were then exposed for five days to one of four diets: (i) no Bt leaves (6% commercial organic maize flour and 6% lyophilized non-Bt maize leaves), (ii) low amount of Bt (9% maize flour and 3% lyophilized Bt maize leaves), (iii) medium amount of Bt (6% maize flour and 6% lyophilized Bt maize leaves), and (iv) high amount of Bt leaves (3% maize flour and 9% lyophilized Bt maize leaves). Treatments were compared in three time periods: L6d0 to L6d1, L6d1 to L6d3 and L6d3 to L6d5. Larvae hemolymph, midgut epithelium, peritrophic membrane and content, as well as frass were analysed for Cry1Ab protein concentrations. ***Results:*** *M. unipuncta* larvae fed on the various diets showed few differences in weight gain, duration of development and pupal weight. The larvae rapidly excreted a large part of the protein ingested. The protein was eliminated, degraded or sequestered inside the peritrophic membrane at a rate that increased with the dose and the duration of feeding. As a consequence, little protein reached the midgut epithelium and therefore its binding sites. Moreover, larvae fed on Bt protein recovered quickly when they were transferred to a non-Bt diet.	The authors concluded that: "*these results explain the low susceptibility of L6 M. unipuncta larvae to Bt maize in the field, where the highly mobile larvae can migrate for feeding from one plant to another, or even from one field to another, thus facilitating the ingestion of sublethal amounts of Bt toxin*".	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Sixth instar (L6) Armyworm moth susceptibility	There are no changes to the conclusions of the safety of the initial risk assessment.

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Gonzalez-Cabrera *et al.*, 2013)	***Objective:*** To determine survival and performance of the moth *Mythimna unipuncta* exposed to a *Bacillus thuringiensis* (Bt) maize expressing Cry1Ab protein (MON 810) and its nearest isogenic line. ***Experimental Design:*** A control colony of *M. unipuncta* was established from egg masses collected in a commercial conventional maize field in Monzón (Spain) in 2009. Two maize varieties were used for the study: DKC6041YG (event MON 810) and the near isogenic conventional line DKC6040. Plants were grown in plastic pots and maintained in a greenhouse under controlled conditions. A laboratory colony of the maize pest *Ostrinia nubilalis* (European corn borer) was used as a positive control to verify the biological activity of pure Cry1Ab protoxin (obtained from *Escherichia coli* cultures) and the Bt maize plants used in the study. The susceptibility of neonates from control (MC) and resistant (MR) strains of *M. unipuncta* was compared performing leaf disc bioassays. Five treatments were carried out: (i) Bt maize, (ii) non-Bt maize, (iii) non-Bt maize + Cry1Ab protoxin, (iv) non-Bt maize + Cry1Ab toxin processed with midgut juice from the MC strain and (v) non-Bt maize + Cry1Ab processed with bovine trypsin. Proteolytic activation of the protoxin and binding of active toxin to brush border membrane vesicles were also investigated in the MC and MR strains. ***Results:*** A reduction in the activity of proteolytic enzymes, correlating with impaired capacity of midgut extracts to activate Cry1Ab protoxin, was observed in the resistant strain. Moreover, resistance in larvae of the MR strain was reverted when treated with Cry1Ab toxin activated with midgut juice from the control strain. These data indicate that resistance in the MR strain is mediated by alteration of toxin activation rather than by an increase in proteolytic degradation of the protein. By contrast, binding assays performed with biotin-labelled Cry1Ab protein suggest that binding to midgut receptors does not play a major role in the resistance to Bt maize.	The authors concluded that: *"the results emphasize the risk of development of resistance in field populations of M. unipuncta and the need to consider this secondary pest in ongoing resistance management programs to avoid the likely negative agronomic and environmental consequences"*.	Environment	No resistance has ever been observed in the field for this secondary pest probably at least partly due to low overall selection pressure (typical for secondary pests) and apparent fitness costs when resistance does occur to MON 810 as shown in current manuscript in lab selection experiments.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Survival and performance of the moth *Mythimna unipuncta*	There are no changes to the conclusions of the safety of the initial risk assessment.

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

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Kuramae *et al.*, 2013)	***Objective:*** To examine the potential impact of four maize cultivars, consisting of two genetically modified (GM) and two near-isogenic non-GM lines, on soil-borne fungal communities. ***Experimental Design:*** The maize cultivars used in this study consisted of Monumental MON 810, DK 3421YG and their near isogenic non-GM lines Monumental and DKC 3420. Intact soil cores collected from an organically managed field in September 2009 were transferred to pots to maintain natural stratification and integrity of fungi inhabiting the soil. The standing crop was a grass-clover mixture which had been sown after maize in 2007 and mown twice a year. After 47 days of plant growth (t1), soil samples were collected. One core (diameter 1 cm) per pot was taken and the part originating from 5-11 cm depth was transferred to dry ice and stored at -80°C. After 104 days (t2), samples were again taken as above. At the end of the experiment (plant age 130 days; at full maturity of the ears), total above and below ground plant biomass was harvested. RNA and DNA were extracted, then DNA and cDNA templates derived from each of the soil pots at t1 and t2 were utilized for nucleic acid amplification and 454 pyrosequencing (18S rRNA). A fungal-specific primer set was used to allow for inclusion of the *Glomeromycota* (Arbuscular Mycorrhizal Fungi - AMF). ***Results:*** No significant differences in soil fungal diversity and community structure associated with different plant cultivars were detected. DNA-based analyses yielded lower fungal "operational taxonomic unit" (OTU) richness as compared to RNA-based analyses. Clear differences in fungal community structure were also observed in relation to sampling time and the nucleic acid pool targeted (DNA versus RNA). The most abundant soil fungi, as recovered by DNA-based methods, did not necessary represent the most "active" fungi (as recovered via RNA). RNA-derived community compositions at t1 were highly similar to DNA-derived communities at t2, based on presence/absence measures of OTUs. Large proportions of fungal sequences belonged to AMF and *Basidiomycota*, especially at the RNA level, suggesting that these fungi are not affected by plant cultivar or by GM trait (Bt protein production).	The authors concluded that: *"soil fungal community proved to be dynamic with changes over time across nucleic acid pools, but did not reveal any detectable impact of the maize cultivar or the GM nature of the plant"*.	Environmental	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Diversity and abundance of soil fungi	There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Insect Resistance Management

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Campagne *et al.*, 2013)	***Objective:*** The survival of maize stalk borer (*Busseola fusca*) progeny originating from controlled crosses of resistant and susceptible individuals on maize was analysed to gather new insights on the possible diversity of mechanisms involved in resistance to *Bacillus thuringiensis* (Bt) maize expressing Cry1Ab protein (MON 810). ***Experimental Design:*** Crosses were performed between an *a priori* susceptible population of *B. fusca* originating from Kenya and a population resistant to genetically engineered MON 810 maize originating from South Africa. Larvae were collected from late-planted Bt maize that showed symptoms of stem borer damage. Approximately 300 larvae were collected and reared in the laboratory until the pupal stage. The offspring of these larvae were used to perform the crosses. Adults were paired as follows: Resistant x Resistant (R x R, 9 pairs), Susceptible x Susceptible (S x S, 23 pairs) or Resistant x Susceptible (R x S, 11 pairs). The progeny of each cross was split into two groups of approximately 30 larvae each: one group was reared on MON 810 maize stems during the entire experiment and the other was reared on the corresponding non-Bt isoline. ***Results:*** The success was not homogenous among the crosses as the R x R type performed better than the two others. The low mating success in R x S crosses resulted in susceptible female and resistant male pairs only. In S x S progeny, survival was significantly higher on non-Bt than on Bt maize, but this was not the case for either the R x R or the R x S progeny. In each R x S progeny, the binomial probability to observe an equal or higher level of larval survival under high-dose expectations was close to zero. Consistent with expectations, the susceptible strain reared on Bt maize died at an early stage of the experiment. By contrast, survival was > 50% after 16 days in both the R x R and R x S progenies reared on Bt maize.	The authors concluded that: *"Results show that resistance of B. fusca to Bt maize is dominant, which refutes the hypothesis of recessive inheritance. Survival on Bt maize was not lower than on non-Bt maize for both resistant larvae and the F_1 progeny from resistant x susceptible parents. Hence, resistance management strategies of B. fusca to Bt maize must address non-recessive resistance"*.	Environment	See notes
			Observed parameter	**Feedback on initial environmental risk assessment**
			Susceptibility of *Busseola fusca*	There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Insect Resistance Management

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Kruger *et al.*, 2014)	***Objective:*** To determine whether there are fitness costs associated with the resistance developed by the noctuid maize stem borer (*Busseola fusca*) to *Bacillus thuringiensis* (Bt) maize (MON 810) in South Africa, in the context of insect resistance management strategies. ***Experimental Design:*** Bt resistant larvae were collected in 2010 in the Valhaart irrigation scheme of South Africa where resistance is common. Approximately 300 larvae were reared in the laboratory on either Bt (VAA10Bt-Bt) or non-Bt maize (VAA10Bt-NBt). A Bt-susceptible control population was collected in 2010 at a locality in the Bronkhorstspruit area where there have been no reports of resistance. 150 larvae were reared to the pupal stage on non-Bt maize (BRO10Con-NBt). A bioassay was conducted to determine fecundity of moths of the field-collected populations and their F1generations. The parameters recorded included total number of eggs and egg batches for each female, moth longevity and fertility. Also, neonate larvae (F1-generation) obtained from field collected individuals were used to assess whether fitness costs were associated with survival of Bt-resistant larvae on non-Bt maize under laboratory conditions. The experiment consisted of four treatments comprising a resistant (VAA10Bt) or a control (BRO10Con) population on each of a Bt (MON 810) and non-Bt near iso-hybrid (CRN 3505). The number and mass of live larvae were determined at 3-5 d intervals up to 66 d after egg hatch. The number of larvae that successfully completed their life cycle and changed into pupae was also recorded at each interval. ***Results:*** Except for LT50 values, no fitness costs were associated with the resistance trait in the highly resistant *B. fusca* population. The absence of fitness costs and presence of resistant populations may promote the use of a multi-gene strategy which would be expected to impact negatively on fitness.	The authors concluded that "*While fitness costs were observed in the control population, no costs were observed in the highly resistant population of B. fusca used in this study. The absence of fitness costs in resistant populations and comparative increased in fitness in terms of survival of more susceptible populations necessitates the use of alternative Bt-resistance management strategies, such as a multigene strategy with non-overlapping targets, which would be expected to decrease fitness. For example, introduction of a stacked event which produces more than one protein active against the resistant target pest, together with compliance to the refuge strategy, is most likely the only solution to managing Bt-resistant stem borer populations in South Africa.*"	Environment	No adverse effects were determined in this study
			Observed parameter	**Feedback on initial environmental risk assessment**
			Fitness parameters of *Busseola fusca*	There are no changes to the conclusions of the safety of the initial risk assessment.

Area of the environmental risk assessment: Insect Resistance Management

Publication	Summary of research and results	Conclusion	Protection Goal	Adverse effects
(Crava *et al.*, 2013)	***Objective:*** To examine the variation in tolerance of the European corn borer (*Ostrinia nubilalis*) to Cry1Ab insecticidal protein through a quantitative genetic approach by using a selection strategy based on single-pair mating isolines derived from larvae collected in Spanish commercial maize fields. To assess the association between *cadherin* (*cdh*) gene and Cry1Ab tolerance. ***Experimental Design:*** *O. nubilalis* populations were obtained from diapausing last instar larvae collected in 2004 from two important Spanish maize growing areas, one located in the Northeast and another in the Southwest. For testing larval tolerance, a threshold Cry1Ab concentration of 40 ng/cm² was chosen to produce ca. 90% mortality and ensure the survival of only the most tolerant individuals. A full sibling experimental design was used to investigate the genetic basis of tolerance to Cry1Ab. F1 offspring was tested for susceptibility to trypsin activated Cry1Ab using a concentration that caused a mean larval mortality of 87% (±17% SD). The progeny of the most tolerant isolines (that had shown mortalities lower than 60%) was crossed to obtain the F2 generation that was exposed to the same Cry1Ab concentration. ***Results:*** A clear reduction in mortality (62±17% SD) was observed. The upper limit for heritability was estimated to range between 0.82 and 0.90, suggesting that a high part of phenotypic variation in tolerance to Cry1Ab was attributable to genetic differences. An estimate of the minimum number of segregating factors indicated that the loci involved in tolerance to Cry1Ab were at least two. The role of the *cdh* gene, which is a *Bacillus thuringiensis* resistance gene in Lepidoptera, was assessed in the most tolerant isolines by using an EPIC-PCR marker specifically developed for this study. Association between *cadherin* and tolerance was obtained in one tolerant isoline; however it could be not confirmed by segregation analysis in the F2 progeny because F2 offspring was not viable.	The authors concluded that: "*the results indicate that the tolerance trait is common in Spanish field populations. Quantitative genetic techniques may be helpful for estimating the influence of genetic factors to Cry1Ab tolerance in O. nubilalis*".	Environment	No resistance to MON 810 maize observed so far in Spain.
			Observed parameter	**Feedback on initial environmental risk assessment**
			Ostrinia nubilalis susceptibility	There are no changes to the conclusions of the safety of the initial risk assessment.

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