

**New Bt toxins in the EU GMO  
application process:  
A review of published research into  
impacts on non-target insects and  
ecosystem services**

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This report was prepared by T. H. Schuler  
for the UK Department for Environment, Food and Rural Affairs

## Summary

The bacterium *Bacillus thuringiensis* (Bt) has been used for approximately twenty years as a source of transgenes for the development of insect resistant genetically modified (GM) crops. The first Bt crops considered by advisory biosafety committees in the EU were the maize events 176, MON810 and Bt11. These events express the Bt toxin Cry1Ab which is targeted at the European corn borer, a moth species that causes economic damage in many maize growing regions.

More recently, companies applied to the regulatory authorities in the EU for approval of a GM maize plant (event 1705) expressing a second Bt toxin active against the European corn borer. Another application received by the EU regulatory authorities relates to a maize plant (event MON 863), which expresses a Bt toxin targeted at a beetle pest. Applications are now being received for the marketing and cultivation of further GM crops expressing Bt toxins, which have not previously been assessed by advisory biosafety committees in the EU.

This desk study provides an overview of the published information on the effects of the new Bt events in the EU application system on non-target arthropods and ecosystem services. Potential risks to non-target organisms are an important part of environmental impact assessments, which have to be conducted for each GM crop on a case by case basis. As Bt toxins target specific pest insects, assessments of the risks they pose to non-target insects and related arthropods are thus one of the priorities of an ERA.

This review focuses primarily on the maize events 1507, MON 863, MON 88017, MIR604 and 59122. Event 1507 expressed the lepidopteran-active Cry1F toxin, which is active against a wide range of species in several families of Lepidoptera (the moths and butterflies). The other four maize events all target the same group of beetle pests (corn root worms, *Diabrotica* spp.). Maize event MON 88017 expresses the same Cry3Bb1 toxin as event MON 863. MIR 604 expresses a version of the Cry3A toxin, which has been modified to extend its activity range. Maize event 59122 expresses two toxins in tandem (designated Cry34Ab1/Cry35Ab1), which belong to a family of binary Bt toxins only recently discovered. In addition to events already in the EU application system, this review also considers UK-relevant Bt crops under development and UK pests which may become targets for novel Bt crops in the future.

Current knowledge indicates that the activity spectrum of Cry3Bb1, mCry3A and Cry34Ab1/Cry35Ab1 is limited to a single beetle family (leaf beetles, Chrysomelidae). The activity spectrum of these toxins is thus narrower than the activity spectrum of Cry1Ab and Cry1F, which are both toxic to species in several families of Lepidoptera. MIR 604 appears to be the first case of a GM crop in the regulatory approval process, which expresses a Bt toxin modified to alter its activity spectrum.

In addition to notifications seeking approval for individual GM events, increasing numbers of notifications are being submitted for hybrids derived from crosses between two or more events. The Bt events involved were either crossed with each other, crossed with herbicide tolerant events or, in one case, with an event transformed for a higher lysine content.

For some of the Bt toxins (Cry1Ab and Cry3Bb1) expressed in maize, extensive information on non-target studies is available in form of refereed scientific papers. For others few (Cry1F) or no (mCry3A and Cry34Ab1/Cry35Ab1) studies appear to have been published so far in the refereed scientific literature. In the latter cases the only sources of information on non-target studies available for this review were studies submitted as part of applications for regulatory approval.

The majority of studies do not show any unexpected negative effects on non-target insects or ecosystem functions. For some of the maize events further information on the susceptibility and potential exposure of non-target insects as well as more extensive field trial data would be desirable to increase confidence in the biosafety of the new events. Of particular concern are the relatively high Bt protein levels in pollen of several of the new Bt maize events. Bt toxin expression in pollen has long been controversial as it increases exposure of non-target insects. Scientists have also expressed concern that understanding of the impacts Bt toxins on soil functions is still limited.

Environmental risk assessment studies carried out by applicants currently differ in many respects, including the scale and scope of field studies conducted, appropriateness of indicator species chosen and the extent to which impacts on soil organisms were investigated. It is recommended that standardised guidance is developed regarding the required approaches and standards for non-target studies for the environmental risk assessment of GM crops in the EU.

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# 1. Introduction

*Bacillus thuringiensis* (Bt) is a common and widespread bacterium which produces a range of insecticidal proteins (Glare and O'Callaghan 2000). This bacterium has been used for close to twenty years as a source of transgenes for the development of insect resistant genetically modified (GM) crops (Vaeck *et al.* 1987). Prior to the advent of genetic engineering, a number of microbial insecticides involving different *B. thuringiensis* strains were already in use for the control of lepidopteran, coleopteran and dipteran pests<sup>1</sup> (Glare and O'Callaghan 2000).

The first transgenic Bt crops considered by biosafety committees in the EU were maize plants, which express the lepidopteran-active Bt toxin Cry1Ab (maize events 176, MON810 and Bt11). More recently, an application was considered for cultivation of a maize plant (event MON863) expressing the coleopteran-active Bt toxin Cry3Bba. Applications are now being received from companies wishing to market and cultivate GM crops containing other Bt genes in the EU, which have not previously been assessed by advisory biosafety committees in Europe.

All applications for cultivation of Bt crops in the EU (past and present) involve maize crops targeted primarily against either the European corn borer (*Ostrinia nubilalis*) or corn rootworms (*Diabrotica* spp.) These insect pests are currently not of economic significance in the UK and the Bt crops they target are thus on their own unlikely to be of interest to UK farmers. However, increasing numbers of applications are received for GM crops that are pest resistant as well as tolerant to herbicides (Tables 2 and 3). Herbicide tolerance (HT) is a trait of commercial interest to UK farmers and an approved HT/Bt crop hybrid may conceivably be grown in the UK for the HT trait only.

An environmental impact assessment (ERA) has to be conducted for each GM crop in the application process. Potential risks to non-target organisms are an important part of each ERA. As Bt toxins target specific pest insects, the risks they pose to non-target herbivores as well as other non-target insects (and related arthropods) are a concern.

This desk study provides an overview of the published information on the effects of the new Bt maize events in the EU application system on non-target insects and how they relate to the UK. In addition, Bt crops of potential relevance to the UK in the future and pests that may become targets for Bt crops in the future are considered.

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<sup>1</sup> Lepidoptera = moths and butterflies, Coleoptera = beetles, Diptera = flies

## 2. New Bt events in the EU GMO application process

The Bt toxin most familiar in Europe is Cry1Ab, which is expressed by the Bt maize crops already approved for cultivation in the EU. Cry1Ab is expressed by maize events MON 810, Bt11 and 176.

The new Bt toxins currently in the EU approval process and the pest insects they target are listed in Table 1. Further details on each toxin are provided in the following sections. All new Bt events currently in the EU application process are maize plants (Tables 2 and 3) (apart from applications for three cotton events, which are for derived products and do not require an ERA<sup>2</sup> (pers. comm. Defra)).

The nomenclature of Bt toxins is based on amino acid identity and allows closely related toxins to be ranked together. Each new toxin is assigned a unique name incorporating four ranks (e.g. Cry3Bb1 or Cry51Aa) although often only three ranks are quoted.<sup>3</sup>

*Table 1. New toxins from Bacillus thuringiensis (Bt) expressed in GM crop events currently in the EU application system. The first Bt crops approved in the EU were Bt maize events expressing the Bt toxin Cry1Ab. The main pest targeted by Cry1Ab maize is the European corn borer.*

Bt toxin	Main insect order susceptible to the toxin	Specific target insects
Cry1F	Lepidoptera (moths and butterflies)	European corn borer and <i>Sesamia</i> spp.
mCry3A	Coleoptera (beetles)	Corn root worm
Cry3Bb1	Coleoptera (beetles)	Corn root worm
<b>Cry34Ab1 /Cry35Ab 1<sup>4</sup></b>	Coleoptera (beetles)	Corn rootworm

In addition to notifications seeking approval for individual GM events, a number of notifications have been submitted for hybrids between two or more events (Table 2). The Bt events involved were either crossed with each other, crossed with herbicide tolerant events or, in one case, with an event transformed for a higher lysine content (Tables 2 and 3). Where more than one Bt toxin is combined in a hybrid a combination of a beetle resistant and a moth resistant event were used (Tables 2 and 3). This choice of toxins indicates that the aim of the applicants was to expand the

<sup>2</sup> MON 531 x MON 1445 Cotton (cotton seed); MON 15985 Cotton and MON 15985 x MON 1445 Cotton; 281-24-236/3006-210-23 Cotton

<sup>3</sup> The *Bacillus thuringiensis* Delta-Endotoxin Nomenclature Committee proposed that the use of the quaternary rank (which distinguishes between toxins that are more than 95% identical) is optional, only being used for the sake of clarity. Note that quaternary ranks are assigned to each independently sequenced toxin gene. So some toxins may be identical despite having different quaternary ranks.

<sup>4</sup> Binary toxin

activity spectrum of their maize plants rather than to delay the onset of pest resistance<sup>5</sup>.

Table 2. Single Bt events and hybrids involving Bt genes currently in the EU application system<sup>6</sup> (IR, insect resistance; HR, herbicide tolerance; QT, quality trait)

	Transformation events	Traits
Single events	1507 maize	IR (Cry1F) & HT (glufosinate)
	MIR604 maize	IR (Cry3A)
	MON88017 maize	IR (Cry3Bb1) & HT (glyphosate)
	59122 maize	IR (Cry34Ab1/Cry35Ab1)
Hybrids of two events	59122 x NK603 maize	IR (Cry34Ab1/Cry35Ab1) X HT (glyphosate)
	1507 x NK603 maize	IR (Cry1F) & HT (glufosinate) X HT (glyphosate)
	NK603 x MON810 maize	IR (Cry1Ab) X HT (glyphosate)
	MON863 x MON810 maize	IR (Cry3Bb1) X IR (Cry1Ab)
	MON863 x NK603 maize	IR (Cry3Bb1) X HT (glyphosate)
	NK603 x MON810 maize	IR (Cry1Ab) X HT (glyphosate)
	LY038 x MON 810 maize	IR (Cry1Ab) X QT (higher lysine content)
	59122 x NK603 maize	IR (Cry 34Ab1/Cry35Ab1) X HT (glyphosate)
	1507 x 59122 maize	IR (Cry1F) & HR (glufosinate) X IR (Cry 34Ab1/Cry35Ab1)
	1507 x NK603 maize	IR (Cry1F) & HT (glufosinate) X HT (glyphosate)
Hybrid of three events	1507 x 59122 maize	IR (Cry1F) & HT (glufosinate) X IR (Cry 34Ab1/Cry35Ab1)
	MON 88017 x MON 810 maize	IR (Cry3Bb1) X IR (Cry1Ab)
	MON863 x MON810 x NK603 maize	IR (Cry 3Bb1) X IR (Cry1Ab) X HT (glyphosate)
	59122 x 1507 x NK603 maize	IR (Cry 34Ab1/Cry35Ab1) X IR (Cry1F) & HT (glufosinate) X HT (glyphosate)
	59122 x 1507 x NK603 maize	IR (Cry 34Ab1/Cry35Ab1) X IR (Cry1F) & HT (glufosinate) X HT (glyphosate)

<sup>5</sup> Modelling has predicted that it is much more difficult for a pest insect to adapt to two Bt toxins with different modes of action simultaneously than to a single Bt toxin (e.g. Roush *et al.* 1998). The stacking of two Bt toxins (targeted at the same pest but with different modes of action) in transgenic crops has therefore been recommended from the point of view of insect resistance management.

<sup>6</sup> Based on information provided by Defra.



Table 3. Status of notifications for single events and hybrids involving Bt toxin genes in the EU (information provided by Defra)

Dossier Reference number	Scope of application	Trait /Species (Brief description of Product)	Insect resistance traits					Higher lysine content	HT traits		Applicant	Status
			Cry1Ab	Cry1F	mCry3A	Cry3Bb1	Cry34Ab1/Cry35Ab1		glufosinate	glyphosate		
EFSA/GMO/UK/2004/01	- Food, feed	NK603 x MON810 Maize (glyphosate X Cry 1Ab)	█						█	Monsanto	Overall opinion published	
EFSA/GMO/NL/2004/02	- Food	1507 Maize (glufosinate; Cry 1F)		█				█		Pioneer Hi-Bred /Mycogen Seeds	Overall opinion published	
EFSA/GMO/DE/2004/03	- Food, feed	MON863 x MON810 Maize (Cry 3Bb1 X Cry 1Ab)	█			█				Monsanto	Overall opinion published	
EFSA/GMO/UK/2004/05	- Food, feed - Import and processing	1507 x NK603 Maize (glufosinate,Cry 1F X glyphosate)		█				█	█	Pioneer Hi-Bred / Mycogen Seeds	Overall opinion published	
EFSA/GMO/UK/2004/06	- Food, feed - Import and processing	MON863 x NK603 Maize (Cry 3Bb1 X glyphosate)				█			█	Monsanto	Overall opinion published	
EFSA/GMO/BE/2004/07	- Food, Feed - Import and processing	MON863 x MON810 x NK603 Maize (Cry 3Bb1XCry1AbXglyphosate)	█			█			█	Monsanto	Overall opinion published	
EFSA/GMO/UK/2005/11	- Food, feed - Import and processing	MIR604 Maize (Cry 3A)			█					Syngenta Seeds	Valid application (16/9/2005)	
EFSA/GMO/NL/2005/12	- Food, feed - Import and processing	59122 Maize (Cry 34Ab1 & Cry35Ab1)					█			Pioneer Hi-Bred /Mycogen Seeds	Valid application (16/9/2005)	
EFSA/GMO/NL/2005/15	- Food, feed - Import and processing	1507 x 59122 Maize (glufosinate & Cry 1F X Cry 34Ab1 & Cry35Ab1)		█				█		Dow AgroSciences / Pioneer Hi-Bred	Under completeness check	
EFSA/GMO/UK/2005/17	- Food, feed - Import and processing - Cultivation	1507 x NK603 maize (glufosinate,Cry 1F X glyphosate)		█				█	█	Pioneer Hi-Bred / Mycogen Seeds	Valid application (10/3/2006)	

Table 3. Status of notifications for single events and hybrids involving Bt toxin genes in the EU (cont.)

Dossier Reference number	Scope of application	Trait /Species (Brief description of Product)	Insect resistance traits					Higher lysine content	HT traits		Applicant	Status
			Cry1Ab	Cry1F	mCry3A	Cry3Bb1	Cry34Ab1/ Cry35Ab1		glufosinate	glyphosate		
EFSA/GMO/UK/2005/20	- Food, feed - Import and processing	59122 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X glyphosate)									Pioneer Hi-Bred	Under completeness check
EFSA/GMO/UK/2005/21	- Food, feed - Import and processing	59122 x 1507 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X Cry1F & glufosinate X glyphosate)									Pioneer Hi-Bred	Under completeness check
EFSA/GMO/NL/2005/23	- Food, feed - Import and processing - Cultivation	59122 maize (Cry 34Ab1 & Cry35Ab1)									Pioneer Hi-Bred /Mycogen Seeds	Under completeness check
EFSA/GMO/NL/2005/26	- Cultivation	NK603 x MON810 maize (glyphosate X Cry1Ab)									Monsanto	Under completeness
EFSA/GMO/CZ/2005/27	- Food, feed - Import and processing	MON 88017 maize (glyphosate & Cry3Bb1)									Monsanto	Under completeness check
EFSA/GMO/NL/2005/28	- Food, feed - Import and processing - Cultivation	1507 x 59122 maize (Cry1F & glufosinate X Cry 34Ab1 & Cry35Ab1)									Dow AgroSciences	Under completeness check
EFSA/GMO/UK/2006/29	- Food, feed - Import and processing - Cultivation	59122 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X glyphosate)									Pioneer Hi-Bred	Under completeness check
EFSA/GMO/UK/2006/30	- Food, feed - Import and processing - Cultivation	59122 x 1507 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X Cry1F & glufosinate X glyphosate)									Pioneer Hi-Bred	Under completeness check
EFSA/GMO/NL/2006/32	- Food, feed - Import and processing	LY038 x MON 810 maize (higher lysine content X Cry1Ab)									Renessen Europe	Under completeness check
EFSA/GMO/CZ/2006/33	- Food, feed - Import and processing	MON 88017 x MON 810 maize (Glyphosate & Cry3Bb1 X Cry1Ab)									Monsanto	Under completeness check

## 3. Published information on the effects of the new Bt events on non-target insects and ecosystem services

### 3.1 Background and non-target studies with Cry1Ac maize

Some of the new Bt events express Bt toxins, such as Cry3A, that have been used for some years in crop protection as components of microbial non-GM insecticides<sup>7</sup>. Others, such as the binary toxin Cry34Ab1/Cry35Ab1, have not been used in such a way before although they are produced naturally by strains of the bacterium. There do not seem to be any reports of unexpected major negative effects of microbial Bt insecticides on non-target insects (Glare and O'Callaghan 2000) and they are used in integrated pest management programme and in organic crops.

In the early days of registration environmental risk assessment of GM crops was mainly based on methodologies developed for the risk assessment of pesticides. Over a decade has passed since and considerable knowledge and experience has been gained, resulting in several research groups recommending improvement to the environmental risk assessment of GM crops (e.g. Schuler *et al.* 2000, Firbank *et al.* 2005, Scholte and Dicke 2005, Andow and Zwahlen 2006, Romeis *et al.* 2006). Not all companies bringing GM crops to market have taken the recommendations on board to the same extent. And only some companies have published their risk assessment studies in refereed scientific journals. There are currently initiatives by OECD<sup>8</sup>, IOBC<sup>9</sup> and COGEM<sup>10</sup> to develop internationally accepted standards for guidelines and methodologies.

Risk is a function of hazard and exposure. Individual Bt toxins have a narrow activity spectrum compared to the majority of synthetic insecticides, but they can present a hazard to non-target insects, especially species related to the target pests. In the case of Cry1Ab and Cry1F such relatives are other moths and butterflies. Moths and butterflies are herbivorous species that can be exposed to the transgene product through feeding on the GM plant itself, on its pollen or on other plants contaminated with pollen from the GM plant. In the case of Cry3A, Cry3Bb1 and the binary toxin Cry34Ab1/Cry35Ab1 close relatives of the target species are other beetle species. Beetles show more variation in their diets than the Lepidoptera and non-target beetle species include not only herbivores but also a wide variety of predatory beetle species. Non-target beetle species can thus be exposed to the transgene product when feeding on the GM plant itself, on its pollen, on other plants contaminated with pollen from the GM plant or on prey that has ingested the transgene product.

The exposure can be estimated by considering the distribution of the crop, the occurrence and distribution of the non-target species, its life cycle and behaviour. Herbivorous non-target species that may be affected by Bt pollen contaminating their food plant, such as butterflies, have received most attention. The most comprehensive risk assessments for these have estimated potential environmental exposure by taking into account levels of pollen expression, timing, duration and intensity of pollen shed, pollen dispersal, distribution of the crop, occurrence, distribution and behaviour of the non-target species and its host plant and the

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<sup>7</sup> Microbial Bt insecticides are produced through mass production of the specific (naturally occurring) Bt strain. Strains of Bt tend to produce a mixture of toxins. E.g. Cry1Ab is one of several toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* strain HD1, the most widely used Bt strain used for control of lepidopteran pests.

<sup>8</sup> <http://www.oecd.org/biotrack/>

<sup>9</sup> [http://www.gmo-guidelines.info/public/about\\_project/index.html](http://www.gmo-guidelines.info/public/about_project/index.html), Birch *et al.* (2004)

<sup>10</sup> <http://www.cogem.net/page.ocl?pageid=46&loc=5&version=&mode=>

susceptibility of the non-target 'relative'. Examples are the assessment of the risk Cry1Ab maize poses for the monarch butterfly in the USA (Sears *et al.* 2001, Dively *et al.* 2004), Cry1F maize poses for a species of blue butterfly in Japan (Wolt *et al.* 2005) and Cry1Ab maize event 176 poses for two species of Lepidoptera in Germany (see below).

Thirty-six species of Lepidoptera were found in maize field margins in south-eastern Germany in a study by the Bavarian State Research Center for Agriculture (LfL). Twenty-eight percent of these were of conservation concern (LfL 2005). Two species selected for further study were the swallowtail (*Papilio machaon*) and the garden tiger moth (*Arctia caja*)<sup>11</sup>. The majority of the populations of both species flies in July when 91% of maize crops are in flower in Bavaria. In laboratory bioassays, the consumption of pollen of maize event 176 had sublethal and lethal effects on swallowtail larvae, depending on pollen dose. No negative effects were observed on larvae of the garden tiger moth. In two field experiments, no effect of Bt maize on swallowtail larvae could be found. However, natural mortality was very high in the field (up to 95%) and the authors of the study suggested that this may have masked any effect of Cry1Ac containing pollen. Analysis of the sample size required to statistically validate a 10% reduction in swallowtail populations indicated that 1728 field margins would have to be sampled. Lang *et al.* suggested that due to the flowering period of maize and the extent of the pollen deposition in field margins, exposure of non-target butterfly larvae appears highly probable. The authors suggested that either only Bt maize plants with low pollen expression (such as MON 810 and Bt11) are planted or, alternatively, that a 10 m wide buffer zone of conventional maize should be grown around Bt maize fields (Lang 2004, Lang *et al.* 2004, LfL 2005, Lang and Vojtech 2006).

The susceptibility of rare non-target species can not be established as their larvae are not available for bioassays (EPA 2001). As it is usually not possible to establish the susceptibility of a whole group of non-target species, the recommended practice is to select some species as indicators. In the case of butterflies, some applicants have addressed this issue by conducting a risk assessment based on the estimated susceptibility of potentially exposed non-target species based primarily on data from pest species (Section 3.2.1).

There is already controversy about whether the levels of Cry1Ab toxin expression in the older Bt events pose a significant risk to non-target species related to the target pest or not (EFSA 2005a, LfL 2005, Wolt *et al.* 2005, Felke 2006, Lang and Vojtech 2006). Somewhat surprisingly, many new Bt events in the EU application system appear to contain relatively high levels of Bt toxin in pollen, often more than event 176 (Annex 1).

There is little evidence that Bt toxins are directly toxic to non-targets in insect orders other than that of the target pests (O'Callaghan *et al.* 2005). However, indirect effects can occur through reduced prey (or host) quantity and quality (Schuler *et al.* 1999b) or changes in management. Impacts caused by changes in management due to the use of the Bt crop are by many scientists judged as positive if the Bt crop replaces sprays of broad spectrum insecticides (Fitt and Wilson 2000, Romeis *et al.* 2006). However, criticism has been voiced regarding the use of Bt maize in years of low

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<sup>11</sup> In the UK, the garden tiger moth (*Arctia caja*) was once a common and widespread moth but this species has declined in recent years due to changes in winter weather conditions (Conrad *et al.* 2002). In the UK, the swallowtail butterfly (*Papilio machaon*) is confined to the Broadland area of Norfolk (Hoole *et al.* 1999).

pest pressure (*O. nubilalis*)<sup>12</sup> (Obrycki *et al.* 2001). However, such side effects should be judged in the context of other control measures employed, such as synthetic insecticides or non-GM insect resistant plants, and are not entirely avoidable.

An extensive body of research data has been assembled on non-target impacts of the Cry1Ab expressing maize events MON810, 176 and Bt11 (e.g. Hilbeck *et al.* 1998a, Hilbeck *et al.* 1998b, Lozzia *et al.* 1998, Losey *et al.* 1999, Lozzia 1999, Saxena *et al.* 1999, Hansen Jesse and Obrycki 2000, Lozzia *et al.* 2000, Manachini 2000, Sears and Stanley-Horn 2000, Zwahlen *et al.* 2000, Head *et al.* 2001, Hellmich *et al.* 2001, Meier and Hilbeck 2001, Obrycki *et al.* 2001, Raps *et al.* 2001, Zangerl *et al.* 2001, Bourguet *et al.* 2002, Dutton *et al.* 2002a, b, Felke *et al.* 2002, Manachini and Lozzia 2002, Dutton *et al.* 2003, Manachini 2003, Musser and Shelton 2003, Zwahlen *et al.* 2003, Birch *et al.* 2004, Dively *et al.* 2004, Manachini and Lozzia 2004, Sanders *et al.* 2004, Saxena *et al.* 2004, Stotzky 2004, LfL 2005, Eder 2006, Lang and Vojtech 2006, Obrist *et al.* 2006, Romeis *et al.* 2006; see also references listed in EFSA (2005a) and LfL (2005)). One important lesson is that minor negative effects observed in the laboratory do not necessarily translate into impacts in the field where many other factors affect the fitness of non-target species (including climate, food availability and predation).

Exposure and impacts on non-target soil organisms are less well understood than impacts on above ground organisms, mostly due to the heterogeneity of the soil environment, the complexity of soil communities and the lack of reliable indicator species for important ecological processes. The same applies to impacts of other agricultural practices on soil organisms. Some studies report rapid degradation of Bt toxins that enter soils through plant residues while others report that Bt toxin can persist in soils. Persistence depends on the interactions between many variables such as biotic activity, soil type, crop management practices as well as environmental conditions, and therefore may vary between sites and seasons (O'Callaghan *et al.* 2005).

Despite persistence in soil in some situations, Cry1Ab from Bt maize appears to have little impact on soil organisms such as earthworms, collembolans and general soil microflora (O'Callaghan *et al.* 2005, Vercesi *et al.* 2006). A recent study reported lower reproduction efficiencies in a bioassay with the bacteriophagous model nematode species *Caenorhabditis elegans* in soil from Bt maize fields, which could be a sign of a direct toxic effect from Cry1Ab. The significance of the results for the field are not yet clear (LfL 2005). Another recent study suggested that short Cry1Ab-maize-induced ecological shifts occur in the microbial communities of croplands' soils (Mulder *et al.* 2006).

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<sup>12</sup> Populations of the European corn borer fluctuate considerably between years and do not cause economic damage every year. It has not yet been possible to predict in advance of planting whether a season is likely to see economic damage to maize crops or not. Farmers therefore use Bt maize preventively rather than based on actual pest pressure.

### 3.2 Cry1F - expressed by events 1507 and 6275

The cry1F gene expressed in maize event 1507<sup>13</sup> (and more recently in maize event 6275<sup>14</sup>) was derived from *Bacillus thuringiensis* var. *aizawai* (Health Canada 2002). Cry1F is known to be active against species in several families of the order Lepidoptera (moths and butterflies) (Wolt *et al.* 2005, Natural Resources Canada 2006). Cry1F is one of a range of Bt toxins present in microbial (non-GM) *Bacillus thuringiensis* insecticides (CABI 1997, Health Canada 2002). The sensitivity of lepidopteran species to Cry1F varies with insect species. A study by Wolt *et al.* (Wolt *et al.* 2005) demonstrated more than three orders of magnitude variation in sensitivity to Cry1F in the different lepidopteran species tested.

The application for maize event 1507 to the EU regulatory authorities has been submitted jointly by Pioneer Hi-bred International and Mycogen Seeds (c/o Dow AgroSciences). EFSA published its opinion on maize event 1507 for cultivation (dossier EFSA/GMO/UK/2004/01) in 2005 (EFSA 2005b). Event 1507 has been crossed with several other GM maize events to produce hybrids that are resistant to a wider range of pest insects and/or herbicides (Table 4). No notification for event 6275 has been received by the EU authorities so far.

Event 1507 has authorisation for cultivation in the US, Canada, Japan and Argentina, and event 6275 for the US and Canada. The hybrids 1507 x 59122 x NK603, 1507 x 59122 and 1507xMON603 have authorisation for cultivation in Japan<sup>15</sup>.

According to Health Canada (2002) the protein encoded by the truncated cry1F gene in event 1507 is nearly identical to the first 605 amino acids of the Cry1F protein protoxin produced by *B. thuringiensis* var. *aizawai*. This protein corresponds to the insecticidally active portion of the delta endotoxin that remains following cleavage in the gut of lepidopteran larvae. The gene sequences for both cry1F (as well as that of the pat selectable marker gene) were optimized for improved expression in plants (Health Canada 2002).

EPA reported in 2001 that Cry1F was expressed throughout the plant. Pollen expression levels of Cry1F in 1507 have been reported to be on average 20.0 ng/mg (dry weight) (EPA 2001) (Annex 1).

Few studies have been published in the refereed scientific literature on the effects of Cry1F on non-target invertebrates nor on persistence and impact of Cry1F in soil.

Cry1F is predominantly a lepidopteran-active Bt toxin, like the well-studied Cry1Ab toxin. To a certain extent results from Cry1Ab can be projected to Cry1F (e.g. in terms of resistance management or indirect impacts on non-targets). However, the two toxins vary in their activity spectrum and more studies with non-target species are recommended to investigate toxicity of Cry1F to European non-target butterflies. It would be unrealistic to assume that low toxicity of Cry1F to the monarch butterfly automatically translates into a low toxicity to other non-target butterflies.

Bt toxins can vary considerably in their activity spectrum (Glare and O'Callaghan 2000) and there is emerging evidence that they may differ in their release from roots (Saxena *et al.* 2004). Caution should therefore be exercised in projecting results from

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<sup>13</sup> OECD identifier DAS-Ø15Ø7-1 (<http://www.agbios.com>)

<sup>14</sup> OECD identifier DAS-Ø6275-8 (<http://www.agbios.com>)

<sup>15</sup> Agbios GM Database, <http://www.agbios.com>, accessed 15 November 2006

Table 4. Dossiers for Cry1F expressing GM crops in the EU

Dossier Reference number	Scope of application	Trait /Species (Brief description of Product)	Insect resistance traits		HT traits		Applicant	Status
			Cry1F	Cry34Ab1/Cry35Ab1	glufosinate	glyphosate		
EFSA/GMO/NL/2004/02	- Food	1507 Maize (glufosinate; Cry 1F)	■		■		Pioneer Hi-Bred /Mycogen Seeds	Overall opinion published
EFSA/GMO/UK/2004/05	- Food, feed - Import and processing	1507 x NK603 Maize (glufosinate,Cry 1F X glyphosate)	■		■	■	Pioneer Hi-Bred / Mycogen	Overall opinion published
EFSA/GMO/NL/2005/15	- Food, feed - Import and processing	1507 x 59122 Maize (glufosinate & Cry 1F X Cry 34Ab1 & Cry35Ab1)	■	■	■		Dow AgroSciences / Pioneer Hi-Bred	Under completeness check
EFSA/GMO/UK/2005/17	- Food, feed - Import and processing - Cultivation	1507 x NK603 maize (glufosinate,Cry 1F X glyphosate)	■		■	■	Pioneer Hi-Bred / Mycogen Seeds	Valid application
EFSA/GMO/UK/2005/21	- Food, feed - Import and processing	59122 x 1507 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X Cry1F & glufosinate X glyphosate)	■	■	■	■	Pioneer Hi-Bred	Under completeness check
EFSA/GMO/NL/2005/28	- Food, feed - Import and processing - Cultivation	1507 x 59122 maize (Cry1F & glufosinate X Cry 34Ab1 & Cry35Ab1)	■	■	■		Dow AgroSciences	Under completeness check
EFSA/GMO/UK/2006/30	- Food, feed - Import and processing - Cultivation	59122 x 1507 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X Cry1F & glufosinate X glyphosate)	■	■	■	■	Pioneer Hi-Bred	Under completeness check

Bt maize events expressing Cry1Ab (176, Bt11 and MON810) to event 1507 and other new events expressing different Bt toxins.

### 3.2.1 Risks to non-target species related to the target pest

Maize pollen is shed in large quantities during the flowering of maize and can contaminate vegetation in and near maize fields where the pollen can be consumed by non-target herbivores. In addition, pollen is collected directly from the maize plant by a wide range of insects, including pollinators, predatory insects and pest insects.

Event 1507 expresses Cry1F in pollen, which extends the range of non-target insects exposed to Cry1F in the field compared to other maize events that have no or negligible transgene expression in pollen (Annex 1). A particular concern is the potential risk to non-target butterflies and moths as they belong to the same insect order as the pest insects targeted by Cry1F. Cry1F levels in pollen of 1507 have been reported to be 31-33 µg/g<sup>16</sup> compared to 1.1-7.1 µg Cry1Ab/g fw pollen in 176 maize (Annex 1). Cry1F levels in 1507 pollen are high enough to cause 100% mortality of larvae of the wax moth (*Galleria mellonella*), a lepidopteran pest of bee hives (Hanley *et al.* 2003).

Another lepidopteran-active Bt toxin, Cry1Ab, has already given rise to concern regarding its potential impact on butterfly larvae feeding on wild plants in and around Bt maize fields. Several well-known butterfly species are susceptible to Cry1Ab, including the peacock (*Inachis io*), the common swallowtail (*Papilio machaon*) (Lang and Vojtech 2006) and the monarch (*Danaus plexippus*) (Sears *et al.* 2001). However, only one event (176) of the registered Cry1Ab-expressing Bt maize events had significant levels of Cry1Ab expression in pollen (this event is not produced anymore).

Cry1F and Cry1Ab differ in their activity spectrum against lepidopteran larvae. For example, Cry1F is far less toxic to larvae of the monarch butterfly than Cry1Ab (Hellmich *et al.* 2001)<sup>17</sup> but more toxic than Cry1Ab to some other species of Lepidoptera (e.g. over eight-fold more active against *Spodoptera littoralis*) (Natural Resources Canada 2006).

No data on toxicity of Cry1F to European non-target Lepidoptera appear to be available. Cry1F appears to be toxic to at least some species of lycaenid butterflies (Herrero *et al.* 2002, Wolt *et al.* 2005) although no lycaenids native to the UK appear to have been tested so far. The Bt Toxin Specificity Database<sup>18</sup> lists test results for Cry1F for 23 species, 22 of which are lepidopteran pest species (the 23<sup>rd</sup> species is the monarch). It is thus not clear which non-target butterfly and moth species present in or near maize fields in the UK may be sensitive to Cry1F.

During the Farm-scale Evaluations of GMHT crops no butterfly species of conservation concern were observed in UK maize fields or their margins. The FSE showed that common butterflies associated with maize field margins in England and Scotland include the large white (*Pieris brassicae*), small white (*Pieris rapae*), green-veined white (*Pieris napi*), small tortoiseshell (*Aglais urticae*), peacock (*Inachis io*), gatekeeper (*Pyronia tithonus*), meadow brown (*Maniola jurtina*) and ringlet (*Aphantopus hyperantus*) (Roy *et al.* 2003). These butterfly species occur also widely in continental Europe.

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<sup>16</sup> The source did not specify if these figures refer to dry or fresh weights.

<sup>17</sup> A study submitted by Dow AgroSciences to EPA showed that Cry1F caused some growth inhibition at 10,000 ng/ml diet in monarch larvae but no mortality. Since pollen doses equivalent to 10,000 ng/ml diet are not likely to occur on leaves of the monarch's host plants in the field, EPA concluded that Cry1F protein will not pose a risk to this species of butterfly (EPA 2001)

<sup>18</sup> [http://www.glfsc.forestry.ca/science/research/netintro99\\_e.html](http://www.glfsc.forestry.ca/science/research/netintro99_e.html) (accessed 11 Oct 2006)

Pollen from another Bt maize, the Cry1Ab-expressing maize event 176, is known to be toxic to larvae of the peacock butterfly (LC<sub>50</sub> = 61 pollen grains per second instar larva<sup>19</sup>) (Felke and Langenbruch 2003).

The applicant seeking registration for 1507 has chosen a modelling approach that used a hypothetical non-target Lepidoptera species. The susceptibility of this hypothetical species to Cry1F was based on the known susceptibility of 15 pest species as well as that of the monarch and chosen to be the 90<sup>th</sup> percentile (LC<sub>50</sub> = 0.67 µg/g) (Dow Agro Sciences 2003). Although this LC<sub>50</sub> is over 15-fold lower than the species geometric mean of 10.1 µg/g Cry1F, it is ten times higher than the LC<sub>50</sub> of the most susceptible species tested (the diamondback moth, *Plutella xylostella*).

Wolt *et al.* (2005) conducted an ecological risk assessment (based on a conceptual model adapted from Sears *et al.* (2001)) with a species of blue butterfly that occurs commonly in Japan. *Pseudaonzeeria maha* (Lycaenidae) was found to be susceptible to Cry1F and Wolt *et al.* investigated the probability for spatial-temporal co-occurrence of the life stages of concern (1<sup>st</sup> and 2<sup>nd</sup> instar) and the stressor (Cry1F protein expressed in pollen) at environmentally relevant concentrations. The authors came to the conclusion that projected levels of exposure to Cry1F pollen were below the toxicity level of concern and that the risk to *P. maha* was probably negligible.

In the US, examination of an overlay map of the distribution of endangered lepidopteran species relative to maize production areas showed that in the US endangered or threatened species of Lepidoptera do not occur in agricultural areas where maize is grown; nor is maize considered a host plant for these species. The overlay map when combined with restricted habitat range indicated that any potential concern for endangered or threatened butterfly species in the US was restricted to the Karner blue butterfly (*Lycaeides melissa samuelis*, Lycaenidae). The toxicity of Cry1F to the Karner blue could not be tested due to its endangered status. Because of the potential for Cry protein containing pollen to affect this butterfly adversely, it was not permitted to grow Cry1F maize near habitats of the Karner blue butterfly in US counties where the butterfly is known to exist in scattered populations. This precaution was taken in view of the lack of adequate data from the field indicating the precise proximity of wild lupine, the butterfly's food plant, to maize fields in those counties (EPA 2001).

### **3.2.2 Risks to other non-target species and ecosystem services – above ground**

The data available for Cry1F indicate that the activity range of this Bt toxin is restricted to the insect order Lepidoptera. Studies reported so far for non-target insects other than Lepidoptera are described below.

Hanley *et al.* (2003) investigated the effects of maize 1507 pollen on the honey bee (*Apis mellifera*). They measured larval and pupal mortalities, pupal weight and haemolymph protein concentration of newly emerged adults after they were fed Cry1F maize pollen as larvae (in comparison to non-transgenic maize pollen, Cry1Ab containing pollen or mixed bee pollen). The authors found no significant differences in the parameters tested between different kinds of maize pollen (Hanley *et al.* 2003). EPA (EPA 2001) also reported that the Agency reviewed a capped honey bee brood cell study in which bee larvae were fed Cry1F corn pollen and pure Cry1F protein. The bees showed normal larval development and emergence of healthy adult honey

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<sup>19</sup> LC<sub>50</sub>= concentration that kills 50% of the test organisms; a measure widely used to express toxicity of a compound

bees. EPA concluded that the data adequately addressed potential toxicity concerns for foraging honey bees exposed to Cry1F protein expressed in maize pollen in the field (EPA 2001).

EPA (2001) further reviewed a study with adult ladybirds (*Hippodamia convergens*) as an indicator species for predatory beetles. The adult beetles were fed a concentration of Cry1F protein at 15x the expected rate found in maize pollen. This resulted in no mortality or signs of toxicity over a 29 day period. Another experiment involved predatory green lacewing larvae, which were also fed a concentration of Cry1F protein at 15x the expected rate found in maize pollen.<sup>20</sup> This dose reportedly did not result in mortality or signs of toxicity due to feeding on Cry1F over a 13 day period (EPA 2001).

The applicant also submitted results of an experiment with a parasitic wasp, the hymenopteran *Brachymeria intermedia*. According to EPA the wasps were fed Cry1F at a concentration “10x the expected rate found in corn pollen”, which showed no mortality or signs of toxicity over a period of twelve days. EPA concluded that no adverse impacts on parasitic wasps were to be expected from field exposure to Cry1F maize (EPA 2001) The choice of indicator species is somewhat surprising as *B. intermedia* is a natural enemy of the gypsy moth (*Lymantria dispar*) (Kerguelen and Carde 1996), a forest pest, and thus not part of the maize ecosystem. Also, parasitoids do not normally feed on pollen. Parasitoid larvae are the most likely stage to be exposed to Cry1F and they only feed on host tissues. Adult parasitoids may be exposed through feeding on nectar or, in some species, through feeding on host haemolymph (Schuler *et al.* 1999b, Groot and Dicke 2002).

EPA reviewed a field study conducted by the applicant and concluded that the GM maize line 1507 does not negatively affect the number of beneficial arthropods in the field. Beneficial insects assessed in this study included several groups and species of predatory arthropods (ladybirds (*Cycloneda munda* and *Coleomegilla maculate*), carabid beetles, brown lacewings (Hemerobiidae), the green lacewing *Chrysoperla plorabunda*, minute pirate bugs (*Orius insidiosus*), assassin bugs (Reduviidae), damsel bugs (Nabidae), damselflies, dragonflies as well as spiders) and parasitic wasps (Ichneumonids and Braconids). EPA, however, recommended that the monitoring of beneficial insects should continue into the first few years of commercial use of Cry1F maize crops to confirm the findings of the single season experiment and to gather data on long range non-target insect effects and abundance (EPA 2001). No field study with Cry1F maize and non-target insects appears to have been published in the refereed scientific literature.

Bt maize events approved previously in the EU do not contain Bt toxin in the phloem (Head *et al.* 2001, Raps *et al.* 2001). Aphids, an important group of prey species for natural enemies, therefore do not ingest Bt toxin when feeding on Bt maize. This lack of phloem expression reduces the exposure of a number of natural enemies present in maize fields to Bt toxin. It is not clear whether Cry1F is present in the phloem of event 1507.<sup>21</sup>

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<sup>20</sup> No details of methodology of the lacewing study were provided by this source. However, the US regulatory agencies criticised the methodology for a similar lacewing study in a later application as inadequate (section 3.5.2) (APHIS 2005).

<sup>21</sup> No published information appears to be available regarding the absence or presence of Bt toxin in the phloem of other novel Bt maize events either.

### 3.2.3 Risks to other non-target species and ecosystem services – below ground

Planting of event 1507 maize will result in Cry1F being added to soils through incorporation of crop debris, pollen or root tissue. In addition 1507 may release Cry protein in root exudates like the earlier Cry1Ab maize events, although it is not clear whether this process occurs in all Bt maize events (Saxena *et al.* 2004). EFSA have reviewed the studies available for event 1507 and other Bt maize events and published its opinion on 1507 in 2005 (EFSA 2005b). No new data appear to have been published on Cry1F expressing plants since.

EPA estimated that the amounts of Cry1F protein added to the soil of fields planted with 1507 maize would be c. 20.5 g/acre and expects the maximum environmental concentration of Cry1F to be 23 mg /kg dry soil (excluding any additional Cry protein released through root exudation). EPA requested from the applicant to provide further data on Bt toxin exudation by roots as well as Cry1F protein expression in plant roots (EPA 2001).

A laboratory study published in 2001 investigated the degradation of full-length Cry1F protein in representative soil from the mid-south cotton-growing area of the United States. The half-life of microbial-produced Cry1F protein was estimated to be less than 1 day under laboratory conditions (Herman *et al.* 2001).<sup>22</sup>

A study submitted by the applicants for the approval of event 1507 in the US indicated that purified Cry1F proteins incorporated into test soils biodegraded with a half-life of approximately 3.13 days (EPA 2001). EPA criticised the study noting that it failed to address adequately the duration and the amount of residual Cry 1F protein in the soil. The agency asked for a soil degradation study to be carried out for a longer period of time to determine the duration and the amount of residual Cry1F protein in agricultural soil. Also, the soil used should be actual field soil containing the microbial flora normally found in the field to give a more accurate rate of degradation of the Cry protein in the agricultural environment (as microbial populations in the rhizosphere are commonly 100-fold higher than in bulk soil). A toxicity study by the applicants for Cry1F impacts on earthworms and the collembolan *Folsomia candida* found no negative effects (EPA 2001).

Blackwood and Buyer (2004) investigated the effects of maize expressing Cry1F (event 1507) or Cry1Ab (event Bt11) on soil microbial community structure in three soil types using growth chamber experiments. They used phospholipids fatty acid profiles from bulk soil and community-level physiological profiles from the rhizosphere community to assess community structure. The authors found that expression of Cry1F and Cry1Ab in maize had very few significant effects on the soil microbial communities in their experiment. Statistically significant effects were only observed in a high-clay soil where the presence of Cry protein seemed to stimulate increased ability of the rhizosphere community to metabolize certain amino acids and other amines. The authors suggested that Bt maize may cause proliferation in the population of certain micro-organisms due to the extra protein present in soil and soils rich in clay increase the retention of Cry protein in soil (Blackwood and Buyer 2004). Blackwood and Buyers (2004) suggest further long-term experiments under a variety of conditions with a variety of assays as small impacts may be amplified in the field over time and more detailed analyses may be able to uncover other important changes in soil microbial communities.

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<sup>22</sup> Bioassay results with tobacco budworm (*Heliothis virescens*) were used to measure the relative titer of the protein in the soil after a series of incubation periods. The half-life of Cry1F was estimated based on the decrease in toxicity over time. Cry1F was derived from transgenic *Pseudomonas fluorescens* modified to express Cry1F. Description of the soil used: commerce silt-loam, fine-silty, mixed, nonacid, thermic aeic fluvaquents.

### 3.3 mCry3A - expressed by event MIR 604

The scope of Syngenta's application for approval of MIR 604 in the EU does not include cultivation (Table 5). In the US the Environmental Protection Agency has conditionally registered mCry3A and the genetic material necessary for its production in event MIR 604<sup>23</sup> in 2006 (EPA 2006a).

Table 5. Dossier for a mCry3A expressing GM crop in the EU

Dossier Reference number	Scope of application	Trait /Species (Brief description of Product)	Insect resistance traits Cry3A	Higher lysine content	HT traits	Applicant	Status
EFSA/GMO/UK/2005/11	Food, feed - Import and processing	MIR604 Maize				Syngenta Seeds	Valid application (16/9/2005)

Maize event MIR604 expresses a modified Cry3A toxin (described as mCry3A or Cry3A055) (Steiner *et al.* 2005). MIR 604 maize is targeted against corn rootworms (*Diabrotica* spp.), which are pests with little sensitivity to the wildtype Cry3A protein (Chen and Stacy 2003, Johnson *et al.* 2003). Modification of the Bt gene expressed by MIR 604 resulted in a protein with activity against *Diabrotica* spp. (Chen and Stacy 2003, Steiner *et al.* 2005). Mir 604 thus appears to be the first case of an application in the EU for a GM crop that expresses a Bt toxin with a significantly changed activity spectrum compared to that of the wildtype Bt toxin.

The wildtype cry3A gene was derived from *Bacillus thuringiensis* subsp. *tenebrionis* (Btt).<sup>24</sup> The data available for Cry3A indicates that this Bt toxin is active against a limited number of beetle species (Coleoptera).

*Bacillus thuringiensis* subsp. *tenebrionis* has a long history of use as a microbial insecticide for the control of the Colorado potato beetle (*Leptinotarsa decemlineata*) and some other beetle pests in the family Chrysomelidae. *Bacillus thuringiensis* subsp. *tenebrionis*-based products have been recommended for integrated pest management regimes due to their safety in respect of natural predators (Beveridge and Elek 1999, Glare and O'Callaghan 2000).

A Cry3A expressing GM potato event, developed by Monsanto, was approved in the USA several years ago for control of the Colorado potato beetle (Perlak *et al.* 1993, Ferro 2000). A considerable number of non-target studies with these Cry3A potato plants were published (e.g. Shieh *et al.* 1994, López and Ferro 1995, Walters and English 1995, Lavrik *et al.* 1995, Dogan *et al.* 1996, Donegan *et al.* 1996, Arpaia *et al.* 1997, Yu *et al.* 1997, Hoy *et al.* 1998, Riddick and Barbosa 1998, Riddick *et al.* 1998, Deml *et al.* 1999, Armer *et al.* 2000, Glare and O'Callaghan 2000, Riddick *et al.* 2000, Reed *et al.* 2001, Saxena *et al.* 2004, Stotzky 2004, Romeis *et al.* 2006).

<sup>23</sup> SYN-IR604-8 (EPA 2006a)

<sup>24</sup> <http://www.agbios.com/dbase.php?action=ShowProd&data=MIR604&format=LONG>

The modification of Cry3A resulted in a change to the activity spectrum amongst pest beetle species and may thus also affect the activity spectrum of the toxin among non-target species (such as beneficial beetles). Results of risk assessment studies conducted with non-modified Cry3A protein (using Bt potatoes or *Bacillus thuringiensis* subsp. *tenebrionis* microbial insecticides) are thus only to a limited extent applicable to MIR 604.

So far no non-target studies with MIR 604 maize or the mCry3A toxin appear to have been published in the scientific literature. Non-target studies submitted by the applicant to the US regulatory authorities included experiments with beneficial insects, which feed on pollen and nectar, and soil invertebrates, particularly beetle species (EPA 2006c). Purified microbially produced mCry3A was used for toxicity studies, to achieve the high doses required by the maximum hazard dose approach required for Tier I tests. This protein appears to have been less toxic than the actual plant-derived mCry3A (EPA 2006c). No field studies investigating impacts on non-target insects seemed to have been submitted.<sup>25</sup>

MIR 604 expresses mCry3A in leaves, roots, silk and kernels but no quantifiable levels of mCry3A were detected in pollen of MIR 604 by the applicant (Syngenta 2005, EPA 2006c). No published information on levels of mCry3A in phloem, if any, appears to be available at present.

### **3.3.1 Risks to non-target species related to the target pest**

The toxicity of mCry3A to non-target species related to the target pest was tested with several non-target beetle species, which also occur in the UK. Species tested included the ladybird *Coccinella septempunctata*, the carabid beetle *Poecilus cupreus* and the rove beetle *Aleochara bilineata*. Reproductive and developmental assessments were made during the experiments with the ladybird and rove beetle. No negative effects of mCry3A were observed. Potential exposure of endangered US species was also considered (EPA 2006c).<sup>26</sup>

### **3.3.2 Risks to other non-target species and ecosystem services – above ground**

In addition to the studies described above, toxicity studies were conducted with honey bees and the predatory anthocorid bug *Orius insidiosus*. No negative effects of mCry3A on mortality and developmental times were reported (EPA 2006c).

As required by the US authorities, the applicants also submitted results of studies with bird and mammal species, which showed no negative effects (EPA 2006c).

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<sup>25</sup> EPA uses a testing system with four tiers. Tier 1 studies use maximum hazard doses and single non-target species are tested, usually in the laboratory. Tiers II-IV encompass longer term greenhouse or field testing and are only carried out when adverse effects are found at the first tier. The applicants followed OPPTS Harmonized Testing Guidelines and guidance for data requirements for microbial toxins as required by EPA (in the absence of specific risk assessment guidance for insect resistant GM crops).

<sup>26</sup> Geographical range overlap of endangered beetle species with maize production in the US was mainly restricted to the American burying beetle (*Nicrophorus americanus*). As this beetle feeds exclusively on carrion the chance that it would be exposed to Bt toxin was considered small.

### **3.3.3 Risks to other non-target species and ecosystem services – below ground**

Toxicity of mCry3A to the earthworm *Eisenia fetida* was assessed. Earthworms were exposed to soil containing mCry3A at a concentration of 370 µg/g dry soil for 14 days. Mortality at the end of the experiment was 5% in the mCry3A treatment compared to 0% in the untreated control. Mean weight loss in the Cry3A treatment was 5.8% compared to 11.4% in the untreated control (EPA 2006c).

A first-order kinetic model submitted by the applicant (based on Colorado potato beetle larvae feeding data) estimated that the time to 50% degradation ( $Dt_{50}$ ) for mCry3A in silty clay loam soil was 7.6 days (EPA 2006c).

### 3.4 Cry3Bb1 - expressed by events MON 863 and MON 88017

The cry3Bb1 gene expressed in maize events MON 863<sup>27</sup> and MON 88017<sup>28</sup> was derived from *Bacillus thuringiensis* var. *kumamotoensis*. The Cry3Bb1 protein variant expressed by MON 88017 shares over 99% sequence identity with the wild type *B. thuringiensis* var. *kumamotoensis* Cry3Bb1 protein and differs only by one amino acid from the Cry3Bb1 protein variant expressed by MON 863 (Monsanto 2004). The structure of Cry3Bb1 has been published (Galitsky *et al.* 2001).

Susceptibility to Cry3Bb1 is most evident in beetle species of the family Chrysomelidae (leaf beetles) (Monsanto 2004). Cry3Bb1 is active against *Diabrotica* spp (Chrysomelidae) as well as the Colorado potato beetle (*L. decemlineata*, Chrysomelidae). In addition to expression in GM maize, Cry3Bb1 is also one of the active ingredients in a microbial (non-GM) insecticide (Monsanto 2004).

Monsanto is seeking approval in the EU for several maize hybrids involving event MON 863. These include MON 863 X MON810 (Cry 3Bb1 plus Cry1Ab), MON 863 X NK603 (Cry 3Bb1 plus glyphosate tolerance) and MON 863 x MON810 x NK603 Maize (Cry3Bb1 plus Cry1Ab plus glyphosate tolerance) (Table 6). The company has approval for cultivation of MON 863 in the US and Canada, and for the MON 863 hybrids in Japan.<sup>29</sup>

Table 6. Dossiers for Cry3Bb1 expressing GM crops in the EU

Dossier Reference number	Scope of application	Trait /Species (Brief description of Product)	Insect resistance traits		HT traits	Applicant	Status
			Cry1Ab	Cry3Bb1	glyphosate		
EFSA/GMO/D E/2004/03	- Food, feed	MON863 x MON810 Maize (Cry 3Bb1 X Cry 1Ab)	■	■		Monsanto	Overall opinion published
EFSA/GMO/U K/2004/06	- Food, feed - Import and processing	MON863 x NK603 Maize (Cry 3Bb1 X glyphosate)		■	■	Monsanto	Overall opinion published
EFSA/GMO/B E/2004/07	- Food, Feed - Import and processing	MON863 x MON810 x NK603 Maize (Cry 3Bb1XCry1AbXglyphosate)	■	■	■	Monsanto	Overall opinion published
EFSA/GMO/C Z/2005/27	- Food, feed - Import and processing	MON 88017 maize (glyphosate & Cry3Bb1)		■	■	Monsanto	Under completeness check
EFSA/GMO/C Z/2006/33	- Food, feed - Import and processing	MON 88017 x MON 810 maize (Glyphosate & Cry3Bb1 X Cry1Ab)	■	■	■	Monsanto	Under completeness check

<sup>27</sup> OECD identifier MON-ØØ863-5 (<http://www.agbios.com>)

<sup>28</sup> OECD identifier MON-88Ø17-3 (<http://www.agbios.com>)

<sup>29</sup> <http://www.agbios.com>, accessed 16 November 2006

In addition, another Cry3Bb1 hybrid in the EU application system is a cross of MON 88017 (which expresses a glyphosate tolerance gene in addition to Cry3Bb1) with MON 810 to produce a maize plant resistant to corn root worms, European corn borer and glyphosate (Table 6). Applications received so far are for food and feed as well as import and processing, not for cultivation (EFSA 2006) (Table 6). MON 88017 has approval for cultivation in the US, Canada and Japan.<sup>30</sup>

ACRE has published advice on the import and use of grain varieties derived from event MON 863 and the MON 863 x MON810 hybrid (scope excluded cultivation) on 12 June 2003, October 2003 and September 2005.<sup>31</sup>

A considerable amount of data on non-target organisms from field and laboratory studies with Cry3Bb1 expressing maize (focussing on event MON 863) has been published in the scientific refereed literature (see below).

Further data will become available over the next two to three years as a network of research projects in Germany is investigating the environmental impact of Cry3Bb1 maize (with a particular focus on MON 88017). These projects were initiated in 2005, are funded by the German Ministry for Education and Science (BMBF)<sup>32</sup> and are investigating the following aspects:

- Impacts of the cultivation of *Diabrotica*-resistant Bt maize on the maize ecosystem (RWTH Aachen) (focuses on foliage-inhabiting arthropods, on species related to *Diabrotica*, pollen feeders and flower-visiting insects)
- Effects on predatory soil arthropods (Carabidae and spiders) and sucking insects (LfL Freising)
- Investigations of the effect of Bt maize (Cry3Bb1) on non-target organisms in the soil (dipteran larvae in soil and their predators) (BBA Braunschweig)
- Test of a nematode bioassay to assess bioavailability and toxicity of Bt toxins in the soil (Institut für Biodiversität - Netzwerk e.V., Regensburg)
- Degradation of Bt toxins and effects on soil microorganisms (FAL Braunschweig)
- Persistence of Bt toxins (Cry3Bb1) in soil (University of Göttingen)
- Production of a Bt toxin standard (Cry3Bb1) and development of detection methods (DLR Neustadt)
- Investigations of the side effects of Bt maize (Cry3Bb1) on above-ground non-target organisms related to *Diabrotica* (BBA Darmstadt)
- Development of resistance to Bt maize in the corn rootworm
- Investigations of the activation of Bt proteins in the corn rootworm (BBA Darmstadt)
- Rearing and characterisation of the corn rootworm (*Diabrotica v. virgifera*)
- Methods for the statistical analysis of Bt maize field trials (Cry3Bb1) (University of Hanover)

These research projects will come to a close in 2008. Information on approaches and methodologies is publicly available on a website ([www.biosicherheit.de](http://www.biosicherheit.de)), which will also contain results as they become available. These new studies with Cry3Bb1 maize build on an earlier set of projects, also funded by the BMBF, which studied the

<sup>30</sup> <http://www.agbios.com>, accessed 16 November 2006

<sup>31</sup> [http://www.defra.gov.uk/Environment/acre/advice/pdf/acre\\_advice68.pdf](http://www.defra.gov.uk/Environment/acre/advice/pdf/acre_advice68.pdf)

<sup>32</sup> Bundesministerium für Bildung und Forschung

environmental impact of Cry1Ab maize. One of the aims of the projects is to develop methods for the monitoring of Bt maize.

Levels of Cry3Bb1 in pollen have been reported to be on average 62 µg/g and 14 µg/g (fw) in MON 863 and MON 88017, respectively (Monsanto 2001, 2004). For comparison: levels of Bt toxin in pollen of have been reported to be 1.1-7.1 µg Cry1Ab per g in event 176 and 31-33 µg Cry1F per g in event 1507 (Annex 1). An independent study is under way to measure expression levels of Cry3Bb1 maize in the field in Germany (Thu *et al.* 2006).

### 3.4.1 Risks to non-target species related to the target pest

Most closely related to the target pests are other beetle species, particularly species in the family Chrysomelidae. Non-target beetles can be exposed to Cry3Bb1 when feeding on maize plants directly, on maize pollen or on herbivorous prey.<sup>33</sup> Some beetle families, particularly the ground beetles (Carabidae) and ladybirds (Coccinellidae), are widely used indicator species for the risk assessment of GM crops (Brooks *et al.* 2003, Bhatti *et al.* 2005b, Lopez *et al.* 2005).

An ongoing laboratory study by Felke (2006) is investigating side effects of MON 88017 pollen on non-target chrysomelid beetles, which occur in habitats next to maize fields in Germany. Species tested so far include *Gastrophysa viridula* (green dock beetle), *Phaedon cochleariae* (mustard beetle) and *L. decemlineata* (Colorado potato beetle). No negative effects were observed when first and second instar larvae of *G. viridula* were fed MON 88017 pollen but purified Cry3Bb1 caused an increase in mortality, a slower weight increase and development of *G. viridula* (Felke 2006).

Toxicity bioassays with Cry3Bb1 toxin submitted by the applicant to USDA for MON 863 indicated that the red flour beetle (*Tribolium castaneum*) and several weevil (Curculionidae) species are not susceptible to Cry3Bb1 (Monsanto 2001). The weevil species tested included the cowpea weevil (*Callosobruchus maculatus*), the cotton boll weevil (*Anthonomus grandis*), the pepper weevil (*Anthonomus eugenii*) and the rice weevil (*Sitophilus oryzae*).

Two US laboratory studies published in 2002 investigated the impact of MON 863 pollen on the ladybird *C. maculata*. Both studies indicate that MON 863 pollen does not have a negative impact on this predatory beetle. Lundgren and Wiedenmann (2002) fed *C. maculata* larvae in their first experiment with mixtures of pollen containing 0, 25, 50, 75, or 100% transgenic pollen or with aphids. The duration of each instar and pupal weight were compared among treatments. In a second experiment Lundgren and Wiedenmann (2002) reared *C. maculata* larvae on one of the pollen mixtures or on artificial diet; and assessed the duration of larval and pupal stages, pupal weight, adult mobility, adult survivorship, and female fecundity. The authors found no differences in any of the fitness parameters among *C. maculata* in the treatments fed different mixtures of pollen. Beetles in the pollen mixture treatments had faster larval development times, greater larval survivorship, and greater pupal weight than the beetles fed only aphids or an artificial diet (Lundgren and Wiedenmann 2002). Duan *et al.* (2002) evaluated the effect of MON 863 pollen on the survival, larval development and reproductive capacity of *C. maculata*. They

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<sup>33</sup> A study by Harwood *et al.* (2005) with the older Cry1Ab expressing maize events confirmed that significant quantities of Bt toxin are not only found in the target pests but also in non-target herbivores which feed on maize. Predatory ladybirds, spiders and predatory bugs of the family Nabidae collected from the Bt maize ecosystem also contained significant quantities of Bt toxin (Harwood *et al.* 2005).

fed first instar *C. maculata* and newly emerged adults individually on a diet mixture containing 50% of MON 863 pollen, non-transgenic (control) maize pollen, bee pollen (a component of normal rearing diet), or potassium arsenate-treated control maize pollen. In the larval tests, 96.7%, 90.0%, and 93.3% of *C. maculata* larvae successfully pupated and then emerged as adults when fed on MON 863 pollen, non-transgenic maize pollen, and bee pollen diets, respectively. Among the larvae completing their development, there were no significant differences in the developmental time to pupation and adult emergence among the transgenic maize pollen, non-transgenic maize pollen, and bee pollen diet treatments. All larvae fed on arsenate treated maize pollen died as larvae. For tests with adults, 83.3%, 80.0%, and 100% of adult *C. maculata* survived for the 30 days of the test period when reared on diets containing 50% of MON 863 pollen, non-transgenic maize pollen and bee pollen, respectively. The adult survival rate on MON 863 pollen diet was significantly less than that on the bee pollen diet but there was no significant difference between the MON 863 and non-transgenic maize pollen treatments. During the period of adult testing, an average of 77, 80, and 89 eggs per female were laid by females fed on the MON 863 pollen, control maize pollen, and bee pollen, respectively; no significant differences were detected in the number of eggs laid among these treatments. These results suggest that at the doses tested MON 863 pollen had no measurable negative effect on the survival and development of *C. maculata* larvae to adulthood nor any adverse effect on adult survival and reproductive capacity. A toxicity bioassay with maize pollen containing Cry3Bb1 submitted by the applicant showed no negative effects of Cry3Bb1 on the ladybird *H. convergens* (Monsanto 2001).

An ongoing study at the Biologische Bundesanstalt für Land und Forstwirtschaft (BBA) in Braunschweig, Germany, investigates the effects of Cry3Bb1 maize on ground beetles and rove beetles. These predatory beetles may be exposed to Bt toxin through predation on detritivorous soil insects (Schlein and Büchs 2006). One such prey species encountered may be sciarid fly larvae, which feed on dead plant material in the soil.

Several refereed publications report the results of US field studies which investigated the impact of Cry3Bb1 expressing maize on non-target arthropods: A publication by Monsanto (Bhatti *et al.* 2005a) reports the results of a field study in Illinois from 2000-2002, which assessed the effect of Bt maize MON 863 on foliage-dwelling beetles and other foliage-dwelling arthropods. Bhatti *et al.* concluded that MON 863 maize had no consistent adverse impact on the relative abundance of any non-target foliage-dwelling beetles. In contrast, insecticide applications of foliar insecticide (permethrin) consistently decreased the abundance of ladybirds (Bhatti *et al.* 2005a). The study employed a split-plot design with four replicates, with MON 863 corn and a conventional maize as the main plots (measuring about 73 m x 18 m each). The study design was sufficient to detect population-level effects of 20-50% with 80% power for all of the abundant taxa. Arthropods were sampled with sticky traps over three growing seasons. Between 6016 and 30,664 individual beetles were collected per year. All taxa collected were identified to at least family level, and to genus or species level where possible. The most abundant beetle taxa collected were chrysomelid maize pests, including *Chaetocnema pulicaria* (corn flea beetle) and *Diabrotica* spp. The corn flea beetle was less abundant in samples collected from MON 863 plots but the authors suggest that this was due to an indirect rather than a direct effect as the corn flea beetle may have been attracted to the non-Bt plots by the damage caused by the target pests (*Diabrotica* spp.) The most abundant predatory beetles encountered were ladybirds (Coccinellidae), particularly *C. maculata*, *Harmonia axyridis* and *Cycloneda munda*. *Hippodamia convergens*, *Coccinella septempunctata* and *Hippodamia parenthesis* were less common. Only *C. maculata* numbers were significantly lower on MON 863 in one year out of three.

Herbivorous beetle species caught by the traps included sap beetles (Nitidulidae), click beetles (Elateridae) and ground beetles (Carabidae).

A second publication by Monsanto from the same year reports the data on ground-dwelling invertebrates collected at the same study site in Illinois from 2000 to 2002 (Bhatti *et al.* 2005b). Ground-dwelling arthropods were sampled with pan and pitfall traps<sup>34</sup> over the three year period. The authors found that MON 863 maize had no consistent adverse impacts on the abundance of any of the non-target beetle species (including ground beetles (Carabidae), rove beetles (Staphylinidae) and sap beetles (Nitidulidae)) compared with the non-Bt maize isolate. In contrast, foliar (permethrin)- and soil (tefluthrin)-applied insecticides significantly decreased the abundance of ground beetles (Bhatti *et al.* 2005b).

Ahmad *et al.* (2005) at Kansas State University published the results of a field study, which evaluated the effects of Cry3Bb1 maize (MON 863) on non-target surface and below-ground arthropods at one location in 2002 and three locations in 2003. The authors found no significant differences in numbers of beetles in plots planted with Cry3Bb1 and non-Bt maize at any of the studied locations. The experiments were designed as a randomised complete block with four replicates. Each plot consisted of twenty 15 m rows (2002) or four 9 m rows (2003) (row spacing c. 76 cm). Families of beetles assessed included ground beetles, click beetles (Elateridae) and rove beetles. The results for other groups of arthropods assessed in this study are described in the following sections.

An earlier field study at Kansas State University, published by Al-Deeb & Wilde (Al-Deeb and Wilde 2003), included visual inspections of adult and immature ladybirds (*C. maculata*, *H. convergens* and *Scymnus* spp.) in Cry3Bb1 maize (event MON 863 as well as the experimental events MON 853 and MON 862) at several locations in Kansas in 2000 and 2001. The authors reported that no significant differences in ladybird numbers could be detected between Bt maize and the non-Bt isolate apart from one occasion where Bt maize had significantly more *H. convergens* adults.

### **3.4.2 Risks to other non-target species and ecosystem services – above ground**

Data from toxicity studies submitted by the applicant to USDA suggests that Cry3Bb1 added to artificial diet or water had no negative effects on larval and adult honey bees (*A. mellifera*), green lacewing larvae (*C. carnea*) or the parasitic wasp *Nasonia vitripennis* (Monsanto 2001). The company's results with honey bees confirm the conclusions of an earlier Italian study which investigated the susceptibility of honey bees to Cry3B. In the latter study the toxin was added to supplemental syrup fed to the bee colonies. No toxic effects on bee larvae were observed and pupal weights were also not significantly affected (Arpaia 1996). However, the choice of *N. vitripennis* for the risk assessment of Bt maize has been criticised as not appropriate as indicator species for the parasitoid community in maize fields (section 3.5.2; see also comment regarding parasitoid toxicity studies in section 3.2.2).

Toxicity bioassay results with Cry3Bb1 toxin submitted by the applicant to the USDA for approval of MON 863 further indicated that the two moth species European cornborer (*O. nubilalis*) and corn earworm (*Helicoverpa zea*) are not susceptible to Cry3Bb1 (Monsanto 2001).

Monsanto's published field study (Bhatti *et al.* 2005a, see section 3.4.1) of the effects of MON 863 maize on foliage-dwelling arthropods reported no consistent adverse

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<sup>34</sup> Pan traps were effective in sampling soil-dwelling organisms, whereas pitfall traps were effective for sampling ground-dwelling organisms (Bhatti *et al.* 2005b).

impact on the relative abundance of any of the non-target foliage-dwelling arthropod taxa encountered. Abundant non-target arthropods included (in addition to the beetle species discussed above) the aphid *Rhopalosiphum maidis*, predatory damsel bugs (Nabidae), minute pirate bugs (*Orius insidiosus*), hover flies (Syrphidae), green lacewings (*Chrysoperla carnea*), the braconid parasitoid *Macrocentrus cingulum* and spiders. Treatment with the foliar insecticide permethrin, however, consistently decreased the abundance not only of ladybirds but also of green lacewings, damsel bugs and the parasitoid (Bhatti *et al.* 2005a).

Monsanto's published field study (Bhatti *et al.* 2005b, see section 3.4.1) of the effects of MON 863 maize on ground-dwelling arthropods found also no consistent adverse impacts of MON 863 on the abundance of any non-target ground-dwelling taxa compared with the non-Bt isolate. The most abundant taxa collected included spiders, ground beetles, rove beetles, scavenger beetles and Japygidae (dipluran insects), ants, centipedes and earthworms. Significant effects of the maize line were seen with two taxa (centipedes and Japygidae) out of the fourteen non-target taxa analysed. The nature of these effects varied within and among years. Pan trap captures of centipedes tended to be higher in MON 863 than in non-Bt maize plots, but pitfall trap captures were higher in non-Bt plots than in MON 863 in one out of three years. More Japygidae were captured in pan traps in non-Bt corn than in MON 863 plots in two out of three years. In contrast, certain insecticide applications had consistent adverse impacts across all three years on several groups of ground-dwelling arthropods such as spiders and Japygidae (Bhatti *et al.* 2005b). Further assessments of soil organisms are described in section 3.4.3.

Ahmad *et al.* (2005), Kansas State University, who conducted the field study of non-target arthropods using several Cry3Bb maize field sites in 2002 and 2003, found no significant effects of Cry3Bb on populations of Cicindelidae, ants, Gryllidae and spiders collected in pitfall traps (in addition to the results for beetles described above).

The field study published by Kansas State University in 2003 by Al-Deeb and Wilde included visual inspections of nymphs and adults of the predatory bug *O. insidiosus* in Cry3Bb1 maize (event MON 863 as well as the experimental events MON 853 and MON 862) at several locations in 2000 and 2001. The authors found no significant differences in numbers of *O. insidiosus* between Bt maize and the non-Bt isolate at nine of ten sites studied. At only one site were the numbers of *O. insidiosus* nymphs lower in Bt maize than in the non-Bt isolate plots (although numbers of nymphs on Bt maize did not differ significantly from the insecticide control in this case) (Al-Deeb and Wilde 2003).

### **3.4.3 Risks to other non-target species and ecosystem services – below ground**

As described above, Bt toxins from GM plants can enter the soil ecosystem in several ways, including through incorporation of crop residues after harvest and through release of root exudates (Bitzer *et al.* 2005). Through these processes, soil organisms can be exposed to Bt toxins and their breakdown products. This exposure can result potentially in a better control of the target pest but there is also the risk of negative impacts on non-target organisms (Saxena *et al.* 2004).

Laboratory bioassays with Cry3Bb1 toxin, submitted by the applicant to USDA for MON 863 approval, indicated that Cry3Bb1 added to soil had no negative effects on the earthworm *Eisenia fetida*. Cry3Bb1-containing plant material fed to *Folsomia candida* also had no negative impact on this species of Collembola (Monsanto, 2001).

A study by Devare *et al.* (2004) at Cornell University assessed the effects of event MON863 on soil microbial biomass, activity (N mineralization potential, short-term nitrification rate, and soil respiration) as well as on the bacterial community structure (as determined by terminal restriction fragment length polymorphism (T-RFLP) analysis) over two seasons in a field experiment. Cry3Bb1 maize had no deleterious effects on microbial activity or bacterial community measures compared to the non-Bt isolate. The T-RFLP analysis indicated that amplifiable bacterial species composition and relative abundance differed substantially between years, but did not differ between rhizosphere and bulk soils. The authors suggested that the release of Cry3Bb1 maize poses little threat to the ecology of the soil microbial community based on parameters measured in their study (Devare *et al.* 2004).

Bitzer *et al.* (2005) at Iowa State University evaluated the effect of MON 863 on the abundance and diversity of surface-active (epedaphic) and subsurface (euedaphic) Collembola (springtails) species at two field sites (Ames, IA, and Monmouth, IL) between 2000 and 2002. Collembola were collected from pitfall traps and soil cores in plots planted with MON 863 and its non-Bt isolate. Few differences were observed in the abundance of individual Collembola species in Bt and non-Bt maize. Nor did the estimated species richness or the Shannon or Simpson diversity indices differ significantly between Bt and non-Bt maize at either location, indicating no effect of Bt maize on diversity of Collembola. In contrast, Collembola were consistently more abundant in soil insecticide-treated plots than in control plots, perhaps because of adverse effects of insecticides on their predators. Soil insecticide also reduced both Shannon and Simpson diversity in the Iowa euedaphic and Illinois epedaphic populations. Such consistent insecticide effects on both abundance and diversity of Collembola provided a positive control by which to assess the power of the experimental design to detect a comparable impact of Bt maize on Collembola (Bitzer *et al.* 2005).

In addition to assessing soil surface arthropods, Ahmad *et al.* (2005) at Kansas State University also levels of Cry3Bb1 in soil planted with Cry3Bb1 maize (MON 863) and the effects on below-ground arthropods. ELISA tests did not detect any Cry3Bb1 in soil samples (15 cm deep, sampling near the maize plant, three sampling occasions during the growing season) taken from plots planted with Cry3Bb1 maize for the first year or planted over three consecutive years near Manhattan, KS. However, some Cry3Bb1 protein (3.38–6.89 ng/g dry soil) was detected in soil samples collected in close proximity to maize plants in a Bt maize field that was planted for the first year near Scandia, KS. No Cry3Bb1 was detectable between the rows of Bt plants. The authors concluded that the Cry3Bb1 protein released from root exudates or decaying plant residues does not persist and is rapidly broken down in the soil. Below-ground microarthropods were sampled from intact soil cores using a high-gradient tullgren type extractor, which allowed the assessment of several groups of soil mites (Astigmata, Prostigmata, Mesostigmata, Oribatids) as well as Collembola (Isotomidae, Entomobryidae, Sminthuridae) (Ahmad *et al.* 2005). The authors found no significant differences in numbers of below-ground arthropods in soil planted with Cry3Bb1 and non-Bt maize at any of the studied locations.

Differences between Cry3Bb1 and Cry1Ab in binding to clay particles in soil is currently being studied at the University of Göttingen in Germany (Pagel-Wieder *et al.* (2006).

### **3.5 Cry34Ab1/Cry35Ab1 - expressed by event 59122**

Event 59122 expresses a novel type of binary Bt toxin. The discovery of this family of binary insecticidal crystal proteins from *B. thuringiensis* was published by Dow AgroSciences in 2002 (Ellis *et al.* 2002, Herman *et al.* 2002). Cry34Ab1 (14 kDa) and Cry35Ab1(44 kDa) were isolated from *B. thuringiensis* strain PS149B1.

Sequence comparisons with other Bt toxins did not show any homology of the genes encoding Cry34Ab1 and Cry35Ab1 with those encoding known other Cry proteins or Cyt and Vip proteins from *B. thuringiensis*. However, there is some evidence that Cry35Ab1 is evolutionarily related to binary dipteran insecticidal proteins from *Bacillus sphaericus* (Ellis *et al.* 2002). Further information on characterisation of Cry34Ab1/Cry35Ab1 and its mode of action has been published by Gao *et al.* (2004) and Masson *et al.* (2004). Herman *et al.* (2003) reported that Cry34Ab1 and Cry35Ab1 were rapidly digested in simulated gastric fluid.

Cry34Ab1 and Cry35Ab1 act together as a binary toxin that is effective against corn rootworms (*Diabrotica* spp.). Cry34Ab1 is the active component and is synergised by Cry35Ab1 (Herman *et al.* 2002). Studies by Dow AgroSciences showed that maize event 59122 expressing Cry34Ab1/Cry35Ab1 can cause close to 100% mortality of the western corn rootworm (*Diabrotica virgifera virgifera*) (Storer *et al.* 2006).

The current dossiers involving event 59122<sup>35</sup> are listed in Table 7. The scope of four of the dossiers includes cultivation. The dossiers relate to event 59122 on its own or to hybrids produced through crosses with event 1507 (which expresses the lepidopteran-active Cry1F toxin) and event NK603 (which expresses glyphosate tolerance only). Event 59122 has regulatory approval for the environment in the US, Canada and Japan.<sup>36</sup>

No studies of the environmental impact of Cry34Ab1/Cry35Ab1 appear to have been published in the refereed scientific literature apart from information relevant to the resistance management of 59122 maize (Storer *et al.* 2006). No refereed publications dealing with the effect of 59122 maize on non-target insects could be located. The information provided in sections 3.5.1 to 3.5.3 is thus solely derived from information published by the US regulatory authorities. Management-related changes have been discussed by APHIS, who suggested that commercial release of event 59122 is likely to have an impact on the use of crop rotation and chemical insecticides (APHIS 2005).

Pollen expression of Bt toxin in 59122 maize has been reported by the applicants to be on average 74.27 µg/g Cry34Ab1 and 0.02 µg/g Cry35Ab1 (dry weight) (Dow Agro Sciences, 2003).

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<sup>35</sup> OECD identifier DAS-59122-7 (<http://www.agbios.com>)

<sup>36</sup> <http://www.agbios.com/dbase.php?action=Submit&evidx=496>

Table 7. Dossiers for Cry34Ab1/Cry35Ab1 expressing GM crops in the EU

Dossier Reference number	Scope of application	Trait /Species (Brief description of Product)	Insect resistance traits		HT traits		Applicant	Status
			Cry1F	Cry34Ab1/Cry35Ab1	glufosinate	glyphosate		
EFSA/GMO/NL/2005/12	- Food, feed - Import and processing	59122 Maize (Cry 34Ab1 & Cry35Ab1)					Pioneer Hi-Bred /Mycogen Seeds	Valid application
EFSA/GMO/NL/2005/15	- Food, feed - Import and processing	1507 x 59122 Maize (glufosinate & Cry 1F X Cry 34Ab1 & Cry35Ab1)					Dow AgroSciences / Pioneer Hi-	Under completeness check
EFSA/GMO/UK/2005/20	- Food, feed - Import and processing	59122 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X glyphosate)					Pioneer Hi-Bred	Under completeness check
EFSA/GMO/UK/2005/21	- Food, feed - Import and processing	59122 x 1507 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X Cry1F & glufosinate X glyphosate)					Pioneer Hi-Bred	Under completeness check
EFSA/GMO/NL/2005/23	- Food, feed - Import and processing - Cultivation	59122 maize (Cry 34Ab1 & Cry35Ab1)					Pioneer Hi-Bred /Mycogen Seeds	Under completeness check
EFSA/GMO/NL/2005/28	- Food, feed - Import and processing - Cultivation	1507 x 59122 maize (Cry1F & glufosinate X Cry 34Ab1 & Cry35Ab1)					Dow AgroSciences	Under completeness check
EFSA/GMO/UK/2006/29	- Food, feed - Import and processing - Cultivation	59122 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X glyphosate)					Pioneer Hi-Bred	Under completeness check
EFSA/GMO/UK/2006/30	- Food, feed - Import and processing - Cultivation	59122 x 1507 x NK603 maize (Cry 34Ab1 & Cry35Ab1 X Cry1F & glufosinate X glyphosate)					Pioneer Hi-Bred	Under completeness check

### 3.5.1 Risks to non-target species related to the target pest

Acute dietary toxicity studies of beneficial arthropods submitted by the applicants showed no adverse effects of Cry34Ab1/Cry35Ab1 on two US predatory ladybird species (*H. convergens* and *C. maculata*) at levels 10 to 100 times the expected field exposure<sup>37</sup> (APHIS 2005).

Potential exposure of threatened and endangered beetle species was considered by EPA and no species at risk from event 59122 were identified (APHIS 2005).<sup>38</sup>

The applicants conducted field trials with event 59122 in the US in 2003 (plot size approximately 390 m<sup>2</sup> during which arthropod populations were sampled by visual observations, sticky traps, pitfall traps and litter bags. Predatory ladybird eggs, larvae and adults were assessed visually. Sticky traps were used to sample staphylinids (rove beetles). Pitfall traps and litter bags assessed staphylinids beetles as well as carabid beetles (*Pterostichus* spp. and *Harpalus* spp.). There were no community-level differences between the Bt and non-Bt fields except for a reduction in staphylinids in the 59122 treatment at one site. Since Cry34 Ab1/35Ab1 is specifically active against chrysomelids and the effect on staphylinids only occurred at the Nebraska site in 2003 and did not occur in the other 2001-2003 field trials, APHIS concluded that the difference was due to normal field variation. However, the applicants informed the US regulatory agencies that they intended to continue sampling to verify that staphylinid beetles are not adversely affected by the binary Cry34Ab1/Cry35Ab1 Bt toxin (APHIS 2005).

The applicants also provided data from small scale field studies conducted in 2001 and 2002 with another event (PS149B1) expressing Cry34Ab1/Cry35Ab1, which had however lower expression levels than 59122.

### 3.5.2 Risks to other non-target species and ecosystem services – above ground

The applicants conducted a series of diet bioassays with microbially-expressed Cry34Ab1/Cry35Ab1 protein to characterize the insecticidal specificity of this binary Bt toxin. Non-target herbivores tested included several moth species (the European corn borer (*O. nubilalis*), corn earworm (*Helicoverpa zea*), black cutworm (*Agrotis ipsilon*)) and the corn leaf aphid (*Rhopalosiphum maidis*). APHIS reported that none of these species showed high mortality when exposed to microbially-expressed Cry34/Cry35 proteins. The field trials with event 59122 also indicated that Cry34/35Ab1 was not active against black cutworm and European corn borer larvae (APHIS 2005).

Acute dietary toxicity studies of beneficial arthropods assessed by APHIS showed no adverse effects at levels 10 to 100 times the expected field exposure. Honey bee survival and development was tested with diets including Bt maize pollen as well as microbially-produced Cry34/35Ab1 protein. Other beneficial insect species tested included the predatory green lacewing (*Chrysoperla carnea*), the parasitic wasp *Nasonia vitripennis* (as well as the ladybird species referred to in section 3.5.1). These non-target insects were fed microbially-produced protein. The applicant's choice of the parasitoid *N. vitripennis* was criticised as an inappropriate indicator

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<sup>37</sup> High end exposure estimates (HEEE)

<sup>38</sup> Geographical range overlap of endangered beetle species with maize production in the US was mainly restricted to the American burying beetle (*Nicrophorus americanus*). As this beetle feeds exclusively on carrion the chance that it would be exposed to Bt toxin was considered small.

species since this species does not typically occur in maize. The green lacewing study was also considered inadequate as the Bt toxins were administered in a moth egg diet that did not allow for exposure of the predator to the proteins. Therefore, a carabid (ground beetle) and anthocorid (insidious flower bug) acute dietary toxicity study were requested from the applicants (APHIS 2005).

The field trials described above also visually monitored lacewing (Chrysopidae) eggs and used sticky traps to assess numbers of non-target herbivores (including aphids, cicadellids, leafhoppers and thrips) and natural enemies (anthocorids (insidious flower bugs), dolichopodids (long-legged flies) and parasitoids (mymarids)). No community-level differences were found between the Bt and non-Bt treatments (APHIS 2005).

### **3.5.3 Risks to other non-target species and ecosystem services – below ground**

Toxicity studies were conducted with earthworms and the collembolan *Folsomia candida* as representative decomposers using microbially-produced Cry34Ab1/Cry35Ab1.<sup>39</sup> All of the organisms evaluated in the dietary toxicity studies were exposed to much greater levels of Cry34Ab1/Cry35Ab1 protein than they would be expected to be exposed to in the field and no adverse effects were observed (APHIS 2005).

A bioassay with southern corn rootworm was used to determine the time to 50% degradation (DT<sub>50</sub>) of Cry34Ab1/Cry35Ab1 in soil. The bioassay established a DT<sub>50</sub> of 3.2 days for event PS149B1. APHIS concluded that the data were satisfactory despite the lower expression levels of Cry34/Cry35Ab1 in this event compared to event 59122. However, EPA asked the applicants to determine a DT<sub>50</sub> under field conditions on large-scale plots in different soil-types and environments following commercial release of event 59122 in the US (APHIS 2005).

The impact of Cry34Ab1/Cry35Ab1 expressing maize on centipedes, spiders, millipedes, oribatid mites and Collembola (incl. entomobryids, isotomids and hypogasturids) was also assessed in the field trials described above (using pitfall traps and litter bags). There were no community-level differences between the Bt and non-Bt treatments (APHIS 2005).

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<sup>39</sup> Other non-target organism tests included a freshwater invertebrate (*Daphnia magna*), rainbow trout fed microbially-produced Cry34Ab1/Cry35Ab1 protein and chickens fed grain from event 59122-7. No adverse effects were observed (APHIS 2005).

### **3.6 Unexpected interactions between stacked traits?**

Several notifications have been submitted in the EU for approval of hybrids between two or more GM crop events (Table 2). The Bt events involved have either been crossed with herbicide tolerant events, with another Bt event or, in one case, with an event transformed to obtain a higher lysine content (Tables 2 and 3). In the hybrids where more than one Bt toxin has been combined, beetle resistant events were crossed with moth resistant events to widen the spectrum of pests to which the crop is resistant (Tables 2 and 3).

Synergism between different Bt toxins have been reported (Herman *et al.* 2002, Wirth *et al.* 2004) and this phenomenon is used intentionally as a means to increase the efficacy of Bt toxins against target pests. An example is event 59122 maize in which Cry35Ab1 synergises the activity of Cry34Ab1 (Herman *et al.* 2002). If such synergism should occur unintentionally when crossing two Bt events, the increase in activity should become obvious at an early stage (i.e. when bioassays are conducted with pest insects).

Accinelli *et al.* (2004) raised the possibility that Bt toxins may affect the degradation of glyphosate or glufosinate in soil when herbicide tolerance traits are expressed alongside Bt genes in hybrids. Glyphosate and glufosinate are non-residual herbicides that are metabolised quickly in soil by microorganisms (Accinelli *et al.* 2004). As described above, Bt toxins from GM crops enter soil via root exudates and when crop residues are incorporated in the soil where they can bind to soil particles (Stotzky 2004). Accinelli *et al.* (2004) conducted a study to investigate the impact of toxins extracted from a commercial microbial *Bacillus thuringiensis* subsp. *kurstaki* (Btk) formulation (Dipel 2X) on the degradation of glyphosate and glufosinate in incubated soil samples from two locations in Italy. The authors found that the *Bacillus thuringiensis* subsp. *kurstaki* toxins did not influence soil microbial carbon and that their insecticidal activity rapidly decreased during the 28 day observation period. However, the addition of a high rate of a mixture of *Bacillus thuringiensis* subsp. *kurstaki* toxins resulted in a significant (about two-fold) increase in persistence of the two herbicides. Accinelli *et al.* (2004) suggested that this increase may be due to a reduction in bio-availability of the two herbicides, modification of the nutrient status of the soil microbial community or other properties, such as soil microbial activity. The authors recommended that potential increases in persistence of herbicides associated with HT crops should be considered in the environmental impact assessment of GM crops expressing both HT and Bt traits.

## 4. Bt crops of potential relevance to the UK in the future

This section can only provide a flavour of Bt crops that may become relevant to UK regulatory authorities, farmers and growers in the future, as only limited information on GM crops under development is publicly available.

*Bacillus thuringiensis*, as a species, is known to produce a much wider range of insecticidal proteins than those used in the presently available Bt crops (Crickmore *et al.* 1998, Maagd *et al.* 2001). A list of the known Bt toxins can be found on the website of The *Bacillus thuringiensis* Delta-Endotoxin Nomenclature Committee<sup>40</sup>. Individual strains of *B. thuringiensis* differ in the range of toxins they produce (Schnepf *et al.* 1998). Corporate, institutional and governmental collections of *B. thuringiensis* contain thousands of isolates from around the world, which are mined as a source of novel active transgene products (Monsanto 2001, Schnepf *et al.* 2005).

Many Bt toxins have shown activity against invertebrates other than species in the Lepidoptera and Coleoptera (e.g. mosquitoes (Zahiri *et al.* 2004) and nematodes (Wei *et al.* 2003)) The review by Glare *et al.* (2000) and the Bt toxin Specificity Database<sup>41</sup> are examples of sources which provide further information on the known activity of each toxin. *Bacillus thuringiensis* is a widespread and common bacterium, and further discovery of new Bt isolates will continue to be a source of potential new transgenes. Bt toxins can also be modified to change their activity range, a new approach that appears likely to be used more widely in the future (Wu *et al.* 2000, Chen and Stacy 2003).

It is also likely that applications for stacked events/hybrids will become more frequent as companies will be interested in (a) combining more traits (HT, IR, etc) in one plant, (b) extending the range of pests against which a plant is resistant, and (c) complying with recommendations for resistance management. The latter refers to the finding that two Bt toxins stacked in a Bt crop are preferable to a single Bt toxin in terms of resistance management as this considerably reduces the risk of development of resistant pest populations. A precondition is that both toxins target the same pest but have different modes of action,(Roush *et al.* 1998).

### *Arable crops*

The main targets for Bt toxins expressed by crops currently in the EU application system are two pests of maize, the European corn borer (*O. nubilalis*) and the western corn rootworm (*Diabrotica virgifera virgifera*). The European corn borer occurs in the UK but is not a serious pest in the UK. The Western corn rootworm, a beetle species native to Central America (Miller *et al.* 2005, Redbond 2006), was first detected in Europe in 1992 near Belgrade and has since spread throughout much of central and south-eastern Europe (Vidal *et al.* 2005). In the UK the first western corn rootworm beetles were caught in 2003 near Heathrow airport but the outbreak was contained. The pest risk assessment conducted by CSL concluded that the western corn rootworm could become established on maize crops in southern England, but is unlikely to cause significant economic damage in the UK (Cannon *et al.* 2005).

<sup>40</sup> [http://www.lifesci.sussex.ac.uk/home/Neil\\_Crickmore/Bt/toxins2.html](http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/toxins2.html)

<sup>41</sup> <http://www.glfrc.forestry.ca/bacillus/>

The following maize events do not target maize pests of economic significance in the UK. However, they are likely to be of interest to farmers in other EU countries and thus may enter the EU approval system in the future. Following EU approval, Bt genes combined with herbicide tolerance traits could be purchased by UK farmers for use of the latter trait.

- Maize event 6275, a second Cry1F maize event developed by Mycogen/Dow Agrosiences for control of a range of moth larvae, has regulatory approval in the US and Canada<sup>42</sup> (EPA 2005). The environmental risk assessment for 6275 was based primarily on the bridging of data submitted to the EPA for event 1507 (EPA 2005) (section 3.2).
- Maize event Pacha, which expresses the vegetative insecticidal protein Vip3A from *Bacillus thuringiensis* in combination with Cry1Ab for the control of a range of moth larvae (Dively 2005). Vip3A differs considerably from Cry1Ab in its toxicity spectrum amongst the Lepidoptera, as neither the monarch (the only butterfly tested) nor the European corn borer or the diamondback moth are sensitive to Vip3A. However, Vip3A is active against the Mediterranean corn-borer (*Sesamia nonagrioides*) (OGTR 2005). Vegetative insecticidal proteins (Vip) are expressed by *Bacillus thuringiensis* during the vegetative stage (as well as during sporulation, the stage at which the Cry proteins (also known as crystal proteins or delta-endotoxins) are expressed)<sup>43</sup>. The mode of action of Vip3A has been described in several refereed publications (Estruch *et al.* 1996, Lee *et al.* 2003, Lee *et al.* 2006). A field study of the effects of the hybrid Pacha X Bt11 on non-target insects has also already been published in a refereed journal (Dively 2005). The risk assessment published by the Australian Office of the Gene Technology Regulator (OGTR 2005) for a limited release of Vip3A cotton includes a list of non-target insects from eight insect orders that were tested for their susceptibility to Vip3A. Of the species tested only lepidopteran species were sensitive to this toxin.
- Maize event MON 89034, expresses the Bt toxins Cry2Ab2 and Cry1A.105 (EPA 2006b). This event appears to be at the field trial stage of development.
- Maize events expressing Cry1B or Cry1B plus Cry1Ab for control of a range of moth larvae (Bohorova *et al.* 2001). These are possibly at an early stage of development.

The novel Bt proteins toxic to chrysomelid beetles (Cry3Bb1, mCry3A and Cry34Ab1/35Ab1) could be expressed in other arable crops where beetle pests are a serious problem. An arable crop where coleopteran pests cause economic damage in the UK is oilseed rape. Attempts have been made before to introduce transgenes for resistance to beetle pests into oilseed rape but the activity of the toxins available at the time was not sufficient (Jouanin *et al.* 2000). The main coleopteran pests of oilseed rape in the UK are the cabbage stem flea beetle (*Psylliodes chrysocephala*, Chrysomelidae), the pollen beetle (*Meligethes aeneus*, Nitidulidae) and two weevil species (*Ceutorhynchus assimilis* and *Ceutorhynchus pallidactylus* Curculionidae). Only the cabbage stem flea beetle is a close relative of *Diabrotica* spp. If one or more of these oilseed rape pests should prove to be susceptible to the novel Bt toxins, a company may be interested in registering a beetle resistant GM oilseed rape event. The invertebrate fauna of oilseed rape in the UK has been extensively studied, including the abundance, diversity and behaviour of beneficial insects (e.g. Alford *et al.* 1995, Murchie *et al.* 1999, Warner *et al.* 2000, Cook *et al.* 2002, Brooks *et al.* 2003, Haughton *et al.* 2003, Hawes *et al.* 2003). These data would provide a good base for environmental risk assessments.

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<sup>42</sup> <http://www.agbios.com/dbase.php?action=Submit&evidx=99>

<sup>43</sup> <http://www.agbios.com/dbase.php> (accessed 11 November 2006)

A wide range of arable crops are attacked by wireworms (the larvae of click beetles, *Agriotes* spp., Elateridae). Another group of pest beetles are stored product beetles, such as the yellow mealworm (*Tenebrio molitor*). As they are not in the family of the Chrysomelidae they are not likely to be sensitive to any of the currently available beetle-active Bt toxins but they may become targets for the development of Bt crops in the future (Wu *et al.* 2000).

In the more distant future, Bt toxins may become a control option for dipteran and nematode pests of crops such as cereals, oilseed rape and potatoes. There is no indication so far that Bt toxins could prove useful in the control of aphid pests of arable crops.

### Vegetables

The commercialization of horticultural GM crops has lagged behind that of arable crops partly due to market reluctance to accept biotech products, to high development and regulatory approval costs as well as restricted access to intellectual property (Clark *et al.* 2004).

Brassicas are an example of a group of vegetable crops for which Bt lines have been under development for a number of years. However, no company has applied for regulatory approval so far. In the UK, cabbages, cauliflower, broccoli and other brassica crops are attacked by a range of lepidopteran pests, including cabbage white butterflies (*Pieris* spp.), cabbage moth (*Mamestra brassicae*) and diamondback moth (*Plutella xylostella*). The latter species is the most serious pest of brassicas on a worldwide scale and has been the main target for the development of several very highly resistant Bt events of *Brassica* spp. The diamondback moth is infamous for developing resistance to a wide range of insecticides and is the only pest that has developed resistance to microbial *Bacillus thuringiensis* subsp. *kurstaki* sprays in open field populations (Talekar and Shelton, 1993; Tabashnik *et al.* 2003, Baxter *et al.* 2005). Single Bt gene events are therefore unlikely to last in the field while Bt brassicas with two stacked Bt genes with different modes of action are predicted to provide durable resistance. In the UK cabbage white butterflies are the more serious target for which UK farmers may wish to purchase Bt seeds, as the diamondback moth only causes economic damage in some years in the UK.

- Cabbage with two unspecified Bt toxins (CIMBAA 2006)
- Cabbage with CRY1Ba1 (Christey *et al.* 2006)
- Broccoli with two unspecified Bt toxins (CIMBAA 2006)
- Broccoli with Cry1Ac and Cry1C (Cao *et al.* 2002)
- Chinese cabbage with Cry1C (Cho *et al.* 2001, Christey *et al.* 2006)

HRI and other institutions have long-term experience with managing the pests of brassicas (e.g. Potting *et al.* 1999, Collier and Minns 2001, Collier *et al.* 2003). A number of in-depth studies have been conducted on the impact of lepidopteran-resistant Bt brassicas on specific beneficial non-target species (Schmaedick and Shelton 1999, Schuler *et al.* 1999a, 2001, 2003, 2004, 2005, Wei *et al.* 2002) and others are underway (CIMBAA 2006).

The novel Bt proteins toxic to leaf beetles (Chrysomelidae) may be expressed in horticultural crops where beetles are serious pests. Examples are flea beetles (*Phyllotreta* spp.) and the lily beetle (*Lilioceris lili*). However, it is not clear if these species are susceptible to any of the novel toxins.

In the future dipteran-active Bt toxins may be available to provide plants with protection against carrot fly (*Psila rosae*) or cabbage root fly (*Hylemya brassicae*). At present Bt toxins do appear to provide a control option for sucking pests such as thrips, whiteflies, aphids or spider mites.

### Trees

Genetic modification of trees may involve traits such as altered wood properties, speeding up of breeding cycles, forests as pharmaceutical factories, remediation, restoration of sensitive landscapes as well as improved resistance to pests and diseases (Gartland *et al.* 2003).

*Populus* is the genus of tree in which genetic modification has been researched most intensively (Boerjan 2005). Bt poplars resistant to lepidopteran or coleopteran pests have been produced. Bt toxins used include Cry1Aa (Kleiner *et al.* 1995), Cry1Ab (Manachini *et al.* 2006), Cry1Ac (Hu *et al.* 2001) and Cry3Aa (Augustin *et al.* 2004). Bt poplar may be of interest in some EU countries (Augustin *et al.* 2004, Manachini *et al.* 2006). In the UK, the planting of poplars is encouraged as short rotation coppice for use as an energy crop<sup>44</sup>. Beetle-resistant poplar may be relevant to UK growers due to damage caused to poplar (and willow) by willow beetles (*Phratora vitellinae*, *Phratora vulgatissima*, Chrysomelidae) (Batley *et al.* 2004) (<http://www.forestry.gov.uk/src>).

Pine is another tree species which has been transformed with the lepidopteran-active Cry1Ac gene (Grace *et al.* 2005). Coleopteran pest species, such as the pine weevil (*Hylobius abietis*, Curculionidae) and the great spruce bark beetle (*Dendroctonus micans*), may become target pests in the future.

The genetic modification of several species of fruit trees, including apple, plum and walnut, has also progressed (Clark *et al.* 2004). Lepidopteran-resistant apple trees could be of interest to UK growers as several moth species are serious pests of apple trees in the UK (including codling moth (*Cydia pomonella*) and several species of tortricids<sup>45</sup>). Apple trees have been transformed with Bt genes as well as with other insect resistance genes (Dandekar *et al.* 1992, Markwick *et al.* 2003) and codling moth resistant apple has advanced to the point of commercial interest in product development (Clark *et al.* 2004).

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<sup>44</sup> <http://www.forestry.gov.uk/src>

<sup>45</sup> Fruitlet Mining Tortrix moth (*Pammene rhediella*), Summer Fruit Tortrix moth (*Adoxophyes orana*) and Fruit Tree Tortrix moth (*Archips podana*) (<http://www.dowagro.com/uk/fruit/tortrix.htm>)

## 5. Conclusions

For some of the Bt toxins (Cry1Ab and Cry3Bb1) expressed in maize, extensive information on non-target studies is available in form of refereed scientific papers while for others few (Cry1F) or no (mCry3A and Cry34Ab1/Cry35Ab1) such papers could be detected in the refereed scientific literature. In the latter cases the only sources of information on non-target studies appear to be studies submitted as part of application dossiers for regulatory approval.

The majority of studies do not show any unexpected negative effects on non-target insects or ecosystem functions. For some of the maize events further data for additional non-target insects as well as more extensive field trial data would be desirable to increase confidence in the biosafety of these new events. Of particular concern are the relatively high expression levels of Bt toxin in pollen of several of the new events. Transgene expression in pollen has long been controversial as it extends the range of non-target insect species exposed to a Bt toxin. Scientists have also expressed concern that understanding of the impacts of Bt toxins on soil biodiversity and functions is still limited.

The non-target studies submitted by the different applicants differ in many respects, including the the scale and scope of field studies conducted, appropriateness of indicator species chosen and the extent to which impacts on soil organisms were investigated. It is recommended that EU-wide guidance is developed regarding the required approaches and standards for non-target studies for the environmental risk assessment of GM crops.

Some Bt crops with more economic relevance to the UK are under development. Novel Bt toxins may prove to be active against pests of more significance to UK farmers than the pests targeted by Bt crops currently in the EU application system.

## 6. Recommendations

- Several of the new maize events express Bt toxins in pollen, which are active against leaf beetles (family Chrysomelidae). Many of the chrysomelid species that occur in the UK are not crop pests. For some of these beetles UK biodiversity action plans have been developed. No information is currently available on the susceptibility of UK non-target chrysomelid species to the novel Bt toxins. A review of the chrysomelid non-target beetles, which occur in habitats next to maize fields in the UK and how their UK distributions overlaps with that of maize cultivation, would help Defra to identify potential species at risk.
- Maize event 1507 expresses relatively high levels of the Bt toxin Cry1F in pollen, which is active against species in several families of Lepidoptera. The susceptibility of non-target European butterflies and moths to Cry1F has been estimated but not measured. Bioassays with UK species would help to define the risk that Cry1F containing maize pollen may pose to non-target Lepidoptera in the UK. Information on the butterfly species that occur in the vicinity of maize fields across much of the country is available from the FSE. Analysis of the presence of larval host plants of moths and butterflies combined with distribution maps of these host plants and maize cultivation in the UK could be used to identify butterfly and moth species potentially at risk. Particular attention should be paid to moth and butterflies species on the UK biodiversity action plan list.
- Environmental risk assessment studies carried out by applicants currently differ in many respects, including the appropriateness of indicator species and exposure method chosen, the scale and scope of field studies conducted as well as the extent to which impacts on soil organisms were investigated. It is recommended that standardised guidance that applies EU-wide is developed regarding the required approaches and standards for non-target studies for the environmental risk assessment of GM crops. Experience with the FSE and assessments of environmental impact of Cry1Ac expressing maize have shown the value of field studies in assessing environmental impacts of GM crops.
- Increasing numbers of applications for approval of GM crops for cultivation in the EU relate to maize plants expressing Bt genes as well as herbicide tolerance genes. It has been recommended by scientists that potential impacts of Bt toxins on the persistence of herbicides should be considered in the environmental impact assessment of GM crops expressing both HT and Bt traits.
- Further research on the impact of Bt toxins in soil is recommended as part of a general push to better understand the impact of agricultural activities on soil function and soil biodiversity. Post market monitoring of the persistence of Bt toxins in soil is recommended and would provide a useful data set for future risk assessments.

## References

- Accinelli, C., C. Screpanti, A. Vicari, and P. Catizone. 2004. Influence of insecticidal toxins from *Bacillus thuringiensis* subsp. *kurstaki* on the degradation of glyphosate and glufosinate-ammonium in soil samples. *Agriculture, Ecosystems & Environment* 103: 497-507.
- Ahmad, A., G. E. Wilde, and K. Y. Zhu. 2005. Detectability of coleopteran-specific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. *Environmental Entomology* 34: 385-394.
- Al-Deeb, M. A., and G. E. Wilde. 2003. Effect of Bt corn expressing the Cry3Bb1 toxin for corn rootworm control on aboveground nontarget arthropods. *Environmental Entomology* 32: 1164-1170.
- Alford, G. V., I. H. Williams, A. K. Murchie, and K. F. A. Walters. 1995. The status and potential of parasitoids of seed weevil and pod midge on winter oilseed rape. HGCA Oilseeds Project Report No. OS14: 89 pp.
- Andow, D. A., and C. Zwahlen. 2006. Assessing environmental risks of transgenic plants. *Ecology Letters* 9: 196-214.
- APHIS. 2005. Environmental Assessment for Dow/Pioneer Rootworm Resistant Corn. Approval of Mycogen Seeds/Dow AgroSciences LLC and Pioneer Hi-Bred International Inc. Request 03-353-01p seeking a Determination of the Non-regulated Status for *Bt* Cry34Ab1/35Ab1 Insect Resistant, Glufosinate Tolerant Corn Line 59122-7, 45 pp, US Animal and Plant Health Inspection Service, USDA, [http://www.aphis.usda.gov/brs/aphisdocs2/03\\_35301p\\_com.pdf](http://www.aphis.usda.gov/brs/aphisdocs2/03_35301p_com.pdf).
- Armer, C. A., R. E. Berry, and M. Kogan. 2000. Longevity of phytophagous heteropteran predators feeding on transgenic Bt-potato plants. *Entomologia Experimentalis et Applicata* 95: 329-333.
- Arpaia, S. 1996. Ecological impact of Bt-transgenic plants: 1. Assessing possible effects of CryIIIB toxin on honey bee (*Apis mellifera* L.) colonies. *J. Genet. Breed.* 50: 315-319.
- Arpaia, S., F. Gould, and G. Kennedy. 1997. Potential impact of *Coleomegilla maculata* predation on adaptation of *Leptinotarsa decemlineata* to Bt-transgenic potatoes. *Entomologia Experimentalis et Applicata* 82: 91-100.
- Augustin, S., C. Courtin, A. Rejasse, P. Lorme, A. Genissel, and D. Bourguet. 2004. Genetics of resistance to transgenic *Bacillus thuringiensis* poplars in *Chrysomela tremulae* (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 97: 1058-1064.
- Batley, J., K. J. Edwards, J. H. Barker, K. J. Dawson, C. W. Wiltshire, D. M. Glen, and A. Karp. 2004. Population structure of the beetle pests *Phyllodecta vulgatissima* and *P. vitellinae* on UK willow plantations. *Insect Molecular Biology* 13: 413-421.
- Baxter, S. W., J.-Z. Zhao, L. J. Gahan, A. M. Shelton, B. E. Tabashnik, and D. G. Heckel. 2005. Novel genetic basis of field-evolved resistance to Bt toxins in *Plutella xylostella*. *Insect Molecular Biology* 14: 327-334.
- Beveridge, N., and J. A. Elek. 1999. *Bacillus thuringiensis* var. *tenebrionis* shows no toxicity to the predator *Chauliognathus lugubris* (F.) (Coleoptera: Cantharidae). *Australian Journal of Entomology* 38: 34-39.
- Bhatti, M. A., J. Duan, G. P. Head, C. Jiang, M. J. McKee, T. E. Nickson, C. L. Pilcher, and C. D. Pilcher. 2005a. Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected Bt corn on foliage-dwelling arthropods. *Environmental Entomology* 34: 1336-1345.
- Bhatti, M. A., J. Duan, G. Head, C. Jiang, M. J. McKee, T. E. Nickson, C. L. Pilcher, and C. D. Pilcher. 2005b. Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected Bt corn on ground-dwelling invertebrates. *Environmental Entomology* 34: 1325-1335.
- Birch, A. N. E., R. Wheatley, B. Anyango, S. Arpaia, D. Capalbo, Emanu Getu D., E. Fontes, P. Kalama, E. Lelmen, G. Løvei, I. S. Melo, F. Muyekho, A. Ngi-Song, D. Ochieno, J. Ogwang, R. Pitelli, T. Schuler, M. Sétamou, S. Sithanatham, J. Smith, N. V. Son, J. Songa, E. S. Q. Tan, F.-H. Wan, and A. Hilbeck. 2004. Biodiversity and Non-Target Impacts: Case Study of Bt Maize in Kenya, pp. 117-185. In D. Andow and A. Hilbeck

- [eds.], Environmental Risk Assessment of Genetically Modified Organisms: A Case Study of Bt Maize in Kenya. CABI International, Wallingford.
- Bitzer, R. J., M. E. Rice, C. D. Pilcher, C. L. Pilcher, and W.-k. f. Lam. 2005. Biodiversity and community structure of epedaphic and euedaphic springtails (Collembola) in transgenic rootworm Bt corn. *Environmental Entomology* 34: 1346–1376.
- Blackwood, C. B., and J. S. Buyer. 2004. Soil microbial communities associated with Bt and non-Bt corn in three soils. *J Environ Qual.* 33: 832-836.
- Boerjan, W. 2005. Biotechnology and the domestication of forest trees. *Current Opinion in Biotechnology* 16: 159-166.
- Bohorova, N., R. Frutos, M. Royer, P. Estanol, M. Pacheco, Q. Rascon, S. McLean, and D. Hoisington. 2001. Novel synthetic *Bacillus thuringiensis* cry1B gene and the cry1B-cry1Ab translational fusion confer resistance to southwestern corn borer, sugarcane borer and fall armyworm in transgenic tropical maize. *Theoretical and Applied Genetics* 103: 817-826.
- Bourguet, D., J. Chaufaux, A. Micoud, M. Delos, B. Naibo, F. Bombarde, G. Marque, N. Eychenne, and C. Pagliari. 2002. *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). *Environmental Biosafety Research* 1: 49-60.
- Brooks, D. R., D. A. Bohan, G. T. Champion, A. J. Houghton, C. Hawes, M. S. Heard, S. J. Clark, A. M. Dewar, L. G. Firbank, J. N. Perry, P. Rothery, R. J. Scott, W. I. P., C. Birchall, M. P. Skellern, J. H. Walker, P. Baker, D. Bell, E. L. Browne, A. J. G. Dewar, C. M. Fairfax, B. H. Garner, L. A. Haylock, S. L. Horne, S. E. Hulmes, N. S. Mason, L. R. Norton, P. Nuttall, Z. Randle, M. J. Rossall, R. J. N. Sands, E. J. Singer, and M. J. Walker. 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* 358: 1847-1862.
- CABI. 1997. Biocontrol News and Information 18.
- Canadian Food Inspection Agency. 2001. Decision Document DD96-09: Determination of Environmental Safety of Event 176 Bt Corn (*Zea mays* L.) Developed by Ciba Seeds and Mycogen Corporation, 10 pp. Canadian Food Inspection Agency, Ontario.
- Cannon, R. J. C., L. Matthews, S. Cheek, R. H. A. Baker, A. MacLeod, P. W. Bartlett, and D. Savage. 2005. Surveying and monitoring western corn rootworm (*Diabrotica virgifera virgifera*) in England & Wales, pp. 155-160. In D. V. Alford and G. F. Backhaus [eds.], Plant protection and plant health in Europe: introduction and spread of invasive species (Proceedings of a conference held on 9-11 June 2005 at Humboldt University, Berlin, Germany).
- Cao, J., J. Z. Zhao, J. D. Tang, A. M. Shelton, and E. D. Earle. 2002. Broccoli plants with pyramided cry1Ac and cry1C Bt genes control diamondback moths resistant to Cry1A and Cry1C proteins. *Theoretical and Applied Genetics* 105: 258-264.
- Chen, E., and C. Stacy. 2003. Modified cry3a toxins and nucleic acid sequences coding therefor. International Application published under the Patent Cooperation Treaty. International Publication number WO03018810. European Patent Office, <http://v3.espacenet.com>.
- Cho, H. S., J. Cao, J. P. Ren, and E. D. Earle. 2001. Control of lepidopteran insect pests in transgenic Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) transformed with a synthetic *Bacillus thuringiensis* cry1C gene. *Cell Biology and Morphogenesis* 20: 1-7.
- Christey, M. C., R. H. Braun, E. L. Conner, J. K. Reader, D. W. R. White, and C. R. Voisey. 2006. Cabbage white butterfly and diamond-back moth resistant *Brassica oleracea* plants transgenic for Cry1Ba1 or Cry1Ca5. In Y. P. Lim [ed.], IV International Symposium on Brassicas and XIV Crucifer Genetics Workshop. ISHS Acta Horticulturae 706, Daejeon, Korea.
- CIMBAA. 2006. Collaboration on Insect Management for Brassicas in Asia and Africa. <http://www.CIMBAA.org> (accessed 11 Nov 2006).
- Clark, D., H. Klee, and A. Dandekar. 2004. Despite benefits, commercialization of transgenic horticultural crops lags. *California Agriculture* 58: 89-98.
- Collier, R., and S. Minns. 2001. Brassicas: Refinement and field validation of forecasts for the caterpillar pests of brassicas. HDC project FV163a. Horticultural Research International, Wellesbourne.

- Collier, R. H., A. Mead, W. E. Parker, and S. A. Ellis. 2003. A risk management system for controlling the foliar pests of *Brassica* crops., pp. 335-340, The BCPC International Congress - Crop Science & Technology, Glasgow.
- Conrad, K. F., I. P. Woiwod, and J. N. Perry. 2002. Long-term decline in abundance and distribution of the garden tiger moth (*Arctia caja*) in Great Britain. *Biological Conservation* 106: 329-337.
- Cook, S. M., E. Bartlett, D. A. Murray, and I. H. Williams. 2002. The role of pollen in the attraction of pollen beetles to oilseed rape flowers. *Entomologia Experimentalis et Applicata* 104: 43-50.
- Crickmore, N., D. R. Zeigler, J. Feitelson, E. Schnepf, J. van Rie, D. Lereclus, J. Baum, and D. H. Dean. 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews* 62: 807-813.
- Dandekar, A. M., G. H. McGranahan, S. L. Uratsu, C. Leslie, P. V. Vail, J. S. Tebbets, D. Hoffmann, J. Driver, P. Viss, and D. J. James. 1992. Engineering for apple and walnut resistance to codling moth, pp. 741-747, Proceedings of the Brighton Crop Protection Conference, Pests and Diseases. British Crop Protection Council, Brighton, UK.
- Deml, R., T. Meise, and K. Dettner. 1999. Effects of *Bacillus thuringiensis* δ-endotoxins on food utilization, growth, and survival of selected phytophagous insects. *Journal of Applied Entomology* 123: 55-64.
- Devare, M., C. Jones, and J. Thies. 2004. Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity, and diversity. *J Environ Qual* 33: 837-843.
- Dively, G. 2005. Impact of transgenic VIP3A x Cry1Ab lepidopteran-resistant field corn on the nontarget arthropod community. *Environmental Entomology* 34: 1267-1291.
- Dively, G. P., R. Rose, M. K. Sears, R. L. Hellmich, D. E. Stanley-Horn, D. D. Calvin, J. M. Russo, and P. L. Anderson. 2004. Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. *Environmental Entomology* 33: 1116-1125.
- Dogan, E. B., R. E. Berry, G. L. Reed, and P. A. Rossignol. 1996. Biological parameters of convergent lady beetle (Coleoptera: Coccinellidae) feeding on aphids (Homoptera: Aphididae) on transgenic potato. *Journal of Economic Entomology* 89: 1105-1108.
- Donegan, K. K., D. L. Schaller, J. K. Stone, L. M. Ganio, G. Reed, P. B. Hamm, and R. J. Seidler. 1996. Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the *Bacillus thuringiensis* var *tenebrionis* endotoxin. *Transgenic Research* 5: 25-35.
- Dow Agro Sciences. 2003. Notification for the Placing on the Market of Insect-tolerant Genetically Modified Maize 1507 for Import, Feed and Industrial Processing and Cultivation, under Part C of Directive 2001/18/EC (C/ES/01/01).
- Dutton, A., J. Romeis, and F. Bigler. 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *BioControl* 48: 611-636.
- Dutton, A., H. Klein, J. Romeis, and F. Bigler. 2002a. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecological Entomology* 27: 441-447.
- Dutton, A., H. Klein, J. Romeis, and F. Bigler. 2002b. Assessing Bt-toxin content of piercing-sucking herbivores on transgenic maize and consequences for *Chrysoperla carnea*. *Antenna (London)* 26: 105-106.
- Eder, J. 2006. Bericht zum Erprobungsanbau mit gentechnisch veraendertem Mais in Bayern 2005. Bayerische Landesanstalt fuer Landwirtschaft, Freising, Germany.
- EFSA. 2005a. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safeguard clause invoked by Hungary according to Article 23 of Directive 2001/18/EC. *The EFSA Journal* 228: 1-14.
- EFSA. 2005b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507 for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds (Question No EFSA-Q-2004-072). *The EFSA Journal* 181: 1-33.

- EFSA. 2006. European Food Safety Authority. Applications under Regulation (EC) 1829/2003 on Genetically Modified Food and Feed. Accessed 20 October 2006. [http://www.efsa.europa.eu/en/science/gmo/gm\\_ff\\_applications.html](http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications.html).
- Ellis, R. T., B. A. Stockhoff, L. Stamp, H. E. Schnepf, G. E. Schwab, M. Knuth, J. Russell, G. A. Cardineau, and K. E. Narva. 2002. Novel *Bacillus thuringiensis* binary insecticidal crystal proteins active on western corn rootworm, *Diabrotica virgifera virgifera* LeConte. *Appl Environ Microbiol.* 68: 1137-1145.
- EPA. 2000a. Biopesticide Fact Sheet 006458: *Bacillus thuringiensis* Cry1Ab Delta-Endotoxin and the Genetic Material Necessary for Its Production (Plasmid Vector pCIB4431) in Corn [Event 176], pp. 24 pp. United States Environmental Protection Agency <http://www.epa.gov/pesticides/biopesticides/factsheets/fs006463.htm>.
- EPA. 2000b. Bt Plant-Pesticides Biopesticides Registration Action Document, United States Environmental Protection Agency, [http://www.epa.gov/oscpmont/sap/meetings/2000/october/brad2\\_scienceassessment.pdf](http://www.epa.gov/oscpmont/sap/meetings/2000/october/brad2_scienceassessment.pdf).
- EPA. 2001. Pesticide Fact Sheet: *Bacillus thuringiensis* subspecies Cry1F Protein and the Genetic Material Necessary for Its Production (Plasmid Insert PHI 8999) in Corn (006481), pp. 28 pp. United States Environmental Protection Agency, [http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet\\_006491.htm](http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_006491.htm).
- EPA. 2005. *Bacillus thuringiensis* var. *aizawai* strain PS811 Cry1F insecticidal crystal protein and the genetic material necessary for its production (plasmid insert PHP12537) in Event DAS-06275-8 corn (006491) Fact sheet. U.S. Environmental Protection Agency.
- EPA. 2006a. mCry3A Fact Sheet. Publication number EPA 70-F-06-001. United States Environmental Protection Agency, [http://www.epa.gov/opppbd1/biopesticides/ingredients/factsheets/factsheet\\_mcry3a.htm](http://www.epa.gov/opppbd1/biopesticides/ingredients/factsheets/factsheet_mcry3a.htm).
- EPA. 2006b. Environmental Protection Agency [EPA-HQ-OPP-2006-0298; FRL-8066-8] Experimental Use Permit - Receipt of Application. *US Federal Register* 71: 30403.
- EPA. 2006c. Environmental risk assessment for modified Cry3A (mCry3A) *Bacillus thuringiensis* protein and the genetic material necessary for its production in event MIR604 corn. Background Document FIFRA Meeting 14-15 March 2006., 21 pp, US Environmental Protection Agency. [http://www.epa.gov/scipoly/sap/meetings/2006/march/mcry3a\\_2\\_9\\_2006\\_env\\_risk\\_assessment.pdf](http://www.epa.gov/scipoly/sap/meetings/2006/march/mcry3a_2_9_2006_env_risk_assessment.pdf).
- Estruch, J. J., G. W. Warren, M. A. Mullins, G. J. Nye, J. A. Craig, and M. G. Koziel. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proceedings of the National Academy of Sciences, USA* 93: 5389-5394.
- Felke, M. 2006. Untersuchungen zu Nebenwirkungen der *Diabrotica*-resistenten, transgenen Maislinie MON88017 auf Nict-Ziel-Chrysomeliden. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 400: 362.
- Felke, M., and G. A. Langenbruch. 2003. Wirkung von Bt-Mais-Pollen auf Raupen des Tagpfauenauges im Laborversuch (Effect of Bt-maize-pollen on caterpillars of *Inachis io* in a laboratory assay). *Gesunde Pflanzen* 55: 1-7.
- Felke, M., N. Lorenz, and G. A. Langenbruch. 2002. Laboratory studies on the effects of pollen from Bt-maize on larvae of some butterfly species. *Journal of Applied Entomology* 126: 320-325.
- Ferro, D. N. 2000. Success and failure of Bt products: Colorado potato beetle - a case study, pp. 177-189. In G. G. Kennedy and T. B. Sutton [eds.], *Emerging Technologies for Integrated Pest Management*. APS Press, St. Paul, MN.
- Firbank, L., M. Lonsdale, and G. Poppy. 2005. Reassessing the environmental risks of GM crops. *Nature Biotechnology* 23: 1475-1476.
- Fitt, G. P., and L. J. Wilson. 2000. Genetic engineering in IPM: Bt cotton, pp. 108-125. In G. G. Kennedy and T. B. Sutton [eds.], *Emerging Technologies for Integrated Pest Management*. APS Press, St. Paul, MN.
- Galitsky, N., V. Cody, A. Wojtczak, D. Ghosh, J. Luft, W. Pangborn, and L. English. 2001. Structure of the insecticidal bacterial delta-endotoxin Cry3Bb1 of *Bacillus thuringiensis*. *Acta Crystallogr D Biol Crystallogr* 57: 1101-1109.
- Gao, Y., B. W. Schafer, R. Collins, and e. al. 2004. Characterization of Cry34Ab1 and Cry35Ab1 insecticidal crystal proteins expressed in transgenic corn plants and

- Pseudomonas fluorescens*. *Journal of Agricultural and Food Chemistry* 52: 8057-8065.
- Gartland, K. M. A., R. M. Crow, T. M. Fenning, and J. S. Gartland. 2003. Genetically modified trees: production, properties, and potential. *Journal of Arboriculture* 29: 259.
- Glare, T. R., and M. O'Callaghan. 2000. *Bacillus thuringiensis*: Biology, Ecology and Safety. John Wiley & Sons, Chichester.
- Grace, L. J., J. A. Charity, B. Gresham, N. Kay, and W. C. 2005. Insect-resistant transgenic *Pinus radiata*. *Plant Cell Rep.* 24: 103-111.
- Groot, A. T., and M. Dicke. 2002. Insect-resistant transgenic plants in a multi-trophic context. *The Plant Journal* 31: 387-406.
- Hanley, A. V., Z. Y. Huang, and W. L. Pett. 2003. Effects of dietary transgenic Bt corn pollen on larvae of *Apis mellifera* and *Galleria mellonella*. *Journal of Apicultural Research* 42: 77-81.
- Hansen Jesse, L. C., and J. J. Obrycki. 2000. Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia* 125: 241-248.
- Harwood, J. D., W. G. Wallin, and J. J. Obrycki. 2005. Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. *Molecular Ecology* 14: 2815-.
- Haughton, A. J., G. T. Champion, C. Hawes, M. S. Heard, D. R. Brooks, D. A. Bohan, S. J. Clark, A. M. Dewar, L. G. Firbank, J. L. Osborne, J. N. Perry, P. Rothery, D. B. Roy, R. J. Scott, W. I. P., C. Birchall, M. P. Skellern, J. H. Walker, P. Baker, E. L. Browne, A. J. G. Dewar, B. H. Garner, L. A. Haylock, S. L. Horne, N. S. Mason, R. J. N. Sands, and M. J. Walker. 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Within-field epical and aerial arthropods. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* 358: 1863-1877.
- Hawes, C., A. J. Haughton, J. L. Osborne, D. B. Roy, S. J. Clark, J. N. Perry, P. Rothery, D. A. Bohan, D. R. Brooks, G. T. Champion, A. M. Dewar, M. S. Heard, I. P. Woiwod, R. E. Daniels, M. W. Young, A. M. Parish, R. J. Scott, L. G. Firbank, and G. R. Squire. 2003. Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* 358: 1899-1913.
- Head, G., C. R. Brown, M. E. Groth, and J. J. Duan. 2001. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. *Entomologia Experimentalis et Applicata* 99: 37-45.
- Health Canada. 2002. Cry1F Insect-resistant/Glufosinate-tolerant Maize Line 1507. Genetically Modified (GM) Foods & Other Novel Foods. Approved Products. Health Products and Food Branch, Ottawa, [http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/cry1f\\_insect\\_e.html](http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/cry1f_insect_e.html)
- Hellmich, R. L., B. D. Siegfried, M. K. Sears, D. E. Stanley-Horn, M. J. Daniels, H. R. Mattila, T. Spencer, K. G. Bidne, and L. C. Lewis. 2001. Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. *Proceedings of the National Academy of Sciences, USA* 98: 11925-11930.
- Herman, R., B. Schafer, V. Korjagin, and A. Ernest. 2003. Rapid digestion of Cry34Ab1 and Cry35Ab1 in simulated gastric fluid. *J Agric Food Chem* 51: 6823-6827.
- Herman, R. A., S. L. Evans, D. M. Shanahan, C. A. Mihaliak, G. A. Bormett, D. L. Young, and J. Buehrer. 2001. Rapid degradation of Cry1F delta-endotoxin in soil. *Environmental Entomology* 30: 642-644.
- Herman, R. A., P. N. Scherer, D. L. Young, C. A. Mihaliak, T. Meade, A. T. Woodsworth, B. A. Stockhoff, and K. E. Narva. 2002. Binary insecticidal crystal protein from *Bacillus thuringiensis*, strain PS149B1: Effects of individual protein components and mixtures in laboratory bioassays. *Journal of Economic Entomology* 95: 635-539.
- Herrero, S., M. Borja, and J. Ferré. 2002. Extent of variation of the *Bacillus thuringiensis* toxin reservoir: the case of the Geranium Bronze, *Cacyreus marshalli* Butler (Lepidoptera: Lycaenidae). *Appl. Environ. Micro.* 68: 4090-4094.
- Hilbeck, A., M. Baumgartner, P. M. Fried, and F. Bigler. 1998a. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* 27: 480-487.

- Hilbeck, A., W. J. Moar, M. PusztaiCarey, A. Filippini, and F. Bigler. 1998b. Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* 27: 1255-1263.
- Hoole, J. C., D. A. Joyce, and A. S. Pullin. 1999. Estimates of gene flow between populations of the swallowtail butterfly, *Papilio machaon* in Broadland, UK and implications for conservation. *Biological Conservation* 89: 293-299.
- Hoy, C. W., J. Feldman, F. Gould, G. G. Kennedy, G. Reed, and J. A. Wyman. 1998. Naturally occurring biological controls in genetically engineered crops, pp. 185-205. In P. Barbosa [ed.], *Conservation Biological Control*. Academic Press, London.
- Hu, J. J., Y. C. Tian, Y. F. Han, L. Li, and B. E. Zhang. 2001. Field evaluation of insect-resistant transgenic *Populus nigra* trees. *Euphytica* 121: 123-127.
- Johnson, T. B., A. C. Slaney, W. Donovan, and M. Rugar. 2003. Insecticidal activity of EG4961, a novel strain of *Bacillus thuringiensis* toxic to larvae and adults of southern corn rootworm (Coleoptera: Chrysomelidae) and Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 86: 330-333.
- Jouanin, L., M. Bonadé-Bottino, C. Girard, J. Lerin, and M.-H. Pham Deleue. 2000. Expression of protease inhibitors in rapeseed, pp. 182-194. In D. Michaud [ed.], *Recombinant protease inhibitors in plants*. Landes Bioscience, USA.
- Kerguelen, V., and R. T. Carde. 1996. Increased host acceptance in experienced females of the parasitoid *Brachymeria intermedia* : which types of oviposition behaviour contribute to experience ? *Entomologia experimentalis et applicata* 78: 95-103.
- Kleiner, K. W., D. D. Ellis, B. H. McCown, and K. F. Raffa. 1995. Field evaluation of transgenic poplar expressing a *Bacillus thuringiensis* cry1A(a)  $\delta$ -endotoxin gene against forest tent caterpillar (Lepidoptera : Lasiocampidae) and gypsy moth (Lepidoptera : Lymantriidae) following winter dormancy. *Environmental Entomology* 24: 1358-1364.
- Lang, A. 2004. Monitoring the impact of Bt maize on butterflies in the field: estimation of required sample sizes. *Environmental Biosafety Research* 3: 55-66.
- Lang, A., and E. Vojtech. 2006. The effects of pollen consumption of transgenic Bt maize on the common swallowtail, *Papilio machaon* L. (Lepidoptera, Papilionidae). *Basic and Applied Ecology* 7: 296-306.
- Lang, A., C. Ludy, and E. Vojtech. 2004. Dispersion and deposition of Bt maize pollen in field margins. *Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz - Journal of Plant Diseases and Protection* 111: 417-428.
- Lavrik, P. B., D. E. Bartnicki, J. Feldman, B. G. Hammond, P. J. Keck, S. L. Love, M. W. Naylor, G. J. Rogan, S. R. Sims, and R. L. Fuchs. 1995. Safety assessment of potatoes resistant to Colorado potato beetle, pp. 148-158. In K. H. Engel, G. R. Takeoka and R. Teranishi [eds.], *ACS Symposium on Genetically Modified Foods: Safety Issues*. American Chemical Society, Washington.
- Lee, M. K., P. Miles, and J.-S. Chen. 2006. Brush border membrane binding properties of *Bacillus thuringiensis* Vip3A toxin from *Heliothis virescens* and *Helicoverpa zea* midguts. *Biochemical and Biophysical Research Communications* 339: 1043-1047.
- Lee, M. K., F. S. Walters, H. Hart, N. Palekar, and J.-S. Chen. 2003. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab  $\delta$ -endotoxin. *Applied and Environmental Microbiology* 69: 4648-4657.
- LfL. 2005. Monitoring of the Environmental Effects of the Bt Gene, pp. 111 pp, Bayerische Landesanstalt fuer Landwirtschaft, Freising, Germany. [http://www.lfl.bayern.de/publikationen/daten/schriftenreihe\\_url\\_1\\_43.pdf](http://www.lfl.bayern.de/publikationen/daten/schriftenreihe_url_1_43.pdf).
- Lopez, M. D., J. R. Prasifka, D. J. Bruck, and L. C. Lewis. 2005. Utility of ground beetle species in field tests of potential nontarget effects of Bt Crops. *Environmental Entomology* 34: 1317-1324.
- López, R., and D. N. Ferro. 1995. Larviposition response of *Myiopharus doryphorae* (Diptera, Tachiridae) to Colorado potato beetle (Coleoptera: Chrysomelidae) larvae treated with lethal and sublethal doses of *Bacillus thuringiensis* Berliner subsp. *tenebrionis*. *Journal of Economic Entomology* 88: 870-874.
- Losey, J. E., L. S. Rayor, and M. E. Carter. 1999. Transgenic pollen harms monarch larvae. *Nature* 399: 214.
- Lozzia, G. C. 1999. Biodiversity and structure of ground beetle assemblages (Coleoptera Carabidae) in Bt corn and its effects on non-target insects. *Bollettino di Zoologia Agraria e di Bachicoltura Serie II* 31: 37-58.

- Lozzia, G. C., C. Furlanis, B. Manachini, and I. E. Rigamonti. 1998. Effects of Bt corn on *Rhopalosiphum padi* L. (Rhynchota Aphididae) and on its predator *Chrysoperla carnea* Stephen (Neuroptera Chrysopidae). *Bollettino di Zoologia Agraria e di Bachicoltura Serie II* 30: 153-164.
- Lozzia, G. C., I. E. Rigamonti, B. Manachini, and R. Rocchetti. 2000. Laboratory studies on the effects of transgenic corn on the spider mite *Tetranychus urticae* Koch. *Bollettino di Zoologia Agraria e di Bachicoltura Serie II* 32: 35-47.
- Lundgren, J. G., and R. N. Wiedenmann. 2002. Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). *Environmental Entomology* 31: 1213-1218.
- Maagd, R. A. d., A. Bravo, and N. Crickmore. 2001. How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. *Trends in Genetics* 17: 193-199.
- Manachini, B. 2000. Ground beetle assemblages (Coleoptera, Carabidae) and plant dwelling non-target arthropods in isogenic and transgenic corn crops. *Bollettino di Zoologia Agraria e di Bachicoltura Serie II* 32: 181-198.
- Manachini, B. 2003. Effects of transgenic corn on *Lydella thompsoni* Herting (Diptera Tachinidae) parasitoid of *Ostrinia nubilalis* Hb. (Lepidoptera Crambidae). *Bollettino di Zoologia Agraria e di Bachicoltura Serie II* 35: 111-125.
- Manachini, B., and G. C. Lozzia. 2002. First investigations into the effects of Bt corn on Nematofauna. *Bollettino di Zoologia Agraria e di Bachicoltura Serie II* 34: 85-96.
- Manachini, B., and G. C. Lozzia. 2004. Studies on the effects of Bt corn expressing Cry1Ab on two parasitoids of *Ostrinia nubilalis* Hb. (Lepidoptera: Crambidae). IOBC WORS Bulletin (GMOs in Integrated Production) 27: 109-116.
- Manachini, B., B. Basso, F. Sala, and H. Yifan. 2006. Transgenic poplar against Lepidoptera: an Italy-China project for biosafety and conservation, Proceedings of the 50th Italian Society of Agricultural Genetics Annual Congress, Ischia, Italy.
- Markwick, N. P., L. C. Docherty, P. M. M., M. T. Lester, C. Murray, J. L. Yao, D. S. Mitra, D. Cohen, L. L. Beuning, S. Kutty-Amma, and J. T. Christeller. 2003. Transgenic tobacco and apple plants expressing biotin-binding proteins are resistant to two cosmopolitan insect pests, potato tuber moth and lightbrown apple moth, respectively. *Transgenic Research* 12: 671-681.
- Masson, L., G. Schwab, A. Mazza, and e. al. 2004. A novel *Bacillus thuringiensis* (PS149B1) containing a Cry34Abl/Cry35Abl binary toxin specific for the western corn rootworm *Diabrotica virgifera virgifera* LeConte forms ion channels in lipid membranes. *Biochemistry* 43: 12349-12357.
- Meier, M. S., and A. Hilbeck. 2001. Influence of transgenic *Bacillus thuringiensis* corn-fed prey on prey preference of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Basic and Applied Ecology* 2: 35-44.
- Miller, N., A. Estoup, S. Toepfer, D. Bourguet, L. Lapchin, S. Derridj, K. Kim, P. Reynaud, L. Furlan, and T. Guillemaud. 2005. Multiple transatlantic introductions of the western corn rootworm. *Science* 310: 992.
- Monsanto. 2001. Petition for the Determination of Nonregulated Status for the Regulated Article: Corn Rootworm Protected Corn Event MON 863, Petition #01-CR-050U, 133 pp, USA, <http://www.agbios.com/docroot/decdocs/04-225-001.pdf>.
- Monsanto. 2004. Petition for the Determination of Nonregulated Status for MON 88017 Corn, Petition # 04-CR-108U, 277 pp, USA.
- Mulder, C., M. Wouterse, M. Raubuch, W. Roelofs, and M. Rutgers. 2006. Can transgenic maize affect soil microbial communities. *PLoS Computational Biology* 2: 1165-1172.
- Murchie, A., I. Williams, and J. Perry. 1999. Edge distributions of *Ceutorhynchus assimilis* and its parasitoid *Trichomalus perfectus* in a crop of winter oilseed rape (*Brassica napus*). *Biocontrol* 44: 379-390.
- Musser, F. R., and A. M. Shelton. 2003. Bt sweet corn and selective insecticides: Impacts on pests and predators. *Journal of Economic Entomology* 96: 71-80.
- Natural Resources Canada. 2006. The *Bacillus thuringiensis* Toxin Specificity Database. Canadian Forest Service. [http://www.glfsc.forestry.ca/science/research/netintro99\\_e.html](http://www.glfsc.forestry.ca/science/research/netintro99_e.html) (accessed 11 Oct 2006)
- O'Callaghan, M., G. T. R., E. P. Burgess, and L. A. Malone. 2005. Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology* 50: 271-292.

- Obrist, L., H. Klein, A. Dutton, and F. Bigler. 2006. Assessing the effects of Bt maize on the predatory mite *Neoseiulus cucumeris*. *Exp Appl Acarol* 38: 125-139.
- Obrycki, J. J., E. J. Losey, O. R. Taylor, and L. C. H. Jesse. 2001. Transgenic insecticidal corn: beyond insecticidal toxicity to ecological complexity. *Bioscience* 51: 353-361.
- OGTR. 2005. Risk assessment and risk management plan for DIR 058/2005. Limited and controlled release of insect resistant (VIP) GM cotton. Applicant: Deltapine Australia Pty Ltd. 89 pp, October 2005. Australian Department of Health and Ageing. Office of the Gene Technology Regulator. <http://www.ogtr.gov.au/rtf/ir/>.
- Pagel-Wieder, S., H. Hunfeld, F. Gessler, J. Niemeyer, and H. Boehnel. 2006. Sorption von Cry1Ab und Cry3Bb1 an Tonfraktionen von Böden unterschiedlicher Freisetzungsfächen. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 400: 359.
- Perlak, F. J., T. B. Stone, Y. M. Muskopf, L. J. Petersen, G. B. Parker, S. A. McPherson, J. Wyman, S. Love, G. Reed, D. Biever, and D. A. Fischhoff. 1993. Genetically improved potatoes: protection from damage by Colorado potato beetles. *Plant Molecular Biology* 22: 313-321.
- Potting, R. P. J., G. M. Poppy, and T. H. Schuler. 1999. The role of volatiles from cruciferous plants and pre-flight experience in the foraging behaviour of the specialist parasitoid *Cotesia plutellae*. *Entomologia Experimentalis et Applicata* 93: 87-95.
- Raps, A., J. Kehr, P. Gugerli, W. J. Moar, F. Bigler, and A. Hilbeck. 2001. Detection of Cry1Ab in phloem sap of *Bacillus thuringiensis* corn and in the non-target herbivores *Rhopalosiphum padi* (Homoptera: Aphidae) and *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Molecular Ecology* 10: 525-533.
- Redbond, M. 2006. The spread of the western corn rootworm to Europe. *International Pest Control* 48: 138-139.
- Reed, G. L., A. S. Jensen, J. Riebe, G. Head, and J. J. Duan. 2001. Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts. *Entomologia Experimentalis et Applicata* 100: 89-100.
- Riddick, E. W., and P. Barbosa. 1998. Impact of Cry3A-intoxicated *Leptinotarsa decemlineata* (Coleoptera : Chrysomelidae) and pollen on consumption, development, and fecundity of *Coleomegilla maculata* (Coleoptera : Coccinellidae). *Annals of the Entomological Society of America* 91: 303-307.
- Riddick, E. W., G. Dively, and P. Barbosa. 1998. Effect of a seed-mix deployment of Cry3A-transgenic and nontransgenic potato on the abundance of *Lebia grandis* (Coleoptera : Carabidae) and *Coleomegilla maculata* (Coleoptera : Coccinellidae). *Annals of the Entomological Society of America* 91: 647-653.
- Riddick, E. W., G. Dively, and P. Barbosa. 2000. Season-long abundance of generalist predators in transgenic versus nontransgenic potato fields. *Journal of Entomological Science* 35: 349-359.
- Romeis, J., M. Meissle, and F. Bigler. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology* 24: 63-71.
- Roush, R. T., G. P. Fitt, N. W. Forrester, and J. C. Daly. 1998. Resistance management for insecticidal transgenic crops: theory and practice, pp. 247-257. In M. P. Zalucki, R. A. I. Drew and G. G. White [eds.], Proceedings of the Sixth Australasian Applied Entomological Research Conference, Brisbane, Australia.
- Roy, D. B., D. A. Bohan, A. J. Haughton, M. O. Hill, J. L. Osborne, S. J. Clark, J. N. Perry, P. Rothery, R. J. Scott, D. R. Brooks, G. T. Champion, C. Hawes, M. S. Heard, and L. G. Firbank. 2003. Invertebrate and vegetation of field margins adjacent to crops subject to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences* 358: 1879-1898.
- Sanders, C. J., T. H. Schuler, J. K. Pell, G. M. Poppy, and A. Raybould. 2004. Survival and development of *Spodoptera frugiperda* and two of its natural enemies on Bt transgenic maize, Abstract, International Congress of Entomology, Brisbane.
- Saxena, D., S. Flores, and G. Stotzky. 1999. Insecticidal toxin in root exudates from Bt corn. *Nature* 402: 480.
- Saxena, D., C. N. Stewart, I. Altosaar, Q. Shu, and G. Stotzky. 2004. Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B.*

- thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco. *Plant Physiol Biochem* 42: 383-387.
- Schlein, O., and W. Büchs. 2006. Fraßleistung und Fitness räuberischer Käfer nach Aufnahme von mit Bt-Mais mit *Diabrotica*-Resistenz ernährten Trauermückenlarven. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 400: 358.
- Schmaedick, M. A., and H. M. Shelton. 1999. Experimental evaluation of arthropod predation on *Pieris rapae* (Lepidoptera: Pieridae) eggs and larvae on cabbage. *Environmental Entomology* 28: 439-444.
- Schnepf, E., N. Crickmore, J. v. Rie, D. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler, and D. H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62: 775-806.
- Schnepf, H. E., S. Lee, J. Dojillo, P. Burmeister, K. Fencil, Morera, L. Nygaard, K. E. Narva, and J. D. Wolt. 2005. Characterization of Cry34/Cry35 binary insecticidal proteins from diverse *Bacillus thuringiensis* strain collections. *Appl Environ Microbiol.* 71: 1765-1774.
- Scholte, E.-J., and M. Dicke. 2005. Effects of insect-resistant transgenic crops on non-target arthropods: first step in pre-market risk assessment studies, Report, pp. 59. Wageningen University and Research Centre, Wageningen, <http://www.cogem.net/ContentFiles/CGM%202005-06.pdf>.
- Schuler, T. H., G. M. Poppy, and I. Denholm. 2000. Recommendations for the assessment of effects of GM crops on non-target organisms, pp. 1221-1228, Proceedings of the Brighton Crop Protection Conference, Pests and Diseases, Brighton.
- Schuler, T. H., R. P. J. Potting, I. Denholm, and G. M. Poppy. 1999a. Parasitoid behaviour and Bt plants. *Nature* 400: 825-826.
- Schuler, T. H., G. M. Poppy, B. R. Kerry, and I. Denholm. 1999b. Potential side effects of insect-resistant transgenic plants on arthropod natural enemies. *Trends in Biotechnology* 17: 210-216.
- Schuler, T. H., I. Denholm, S. J. Clark, C. N. Stewart, and G. M. Poppy. 2004. Effects of Bt plants on the development and survival of the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) in susceptible and Bt-resistant larvae of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Insect Physiology* 50: 435-443.
- Schuler, T. H., I. Denholm, L. Jouanin, S. J. Clark, A. J. Clark, and G. M. Poppy. 2001. Population-scale laboratory studies of the effect of transgenic plants on non-target insects. *Molecular Ecology* 10: 1845-1853.
- Schuler, T. H., A. J. Clark, S. J. Clark, G. M. Poppy, C. N. Stewart, and I. Denholm. 2005. Laboratory studies of the effects of reduced prey choice caused by Bt plants on a predatory insect. *Bulletin of Entomological Research* 95: 243-247.
- Schuler, T. H., R. P. J. Potting, I. Denholm, S. J. Clark, A. J. Clark, C. N. Stewart, and G. M. Poppy. 2003. Tritrophic choice experiments with Bt plants, the diamondback moth (*Plutella xylostella*) and the parasitoid *Cotesia plutellae*. *Transgenic Research* 12: 351-361.
- Sears, M. K., and D. Stanley-Horn. 2000. Impact of Bt corn pollen on monarch butterfly populations, pp. 120-130. In C. Fairbairn, G. Scoles and A. McHughen [eds.], Proceedings of the 6th International Symposium on the Biosafety of Genetically Modified Organisms, Saskatoon, Canada.
- Sears, M. K., R. L. Hellmich, D. E. Stanley-Horn, K. S. Oberhauser, J. M. Pleasants, H. R. Mattila, B. D. Siegfried, and G. P. Dively. 2001. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. *Proceedings of the National Academy of Sciences of the U.S.A.* 98: 11937-11942.
- Shieh, J.-N., R. E. Berry, G. L. Reed, and P. A. Rossignol. 1994. Feeding activity of green peach aphid (Homoptera: Aphididae) on transgenic potato expressing a *Bacillus thuringiensis* ssp. *tenebrionis* d-endotoxin gene. 87: 618 - 622.
- Steiner, H., E. Chen, and M. Meghui. 2005. Corn event MIR604. International Application Published under the Patent Cooperation Treaty. International Publication Number WO2005103301. European Patent Office, <http://v3.espacenet.com>.
- Storer, N., J. M. Babcock, and J. M. Edwards. 2006. Field measures of western corn rootworm (Coleoptera : Chrysomelidae) mortality caused by Cry34/35Ab1 proteins

- expressed in maize event 59122 and implications for trait durability. *Journal of Economic Entomology* 99: 1381-1387.
- Stotzky, G. 2004. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. *Plant and Soil* 266: 77-89.
- Syngenta. 2005. Application for import and use of Event MIR604 maize derived products. Part II: Summary, 28 pp. European Food Safety Authority, [http://www.efsa.europa.eu/en/science/gmo/gm\\_ff\\_applications/more\\_info/818.html](http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications/more_info/818.html).
- Tabashnik, B. E., Y. Carrière, T. J. Dennehy, S. Morin M. S. Sisterson, R. T. Roush, A. M. Shelton, and J. Z. Zhao. 2003. Insect resistance to transgenic *Bacillus thuringiensis* crops: lessons from the laboratory and field. *Journal of Economic Entomology* 96: 1031-1038.
- Talekar, N. S. and A. M. Shelton. 1993. Biology, ecology and management of the diamondback moth. *Annual Review of Entomology* 38: 275-301.
- Thu, H. N., A. Rosisko, and J. Jehle. 2006. Cry3Bb1-Expression der transgenen Maislinie MON88017 im Freiland. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 400: 360.
- Vaeck, M., A. Reynaerts, H. Höfte, S. Jansens, M. D. Beuckeleer, C. Dean, M. Zabeau, M. V. Montagu, and J. Leemans. 1987. Transgenic plants protected from insect attack. *Nature* 328: 33-37.
- Vercesi, M. L., P. H. Krogh, and M. Holmstrup. 2006. Can *Bacillus thuringiensis* (Bt) corn residues and Bt-corn plants affect life-history traits in the earthworm *Aporrectodea caliginosa*? *Applied Soil Ecology* 32: 180-187.
- Vidal, S., U. Kuhlmann, and C. R. Edwards [eds.]. 2005. Western Corn Rootworm Ecology and Management. CABI Publishing, Wallingford, UK.
- Walters, F. S., and L. H. English. 1995. Toxicity of *Bacillus thuringiensis* delta-endotoxin toward the potato aphid in an artificial diet bioassay. *Entomologia Experimentalis et Applicata* 77: 211-216.
- Warner, D., L. Allen-Williams, A. Ferguson, and I. Williams. 2000. Pest-predator spatial relationships in winter rape: implications for integrated crop management. *Pest Management Science* 56: 977-982.
- Wei, J.-Z., K. Hale, L. Carta, E. Platzer, C. Wong, S.-C. Fang, and R. V. Aroian. 2003. *Bacillus thuringiensis* crystal proteins that target nematodes. *Proceedings of the National Academy of Sciences USA* 100: 2760-2765.
- Wei, W., T. H. Schuler, S. J. Clark, C. N. Stewart, and G. M. Poppy. 2002. The variational expression of Bt proteins within transgenic canola and its movement through trophic levels, 7th International Symposium on the Biosafety of Genetically Modified Organisms, Beijing, China.
- Wirth, M. C., J. A. Jiannino, B. A. Federici, and W. E. Walton. 2004. Synergy between toxins of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus*. *J Med Entomol.* 41: 935-941.
- Wolt, J., C. Conlan, and K. Majima. 2005. An ecological risk assessment of Cry1F maize pollen impact to pale grass blue butterfly. *Environ Biosafety Res.* 4: 243-251.
- Wraight, C. L., A. R. Zangerl, M. J. Carroll, and M.R. Berenbaum. 2000. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proceedings of the National Academy of Sciences USA* 97: 7700-7703.
- Wu, S.-J., C. N. Koller, D. L. Miller, L. S. Bauer, and D. H. Dean. 2000. Enhanced toxicity of *Bacillus thuringiensis* Cry3A d-endotoxin in coleopterans by mutagenesis in a receptor binding loop. *FEBS Letters* 473: 227-232.
- Yu, L., R. E. Berry, and B. A. Croft. 1997. Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: Oribatidae). *Journal of Economic Entomology* 90: 113-118.
- Zahiri, N., B. Federici, and M. Mulla. 2004. Laboratory and simulated field evaluation of a new recombinant of *Bacillus thuringiensis* ssp. *israelensis* and *Bacillus sphaericus* against *Culex* mosquito larvae (Diptera: Culicidae). *J Med Entomol.* 41: 423-429.
- Zangerl, A. R., D. McKenna, C. Wraight, M. Carroll, P. Ficarelo, R. Warner, and M. R. Berenbaum. 2001. Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. *Proceedings of the National Academy of Sciences of the USA* 98: 11908-11912.
- Zwahlen, C., W. Nentwig, F. Bigler, and A. Hilbeck. 2000. Tritrophic interactions of transgenic *Bacillus thuringiensis* corn, *Anaphothrips obscurus* (Thysanoptera: Thripidae), and

the predator *Orius majusculus* (Heteroptera: Anthocoridae). *Environmental Entomology* 29: 846-850.

Zwahlen, C., A. Hilbeck, P. Gugerli, and W. Nentwig. 2003. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Molecular Ecology* 12: 765-775.

## Annex 1. Levels of Bt toxins in pollen of some transgenic maize events

Event	Protein	fresh weight	dry weight	? (fw or dw)	total protein	? (fw or total protein)	Source
176	Cry1Ab	range = 1.1-2.3 µg/g max = 7.1 µg/g mean = 2.59 µg/g				mean = 90.5 ng/g	(Canadian Food Inspection Agency 2001) (EPA 2000a) (Lfl 2005) (Wraight 2000)
MON810	Cry1Ab	mean = 0.25 µg/g	< 90 ng/g			mean = 2.1 ng/g	(EPA 2000b) (Lfl 2005) (Wraight 2000)
Bt11	Cry1Ab		< 90 ng/g				(EPA 2000b)
1507	Cry1F		mean = 20.0 ng/mg max=29.3 ng/mg	31-33 ng/mg (hybrid)	135.5 (113.4-168.2) pg/µg (hybrid) 207.5 (186.3-231.1) pg/µg (inbred line)		(EFSA 2005b) (EFSA 2005b) (EPA 2001) (EPA 2001)
MIR 604	mCry3A	below dection levels	below dection levels				(Syngenta 2005)
MON 863	Cry3Bb1	mean = 62 µg/g range = 30-93 µg/g					(Monsanto 2001) (Monsanto 2001)
88017	Cry3Bb1	mean = 14 µg/g range = 11-20 µg/g	mean = 25 µg/g range = 17-32 µg/g				(Monsanto 2004) (Monsanto 2004)
59122	Cry34Ab1/35Ab1		mean=74.27 µg/g Cry34Ab1 mean=0.02 µg/g Cry35Ab1				(Dow Agro Sciences 2004) (Dow Agro Sciences 2004)

