

BUNDESMINISTERIUM FÜR
GESUNDHEIT UND FRAUEN



**Ecological effects of genetically
modified maize with insect resistance
and/or herbicide tolerance**

**Ökologische Effekte von gentechnisch
verändertem Mais mit Insekten-
resistenz und/oder Herbizidresistenz**



**Forschungsberichte der
Sektion IV**



Band 6/2005

**Ecological effects of genetically
modified maize with insect resistance
and/or herbicide tolerance**

**Ökologische Effekte von gentechnisch
verändertem Mais mit Insekten-
resistenz und/oder Herbizidresistenz**

**Forschungsberichte der
Sektion IV**

Band 6/2005

Impressum:

Herausgeber, Medieninhaber und Hersteller:

Bundesministerium für Gesundheit und Frauen, Sektion IV
Radetzkystraße 2, 1031 Wien

Für den Inhalt verantwortlich:

Sektionschef DI Harald Gaugg

Erscheinungstermin: Jänner 2006

Projektleitung:

Mag. Marion Dolezel

Projektmitarbeiter:

Dr. Andreas Heissenberger

Dr. Helmut Gaugitsch

Umweltbundesamt

Druck: Kopierstelle des BMGF, Radetzkystraße 2, 1031 Wien

Bestellmöglichkeiten:

Telefon: +43-1/711 00-4700 DW

Fax: +43-1/715 58 30

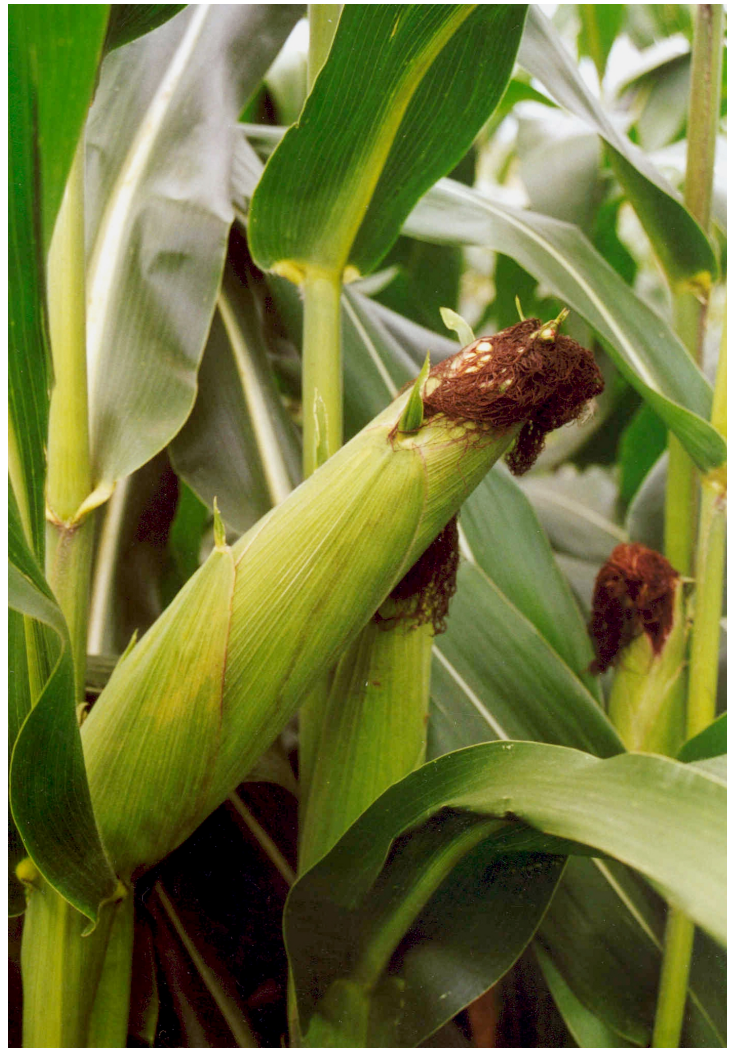
E-Mail: broschuerenservice.bmgf@bmgf.gv.at

Internet: <http://www.bmgf.gv.at>

ISBN 3-900019-63-0

Diese Studie/Broschüre ist kostenlos beim Bundesministerium für Gesundheit und Frauen, Radetzkystraße 2, 1031 Wien, erhältlich.

Ecological effects of genetically modified maize with insect resistance and/or herbicide tolerance





**ECOLOGICAL EFFECTS OF GENETICALLY
MODIFIED MAIZE WITH INSECT RESISTANCE
AND/OR HERBICIDE TOLERANCE**

**ÖKOLOGISCHE EFFEKTE VON
GENTECHNISCH VERÄNDERTEM MAIS MIT
INSEKTENRESISTENZ UND/ODER
HERBIZIDRESISTENZ**

Mag. Marion Dolezel
Dr. Andreas Heissenberger
Dr. Helmut Gaugitsch

**BUNDESMINISTERIUM FÜR
GESUNDHEIT UND FRAUEN**



Wien, 2005



Projektleitung

Mag. Marion Dolezel

Projektmitarbeiter

Dr. Andreas Heissenberger

Dr. Helmut Gaugitsch

Inhaltsverzeichnis

Zusammenfassung	4
Short summary	6
Summary and Conclusion	8
Overview of the studies	12
Bt maize containing the Cry1Ab toxin	12
Effects on non-target Lepidoptera	12
Effects on pollinators	17
Effects on non-target herbivores	18
Effects on predators and parasitoids	24
Effects on soil organisms and processes	33
Bt maize containing other than the Cry1Ab toxin	41
Effects on non-target Lepidoptera	41
Effects on pollinators	41
Effects on other non-target organisms	41
Effects on soil organisms and processes	43
Effects of herbicide tolerant maize	47
Effects on weeds	47
Other effects on plants (non-weeds)	48
Effects on the bacterial community	48
Effects on non-target arthropods	48
Other effects	49
Sequence of Bt176 maize	51
References	52

Zusammenfassung

In der vorliegenden Literaturübersicht werden Studien zusammengefasst und evaluiert, die in wissenschaftlichen Journalen innerhalb der letzten 3-4 Jahre veröffentlicht wurden und ökologische Effekte von insektenresistentem (Bt) bzw. herbizidtolerantem Mais berücksichtigen. Der Großteil dieser Studien behandelt ökologische Effekte von Bt Mais, der das Cry1Ab Toxin enthält. Nur wenige Studien sind verfügbar, die andere Cry Toxine berücksichtigen, wie z. B. Cry3Bb1 oder Cry1F. Einige der Studien beziehen sich auf ökologische Auswirkungen von Bt Mais, der das Cry1Ab Toxin enthält, auf Tagfalter, jene Gruppe von Nichtzielorganismen, für die erstmals negative Effekte durch den gentechnisch veränderten Mais Bt176 beschrieben wurden. Neuere Studien bestätigen diese nachteiligen Effekte von Bt176 Maispollen für Tagfalter als Nichtzielorganismen. Weitere Studien zeigen, dass auch Maispollen oder Antheren von MON810 und Bt11 Mais Tagfalterlarven negativ beeinträchtigen können, vor allem bei längerer Exposition. Additive Effekte können erwartet werden, wenn Falterlarven einer Kombination von Bt Maispollen und Antheren, die das Cry1Ab Toxin enthalten, ausgesetzt sind. Generell sind die nachteiligen Effekte auf Tagfalterlarven art- und altersspezifisch. Unter Feldbedingungen kann die Exposition der Larven hinsichtlich Bt Maispollen oder Antheren extrem variabel sein und ist für den Großteil europäischer Schmetterlingsarten nicht bekannt. Publierte Studien zu ökologischen Effekten von Bt Mais, der das Cry1Ab Toxin enthält, behandeln zudem auch Auswirkungen auf andere Nichtzielorganismen als Tagfalter, wie z. B. herbivore und räuberische Arthropoden. Das Cry1Ab Toxin wurde in mehreren herbivoren und räuberischen Nichtzielorganismen nachgewiesen, wenn diese Organismen dem Bt Mais unter Feldbedingungen ausgesetzt sind. In Laborstudien wurden nachteilige Effekte für einige herbivore oder räuberische Nichtzielorganismen nachgewiesen. Eine Standardisierung von Fütterungsstudien oder tritrophischen Experimenten von Nichtzielorganismen im Labor ist jedoch dringend vonnöten, um die Vergleichbarkeit dieser Studien zu gewährleisten. Unklar ist, ob die im Labor nachgewiesenen, nachteiligen Effekte auch unter Freilandbedingungen auftreten. Die Ergebnisse von Studien, die die Häufigkeit der Arthropoden in Bt und nicht-Bt Maisfeldern unter Feldbedingungen untersuchten, sind sehr unterschiedlich und negative Effekte sind meist auf einzelne Jahre, Standorte oder spezifische Arten beschränkt. Allerdings kann aufgrund von methodischen Mängeln im experimentellen Design und geringer Replikation bei diesen Freilandstudien nicht erwartet werden, dass geringe Effekte auf diese Nichtzielorganismen erkannt werden können. Gemäß jenen Studien, die derzeit publiziert sind, scheinen starke nachteilige Effekte für herbivore oder räuberische Nichtzielorganismen aufgrund von Bt Mais, der das Cry1Ab Toxin enthält, derzeit eher unwahrscheinlich. Deshalb sollte in zukünftigen Studien der Schwerpunkt eher auf der Detektion geringfügiger Effekte liegen, die sich über einen längeren Zeitraum hinweg manifestieren. Nachteilige Auswirkungen von Bt Mais mit dem Cry1Ab Toxin auf Parasitoide und Hyperparasitoide wurden in Studien bestätigt und sind am ehesten auf einen indirekten und wirtsvermittelten Wirkungsmechanismus zurückzuführen. Die Persistenz und insektizide Aktivität von Cry1Ab Toxinen im Boden wird derzeit kontroversiell diskutiert, obwohl Unterschiede im experimentellen Ansatz oder verwendete Methoden die unterschiedlichen Ergebnisse zu einem bestimmten Ausmaß erklären können. Bezüglich nachteiliger Effekte auf verschiedene Bodenorganismen sind derzeit nur Hinweise vorhanden, eine Bestätigung dieser ersten Indizien ist notwendig. Es gilt allerdings als gesichert, dass der Abbau von Bt Mais mit dem Cry1Ab Toxin und nicht-Bt Mais unterschiedlich ist. Dies wurde nicht nur für Mais, sondern auch für unterschiedliche Bt Pflanzenarten nachgewiesen. Ursache dafür sind vermutlich Unterschiede in der Lignifizierung von Bt und nicht-Bt Pflanzen. Im Gegensatz zu Studien zu ökologischen Effekten von Bt Mais, der das Cry1Ab Toxin enthält, sind erst relativ wenige Studien erhältlich, die im Detail Auswirkungen von Mais mit dem Cry3Bb1 Toxin evaluierten. Diese Studien geben nur wenige Hinweise für konsistente oder größere nachteilige Effekte dieses Toxins auf Nichtzielorganismen. Weder Labor- noch Feldstudien, die Auswirkungen des Cry1F Toxins oder der Cry34Ab1 und Cry35Ab1 Toxine auf Nichtzielherbivoren, -räuber, Parasitoide oder Bodenorganismen evaluieren, wurden

bisher publiziert. Nachteilige Auswirkungen des Cry1Ab, Cry3Bb1 und Cry1F Toxins auf Honigbienen als wichtige Bestäubergruppe konnten bisher nicht bestätigt werden. Nur wenige aktuelle Studien zu herbizidtolerantem Mais sind derzeit verfügbar. Die Ergebnisse der Britischen „Farm Scale Evaluations“ wurden erst kürzlich erneut analysiert und jene Felder, die Atrazin im Voraufbau verwendeten, aus der Analyse ausgeschlossen. Dadurch zeigte sich, dass der Unterschied in der Biodiversität von Beikräutern und Arthropoden zwischen herbizidtoleranten und konventionellen Maisfeldern geringer ausfiel als in der ursprünglichen Analyse, die jene Felder mit Atrazinbehandlungen im Voraufbau mitberücksichtigte, obwohl die Biodiversität in den Feldern mit herbizidtolerantem Mais noch immer geringfügig höher war. Andere Studien zeigten, dass die fortdauernde Anwendung eines Totalherbizids wie Glyphosat Änderungen in Beikrautgesellschaften nach sich ziehen kann. Weiters werden durch die großflächige Anwendung von Totalherbiziden mehr Infektionen der Pflanzen mit Wurzelpathogenen aufgrund der verzögerten Herbizidaufbringung vermutet. Das Auftreten von herbizidtoleranten Beikräutern wurde seit der intensiven Anwendung herbizidtoleranter Kulturpflanzen beobachtet und ein weiterer Anstieg dieser herbizidtoleranten Beikräuter in Häufigkeit und Frequenz wird vermutet.

Short summary

This review evaluates scientific studies published in peer-review journals during the last 3-4 years that considered ecological effects of insect resistant (Bt) and herbicide tolerant maize. The majority of the scientific studies deal with ecological effects of maize containing the Cry1Ab toxin. In contrast, very few studies are available that considered other Cry toxins used in Bt maize such as Cry3Bb1 or Cry1F. Studies dealing with the Cry1Ab toxin relate to effects on non-target Lepidoptera which were the first non-target effects confirmed for Bt176 maize. New studies confirm these adverse effects of Bt176 pollen to non-target Lepidoptera but show that also MON810 and Bt11 maize pollen or anthers may adversely affect lepidopteran larvae especially under prolonged exposure. Additive effects can be expected when larvae are exposed to a combination of Bt pollen and anthers containing the Cry1Ab toxin. Generally, effects on lepidopteran larvae are shown to be species and age-specific. Exposure of non-target lepidopteran larvae to Bt maize pollen under field conditions can be highly variable and is still unknown for the majority of European butterfly species. Published studies on ecological effects of Bt maize containing the Cry1Ab toxin also deal with impacts on other non-target organisms than Lepidoptera, such as herbivorous and predatory arthropods. A large range of herbivorous or predatory species have been shown to contain the Cry1Ab toxin when exposed to Bt maize in the field and adverse impacts on some species were confirmed mostly in laboratory studies. There is a definite need for standardization of laboratory feeding assays or tritrophic experiments with non-target herbivores and predators in order to enable the comparability of these studies. It is unclear if these adverse effects which were observed in the laboratory can be also translated to field conditions. Results on non-target arthropod abundance in Bt and non-Bt maize fields are inconsistent and adverse effects are mostly restricted to single years or locations or certain species. Methodological flaws in the experimental design and few replications make it unlikely to detect small abundance effects of these non-target organisms in most field studies. According to the studies currently available major effects on non-target species abundance due to Bt maize cultivation seem to be rather unlikely. Therefore the emphasis of further studies should be on the detection of subtle and long-term effects to non-target organisms. Adverse effects of Bt maize containing the Cry1Ab toxin on parasitoids and hyperparasitoids have been shown and are most likely due to indirect and host-mediated effects. Reports on soil persistence and insecticidal activity of Cry1Ab toxins are still controversial although differences in the experimental design or methods used explain to some extent the different results obtained. Some adverse effects of Bt maize on different soil organisms are indicated but confirmation of these indications is still needed. Nevertheless decomposition is most likely different between Bt and non-Bt maize containing the Cry1Ab toxin and was confirmed even for different Bt plant species which is probably the result of differences in lignification patterns between Bt and non-Bt maize. In contrast to non-target studies of Bt maize containing the Cry1Ab toxin only few studies have so far evaluated in depth non-target effects of the Cry3Bb1 containing maize. These studies give only few indications for consistent or major effects of this toxin on non-target organisms. Neither laboratory studies nor field experiments considering effects of the Cry1F toxin or the insecticidal toxins Cry34Ab1 and Cry35Ab1 on non-target herbivores, predators or soil organisms are so far available. Currently no evidence is available confirming negative impacts of Cry1Ab, Cry3Bb1 and Cry1F toxins on pollinators such as honey bees. Only few studies are currently available that consider ecological effects of herbicide tolerant maize. The results of the British Farm Scale Evaluations were re-analysed recently. Exclusion of pre-emergence atrazine treatments from the analyses resulted in fewer positive effects of the herbicide tolerant maize fields compared to the non transgenic maize fields than reported previously both for weeds and arthropods. Other studies have, however, shown that the continuous use of glyphosate can change weed communities. Other studies considering herbicide tolerant maize predict the increase of infections by root pathogens due to the delay of the herbicide application. The occurrence of herbicide tolerant weeds has been observed since the large-scale introduction of

herbicide tolerant crops and a further increase in abundance and frequency of herbicide resistant weeds is expected to occur.

Summary and Conclusion

The majority of the publications on ecological effects of Bt maize which are currently available in the scientific literature consider effects of Bt maize containing the Cry1Ab toxin on non-target species or on soil processes and soil organisms. Only a limited number of studies deal with other Cry toxins which are currently used in Bt maize and are mainly restricted to the Cry3Bb1 toxin. Almost no studies considering the Cry1F toxin or the binary insecticidal toxin Cry34Ab1/Cry35Ab1 are available.

Effects of the Cry1Ab toxin

Non-target Lepidoptera (butterflies) are important non-target organisms for which the effect of Bt corn is studied in the laboratory. Adverse effects of Bt176 maize pollen on lepidopteran larvae suspected already in earlier studies for the monarch butterfly were further confirmed also for other species and also for low pollen exposure scenarios. Negative effects of pollen of other Bt maize events containing the Cry1Ab toxin (MON810 and Bt11) on monarch butterfly larvae were also shown recently, especially after prolonged exposure of the larvae to the Bt corn pollen. The higher sensitivity of younger larval stages to Bt corn pollen compared to older larvae seems to be generally valid for several lepidopteran species. The exposure of lepidopteran larvae not only to Bt corn pollen but also to corn anthers resulting in adverse effects as well as additive adverse effects of the combined consumption of Bt corn anthers and pollen have been reported recently and should be considered in studies with other lepidopteran larvae. Effects of Bt corn pollen or anthers were also shown to be species-specific. However, there is a necessity to standardize laboratory feeding assays for lepidopteran larvae as the results obtained largely depend on the butterfly species, the larval stage and the pollen density fed in a certain feeding assay. Also the plant species used on which the Bt corn pollen are deposited should be considered in feeding studies as different leaf structures, palatability or composition may additionally influence disadvantageous effects of Bt corn pollen on non-target Lepidoptera. Generally, under field conditions the overall exposure of non-target Lepidoptera to Bt pollen seems to be low although it has been shown to be highly variable and depending on local parameters and sampling methodology. In and next to maize fields pollen densities can reach values that trigger at least sublethal effects in some non-target butterfly species. As only very few species have been studied so far, it is unknown to what extent single species are exposed under field conditions in Europe. Standardization for field sampling procedures of pollen deposition is therefore also required to elucidate general patterns of pollen deposition and exposure under different conditions. As a consequence even slight sublethal effects during the larval phase resulting from exposure to Bt corn pollen or anthers could result in lower pupal or adult weights and consequently in lower egg production or shorter life-span of adult non-target butterflies.

Negative impacts of Bt pollen containing the Cry1Ab toxin on **pollinators** were so far not confirmed for a series of parameters tested which is most likely explained by the selectivity of the toxins used and the low level of these toxins in the food fractions that are ingested by bees or bee larvae. However, caution must be used to extrapolate these results to other Cry toxins as adverse effects on bees have been shown for other insecticidal proteins.

Effects on **non-target phytophagous** species depend on the route of Bt exposure to these organisms when directly feeding on Bt maize. Different feeding mechanisms will lead to different exposure scenarios of the Bt toxin to a non-target herbivore. Studies on the exposure of non-target herbivores are, however, still rare. So far, only few studies evaluated the exposure of non-target herbivorous species by examining the amount of Bt toxin contained in these species. While it has been shown that aphids do not take up and contain the Bt toxin, presence of the Bt toxin has been shown for other phytophagous species such as spider mites, thrips, and leaf chewing herbivores such as leaf hoppers or corn flea beetles. However, their exposure should be evaluated for different developmental stages and species as it might differ due to differences in feeding behaviour and metabolic processes. Similarly, exposure of herbivores will differ for leaf chewing herbivores depending on the leaf section which is consumed as different parts of

Bt maize leaves contain different toxin levels. However, while some species might not be able to detect these differences in toxin concentrations or might be able to compensate sublethal effects other species might be adversely affected or change their feeding pattern. The results of the studies evaluating herbivore abundance under field conditions are controversial with some studies finding differences in the abundance of certain non-target herbivores between Bt and non-Bt maize while other do not confirm differences or do not find any consistent effects over locations or years. In field experiments small effects of Bt maize on herbivore abundance cannot be detected with the low replication used in most of the studies. Also experimental design such as plot size, isolation or field border treatment considerably influence the abundance of foliar arthropods which are highly mobile and escape easily when food or habitat conditions worsen. Furthermore, it is unrealistic to expect major differences in the abundance of most non-target species under field conditions. Therefore sampling designs must be adjusted in order to be able to detect also rather small differences in abundance. Moreover, it is so far unclear if and how exposure to Bt toxins and the results of laboratory studies can be translated into effects on abundance in field scenarios. Laboratory feeding assays can give more detailed indications of cause-effect relationships than field studies but do not always represent realistic exposure scenarios as certain behavioural and environmental parameters (e.g. food or habitat choice) cannot be considered. A discrepancy between laboratory and field experiments can be shown for some herbivorous groups where consistence between exposure to Bt toxin and effects observed in the field is lacking. While in laboratory assays phloem feeding aphids do not ingest the Bt toxin, in field experiments their abundance has been reported to be higher in Bt maize fields by some authors. Apart from aphids also leafhoppers and corn flea beetles have been reported to increase in abundance by some authors while others find no clear evidence for such an effect. Although it is unclear whether this effect is due to plant-related factors such as changed plant quality or reduced predator abundance it shows the necessity of field experiments to complement laboratory studies. In contrast, other herbivores have been shown to contain high amounts of the Bt toxin but no abundance effects in the field were reported. The example of aphid abundance in the field can also be used to emphasize the necessity to evaluate different developmental stages and sampling periods as effects may occur temporarily and may not be seen when the whole vegetation period is exclusively considered.

Under field conditions the Cry1Ab toxin is mediated from the maize plant through herbivorous species to the third and the fourth trophic level in the non-target food chain. Cry toxins are ingested by some **predators** while other predators contain no or only trace amounts depending on the presence of food sources containing the toxin. Similarly to herbivores, discrepancies between laboratory and field experiments of predator species are evident. This was shown for green lacewing larvae where adverse effects reported under laboratory conditions were shown to be absent under field conditions due to a certain flexibility in feeding and avoidance behaviour of the chrysopid larvae. Up to date evidence is lacking that higher order predators such as coccinellids, heteropterans, carabids or spiders are negatively affected by exposure to transgenic insect resistant maize although the presence of the Bt toxin in these species has been confirmed. Feeding on Bt-fed prey has been shown to be detrimental on predatory species in some cases in laboratory assays but negative effects were not consistently reported from field studies of these species. Generally, abundance differences of predators between Bt and non-Bt maize fields observed under field conditions are restricted to certain years or locations. However, in field studies methodological difficulties may mask effects on non-target predators. Several studies admit that low power estimates in their studies indicate problems in experimental designs. Data of field studies indicate that only effects of 20-50% were detectable with 80% power for all abundant taxa. For example, in order to detect a 30% difference in spider abundance at a probability of 80% between the two treatments (Bt and non-Bt) the sample size, corresponding to the number of fields per treatment, would have to be 14 fields per treatment when using suction sampling as the preferred method. In order to detect a 10% abundance difference this value would increase to approximately 110 fields. The sample size used in most non-target field studies (mostly 4 replications) would however only reveal an effect on spider abundance of 70% (with 80% probability) using the suction sampling method. Additionally, the

majority of species of non-target organisms are present at numbers too low for statistical evaluation while the minority of species are present in abundances sufficient for statistical evaluation. Generally, clarification is needed what adverse effects on non-target predators under field conditions are. Currently, abundance of certain non-target organisms is the only parameter which is evaluated under field conditions and it is unclear whether increased abundance of certain common predator species should be the conservation goal or whether the emphasis should rather be on less common, rare or even endangered species. Other methodological flaws are evident in many field studies including short sampling periods or the lack of determination of taxa to the genera or species level. Finally, taxon-level effects of plot size, border treatment and isolation have to be considered when studying non-target predator species with high mobility.

Some effects of Bt maize fed hosts on **parasitoids** were shown to be indirect due to a lower survival of the host. Most of the other effects reported are to a large extent controversial and depend on the methodology or species used and the life cycle parameters measured. In contrast, the observation of a lower abundance of parasitoids and lower parasitism of European corn borer in Bt maize fields seems to be consistent and may be explained by reduced host density or plant damage in Bt maize fields. Also effects on hyperparasitoids of Bt feeding larvae were reported. The implications of these effects for parasitoid or hyperparasitoid populations are so far unclear.

Studies on the fate and effect of Bt toxins in the environmental compartments comprise only the fate of these toxins in the **soil ecosystem**. So far, no study is available analysing the fate of Bt toxins in the groundwater compartment. Toxin release from root exudates of Bt plants is common and similar for different Bt-maize hybrids. In contrast, estimates on decomposition rates of Bt toxins in the field are to some extent controversial with some authors reporting fast decomposition and no larvicidal activity while others reporting persistence of these toxins for several months accompanied with residual larvicidal activity of these toxins. Differences in the plant material used (roots, stems, leaves), degree of pre-destruction of the plant material, soil type and the experimental conditions, mainly temperature, explain at least some of these differences. Standardization of methods and parameters is highly necessary in order to achieve consistent results of decomposition of Cry toxins under natural conditions. Decomposition of Bt maize was found to be significantly lower than of non-Bt maize, even for different maize varieties and different Bt plant species although this was not confirmed by some authors. However, there is increasing evidence that structural differences in lignin composition between Bt and non-Bt plants are the main cause for the observed slower decomposition of Bt maize.

Effects of Bt maize on **microbial populations** seem to be small and less than effects due to different cultivars and crops. It has been shown that the analysis of culturable micro-organisms is less sensitive to bacterial community shifts than molecular fingerprinting methods or community-level physiological profiling which were able to reveal some differences between microbial populations of Bt and non-Bt maize in some instances.

So far reports on effects of Bt corn on **mycorrhizal fungi** are rare but such an effect is indicated by one single study and merits further investigation. Studies evaluating effects on **higher soil organisms** such as earthworms, isopods, nematodes or soil Collembola are currently rare. Although soil organisms might be exposed to the Bt toxins in soil there are so far only indications that these toxins might affect them adversely (e.g. earthworms, isopods) and the implications of these studies are not clear. Additionally, studies of Bt maize effects on other soil organisms such as nematodes or Collembola are still inconclusive.

Further studies are necessary to evaluate risks for these soil organisms and to understand the implications of these studies.

Other than Cry1Ab toxins

Among studies addressing other Cry toxins used for Bt maize reports on the Cry3Bb1 toxin are more frequent than reports on the Cry1F toxin or the binary toxin Cry34Ab1/Cry35Ab1. The Cry1F toxin was shown to be almost non-toxic to neonate monarch larvae and also sublethal effects such as decreased weight gain were absent. The pollen of MON863 containing the coleopteran specific toxin Cry3Bb1 did also not result in any kind of adverse effect on one **non-target lepidopteran species**. Available

studies on effects of Cry toxins other than the Cry1Ab toxin on other **non-target arthropods** than Lepidoptera are restricted to the Bt maize event MON863 containing the Cry3Bb1 toxin. No studies are available that deal with non-target effects on arthropods of the Cry1F toxin. Laboratory feeding studies which evaluate lethal or sublethal effects of the Cry3Bb1 toxin on a range of life-cycle parameters of several non-target organisms, comparable to those of the Cry1Ab toxin, are still rare. In field conditions consistent effects of this toxin on the abundance of foliage or ground dwelling arthropods could so far not be confirmed although some significant effects of the Bt maize were shown but were restricted to some predator groups or years. First indications for adverse impacts of MON863 maize on ladybird beetles and a soil inhabiting dipluran species are provided but necessitate further detailed assessment. However, for field studies with MON863 maize the same criticism regarding the methodology is valid as mentioned above for the field studies of Bt maize containing the Cry1Ab toxin. Similarly to field experiments of Cry1Ab maize, in most field experiments of Cry3Bb1 only population-level effects of more than 20% and only effects on abundant taxa would be detectable.

The few available studies of other Cry toxins than the Cry1Ab toxin on **soil ecosystems** showed that also Cry3Bb1 toxins bind largely to clay particles depending on the clay concentration of the soil type, thus being protected from microbial degradation, similarly to the Cry1Ab toxin. However, no effects of the Cry3Bb1 toxin either on microbial biomass and activity or on soil microarthropods and nematodes could be shown so far, although methodological flaws of these studies are evident. The only study available considering the Cry1F toxin in soil ecosystems showed that the toxin degraded within a relatively short time and consequently toxicity to target organisms declined rapidly. Similarly, the only study available on the binary insecticidal crystal proteins Cry34Ab1 and Cry35Ab1 was conducted by the same author and found very short retention times for these proteins in the soil. In conclusion, detailed evaluations of these Cry toxins and their behaviour in soil systems as well as their effects on non-target arthropods, herbivores and soil organisms are so far lacking.

Herbicide tolerant maize

Regarding ecological effects of herbicide tolerant maize recent studies are rare. In the year 2003 the results of the British Farm Scale Evaluations became available which analysed non-target effects of herbicide tolerant (glyphosate and glufosinate) maize. Due to heavy criticism regarding the use of atrazine-treated maize fields as controls in the experiments, the authors re-evaluated the data of these large scale field trials. Excluding the use of pre-emergence atrazines resulted in consistent reductions of the positive effects of the herbicide tolerant maize fields compared to the non transgenic fields, both for weed and arthropod abundances. If pre-emergence atrazine sites were excluded from the analysis the reduction of the effects was approximately 2/3 for weeds and 1/10 for arthropods. An increased abundance of weeds or non-target arthropods in fields of herbicide tolerant maize was evident although this effect was reduced when the atrazine treatment was not considered. However, these favourable effects were only relevant for final plant density but not for other parameters such as weed biomass or seed rain. In contrast, other studies have shown that continuous glyphosate treatments induce changes in the weed community independent of crop-rotations and that weed biomass was lowest when glyphosate was applied late in the season. The implications of weed biomass on arthropod abundance and diversity are to some extent well known, although this is not true for the response of single species. Additionally, there are some concerns that the delay of the herbicide application could lead to increased infections by root pathogens. Other studies on herbicide tolerant maize mostly refer to herbicide resistant weeds which are predicted to increase under continuous application of the total herbicides. Additionally, spray drift of these herbicides could result in changes and selection of non-weed species next to farmland. No adverse effects of herbicide tolerant maize on bacterial communities of rhizospheres have been shown.

Overview of the studies

Bt maize containing the Cry1Ab toxin

Effects on non-target Lepidoptera

In total eight recent publications consider the possible effects of Cry toxins in Bt maize on non-target lepidoptera. Five of them dealt with either MON810 or Bt11 or both of them (DIVELY et al. 2004, ANDERSON et al. 2004, ANDERSON et al. 2005, HELLMICH et al. 2001, WENDONG et al. 2005) while another four dealt with Bt176 (HELLMICH et al. 2001, SHIRAI & TAKAHASHI 2005, FELKE et al. 2002 and FELKE & LANGENBRUCH 2005, in press). These studies will be distinguished regarding the effects of corn pollen alone, corn anthers alone or a combination of corn pollen and anthers on non-target Lepidoptera.

Effects of Bt corn pollen alone

The transgenic maize which first created considerable debate about the effects of Bt corn pollen on non-target Lepidoptera was Bt176. LOSEY et al. (1999) reported toxicity to the monarch butterfly larvae, *Danaus plexippus*, feeding on Bt corn pollen deposited on milkweed leaves. In laboratory assays they observed less consumption, slower growth and higher mortality of 2nd and 3rd instar monarch larvae. LOSEY's results were challenged by many scientists because the pollen density on the leaves and the amount of pollen consumed was not quantified, because of the extrapolation from the laboratory assays to the field and the extrapolation of the one type of pollen to others types of Bt pollen (MINORSKY 2001, FELKE et al. 2002). However, later also HANSEN-JESSE & OBRYCKI (2000), STANLEY-HORN et al. (2001) and ZANGERL et al. (2001) reported adverse effects of corn pollen of event 176 to the monarch *D. plexippus* or black swallowtails *Papilio polyxenes* feeding on *Asclepias syriaca* or *Pastinaca sativa* even under field conditions. The adverse effects of Bt176 pollen on different lepidopteran species were also confirmed by three studies which appeared later.

In laboratory bioassays FELKE et al. (2002) tested different lepidopteran species for their sensitivity to Bt pollen densities. Reduced consumption was observed for 4th instar larvae of *Plutella xylostella* feeding on less than 20 grains/cm² and for 2nd instar larvae of *Pieris rapae* feeding on 21-30 grains. Reduced consumption of 2nd instar of *Pieris brassicae* was not observed up to 90 pollen grains. Weight increase was also affected by Bt pollen consumption. Less weight increase was observed for 2nd instar *P. rapae* already from 10 grains/cm² onwards and for 2nd instars of *P. brassicae* from 20 pollen grains onwards. Weight increase was also reduced for 3rd instar larvae of *P. brassicae* but only at 140 grains. Mortality was also affected. An increased mortality rate for 4th instars of *P. xylostella* with increasing grain densities was observed. Increased mortality for 2nd instar larvae of *P. rapae* and *P. brassicae* was also observed. However, no increased mortality of 3rd instar larvae of *P. brassicae* was observed even at 130 grains.

Similar patterns were shown for larvae of *Inachis io* in laboratory bioassays (FELKE & LANGENBRUCH 2005, in press). Younger larvae (2nd instar) showed significantly increased mortality when consuming 80 Bt176 pollen grains and significantly reduced weight gain already at 10 grains/cm² (72% of control group). At the highest pollen dose fed (320 grains/cm²) they had only 14% of the weight gain of the control group. In contrast older 3rd and 4th instar larvae showed no increased mortality up to 80 pollen grains fed but had a reduced weight gain compared to that of the control group for all pollen densities tested.

SHIRAI & TAKAHASHI (2005) found in laboratory assays a significant negative correlation between survival rate of the 1st instar of a non-target lycaenid butterfly (*Pseudozizeeria maha*) and Bt176 corn pollen dose, the survival of the larvae after 6 days feeding decreased significantly at more than 20 grains/cm². Additionally, the development of *P. maha* larvae to the 2nd instar was significantly lower at a pollen dose of more than 60 grains/cm². SHIRAI & TAKAHASHI (2005) used pollen trap plants in order to quantify the deposition of Bt176 maize pollen under field conditions and found 150-155 grains/cm² as

the highest pollen density at 1 m from the field edge during 9-12 days after the beginning of anthesis in one wind direction. The authors conclude that larvae of *P. maha* might encounter a risk within 5 m from the edge of a cornfield due to the consumption of Bt176 corn pollen but not at a distance of more than 10 m from the field edge.

Toxicity of Cry1Ab toxins to non-target butterflies depends mainly on the transformation event. As shown with the purified toxin as well as with pollen bioassays the toxicity of Bt176 maize pollen is considerably higher than that of other events containing Cry1Ab toxins (HELLMICH et al. 2001). While it has been recognized that event 176 corn pollen might put certain non-target lepidopteran species at risk due to its high expression of the Bt toxin in the pollen, the risks from other Bt maize varieties expressing Cry1Ab toxin (MON810, Bt11) was less clear. A series of publications in the year 2001 showed that effects of Cry1Ab Bt corn pollen of the transgenic events MON810 and Bt11 on the monarch butterfly *Danaus plexippus* or the black swallowtail *Papilio polyxenes* were negligible (WRIGHT et al. 2001, STANLEY-HORN et al. 2001, SEARS et al. 2001) and that larval exposure to Bt corn pollen was low (OBERHAUSER et al. 2001, PLEASANTS et al. 2001). Less than 0,8% of the monarch population was estimated to be exposed to Bt corn pollen (SEARS et al. 2001) and acute toxic or sublethal effects of pollen from Bt corn events MON810 and Bt11 were considered to be unlikely in laboratory bioassays (HELLMICH et al. 2001). Also field studies showed that growth and survival of monarch larvae were unaffected after exposure to Cry1Ab expressing corn pollen of MON810 and Bt11 (STANLEY-HORN et al. 2001). However, these studies examined acute and sublethal effects after a short term exposure of 4-5 days but not long term exposure.

Since the publication series in 2001 only two new studies (DIVELY et al. 2004, WENDONG et al. 2005) have been carried out considering adverse effects of Bt corn pollen of the transgenic events MON810 and Bt11 on non-target Lepidoptera.

DIVELY et al. (2004) reported on the effects of Cry1Ab expressing Bt corn after prolonged exposure of monarch larvae to natural deposits of corn pollen in the field. They used greenhouse grown milkweed plants which they placed within a maize field. Then they exposed monarch butterfly cohorts of 1st, 2nd and 3rd instar larvae to naturally deposited levels of Bt and non-Bt pollen at two times during anthesis, 3-4 days after the onset of anthesis and 6-7 days after anthesis onset. They found that the larvae needed significant more days to pupation and to eclosion when exposed to Bt corn pollen 3-4 days after anthesis and also their developmental time was prolonged by 0,6-1,2 days for both Bt corn events. When exposed 6-7 days to Bt corn (Bt11) pollen the larvae needed more days to pupation and eclosion and their developmental time was prolonged by 0,2-2,4 days. Both Bt corn events reduced the percentage survival to pupation and to eclosion for both exposures (3-4 days and 6-7 days after anthesis). Overall, the authors calculated 25% fewer larvae surviving to adults in the first exposure scenario (3-4 days) and 22% fewer larvae surviving to adults in the second exposure scenario (6-7 days). However, in this study not only larvae were affected but also pupae and adults. Pupal weights were significantly lower for pooled Bt events Bt11 and MON810 in both exposure scenarios (3-4 days and 6-7 days after anthesis) and adult weight was significantly lower for pooled Bt events for the longer term exposure of 6-7 days but not for a 3-4 day exposure (adults weighed 7,9% less when exposed to Bt pollen than non-Bt pollen). Wing length of adult monarchs was not significantly different between butterflies reared on Bt and non-Bt corn pollen although Bt pollen exposed adults had generally shorter wing lengths. Also sex ratio of adults was not affected by Bt corn pollen exposure during larval stage.

WENDONG et al. (2005) used pollen bioassays with three different densities of Bt and non-Bt pollen applied on leaf disks of the food plant *Q. liaotungensis* of the Chinese Tussah Silkworm *Antheraea pernyi* (100, 1000, 10.000 pollen grains/cm²). They fed neonate silkworm larvae for five days with the pollen-dusted leaf disks and then fed clean leaves until the larvae reached the 3rd instar stage. They observed adverse effects on the weight of the larvae only for highest pollen density (10.000 grains/cm²) for the 1st to 3rd instars but found no effects on mortality of these larvae. The authors conclude that pollen densities observed under field conditions do not represent a significant risk to the larvae of the Chinese Tussah Silkworm as the accumulative pollen deposition did not exceed 1000 grains/cm².

Effects of Bt corn anthers

Already HANSEN-JESSE & OBRYCKI (2000) suggested adverse effects not only of corn pollen of Bt11 to the monarch *Danaus plexippus* feeding on *Asclepias syriaca* but also of corn anthers. They found a similar mortality of lepidopteran larvae when fed with pollen of two Bt events at the same grain density despite the fact that Bt176 had fourfold higher levels of Bt toxin in the pollen than Bt11. They suggested that anther material had contaminated the pollen fraction of Bt11 thus explaining the similarity in toxicity. Also HELLMICH et al. (2001) had already shown that contamination of pollen with a non-pollen fraction (7-8% fractured anthers in the pollen sample) was the most likely cause of mortality attributed to Bt11 pollen shown by HANSEN-JESSE & OBRYCKI (2000) as finely sifted Bt pollen did not adversely impact larvae while the siftings caused reduced larval weights and higher mortality in larvae that consumed Bt11 pollen with contaminants.

The laboratory studies of ANDERSON et al. (2004) confirmed the negative effects of corn anthers reported previously and showed that monarch butterfly larvae (1st to 5th instar) fed on 40% less leaf material, weighed 27% and 11% less after 4 and 10 days feeding, respectively, needed on average 1,1 days more to pupation or to eclosion and had approximately 30% less survival to pupation or to eclosion when exposed to a single density of Bt11 corn anthers (0,9 anthers/cm²) on milkweed leaves. Days to pupation or pupal weight were unaffected. For the 3rd to 5th instars larval weight gain was reduced only for the highest Bt anther density, no significant differences for development time, pupal or adult weight were observed. The authors suggest that some of the observed effects may be the result of avoidance behaviour of the larvae since feeding of anthers was observed to cease after some days, although no exact data is given on these assumptions. The fact that older larvae (3rd-5th instar) were only affected by high densities (1,2 anthers/cm²) of anthers is consistent with HELLMICH et al. (2001) who used the purified toxin. Larvae are most sensitive to Bt during the first four days of their development (1st and 2nd instars). When looking at anther distribution on naturally occurring milkweed within cornfields ANDERSON et al. (2004) found that the anther densities rapidly declined on milkweed leaves. At peak anther shed (7-9 days after initiation of anther shed) an average of 0,06-0,09 anthers per cm² leaf occurred. Most anthers and pollen were found in the middle third of milkweed plants while most larval feeding took place in the upper eight leaves of the plants. However, they also observed some kind of avoidance behaviour of the larvae. The authors calculated that toxic anther densities were rare on milkweed leaves in cornfields representing only 0,4% of leaves examined.

Combined effects of Bt corn pollen and anthers

Only recently combined effects of Bt corn anthers and Bt corn pollen were studied by ANDERSON et al. (2005). They showed that combined exposure to pollen and anthers of Bt-maize MON810 resulted in reduced leaf feeding of monarch butterfly neonates while feeding on either Bt pollen or Bt anthers alone did not reduce feeding activity after four days of exposure. Also fewer larvae that fed on Bt anthers and pollen survived to pupation and eclosion although this was not observed for Bt anthers or pollen alone. However, when feeding another six days the leaf area consumed was still smaller when fed Bt anthers and pollen than for non-Bt anthers and pollen, but not significantly. This reduced feeding activity did not translate into a reduced larval or pupal weight or prolonged developmental time but into reduced survival to pupation and eclosion. This result shows that exposure to both, Bt anthers and Bt pollen, can have an additive effect on butterfly larvae. When both Bt anthers and Bt pollen were present, fewer larvae fed on Bt-anthers than on non-Bt anthers, probably avoiding them due to increased Bt intoxication. The same authors also used cage experiments to simulate a more realistic exposure to anthers and pollen. Additive effects of Bt anthers and pollen (Bt11) compared to non-Bt anthers and pollen were seen when using two species of milkweed, the common milkweed and a tropical milkweed. Effects were seen as reduced larval and pupal weight or more days to pupation and eclosion. However, in these experiments also

significant effects of Bt anthers or Bt pollen when fed alone were seen. The authors suggest that the intact leaves of the milkweed plants are likely to have higher levels of latex and cardiac glycosides than the leaf discs in the Petri dish studies. Moreover, the effects were stronger with the tropical than with the common milkweed, suggesting that species characteristics such as leaf size or thickness may play also an important role.

Exposure of non-target butterflies to Bt corn pollen

Expose of non-target butterflies to Bt corn pollen has been discussed controversially since the publication of LOSEY et al. (1999) which was criticised because the pollen doses in the lab assays were not quantitatively measured. PLEASANTS et al. (2001) was the first to measure the density of maize pollen on milkweed plants inside and outside maize fields and showed that pollen density was highest within maize fields, falling with distance from the field edge. They also observed on average 30-50% less pollen on younger milkweed leaves which are preferred by monarch larvae compared to leaves from the middle of the plants due to higher exposure to rain. They reported an average within-field density of 170,6 grains/cm² based on different localities. Also DIVELEY et al. (2004) assessed pollen deposition on milkweed plants in cornfields and found mean densities ranging from 122-249 grains/cm² depending on the location a range of 8 to 651 grains/cm². SHIRAI & TAKAHASHI (2005) also studied pollen deposition on leaves of pollen trap plants *Helianthus annuus* and *Solanum nigrum* placed at the edge of the corn field and found the highest grain depositions of 150 grains/cm² at one meter from the field edge. WENDONG et al. (2005) studied pollen dispersal at corn pollen anthesis of MON810 maize within the maize fields at different heights and up to 20 m from the field edge over 7 days after the onset of anthesis. Their cumulative pollen deposition evaluation over 7 days showed 411-995 grains/cm² inside the corn fields depending on position in field to the main wind direction. At 20 m from the field edge the amount of pollen grains deposited fell to 5,4-27,6 grains/cm². ANDERSON et al. (2004) evaluated the density of corn anthers on naturally occurring milkweed within cornfields and found average numbers of 0,06-0,09 anthers/cm² seven or nine days after onset of anther shed. DIVELEY et al. (2004) found average anther deposition values on milkweed leaves placed within cornfields of 1,1-1,8 anthers/leaf for different maize hybrids with the number of anthers deposited being positively correlated with pollen density. LANG et al. (2004) studied the temporal and spatial dispersion of Bt maize pollen of Bt176 and MON810 maize fields over three years. They measured deposition of Bt maize pollen within the maize fields or until 10 m distance from the field edge using Vaseline coated microscope slides at a height of 6-20 cm or pollen trap plants (*Daucus carota*) during full corn anthesis. They found that pollen deposition in field margins was highly variable and decreased with distance from the field edge but was also influenced by other factors such as humidity and growth stage of the maize plants. Approximately one third of the pollen drifting to field margins was found on leaf surfaces of wild carrots. When using slides they found on average 65,9±70 pollen grains/cm² while when using pollen trap plants they found 33,5±25 pollen grains/cm² at a height of 20 cm next to the cornfields.

Summary: Effects on non-target Lepidoptera

Adverse effects of Bt176 **corn pollen alone** which were earlier confirmed for larvae of the monarch butterfly *Danaus plexippus* and black swallowtails *Papilio polyxenes* were later also confirmed for two *Pieris* species, *P. rapae* and *P. brassicae*, *Inachis io* and *Pseudaizeeria maha* when exposed to Bt corn pollen of Bt176 maize on their food plants under laboratory conditions (FELKE et al. 2002, FELKE & LANGENBRUCH 2005 in press, SHIRAI & TAKAHASHI 2005). However, there is a relation between the amount of pollen of Bt maize consumed and larval food consumption, weight increase and mortality which is species and age specific. Generally, older larvae are less sensitive and have a higher tolerance against Bt maize pollen than younger larvae and adverse effects can be observed at exposures to higher pollen densities only. However, sublethal effects such as reduced consumption or weight gain were also observed for older larval stages and for certain species even at low pollen densities. Interestingly, some authors report a

behavioural change of the butterfly larvae after Bt pollen consumption. Larvae seem to be lethargic or stayed on the upside of the plant leaf instead of feeding on the underside which could have implications for predator avoidance of lepidopteran larvae (FELKE et al. 2002). Even slight sublethal effects during the larval phase resulting from exposure to Bt corn pollen could result in lower pupal or adult weights and consequently in lower egg production or shorter longevity of the adult butterfly.

The effects of corn pollen of the Bt events MON810 or Bt11 are reported in two new studies (DIVELY et al. 2004 and WENDONG et al. 2005). DIVELY et al. (2004) found adverse effects of corn pollen of both Bt events on developmental time of lepidopteran larvae already after short-term exposure and for Bt11 pollen during long-term exposure. Survival was also negatively affected for both, short- and long term exposure and both Bt corn events. Moreover, negative effects on pupae and adults were confirmed. In contrast the study of WENDONG et al. (2005) found adverse effects of Bt corn pollen on Chinese silkworm larvae only at very high pollen densities which were not observed under field conditions. The laboratory study of ANDERSON et al. (2004) confirmed negative effects of Bt11 **corn anthers** on leaf feeding, larval weight and developmental time and survival of lepidopteran larvae which had been suspected in earlier studies (HANSEN-JESSE & OBRYCKI 2000, HELLMICH et al. 2001). Consistent with effects of Bt corn pollen alone they also confirmed age-dependent effects with younger larvae being more sensitive to Bt corn anthers and observed also an avoidance behaviour of the larvae. However, under field conditions exposure of lepidopteran larvae to Bt corn anthers is estimated to be low. Additive adverse effects of combined exposure of **Bt corn pollen and anthers** of MON810 maize to non-target Lepidoptera were shown by one recent study (ANDERSON et al. 2005) confirming reduced feeding, feeding avoidance and reduced survival while this was not observed when larvae were exposed to pollen or anthers alone. Furthermore, the same authors showed the significance of the food plant species used in risk assessment studies of non-target Lepidoptera.

Results of studies which evaluated **corn pollen densities** under field conditions vary considerably. Some authors report pollen densities being high within cornfields (DIVELY et al. 2004) while others find similar quantities of corn pollen also at a distance of one meter from the field edge (SHIRAI & TAKAHASHI 2005). However, pollen deposition on field margins is highly variable and dependent on different environmental variables as well as the sampling method. Anther densities within cornfields were studied by ANDERSON et al. (2004) and DIVELY et al. (2005) and found to be positively correlated with pollen density.

Effects on pollinators

Adverse effects of Bt corn pollen containing the Cry1Ab toxin on pollinators could so far not be confirmed. In the feeding studies of HANLEY et al. (2003) evaluating the effect of Bt11 maize pollen on worker larvae of the honey bee it was shown that no differences in larval mortality, pupal mortality or pupal weight between Bt pollen fed (Cry1Ab) and non-Bt pollen fed worker larvae occurred. Also when they measured the haemolymph protein concentration of newly-emerged adult bees, an indicator for the quality of the diet, they found no effect due to Bt-pollen fed to the larvae. Also MALONE et al. (2005) review effects of insecticidal proteins to honey bees and conclude that Bt pollen does not pose any harm to honey bee colonies. They base their argumentation on the low levels of proteins in the food fractions that bees ingest (pollen, nectar, resins) and the selectivity of the toxins used so far in transgenic plants but asks for precaution for newer, less-specific insecticidal proteins, such as protease inhibitors or biotin-binding proteins such as avidin.

Effects on non-target herbivores

Exposure of Cry1Ab to non-target herbivores

HARWOOD et al. (2005) quantified the concentration of the Bt endotoxin (Cry1Ab) within non-target herbivores of nine orders feeding within a Bt corn (Bt11) agroecosystem. They showed that the toxin was present in five species within the Coleoptera and Homoptera (Chrysomelidae, Scarabeidae, Curculionidae and Cicadellidae). The majority of the corn flea beetles *Chaetocnema pulicaria* (Coleoptera: Chrysomelidae, 87,3%) screened positive for the toxin, followed by a scarabeid (*Popillia japonica*, 23,5%) and another chrysomelid (*Diabrotica undecimpunctata*, 21,3%). Mean concentrations of the Cry1Ab toxin in these species ranged from 0,4 to 2,6 µg/g. Although two of the species tested (a chrysomelid and a cicadellid) had a low percentage of positives (only approx. 2% of the individuals corresponding to a single individual) these individuals had relatively high mean toxin concentrations of 2-2,6 µg/g.

However, exposure to the Cry toxin may differ depending on which plant part a potential non-target herbivorous organism is actually feeding. ABEL & ADAMCZYK (2004) evaluated maize plants expressing the Cry1Ab toxin (MON810) for the production of the Bt toxin in various parts of the maize plants which had visibly different levels of chlorophyll. They found significant differences of Cry1Ab concentration between the middle versus the distal tip of the maize leaf and three leaf locations (middle V7 leaf versus middle V9 leaf versus distal V7 leaf tip). The distal tips of the V7 leaves produced more Cry1Ab toxins than the middle portions of the V7 and V9 leaves. Significant differences in Cry1Ab concentration were also found between white-yellow, yellow-green and green portions of V9 leaves with green tissues having the highest toxin amount.

Quantification of the Bt toxin of Bt11 maize also showed that most variation of concentration was present in the youngest leaves (leaf 9) with the highest amount in the point section, the lowest concentration was measured in the growing section of the leaf while consistent amounts were present in older leaves (leaves 5 and 7, DUTTON et al. 2005). Also toxin expression of MON810 transgenic maize at two developmental stages (5-6 developed leaves and at anthesis) under different environmental conditions (close and open glasshouse, field conditions) was investigated by DUTTON et al. (2004b). They found significant differences in Bt-toxin concentrations between young and old plants with younger plants having approximately double amounts of the toxin compared to older ones. Older plants also had lower nitrogen values. Closed glasshouse plants had higher Bt-toxin values than open glasshouse plants, mainly in young leaves. Open glasshouse plants had the lowest Bt-toxin values in young leaves and also the lowest nitrogen levels. Although the authors do not exclude differences in nitrogen fertilization for the results, they also suggest differences in temperature as a reason which might influence (lower or silence) transgene expression.

Effects on aphids

RAPS et al. (2001) showed that the phloem sap of Bt corn expressing delta-endotoxin Cry1Ab (Bt11 and Bt176) did not contain any measurable concentrations of the Cry1Ab toxin. Additionally, they could not detect any toxin in apterous adults of the aphid *Rhopalosiphum padi* and their honeydew thus concluding that *R. padi* ingests or contains no or only very low concentrations of the endotoxin. This was also confirmed by DUTTON et al. (2002) who found only trace amounts of the toxin (Bt11, 0,02 µg/g) in the same aphid species. Also HEAD et al. (2001) evaluated levels of Cry1Ab toxin (MON810) in aphids after feeding on transgenic Bt corn plants expressing the protein or feeding on artificial diets containing the protein. No Cry1Ab toxin was detected by ELISA in aphids feeding on transgenic Bt corn plants. DUTTON et al. (2004a) evaluated Cry1Ab toxin levels in the same aphid species (*R. padi*) feeding on Bt11 maize. They found only trace

amounts of the Bt-toxin in *R. padi* suggesting that exposure of this species to the Cry1Ab toxin was minimal.

The absence of any adverse effect on aphids in laboratory bioassays was confirmed in field studies evaluating the abundance of aphids in Bt corn compared to non-Bt corn. For example, BOURGUET et al. (2002) found no significant effects in the abundance of the aphid species *Metopolophium dirhodum*, *Rhopalosiphum padi* and *Sitobion avenae* under field conditions in France which is consistent with the results of MUSSER & SHELTON (2003) who also found no difference in aphid infestation between Bt and non-Bt corn. This is in contrast to the field studies of PONS et al. (2005) reporting a higher density of *Rhopalosiphum padi* on Bt plants, especially of some developmental stages, e.g. such as alate and apterous adults and 1st-3rd instar nymphs. The authors find no definite explanation of the observed effects but note that the higher incidence of these species on Bt maize did not affect the yield of this variety. A more detailed analysis was made recently by LUMBIERRES et al. (2004) who evaluated the effects of Bt maize (Bt176) on abundance and age structure of the most common aphid in maize, *Rhopalosiphum padi*, in the field and on mortality, development and reproduction of this aphid in laboratory experiments over several generations. Significantly higher densities of aphids were found in Bt maize in May in all three years and in June in the second and third year relating to adult alate and young nymphs. In the laboratory experiments offspring of alate adults performed significantly better on Bt maize whereas the offspring of apterous adults performed significantly worse on Bt maize than on non-Bt maize regarding mortality and reproductive parameters. Although some significant differences in reproductive parameters after 3 and 10 weeks on Bt maize were found, no differences between aphids reared on Bt and non-Bt maize for 18 weeks were found. The higher abundance of aphids on Bt corn (MON 810) in early summer was also reported by SEHNAL et al. (2004) who evaluated aphid abundance over two years by visual inspection. They also report that aphid abundance at one date (June) is higher in Bt maize but found no differences over the season of both aphid species examined (*Rhopalosiphum padi*, *Metopolophium dirhodum*). PONS et al. (2005) evaluated species composition and abundance of aphids in Bt and non-Bt maize fields and found no difference in species composition between the two treatments. Both year and cultivar had significant effects on total aphid species abundance with a higher abundance of all developmental stages of the aphid species *S. avenae* and for different developmental stages of other aphid species in Bt maize. Aphid densities were consistently higher in Bt maize fields with respect to all species and ages.

Effects on spider mites

Reports on toxin levels in spider mites showed that the mite species *Tetranychus urticae* contained the Cry1Ab Bt-toxin at levels (2,5 µg/g) approximating those of the transgenic maize (3,4 µg/g fresh weight, DUTTON et al. 2002). These results contrast the preliminary results of OBRIST et al. (2005b) who found high toxin concentrations in this species when feeding on Bt-maize (event 176) with toxin concentrations being almost three times higher in the mite compared to those of Bt maize leaves indicating an accumulation effect of the toxin in the herbivore. DUTTON et al. (2004a) found the highest amounts of Bt toxin in the mite species *Tetranychus urticae* when evaluating different herbivorous species (mites, thrips, aphids and leafhoppers) for the presence of the Cry1Ab toxin (Bt11). By fluorescent antibody staining they showed that the expression of the toxin occurred mainly in mesophyll cells. *Tetranychus urticae* is a mesophyll cell feeder but contained 33 times higher levels of the Bt toxin compared to a mesophyll cell feeding cicada.

Effects on thrips

DUTTON et al. (2004a) showed that exposure to the Cry1Ab toxin (Bt11) in Bt maize was lower in the thrip species *Frankliniella tenuicornis* (Thysanoptera) than in the spider mite *T. urticae*. In field studies BOURGUET et al. (2002) found differences in thrips abundance

with significantly more thrips in Bt corn at one site in their field studies. No differences over the season were found in the abundance of thrips *Franklinella occidentalis* by SEHNAL et al. (2004). ECKERT et al. (2004) showed under field conditions that thrips were more abundant at the start of vegetation period and found no significant differences in thrips abundance between Bt and non-Bt maize panicles (MON810) while less thrips occurred on insecticide treated non-Bt maize one month after insecticide treatment. When sampling maize cobs they found more thrips in non-Bt maize than on Bt maize but this difference was not significant. In other studies differences in abundance of thrips between Bt and non-Bt fields were inconsistent (FREIER et al. 2004) and significantly more thrips were reported in Bt fields in some years and locations (DALY & BUNTIN 2005). OBRIST et al. (2005a) studied the effect of Bt maize (Bt11) on different life table parameters of the thrips species *Frankliniella tenuicornis*. They found no negative effects of Bt maize on life table parameters of the thrips and a low persistence of the toxin in the different developmental stages of the thrips which 97% of the toxin being lost within 24 hours. Larvae contained the highest toxin concentrations followed by adult thrips. However, very low levels of the toxin were found in the non-feeding stages of prepupae and pupae and the toxin was not detectable in newly emerged adults. This and the fact that the faeces contained very high but variable amounts of the toxin indicated that the thrips are able to excrete the toxin successfully and are not impaired by the toxin ingestion via the Bt plant material. Additionally, they observed a differential predation success by *Chrysoperla carnea* larvae depending on the developmental stage of the prey with prepupae and pupae of thrips being more successfully attacked within a short time period indicating that exposure of predators to Bt toxin can additionally depend on the prey stage.

Leaf chewing herbivores

In contrast to the phloem sucking herbivorous species, the Cry1Ab toxin can clearly be detected in larvae of the leaf chewing herbivore *Spodoptera littoralis* and their faeces, showing that the toxin is detectable after ingestion and excretion by herbivores (RAPS et al. 2001). This was also confirmed by DUTTON et al. (2002) who report Cry1Ab toxin levels in *Spodoptera littoralis* larvae being intermediate between those of aphids and spider mites. HEAD et al. (2001) evaluated levels of Cry1Ab toxin in three different lepidopteran species after feeding on transgenic Bt corn plants expressing Cry1Ab protein or artificial diets containing Cry1Ab protein. They showed that prey insects vary in how much Bt toxin they assimilate. Levels of Cry1Ab in larvae varied significantly in *Ostrinia nubilalis*, *Helicoverpa zea* and *Agrotis ipsilon*. When feeding for 24 hours on artificial diets containing 20 and 100 ppm of Cry1Ab, the level of the Cry1Ab toxin in the *O. nubilalis* larvae was about 57 and 142 times, respectively, lower than the original protein level in the diet, 20 and 34 times lower in *H. zea* larvae and 10 to 14 times lower in *A. ipsilon* larvae.

DUTTON et al. (2004a, 2004b) found that European corn borer larvae that fed on younger Bt leaves with higher Cry1Ab toxin amounts had a significantly lower larval weight compared to those fed on older Bt-leaves and established a correlation between the Cry1Ab toxin from leaves and larval weight of neonate *O. nubilalis*. In bioassays DUTTON et al. (2005) showed higher mortality and longer developmental time for young *Spodoptera littoralis* larvae (1st and 2nd instar) when reared on Bt-maize compared to non-Bt plants. Although the larvae seemed to show some feeding avoidance behaviour there was no difference for 1st and 3rd instar larvae consumption of leaf area of Bt-maize (although there was a tendency for more consumption of 3rd instar larvae). In contrast, 2nd instar larvae consumed less leaf area of Bt maize than of non-Bt maize. Consequently, possibly due to the lower food consumption, older larvae (3rd instars) reared on Bt maize were lighter than larvae reared on non-Bt or Bt-sprayed maize while 2nd instar larvae or pupae did not show any effect of Bt-maize consumption on their weights. 3rd instar larvae which were reared on Bt-maize needed significantly longer to adult emergence than when reared on non-Bt or Bt-sprayed maize. Mortality of larvae on Bt-sprayed maize was intermediate but not different from Bt maize or non-Bt maize. The authors explain these effects with younger larvae being more sensitive to the Cry-toxin,

possibly via changes in gut protease enzyme activity. Possibly, as 2nd instar larvae consumed less on Bt-maize (or Bt-sprayed maize) than on the control maize, the weight of 3rd instar larvae on Bt-maize was significantly reduced. This weight disadvantage seems to be compensated during feeding of 3rd instar larvae on Bt-maize which feed more on Bt maize (not significantly but tendency) therefore reaching the same weight when pupated as the larvae reared on the other two treatments. However, no significant differences in amount of leaves consumed as well as no differences between different sections of leaf 9 by the herbivore lepidopteran *Spodoptera littoralis* could be detected although quantification of the Bt toxin showed that variation of the toxin concentration was present in the different leaves and different part of leaves of Bt maize plants (DUTTON et al. 2005).

ABEL & ADAMCZYK (2004) could not relate higher toxin values in different leaf portions to higher mortality of armyworm larvae but significant differences in Cry1Ab concentration between white-yellow, yellow-green and green portions of V9 leaves lead to significant correlations with toxin concentration and armyworm larval weights. Larval mortality of southwestern corn borer was completely independent of the location of the maize leaf or the developmental stage suggesting that differential amounts of the toxin in the developing leaves did not change the susceptibility of the corn borer to the Cry1Ab toxin. The results show that the maize plants did not fully express the Cry1Ab toxin in the tissue that had low chlorophyll contents and suggest that factors that limit photosynthesis (e.g. low available water, high or low temperatures, unbalanced O₂/CO₂ ratios etc.) may limit Cry1Ab production in this transgenic maize. Toxin concentrations in younger distal leaves (V7) were double to three times the values of middle leaves V9 and middle leaf concentrations of older V9 leaves were in the range of 60-70% of the middle leaf concentrations of younger V7 plants.

VOJTECH et al. (2005) found that larvae of the non-target Lepidopteran species *S. littoralis* are negatively affected by Bt maize (MON810) in terms of survival and developmental time. Neonate larvae fed with Bt maize experienced a significantly lower survival than larvae fed non-Bt maize for 3 and 4 days. Bt maize also had an adverse effect on later instar larvae weight which was significantly lower as well as time to pupation and adult emergence which were both significantly prolonged. However, those larvae that pupated had a similar or even higher weight compared to the control and pupal mortality was unaffected. Similarly, wing width and length of pupae developing into adults were also unaffected. Also survival until pupation was significantly higher in 2nd instar non-Bt fed larvae, but only in one of the two trials and no differences in pupal mortality were observed.

Effects on leaf hoppers

The results of DUTTON et al. (2004a) who analysed Cry1Ab toxin levels of the cicada species *Zyginidia scutellaris* (Cicadellidae) which was allowed to feed on either Bt or the near isogenic line showed that toxin levels of *Z. scutellaris* were lower than in other herbivorous species (mites, thrips). Both mesophyll cell feeders, the mite *Tetranychus urticae* and the cicadellid *Zyginidia scutellaris* contained very different Bt toxin levels, with *T. urticae* having 33 times higher levels of the Bt toxin compared to the cicadellid. RAUSCHEN et al. (2004) evaluated the abundance and diversity of cicadas on Bt maize in field experiments. While the majority of the catches belonged to one cicada species (*Zyginidia scutellaris*), there was no difference in abundance of this species between Bt and non-Bt maize. However, in the non-Bt maize treated with a conventional insecticide significantly fewer individuals of this species were reported.

PONS et al. (2005) evaluated leafhopper abundance directly by visual observation and found that the cultivar had a significant effect on the total number of mature nymphs with a higher density of leafhoppers in Bt maize fields. However, when they measured leafhopper damage by the use of a chlorophyll meter they did not record a higher level of damage in Bt maize fields. Generally they found significant effects for year, cultivar, leaf-stratum and sampling date in relation to leafhopper abundance. Other authors found no evidence for a clear effect of Bt maize on the abundance of leafhoppers in the field (DELRIO et al. 2004, ARPAS et al. 2004).

Effects on other herbivores

An indirect method of evaluating herbivore abundance in maize fields was chosen by ARPAS et al. (2004) who evaluated the abundance of prey items in spider webs of *Theridion impressum* (Araneae: Theridiidae) over two years. They found that herbivores were significantly more abundant in non-Bt maize fields in one year although the tendency was also observed in the other year. Interestingly, one of the most abundant herbivorous group were the flea beetles (Coleoptera: Chrysomelidae), which composed 75-83% of the Coleopteran catches in the spider webs but did not differ significantly between Bt and non-Bt maize fields. By visual counting DALY & BUNTIN (2005) found more flea beetles in Bt fields than in non-Bt fields combined over several years and locations. However, they question whether this effect was real or a sampling artefact as the single comparisons of years and locations did not result in statistically significant differences between Bt and non-Bt maize fields.

Summary: Effects on non-target herbivores

A recent study gives clear evidence that a large range of herbivorous organisms are exposed to the Bt toxin when feeding on Bt maize under field conditions (HARWOOD et al. 2005). The results of the studies on non-target herbivorous organisms have so far shown that the route of exposure determines the susceptibility of an herbivore to the toxin. Exposure to the Cry toxin will depend on the part of the plant on which an herbivorous organism is actually feeding because toxin levels differ between or even within plant organs. So far two studies have shown that in both, MON810 and Bt11 maize, the Cry1Ab concentration is higher in younger leaves or younger plants compared to older ones and are highest in the leaf tips (ABEL & ADAMCZYK 2004, DUTTON et al. 2005).

An earlier study had already suggested that **aphids**, being phloem sap feeders, are unlikely to be exposed to the Bt toxin as it is not contained in the phloem (RAPS et al. 2001). Consequently it has been confirmed that the toxin is not present in aphids (RAPS et al. 2001, DUTTON et al. 2002, HEAD et al. 2001 and DUTTON et al. 2004a).

Evaluations of aphid abundance in field studies reported that aphid abundance in Bt maize fields was similar (BOURGUET et al. 2002, MUSSER & SHELTON 2003, DELRIO et al. 2004) or higher (PONS et al. 2005, LUMBIERRES et al. 2004, SEHNAL et al. 2004) to than in non-Bt maize fields. Apart from one study (PONS et al. 2005) which reports consistently higher aphid densities in Bt maize fields with respect to all species and ages, other studies show that these higher abundances are restricted to certain developmental stages and that the difference levels off if the whole planting period is considered. In other studies differences in aphid abundance between Bt and non-Bt maize fields are restricted to a certain year or location (FREIER et al. 2004, ARPAS et al. 2004). One study shows beneficial effects of Bt maize on the aphid *R. padi* at very young developmental stages which is reflected in reproductive parameters in the laboratory experiments as well as higher densities of aphids in the first developmental stages of the maize plant (LUMBIERRES et al. 2004). However, these effects level off if the whole planting period is considered and no long-term effects could be shown.

In contrast to aphids the Bt toxin was detected in **spider mites** but in different quantities and with an indication for accumulation of the toxin in this non-target organisms (DUTTON et al. 2002, DUTTON et al. 2004a, OBRIST et al. 2005b). Spider mites seem to contain the highest amounts of Cry toxins compared to other herbivorous species. Differences in feeding behaviour (amount of plant material ingested), in metabolic processes (toxin excretion, digestion) between the species but also between developmental stages could explain these results. However, to what extent this high exposure of mites to the Bt toxin is translated into effects on abundance of these phytophagous group and/or tritrophic effects on natural predators is so far unclear. Besides aphids and mites, also **thrips**, being epidermis or mesophyll cell feeder, contain the Cry toxin when feeding on Bt maize (DUTTON et al. 2004a). However, the persistence of the toxin is low and thrips are able to excrete the toxin successfully and are apparently not impaired by the toxin ingestion via the Bt plant material (OBRIST et al. 2005a). The

results of studies evaluating the abundance of thrips under field conditions are controversial and inconsistent over years and locations (BOURGUET et al. 2002, DALY & BUNTIN 2005, FREIER et al. 2004, SEHNAL et al. 2004, ECKERT et al. 2004, DELRIO et al. 2004, ARPAS et al. 2004).

In contrast to phloem sucking or mesophyll cell feeding herbivorous species, it has been shown that larvae of the **leaf chewing herbivore** *Spodoptera littoralis* are exposed to the Cry1Ab toxin (RAPS et al. 2001, DUTTON et al. 2002). Studies have shown that the consumption of Bt maize leaves has sublethal and lethal effects on larvae of *Spodoptera littoralis* and also induces some feeding avoidance behaviour (DUTTON et al. 2005, VOJTECH et al. 2005). However, surviving larvae seem to be able to compensate sublethal effects to some extent. One earlier study showed that lepidopteran prey insects vary in how much Bt toxin they assimilate (HEAD et al. 2001). However, variations in Bt toxin contents in different leaves or different part of leaves do not seem to affect leaf area consumption of lepidopteran larvae. Apparently differences in Bt toxin between leaf sections are below the levels that larvae can detect leading to differences in leaf consumption (DUTTON et al. 2005). Other studies found sublethal effects of different leaf portions on armyworm, Southwestern corn borer or European corn borer with lower weight of these lepidopteran larvae when feeding on younger Bt leaves (ABEL & ADAMCZYK 2004, DUTTON et al. 2004a, 2004b). While consequences for other leaf feeding non-target species are so far unclear the study of ABEL & ADAMCZYK (2004) shows the importance of the leaf section sampled when evaluating effects of Bt maize on non-target phytophagous species in field trials.

Other non-lepidopteran non-target organisms feeding directly on Bt maize plants are represented by the group of **leafhoppers**. Toxin levels in this group of non-target organism seem to be generally lower than levels in other herbivores (DUTTON et al. 2004a). The results of field studies examining possible effects of Bt maize on the abundance of this non-target species are somehow contrasting with some authors finding no clear effect on abundance (RAUSCHEN et al. 2004, DELRIO et al. 2004, ARPAS et al. 2004) and others finding a higher abundance in Bt maize fields (PONS et al. 2005). There are also some indications that **corn flea beetles**, generally a very abundant herbivorous group in maize, increase in abundance in Bt fields as compared to non-Bt maize fields.

Other studies have used indirect methods evaluating species abundance in maize fields such as the use of spider web analysis (ARPAS et al. 2004) suggesting that there is a tendency that **herbivores are generally more abundant** in non-Bt maize fields. Generally, evaluations of herbivore abundance under field conditions should consider that the abundance of foliar arthropods is influenced by plot size, field border treatment and isolation (PRASIFKA et al. 2005). For example, comparisons between plots which received insecticide applications with or without surrounding vegetation showed a significant effect of isolation on the number of leafhoppers.

Effects on predators and parasitoids

Effects on lacewings

Tri-trophic effects of Bt maize have first been reported for the green lacewing *Chrysoperla carnea* in the laboratory (HILBECK et al. 1998a, 1998b, 1999). Controversies arose about the prey fed to the chrysopid larvae and if the type of effects observed in these studies was a direct toxin effect or an indirect, prey-mediated effect. Subsequently, several studies have been carried out in order to clarify the effects observed by HILBECK et al. (1998a, 1998b, 1999). DUTTON et al. (2002) found no adverse effect on the larvae of *Chrysoperla carnea* when fed with the aphid *Rhopalosiphum padi* or the spider mite *Tetranychus urticae* which were previously reared on Bt maize. However, when the lacewing larvae were fed with *Spodoptera littoralis* reared on Bt maize, the specific and overall survival was lower, developmental time for 1st and 2nd instars and total development time to reach the adult stage of *Chrysoperla carnea* larvae was significantly prolonged. No effects on larval development of 3rd instar larvae were observed and a lower larval weight was only observed for 1st instar larvae. Absence of direct toxic effects of the Cry1Ab toxin mediated through prey species was also shown by ROMEIS et al. (2004). In bioassays they found no significant effects when feeding a Cry1Ab-sucrose solution on the weight gain, larval survival, development or mortality of *Chrysoperla carnea* larvae. They conclude that *Chrysoperla carnea* larvae are not sensitive to Cry1Ab toxin and are unlikely to contain Cry1Ab when feeding on prey herbivores. The study of ROMEIS et al. (2004) also showed that the effect of the toxin to the larvae was not enhanced when the predator was set under nutritional stress by using a low quality prey prior to ingestion of the toxin.

An indication that directly toxic effects are absent for the green lacewing when fed on Bt-plant fed prey was further confirmed by the studies of SCHULER et al. (2005) who assessed the possibly adverse effect of target pest reduction on *Chrysoperla carnea* in the presence of alternative prey using Bt oilseed rape. They found that numbers of lacewings did not change when aphid densities were high. However, significantly fewer lacewings were recovered from Bt plants as aphid densities were lowered indicating that lacewings largely rely on aphid populations as a prey species. Choice tests have shown that *Chrysoperla carnea* larvae of all developmental stages preferred aphids over lepidopteran larvae (MEIER & HILBECK 2001) therefore lepidopteran larvae might not be a common prey in the field, depending on the composition of the herbivore community. This study also showed that when given a choice 3rd instar larvae had a significant preference for *Spodoptera littoralis* fed non-Bt corn compared to Bt-corn fed larvae. The same trend was observed for 2nd instar larvae although here the effect was not significant. In contrast, no preference was observed when *Rhopalosiphum padi* which was either fed on Bt or on non-Bt was offered. Combinations of prey species, *R. padi* or *S. littoralis* showed a clear preference of all larval stages of *C. carnea* for *R. padi* regardless if fed on Bt or non-Bt maize. Other tritrophic laboratory assays have also shown the absence of adverse effects of Bt-fed prey on *C. carnea* (AVILLA et al. 2005). In field studies either no effects on the abundance of lacewings in Bt maize fields (DELRIO et al. 2004, SEHNAL et al. 2004) or no consistent effects over years have been shown (ARPAS et al. 2004, significantly more lacewings in non-Bt maize in one year). PILCHER et al. (2005) evaluated abundance of *Chrysoperla carnea* in two different Bt events (Bt176, Bt11) in early, middle and late sown corn and found no significant Bt effect. They showed that this species was least impacted by both corn phenology and corn type compared with four other predator species.

Also the exposure analysis of several predator species occurring in Bt-maize of HARWOOD et al. (2005) has not revealed any risk scenario for the green lacewing *Chrysoperla carnea*. Of the two lacewing individuals tested none screened positive for the presence of the Cry1Ab toxin (HARWOOD et al. 2005).

Effects on parasitoids and hyperparasitoids

Since the laboratory studies of SCHULER et al. (1999, 2001, 2003 and 2004) effects of Bt oilseed rape on parasitoids were considered to be negligible. The study of SCHULER et al. (2004) showed that when eggs of an endoparasitoid were laid in a susceptible host (*Plutella xylostella*) which fed on Bt leaves the endoparasitoid hatched but premature host mortality did not allow the endoparasitoid to complete its development. In Bt-resistant hosts the endoparasitoid was able to develop to maturity and there was no effect of Bt plants on percentage parasitism, time to emergence from hosts, time to adult emergence and percentage adult emergence from cocoons.

PRÜTZ & DETTNER (2004) conducted laboratory experiments in order to assess the potential effect of Bt maize leaf material on the parasitized lepidopteran herbivore *Chilo partellus* and on its parasitoid *Cotesia flavipes*. Bt corn leaf material fed hosts experienced a significantly higher mortality prior to parasitoid emergence and their food consumption was significantly lower. Due to the lower number of hosts which survived, emergence of parasitoids from Bt hosts was only 23% compared to 83% of the control group. Other life cycle parameters such as cocoon weight, pupa and adult weight were also negatively affected in the Bt group. Only in the Bt group a strong negative correlation was found between the food intake by the parasitized host *C. partellus* and the number of parasitoid cocoons indicating that less cocoons were produced with increasing Bt toxin intake of the host.

Using laboratory bioassays, MEISSE et al. (2003) showed that the toxin Cry1Ab was present in the larvae and cocoons of the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) of *Spodoptera littoralis* when fed with Bt maize leaves. The emergence of adult parasitoids was lower in Bt than non-Bt maize. The parasitoid needed significantly more time from parasitisation to pupation in hosts feeding on Bt maize. However, no effects on the weights of parasitoid cocoons and on the time from pupation to emergence, sex ratio or survival of the parasitoid were observed.

VOJTECH et al. (2005) analysed the effect of Bt-maize reared *Spodoptera littoralis* on the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). The weight of parasitized *S. littoralis* larvae (Bt) was significantly lower than that of the control group on three and six days after parasitization. Also the weight of the cocoons was lower and development was slower when the lepidopteran host was fed the Bt maize. In the Bt group significantly fewer males emerged and survival of larvae from cocoon formation until emergence of adults was also lower than in control group. In contrast, no difference in mortality of parasitoid pupae was observed. When analysing the Cry1Ab toxin levels in the host they found that approximately 0,6 µg toxin/g fresh weight were contained in the lepidopteran larvae, corresponding to about 40% of the content of the maize while the faeces contained only traces of the toxin. No toxin was found in adult hosts and also not in the adult parasitoid or its cocoons containing pupae while traces were detected in empty cocoons. The latter could be explained by the fact that either the parasitoid larvae do not take up the toxin, metabolize it or excrete it.

MANACHINI & LOZZIA (2004) analysed two parasitoids of the European corn borer, *Lydella thompsoni* and *Trichogramma brassicae* and found that percentage parasitism was higher in European corn borer in non-Bt corn compared to Bt corn probably due to the fact that the parasitized hosts die before the parasitoids can pupate outside the host. No effects on developmental and emergence parameters of the parasitoid *Cotesia marginiventris* parasitizing the lepidopteran larvae *Spodoptera frugiperda* were observed by RAMIREZ-ROMERO et al. (2004). Neither the proportion of parasitized larvae nor the emergence of parasitoids was different between Bt and non-Bt protoxin fed hosts and also developmental periods, size or longevity of adult parasitoids were unaffected.

Effects on the 4th trophic level, i.e. on hyperparasitoids of parasitoids infesting larvae that feed on Bt or non-Bt maize have been shown (BRINK et al. 2004, PRÜTZ et al. 2004). PRÜTZ et al. (2004) found that transgenic insect-resistant plants could affect hyperparasitoids indirectly. They analyzed the impact of Bt plants on the hyperparasitoid *Tetrastichus howardi* mediated through the herbivore *Chilo partellus* and its primary parasitoid *Cotesia flavipes*. Females of the hyperparasitoid in the Bt-group parasitized

significantly less cocoon clusters of the primary parasitoid than in the control. Moreover, significantly fewer *C. flavipes* cocoons were successfully parasitized. As a consequence, the hyperparasitoid females of the control had more offspring than in the Bt-group. Adult female hyperparasitoid offspring of the Bt-group weighed significantly less than in the control. However, no differences in developmental time, cocoon mortality or sex ratio of the hyperparasitoids were noted. However, it remains to be determined whether facultative hyperparasitoids prefer to develop as primary or secondary parasitoids under field conditions.

BRINK et al. (2004) showed that toxin concentrations of 1/5 or 1/10 of the concentrations of the toxin contained in maize leaves of the 7th-8th leaf stage had a significant effect on the parasitoid *Cotesia flavipes* reared on Bt or non-Bt fed *Chilo partellus* larvae with significantly lower weight of cocoon clusters for both Bt concentrations. Additionally, these cocoon clusters of *C. flavipes* were less successfully parasitized by the hyperparasitoid *Tetrastichus howardi* resulting in significantly less adult *T. howardi* emerging from cocoon clusters of the Bt group at the 1/5 concentration. Also adult *T. howardi* females emerging from these cocoons weighed significantly less thus influencing the fitness of the female hyperparasitoid. So far it is unclear whether the lower parasitization of the cocoons is due to any Bt-related direct effect or due to indirect effects such as smaller and therefore lighter cocoons.

BOURGUET et al. (2002) evaluated European corn borer (ECB) parasitism by tachinids under field conditions in France. They found significant fewer tachinid parasites in ECB collected from Bt corn (Bt176) than from non-Bt corn. The most likely explanation for the effect on parasitism is reduced host density. The authors conclude that surviving individuals of ECB larvae on Bt corn would be less likely to be parasitized which is not favourable for pest resistance management. Also VENDITTI & STEFFEY (2002) evaluated in field experiments the ECB parasitoid *M. grandii*. For both European corn borer generations the level of parasitism by this parasitoid was not different between Bt and non-Bt corn fields. Other authors found higher numbers of the corn borer parasitoid *Lydella thompsoni* collected on sample plants in non-Bt fields (DELRIO et al. 2004). No difference in the proportion of parasitism of four different aphid species in Bt maize fields were observed (PONS & STARY 2003). PILCHER et al. (2005) showed in field evaluations that the European corn borer specialist parasitoid *Macrocentrus cingulum* responded to both crop phenology and corn type (Bt and non-Bt). This parasitoid was shown to be significantly less abundant in Bt corn fields for 1st and 2nd ECB generations, although this effect was stronger for event Bt176 than for Bt11. Also adult numbers were highly correlated with ECB egg mass density. This effect is explained by the lack of ECB larvae or plant damage in Bt corn as compared to non-Bt corn.

SISTERSON & TABASHNIK (2005) simulated the loss of a specialist parasitoid in a model region with 900 fields of 10 ha each planted with Bt or non-Bt crop starting with each single field with 500.000 hosts and 200 000 parasitoids for 250 generations of colonization. Biological parameters such as host reproductive rate, parasitoid attack rate, percentage of hosts and parasitoids leaving their natal field and insecticide use parameters were drawn randomly from even distributions. For each simulation the model was run for 500 generations. The modelling showed that the effect of Bt crops on specialist parasitoids depends on the percentage of Bt fields, temporal field distribution (fixed or rotated), life history traits of the host and parasitoid and insecticide use. They suggest that parasitoid loss could be reduced by increasing the percentage of refuge fields, fixing refuge locations and increasing the insecticide spray threshold for target pests in refuges.

Effects of parasitoids may also influence resistance development of the target organism of Bt maize. Although Bt tolerance of lepidopteran hosts may not affect parasitism success of hymenopteran parasitoids, longer parasitoid wasp development time in Bt tolerant hosts compared to Bt susceptible hosts was observed (RAHMAN et al. 2004). Moreover wasps developing in Bt tolerant hosts were larger than those developing in Bt susceptible hosts. The results show that the increased immune status associated with the Bt tolerance did not provide the host a cross-protection against parasitism and possibly a partial inhibition of parasitoid development in Bt tolerant hosts.

Effects on heteropteran predators

Already ZWAHLEN et al. (2000) showed in a tritrophic feeding experiment that no lethal or sublethal effects were observed when nymphs of the heteropteran predator *Orius majusculus* were reared on Bt-fed prey, *Anaphothrips obscurus*, a thysanopteran pest of corn. Also other authors did not find any adverse effects of Cry1Ab toxins on several life history parameters of heteropteran predators such as *Orius majusculus* (PONS et al. 2004). However, in this study the anthers were removed from the pollen fed to the bug nymphs which might have removed a significant source of Bt toxin. Also, in this study alternate food sources were provided to the nymphs. It is unclear whether the bugs actually fed mainly on the eggs of the alternate prey or on the maize leaves and pollen provided. The amount of Bt toxin was not quantified in the leaves and the pollen and different developmental stages of leaves (5th or 6th) were provided to the nymphs. Other authors have shown the amount of Bt toxin in maize leaves can vary considerably up to 2-3 times depending on the developmental stage of the leaf (ABEL & ADAMCZYK 2004). ECKERT et al. (2004) sampled thrips and bugs from Bt maize fields and did not find any differences in the abundance of bugs (*Orius* sp., *Nabis* sp.) between Bt and non-Bt maize (in panicles or cobs). However, they found that the bugs were more abundant towards end of the vegetation period (from September onwards) and there was a tendency for fewer bugs on insecticide treated non-Bt maize plants. No differences between Bt and non-Bt fields in bug abundance as determined by visual counts on plants (Miridae, Anthocoridae) or by the use of yellow traps (Anthocoridae) have been reported by DELRIO et al. (2004) or by ARPAS et al. (2004) examining web contents of spiders. In some studies differences in bug abundance between Bt and non-Bt fields are inconsistent over years and locations with no clear preference found (FREIER et al. 2004, SEHNAL et al. 2004). MUSSER & SHELTON (2003) found no difference for *O. insidiosus* nymphs or adults between Bt and non-Bt fields in one year, while in the following year significantly more adult *O. insidiosus* were found on Bt maize than in the control field. When evaluating predation rate of egg masses of *O. insidiosus* in the field they found no significant reduction of egg predation in Bt corn compared to control. They explain the higher bug abundance in Bt corn with a possible preference of *O. insidiosus* for ears and silks being free from lepidopteran feeding. PILCHER et al. (2005) evaluated abundance of the bug species *Orius insidiosus* in two different Bt events (Bt176, Bt11) in early, middle and late sown corn and found no Bt effect on this predator species combined over years and locations. However, a significant correlation of bug abundance with ECB egg density was observed. However, *O. insidiosus* population dynamics were highly variable during the 2nd ECB generation and no particular pattern could be observed possibly indicating that *O. insidiosus* prefers also other prey beside ECB populations. Also DALY & BUNTIN (2005) evaluated bugs in visual counts in Bt and non-Bt maize fields and found more *Geocoris* bugs in Bt fields in one location and one year but not consistently over years and locations. However, the abundance of *Nabis* spp. was significantly reduced in Bt maize fields compared to non-Bt maize fields combined over years and locations. The abundances of *Orius* sp. was generally higher in Bt maize field in the combined analysis although this effect was not but almost significant.

It has been shown that nymphs of the common heteropteran predator *Nabis roseipennis* contained the toxin in 77% of 30 individuals tested and also other bug species tested (*Orius insidiosus*, Anthocoridae) screened positive for the toxin (HARWOOD et al. 2005). Toxin concentrations in the bug *Orius insidiosus* were highest of all species tested (2,53 µg/g), followed by the other Heteropteran species *Nabis roseipennis* (1,85 µg/g), a plant feeding species. These nymphs are reported to be readily consumed by some spiders (see references in HARWOOD et al. 2005) thus partly responsible for the toxin flow to predator communities. Also *Orius* is an important predator of many pests but supplements the diet by feeding on plant material. The toxin concentration of the latter was significantly higher than that in spiders and lower than in corn flea beetles.

Effects on carabid predators

A recent study using an abundant carabid predator in maize fields showed that these predators can be negatively affected when their larvae feed on lepidopteran larvae which in turn are feeding on Bt maize depending on the predator's larval stage (MEISSLE et al. 2005). In larval no-choice trials they evaluated the effect of Bt-maize fed prey species (*Spodoptera littoralis*) on the coleopteran predator *Poecilus cupreus*. Neonate larvae of this predator experience a significantly lower survival from day 11 onwards when fed on the Bt-maize reared prey species with none of the larvae surviving beyond day 20. When older predator larvae were used (10 days old) the larvae had still a significantly lower survival after 20 days than when preying upon non-Bt prey with only 77% survival. In adult paired-choice test they showed that dead or frozen Bt-fed *S. littoralis* larvae were preferred by *P. cupreus* over dead or frozen non-Bt fed larvae. Also living Bt or non-Bt fed *S. littoralis* larvae were preferred over frozen *Calliophora* pupae or living aphids. Frozen cereal aphids were preferred over frozen Bt-fed larvae but not non-Bt fed larvae, indicating that prey condition and prey type were important parameters for prey selection. Living prey was generally preferred over dead and frozen prey and *S. littoralis* was only preferred over *Calliophora* and aphids when living. Bt or non-Bt treatment was less important for prey selection. The toxin analysis of the beetle larvae showed that the highest concentration of the toxin was present in 10 days old *P. cupreus* larvae (80 ng/g fresh weight), followed by 33-43 days old larvae (42 ng/g) and 20 days old larvae (35 ng/g). The amount of Bt toxin in 10 days old larvae was approximately one tenth of the toxin amount in the prey species. The authors suggest that the adverse effects observed might be due to indirect effects on the nutritional quality of the prey, probably due to changed haemolymph composition.

This study and also others have shown that predators can contain a significant amount of the Bt toxin even under field conditions. HARWOOD et al. (2005) quantified the concentration of the Bt endotoxin (Cry1Ab) within natural enemies of non-target herbivores in a corn agroecosystem. The screening of predators showed that three orders (Araneae, Coleoptera and Heteroptera) contained the Cry1Ab endotoxin. Toxin concentrations ranged from 0,42 to 2,53 µg/g.

In contrast to the laboratory studies two recent study found no effect of Bt maize on the abundance of carabid species in corn fields (LOPEZ et al. 2005, DALY & BUNTIN 2005). However, the retrospective power analysis in one of the studies (LOPEZ et al. 2005) showed that the likelihood of detecting any existing Bt treatment difference was less than 0,20 for the three most common carabid species which were evaluated. Also the preliminary results of SZEKERES et al. (2005) showed no differences in the seasonal activity patterns of carabids in Bt and non-Bt maize fields.

Effects on spiders

As HARWOOD et al. (2005) have shown in their field study, spiders in Bt maize fields contained individuals from different families (Linyphiidae, Tetragnathidae, Theridiidae, Thomisidae and Lycosidae) and 7,7% of the spiders sampled contained the Cry1Ab toxin. The results of MEISSLE & LANG (2005) could not confirm a negative effect of Bt maize on this predator group. They evaluated the spider community in Bt maize fields (Bt176) compared to non-Bt maize fields, both treated once with or without a pyrethroid insecticide. In total they found 29 spider species in 32 genera and 14 families, predominantly web builders. They found significant differences in the collection efficiency of different collection methods (beating sheet, suction sampling, plant removal, stem elector). None of the four sampling methods detected any effect of the Bt maize. The results of MEISSLE & LANG (2005) are consistent with those of VOLKMAR & FREIER (2003). Both studies found no difference in spider species abundance or diversity between Bt and non Bt maize but negative effects of insecticide application on the parameters measured. VOLKMAR et al. (2003) showed that most of the variation in a spider community was explained by environmental variable while only 2,4% of the variation was explained by the maize variety. However, one species *Bathyhantes gracilis* was less abundant in Bt maize compared to non-Bt maize. No effect of Bt maize on spider

abundance under field conditions as determined by counts on sample plants was shown by DELRIO et al. (2004). Also the field samplings of DALY & BUNTIN (2005) showed no consistent effect of Bt maize on spider abundance in any location or year.

Effects on coccinellids

Around 6% of the common and abundant coccinellid predator *Coleomegilla maculata* contained the Bt toxin while of the less abundant coccinellid species *Cycloneda munda* and *Coccinella septempunctata* 33-50% of the individuals sampled screened positive for the Bt toxin (HARWOOD et al. 2005). The larvae of *C. septempunctata* did not contain the toxin. However, only the foreguts of the coccinellids contained the toxin, the bodies of all species did not contain any recognizable quantities of the Cry1Ab toxins. Coccinellids are known to feed on pollen and are important generalist predators. Also preliminary results of the EU funded project Bt-BioNoTa (SCHMIDT et al. 2004) show that the Cry1Ab toxin solutions sprayed in different concentrations on *Ephestia* eggs resulted in a significantly higher mortality of the coccinellid *Adalia bipunctata* at a range of the activated toxin of 5-50 µg/ml compared to the non-Bt isoline. However, although developmental time for this species was slightly reduced with higher concentrations, this effect was not significant. In their tritrophic feeding assays with *Stethorus punctillum* (Coleoptera: Coccinellidae) feeding on the mite *Tetranychus urticae* (Acarina: Tetranychidae) which were reared on either Bt or non-Bt maize plants the same authors report a slightly higher mortality of the predator on Bt maize but this effect was not significant. Similarly, developmental time was not affected. By using yellow traps in field experiments DELRIO et al. (2004) found no differences for most predator species in Bt maize fields, except for the total number of Coccinellidae and the single dominant coccinellid species *Propylea quatuordecimpunctata* which was significantly more abundant in the non-Bt maize field. MUSSER & SHELTON (2003) found no effects on adult or nymphal coccinellid abundance between Bt and non-Bt maize in one year but reported more adult *C. maculata* on late sown Bt maize than in the control of the other year. Also PILCHER et al. (2005) evaluated abundance of two coccinellid species (*Coleomegilla maculata*, *Cycloneda munda*) in two different Bt events (Bt176, Bt11) in early, middle and late sown corn and found no significant Bt effects when years and locations were combined. However, the populations of the coccinellids were larger in Bt maize (*C. munda*) and in some cases significantly more individuals were found in Bt maize (*C. maculata*). Both species preferred the early-planted corn and trends for a greater recruitment over time to Bt corn during the 2nd ECB generations were observed possibly due to differences in plant health. Also DALY & BUNTIN (2005) recorded more *Coleomegilla maculata* in Bt corn in one year and one location, although consistent effects over years and locations were absent.

Effects on predators under field conditions

Reports on non-target predator abundance under field conditions are controversial. Most authors do not find any effect of Bt-corn on predator species (DE LA POZA et al. 2005, JASINSKI et al. 2003, PONS & STARY 2003, CANDOLFI et al. 2004, DIVEY & ROSE 2002). DE LA POZA et al. (2005) and JASINSKI et al. (2003) studied non-target arthropods in Bt cornfields and found no significant differences of non-target arthropods consistently over locations and years when they pooled the number of arthropods of all sites. However, the study of JASINSKI et al. (2003) can be considered as of minor importance. The authors do not state which Bt hybrid (event) was chosen and if the non-Bt fields were the isogenic cultivar. Additionally, they found, after the study had started, that one of the non-Bt corn fields was a Roundup Ready cornfield. Additionally, this non-Bt cornfield was treated with another pesticide than the Bt corn which was also the case for two other of the six locations which makes the attribution of the observed effects to the transgenic event impossible because pesticide application might have interfered with arthropod abundance. Moreover, they only use two yellow sticky traps in the cornfields over a relatively short period (1,5 months). Only four of the non-target arthropods were determined to species level, three to generic level and five to family level. Also

BOURGUET et al. (2002) found no significant effects in the abundance some predators of aphids (*Orius insidiosus*, *Syrphus corollae*, *Coccinella septempunctata*) but they admit that small effects of Bt corn cannot be detected with low replication and that it is unrealistic to expect a difference of 10-20% in the abundance of most non-target species. ARPAS et al. (2004) evaluated in field experiments the abundance of prey items in spider webs of *Theridion impressum* (Araneae: Theridiidae) in Bt maize over two years. They found generally a higher numbers of prey items of this spider species in isogenic maize but this effect was not significant. Herbivores were significantly more abundant in non-Bt maize but only in one year although the tendency was also observed in the other year. Predators, parasitoids and omnivores were similarly contained in the webs in both years with no clear differences for either Bt or non-Bt maize. SEHNAL et al. (2004) evaluated epigeic beetles and spiders caught in pitfall traps. The numbers of species as well as of individuals was comparable although the authors did not statistically evaluate the catches. They conclude that the dominant and abundant species are present in all plots over both years while the less abundant species were distinctly different in the two years. PILCHER et al. (2005) evaluated four non-target predators in two Bt events (176 and Bt11) in early, middle and late sown maize and found no consistent negative Bt effects on these species although in some cases abundances of coccinellids were higher in Bt corn. Similarly, DALY & BUNTIN (2005) evaluated predacious arthropod abundance in Bt fields and found few significant effects on abundance which were mainly restricted to individual years and locations when predators where either collected by pitfall traps or counted visually. However, at one location and in one year significantly fewer Staphylinids, Anthicids and ants in the Bt fields were sampled by pitfall trapping. The combined analysis of these taxa over years and locations did not result in a statistical significant difference.

Summary: Effects on predators and parasitoids

Several studies have shown that adverse effects of Bt maize on larvae of the **green lacewing** are rather prey-mediated than direct toxic effects (ROMEIS et al. 2004, DUTTON et al. 2002, AVILLA et al. 2005). Moreover it was shown that under field conditions lacewing larvae prefer and rely on aphids rather than on lepidopteran larvae (SCHULER et al. 2005) which was also confirmed by choice tests in the laboratory (MEIER & HILBECK 2001). When given a choice *C. carnea* switches to phloem feeders in the field in order to avoid detrimental effects and therefore reduce the predator pressure on prey containing the Bt toxin. The absence of consistently adverse effects of Bt maize on *C. carnea* under field conditions was further confirmed by several studies (DELRIO et al. 2004, SEHNAL et al. 2004, ARPAS et al. 2004 PILCHER et al. 2005) and also no exposure of this predatory species to the Bt toxin has so far been confirmed (HARWOOD et al. 2005).

So far, five laboratory studies evaluated effects of Bt maize on **parasitoids**. Two studies evaluated the presence of the Bt toxin in parasitoid larvae, adults or cocoons (MEISSLE et al. 2005, VOJTECH et al. 2005) with contrasting results. Therefore it is still unclear whether a parasitoid actually takes up the toxin or if it is immediately metabolised or excreted. Reported negative effects of Bt fed hosts on parasitoids were mainly due to host-mediated effects such as reduced survival or food consumption resulting in a significant lower emergence of parasitoids from Bt-fed hosts (PRÜTZ & DETTNER 2004, MEISSLE et al. 2003, VOJTECH et al. 2005). Effects on other life cycle parameters of parasitoids are controversial. While some authors did not find any effects on the parasitoid's developmental time (PRÜTZ & DETTNER 2004, MANACHINI & LOZZIA 2004, RAMIREZ-ROMERO et al. 2004) others report on longer developmental time from parasitization to pupation in hosts fed with Bt maize (MEISSLE et al. 2003, VOJTECH et al. 2005). Time from pupation to emergence was unaffected (MEISSLE et al. 2003). Also parasitoid cocoon weight has been reported to be affected with significantly lower cocoon weight in the Bt group (PRÜTZ & DETTNER 2004, VOJTECH et al. 2005) or not been negatively affected (MEISSLE et al. 2003). Pupa and adult weight of parasitoids arising from the Bt group may also be negatively affected (PRÜTZ & DETTNER 2004). Effects on size of adult parasitoids might significantly influence parasitoid fitness as larger

parasitoid females have a higher fecundity, tend to live longer and have higher searching efficiency and egg load than smaller females. Sex ratio or survival of the pupal or adult parasitoids seem to be unaffected in some studies (MEISSLE et al. 2003, PRÜTZ & DETTNER 2004, MANACHINI & LOZZIA 2004) while other found effects on sex ratio with significant fewer males in the Bt group and a higher survival in the control group (VOJTECH et al. 2005). In field studies a lower proportion of parasitism for some parasitoids of the European corn borer was recorded in Bt corn fields (BOURGUET et al. 2002, MANACHINI & LOZZIA 2004, DELRIO et al. 2004, PILCHER et al. 2005) while one other study does not find any difference in parasitism between Bt and non-Bt fields (VENDITTI & STEFFEY 2002). The effect of a lower abundance of parasitoids of the European corn borer can most probably be explained by reduced host density (lack of ECB larvae) or plant damage in Bt corn as compared to non-Bt corn. Effects of Bt maize on **hyperparasitoids** of Bt feeding larvae have been evaluated by two studies (BRINK et al. 2004, PRÜTZ et al. 2004) reporting indirect effects on the hyperparasitoid with less hyperparasitization of the primary parasitoid, lower hyperparasitization success and fewer and lighter offspring of hyperparasitoids from Bt-fed hosts. One study showed (RAHMAN et al. 2004) that Bt tolerance of a host did not provide a cross-protection against parasitism but possibly a partial inhibition of parasitoid development in Bt tolerant hosts. Exposure analysis has shown that both **bug species** *Nabis roseipennis* and *Orius insidiosus* (Heteroptera) contained the Bt toxin when captured from a Bt maize field therefore enabling a toxin flow to higher predator communities (HARWOOD et al. 2005). Despite of the presence of the toxin in this arthropod group no lethal or sublethal effects of Bt maize on heteropteran predators such as *Orius* sp. could be confirmed in laboratory assays (ZWAHLEN et al. 2000, PONS et al. 2004) although the effective exposure of the bugs in these studies was not always evident. In field studies either no difference in abundance of bugs was found when abundance was determined by visual counts, yellow traps or web contents of spiders (ECKERT et al. 2004, DELRIO et al. 2004, ARPAS et al. 2004, PILCHER et al. 2005) or inconsistent results with differences in bug abundance in some years and locations are reported (FREIER et al. 2004, SEHNAL et al. 2004, MUSSER & SHELTON 2003). In some cases and for some species (e.g. *Orius* sp.) higher bug abundances were recorded in Bt corn (see e.g. MUSSER & SHELTON 2003, DALY & BUNTIN 2005) while for other species (e.g. *Nabis* sp.) lower abundances were recorded in Bt corn (DALY & BUNTIN 2005).

While it has been shown that natural enemies such as **carabids** take up and contain the Bt toxin (HARWOOD et al. 2005, MEISSLE et al. 2005) and that feeding of carabid larvae on Bt-fed lepidopteran larvae resulted in lower survival (MEISSLE et al. 2005) these adverse effects do not translate into adverse effects on the abundance of this predator group in the field as shown by recent studies (LOPEZ et al. 2005, DALY & BUNTIN 2005, SZEKERES et al. 2005).

Also **spiders** have been shown to be exposed to the Bt toxin under field conditions (HARWOOD et al. 2005) but consistent adverse effects on abundance of this predator group have so far not been confirmed (MEISSLE & LANG 2005, VOLKMAR & FREIER 2003, DELRIO et al. 2004, DALY & BUNTIN 2005). In one study one species of Araneae was less abundant in Bt maize compared to non-Bt maize (VOLKMAR et al. 2003).

The presence of Bt toxin has been shown in adult **coccinellids** but was absent in larval coccinellids (HARWOOD et al. 2005). Apparently the beetles take up and digest the toxin but it is not incorporated into the body as it was only detectable in the foreguts of the beetles. In laboratory assays adverse effects on coccinellids feeding directly on Cry1Ab toxin solutions have been confirmed while coccinellid mortality and development was only slightly affected in tritrophic feeding assays (SCHMIDT et al. 2005). In field studies so far consistent effects over years and locations of Bt maize on coccinellid abundance could not be confirmed (PILCHER et al. 2005) or coccinellids have been reported to be more abundant in non-Bt maize (DELRIO et al. 2004) or more abundant in Bt maize in some years or locations (MUSSER & SHELTON 2003, PILCHER et al. 2005, DALY & BUNTIN 2005).

Generally, effects of Bt maize on predator species evaluated in field experiments give controversial results for a variety of organisms and consistent differences between Bt and non-Bt maize fields could so far not be proven for most species. Differences observed

under field conditions are restricted to certain species, years or locations (e.g. PILCHER et al. 2005, DALY & BUNTIN 2005). Predators often respond to a greater degree to different phenological stages of corn development than to the presence of the European corn borer or corn type (PILCHER et al. 2005). However, the detection of treatment effects of Bt maize on predator or parasitoid abundance in field studies is largely dependent on sample size and replication. Several studies admit that low power estimates in their studies indicate problems in experimental designs. Data of field studies indicate that only effects of 20-50% were detectable with 80% power for all abundant taxa (e.g. BHATTI et al. 2005a, 2005b). MEISSLE & LANG (2005) calculate that in order to detect a 30% difference in spider abundance the sample size (number of fields per treatment) should be 14 fields per treatment (probability of 80%) when using suction sampling as the preferred method. In order to detect a 10% abundance difference this value would increase to approximately 110 fields. The sample size used in their study ($n = 4$) would however only reveal an effect on spider abundance of 70% (with 80% probability) using the suction sampling method. LOPEZ et al. (2005) used carabid beetle abundance in corn fields and evaluated statistical power thereby showing that when treatment differences are not detected, insufficient replication is a likely cause. They also suggest that moderate to large treatment effects require much more replication than is commonly used to have a more than 0,50 likelihood of detection (= statistical power). Statistical power of 0,70 or higher is often suggested for non-target studies but depends on what effects size is considered probable or biologically significant (see LOPEZ et al. 2005 and references therein). Moreover it has to be considered that only some of the species present in a maize field are available in sufficient numbers for statistical evaluation while many species are present in low numbers only. LOPEZ et al. (2005) have shown that the relative species abundance of most ground beetles collected in pitfall traps was less than 1% while only 1-4 species comprised the majority (80% or more) of beetles present. Additionally, other methodological flaws are evident in many field evaluations of non-target arthropod abundance such as unknown maize hybrids used, short sampling periods or determination of the arthropods to the generic level only (e.g. JASINSKI et al. 2003). Also small plot size or different field borders influence the detection of significant differences in arthropod abundance. PRASIFKA et al. (2005) showed taxon-level effects of plot size, border treatment or isolation with a trend of higher populations of epigeal arthropods in smaller plots which is probably based on variation in mobility and behaviour.

Effects on soil organisms and processes

There is considerable concern that Bt-toxins could accumulate in the environment to concentrations that adversely affect soil organisms such as soil microbiota, soil processes or even higher soil organisms. The ecotoxicity of transgenic Bt toxins will largely depend on their persistence and their retained biological activity in the soil. Therefore the examination of the release, the persistence, accumulation and degradation of these toxins is necessary.

The results of the earlier studies of TAPP & STOTZKY (1998), SAXENA et al. (1999), STOTZKY (2000), SAXENA & STOTZKY (2000) as well as SAXENA & STOTZKY (2001a) regarding Bt toxins in soils can be summarized as follows:

- The toxin is released in root exudates from Bt corn.
- Bt toxins absorb rapidly (within less than 30 minutes) on clay minerals, humic acids or clay-humic acid complexes.
- Only 10-30% of the Bt toxins desorb again after absorption.
- Binding of the purified toxins on the clays does not alter their structure.
- The binding of the toxins reduces their availability to microbes.
- Neither free nor bound toxins have an effect on the growth on gram-positive or gram-negative bacteria, fungi and algae, nematodes or protozoa.
- The toxins remains larvicidal to their target insects for months (at least 234 days) even after subsequent frost and death of Bt corn plants under field conditions.
- Persistence of larvicidal activity is independent of the clay mineralogy and other physiochemical characteristics of the soils.
- Mortality and weight of earthworms exposed to Bt toxins is not affected after 40 days in soil planted with Bt-corn although the toxin is present in the gut of the earthworms.

Toxin release via root exudates

Regarding the reports on Bt toxin release in root exudates further studies (SAXENA et al. 2002, SAXENA et al. 2004) confirmed the previous hypothesis. The results of the studies of SAXENA & STOTZKY (2000, 2001) on Bt toxin release in root exudates were confirmed by further studies of SAXENA et al. (2002) who showed that toxin release from root exudates is common and similar for twelve different Bt maize hybrids representing three different Bt-maize events (Bt11, MON810, Bt176). Furthermore, SAXENA et al. (2004) showed the release via root exudates was different for different transgenic plants. While Cry1Ab toxins were released in root exudates from corn and rice and Cry3A from potato this was not found for Cry1Ac from canola, cotton and tobacco. Moreover, the Bt toxins released from Bt corn were more toxic than those released by Bt rice or Bt potato. The transformation method, somaclonal variation, differences in protein expression levels or the location of endoplasmatic reticulum relative to plasma membrane in corn may be the reason for this effect observed (SAXENA et al. 2004).

Persistence, degradation and biological activity of Bt toxins in soil

Persistence of Bt toxins released into the soil is a function of the amount of the toxin present, the rate of consumption and inactivation by insect larvae, the rate of degradation by microorganisms and the rate of abiotic inactivation (STOTZKY 2004). Several studies report on persistence, binding to soil particles, degradation and biological activity of Bt toxins in the soil.

SAXENA & STOTZKY (2002) report on the persistence and larvicidal activity of the Cry toxin in the soil after the addition of the purified toxin (approx. 3 µg/g soil) to different soils which was still lethal to the target organism *M. sexta* after 90 or after 120 and 180 days after the soils had been amended either with rhizosphere soil or ground biomass (mixture of leaves, stems and roots) of Bt corn. The authors also found that the toxin was more toxic to *Manduca sexta* larvae in larvicidal bioassays when a soil was amended to an amount of 9% Kaolinite or Montmorillonite than when the soil was not amended or

amended to only 3% of clay minerals. Also, larvicidal activity was greater in soil amended with Montmorillonite than with Kaolinite, probably because the former has a higher cation-exchange capacity and specific surface than the latter thus binding more toxin. ZWAHLEN et al. (2003a) studied degradation of Bt leaves under field conditions (fresh leaves before and after pollen shed). When leaf litter was incorporated into soil they observed a non-linear degradation over a time period of 8 months. The toxin concentrations did not decrease significantly over the first month possibly due to a necessary breakdown of the litter by soil organisms. After 7 months 1,5 % of the initial Bt toxin concentration was still present in the leaves. When they added leaf litter on top of the soil they found that approximately 62 % of the initial toxin concentrations already degraded after 40 days and continued gradually over the winter. However, at the end of the experiment, after 200 days, 0,33 % of the initial concentration was still present in leaves. The authors also showed that this remaining Bt toxin had still lethal effects on the target organism *O. nubilalis* at least for the first 3 months but also after 7 months while sublethal effects were shown for a longer time period.

In another study ZWAHLEN et al. (2003b) found that Bt toxin degradation of Bt leaves (collected 3 weeks after pollen shed) was significantly different in the lab and in the field, with significantly slower degradation in the field than in the lab. Concentrations were approximately 5-6 times higher in field degrading leaves than in lab degrading leaves. At the end of the experiment the Bt concentration was higher in the lab than in the field indicating that degradation continued in the field while it stagnated in the lab. Average temperature in the field (8,5°C) was below the temperature in the lab (constantly 10°C). Larvicidal activity of the leaf litter remains was also different between lab and field with longer activity in the field (80 days) compared to the lab (until 40 days). In contrast, sublethal effects were observed in lab until 120 days, but not in field.

HOPKINS & GREGORICH (2003) examined the amount of Bt toxin in soils from fields where Bt maize was grown over four years as well as the decay of plant material and the toxin in soil. In their study the Cry toxin could not be detected anymore in the soil amended with Bt leaves after 14 days. They examined different soil fractions and found that most of the toxin was concentrated in the $\geq 2000 \mu\text{m}$ soil fraction which was a mixture of light-and dark coloured organic material and mineral material. Most of the toxin was present in the light coloured organic material which consisted mainly of roots although some toxin could also be detected in the dark coloured organic material which represented decaying residues, probably from the previous years. This is again in agreement with the results of BAUMGARTE & TEBBE (2005) who found the majority of the Cry toxin in the roots of the maize residues. After 43 days approximately 40% of the added plant carbon had been degraded. These results indicate that some of the toxins are protected from decay in the more stable organic material as compared to toxins contained in more labile plant material. However, HOPKINS & GREGORICH (2003) detected no difference in the rate of decomposition of Bt maize and non-Bt maize leaves. The fact that also other Bt toxins bind rapidly on clay and do not desorb was also confirmed by LEE et al. (2003) who studied clay adsorption of solubilized and lyophilized Bti toxins obtained from Bti microbial populations. They found that 88-98% of the adsorbed toxins was tightly bound to the clays and that adsorption was rapid and greater on montmorillonite than on kaolinite. Also no saturation for the maximum amount of protein added (500 μg to 100 μg soil) was observed. The authors also observed no changes in protein structure due to binding to the clay and the toxicity of the toxin to the dipteran target species was significantly higher of clay-bound toxins than that of free toxins after 45 days of incubation.

The persistence of the Bt toxin over a long period of time was also confirmed by BAUMGARTE & TEBBE (2005) who analysed bulk and rhizosphere soil samples of four growth stages of Bt maize (MON810) over two growing seasons and at two different sites as well as post-harvest residues for the presence of the Cry1Ab toxin. They found small amounts of the Cry toxin (0,21 ng/g soil) seven months after the harvest of the previous Bt maize cultivation. The mean concentrations of the Cry toxin ranged from 0,1-10 ng/g soil from rhizosphere or bulk soil depending on year and location. Their results indicated that no accumulation of the toxin in the soil had occurred as toxin concentrations in rhizosphere were consistently higher than in bulk soil. However, they found that plant residues in the fields represent the major reservoir of the toxin after harvest. In root

residues they found 151,5 -182,5 ng/g fresh weight (approx. 12% of initial concentration) seven months after cultivation of Bt maize decreasing to 70 ng/g another two months later. In contrast, leaf residues had much lower Cry toxin concentrations (0,2% of the initial concentration) which is similar to the results of ZWAHLEN et al. (2003a, 2003b) who found 0,33% of the initial concentration still present in leaves from the soil top degraded for seven months. These results suggest that persistence in leaves is much lower than in roots as the expression levels of the toxin in Bt maize MON810 is four to seven times higher in leaves than in roots. However, BAUMGARTE & TEBBE (2005) used no insect bioassays in order to determine the larvicidal activity of the remaining Cry toxin.

PAGEL-WIEDER et al. (2004) found no indication for a saturation effect in the soil of Bt-toxin (Cry1Ab) adsorption for concentrations in the range of 0-80 ng/ml. However, they observed different adsorption affinities of soils for the Bt-toxin despite of similar mineralogical composition of the soils which the authors explain by the differences in organic carbon content of the soils. A correlation between the organic carbon content and the absorption affinity was observed. The lower the organic carbon content was the higher was the adsorption affinity which the authors explain by soil organic matter coatings on particles hindering the Bt toxin adsorption as well as by the blocking of binding sites for Bt-toxin by organic matter.

Lignification and composition of Bt plants was studied by several authors (DINEL et al. 2003, POERSCHMANN et al. 2005, JUNG & SHEAFFER (2004).

DINEL et al. (2003) used the same plants as HOPKINS & GREGORICH (2003) and evaluated extractable lipids as indicators of organic matter quality not only in composts but also in soils under different cropping practices. Concentrations of total alkenes in non-Bt maize shoots and soils were 47,9 and 21,3 % higher than in Bt maize while concentrations of n-alkanes were of similar orders of magnitude in Bt and non-Bt shoots but were still 28,6% higher in Bt than in non-Bt soils. Bt maize shoots contained almost 30% more n-fatty acids than non-Bt maize shoots, similarly to Bt soils with n-fatty acid concentrations twice as high as those in non-Bt soil. The concentrations of unsaturated fatty acids were 22% higher in Bt maize shoots while in soils they were 25,5% higher in non-Bt than in Bt soils. In summary, they showed that Bt maize shoots were significantly lower in alkenes but richer in n-alkanes, n-fatty and unsaturated fatty acids than non-Bt shoots. Similarly, Bt soils were lower in alkenes and unsaturated fatty acids but richer in n-alkanes and n-fatty acids than non-Bt soils. Additionally, Bt shoots and soils were lower in pristene, an isoprenoid hydrocarbon, than non-Bt shoots. The authors conclude that Bt maize significantly increase the ratio saturated to unsaturated lipids in soils thus affecting microbial activity. The authors of this study also found, in agreement with other studies, slower decomposition rates of Bt maize compared to non-Bt maize as measured by lower CO₂-carbon evolution of Bt soils and non-Bt soils amended with Bt shoots, ranging from 16,5% to 30,5% lower CO₂ development.

POERSCHMANN et al. (2005) evaluated total lignin contents and lignin patterns of stem samples by thermochemolysis and GC-MS analysis and found higher lignin contents in Bt maize in stems but not in leaves with mainly G-type (G18 subunit) but also P-type monomeric units being responsible for the increase of lignin in Bt corn. They showed that the G-type lignin was mainly responsible for the higher lignin content of Bt maize stems. This structural component is assumed to be more resistant to chemical and biological breakdown than other lignin-types. When they used the Klason method for lignin determination (similarly to JUNG & SHEAFFER 2004) their previous results were confirmed.

FLORES et al. (2005) studied recently the decomposition of Bt plant (Bt corn, Bt rice, Bt potato, Bt cotton, Bt canola and Bt tobacco) biomass by carbon mineralization in order to determine gross metabolic activity of soils and report that the biomass of these plants decomposed consistently less than the biomass of near isogenic non-Bt plants as measured by CO₂ evolution. Although the authors can exclude inhibition of soil microbiota activity as a reason for this lower biodegradation they conclude that the consistently higher lignin content in all Bt plants could account for the effect observed. The effect was apparently due to the Cry-protein added as no such effects (higher lignin content, lower metabolic activity) were observed for GFP transgenic plants.

JUNG & SHEAFFER (2004) studied lignin contents of six different Bt-maize varieties with three different analysis methods and did not find any consistent effects of the Bt trait on lignification although they found some location specific significant differences between Bt maize and their isolines. However, when considering only the acetyl bromide analysis (as did e.g. SAXENA & STOTZKY 2001b) the lignin values for the Bt corn internodes were higher in five of the six varieties analysed.

Other effects than larvicidal activity for target organisms or persistence of Bt toxins in the soil ecosystems have been shown by ACCINELLI et al. (2004). They found that Bt toxins are able to enhance the persistence of the herbicides glyphosate and glufosinate in soil. The persistence of both herbicides was significantly prolonged when Bt-toxin was added to the soil and was approximately twofold higher than without the Bt toxin independent of soil type.

Effects on microbial populations

Microbial populations come into contact with proteins released from transgenic plants. Results on studies examining effects of Bt maize on soil microbial populations are so far available by BRUSETTI et al. (2004), BAUMGARTE & TEBBE (2005) and BLACKWOOD & BUYER (2004) as well as some preliminary results from the EU-funded project ECOGEN. BRUSETTI et al. (2004) used different approaches in order to measure effects of Bt maize (event 176) on soil bacterial communities. Soil and rhizosphere samples from greenhouse grown plants were used for bacterial plate counts of aerobic copiotrophic, oligotrophic, sporeforming bacteria, community level catabolic profiling (CLCP) of rhizosphere soil, rhizoplane fraction (obtained when roots were washed and shaken in a sterile saline solution) and bulk soil and ARISA fingerprinting of DNA of total eubacterial communities. Moreover, they measured the effect of root growth solution of Bt maize in hydroponic culture with ARISA. In the bacterial plate counts they found significant differences for copiotrophic and oligotrophic bacteria only sporadically and not consistently. This result was also confirmed by CLCP which revealed no significant differences in functional diversity of microbial populations of rhizosphere soil or rhizoplane between Bt and non-Bt maize but between both treatments (Bt and non-Bt) and bulk soil. However, they also used a molecular population fingerprinting approach which targets the hypervariable 16S-23S intergenic transcribed spacers and is useful for discrimination of bacteria at subspecies level. With this method they found not only greater variability among the same plant type than between different plant types (especially after 100 days) and rhizosphere-induced bacterial population shifts and plant age-dependent population patterns but also time dependent bacterial community differences between Bt and non-Bt maize (mainly after 30 days). When they analyzed root growth solutions these significant community differences between Bt and non-Bt maize or bulk soil were confirmed. The authors conclude that the different maize types (Bt and non-Bt) select different rhizosphere communities, especially shortly after cultivation and plant growth, probably by differences in the exudation of roots.

BAUMGARTE & TEBBE (2005) used partial 16S rRNA amplification and single-strand conformation polymorphism analysis (SSCP) in order to analyse the structural bacterial community in rhizospheres over three years and found that plant age and field heterogeneity had a strong influence on the bacterial communities. However, in two years they found a clustering effect of the Bt-variety on the bacterial communities. BLACKWOOD & BUYER (2004) found no consistent effects of Bt corn of two different events (Bt11 and TC1507) on phospholipid fatty acid profiles (PLFA) from bulk soil or on fungal and bacterial community-level physiological profiles (CLPP) from rhizospheres. Only in one soil type (high clay soil with 42% clay) the effect of the Cry protein on bacterial CLPP was statistically significant. In this soil the Cry protein seemed to stimulate the ability of the rhizosphere community to metabolize certain amino acids and other amines. This method characterizes those members of a community that are fast-growing aerobic heterotrophs which are an important component of the rhizosphere while phospholipid fatty acid profiles from bulk soil provides an analysis of all organisms present. The significant reduction in eukaryotic PLFA biomarkers in bulk soils from Bt

plants suggested that the Cry protein may have significantly reduced populations of soil microarthropods or other invertebrates as there was no decrease in fungal, fungal or protozoan biomarkers.

First results of the EU-funded ECOGEN project (www.ecogen.dk) studying the effects of Bt expressing crops on the soil biology also indicate that changes in the microbial communities due to effects of Bt maize (Cry1Ab) are small and less than due to effects of different cultivars and crops (GRIFFITHS et al. 2005a, 2005b, submitted). CORTET et al. (2005, submitted) showed that decomposition of wheat straw was unaffected by the Bt trait under three different European climatic conditions after four months incubation in the field.

Effects on mycorrhizal fungi

So far there is only one publication that studied the effect of Bt corn on mycorrhizal fungi. TURRINI et al. (2004) were the first that studied an arbuscular mycorrhizal fungi species and its pre-symbiotic mycelial growth, host recognition and root infectivity of Bt176 and Bt11 maize. While host recognition was unaffected by Bt maize, Bt176 roots reduced pre-symbiotic hyphal growth and a high number of infection structures failed to produce viable infection units and were not able to colonise roots.

Mycorrhizal symbiosis of crop plants is an essential ecological parameter and should be included in every risk assessment. For example it was shown that arbuscular mycorrhizal symbiosis can make crops plants more attractive towards aphid parasitoids (GUERRIERI et al. 2004). Significantly fewer aphids completed their development and reproduced on arbuscular mycorrhizal plants in comparison to non-mycorrhizal plants. Moreover, more parasitic wasps were attracted to mycorrhizal plants either infested or uninfested by aphids suggesting that parasitoids arrive on mycorrhizal plants either with or without aphid hosts. As possible explanation the authors suggest that the symbiosis may allow the parasitoids to locate hosts already at very low population densities.

Effects on earthworms

Earlier studies could not confirm that Bt toxins deriving from root exudates or from degradation of Bt maize biomass are toxic to several soil organisms including earthworms in (TAPP & STOTZKY 1998, SAXENA et al. 1999, STOTZKY 2000, SAXENA & STOTZKY 2000, SAXENA & STOTZKY 2001a). SAXENA & STOTZKY (2001a) reported the absence of toxic effects of the Bt toxin on earthworms and showed that the toxin was present in the guts and casts of earthworms. They suggested that soil particles protect the toxins from biodegradation due to binding of the toxins to the surface-active particles similarly to what was observed in other studies.

Also the study of ZWAHLEN et al. (2003b) found no significant mortality for Bt maize leaves fed earthworms *Lumbricus terrestris* both in laboratory and field experiments over a period of 200 days. However, in their study ZWAHLEN et al. (2003b) observed a significant weight loss of the earthworms of approximately 18% of their initial weight after 200 days in the laboratory although this effect could not be observed in the field trials. The cause of this effect was unknown and the authors were not sure if it was due to the Bt toxin exposure or to changes in the plant quality.

A study funded by the EU-project ECOGEN (VERCESI et al. 2005, in press) investigated the effects of Bt corn on important life-history traits (survival, reproduction and growth) of one earthworm species but found no detrimental effects on survival, growth, development or reproduction of juvenile earthworms. However, a slight, but statistically significant, negative effect of Bt-corn residues on cocoon hatchability was observed.

Effects on isopods

Effects on other higher soil organisms are also reported for isopods. Already ESCHER et al. (2000) examined the effect of Bt leaves on the consumption of the decomposer *Porcellio scaber* (Crustacea: Isopoda) and found no effect of Bt maize leaves on weekly

consumption. However, the age of the leaves in these experiments is unknown, but leaves were decomposed for three weeks before being used in the experiments. Also a significantly higher mortality in juveniles fed on non-Bt leaves was observed, as well as a significantly higher weight increase of adult *P. scaber* fed on Bt leaves. In contrast, weight gain of juvenile isopods was significantly faster when fed on non-Bt leaves. Regarding decomposition the bacterial density was up to 60% lower on faeces of Bt maize fed isopods than on non-Bt fed individuals. The authors speculate that differences in nutritional quality of the plants might explain the differences observed.

Also WANDELER et al. (2002) examined the effect of Bt11 or Bt176 leaf consumption on the isopod *Porcellio scaber* and found significantly less consumption on Bt11 senescent leaves than on the isogenic control and lower consumption of Bt11 leaves than of Bt176 leaves. However, no consistent effects of the transgenic varieties when compared to conventional maize varieties could be shown. The authors could not conclude whether the observed effects were due to direct effects of the toxin or indirectly mediated but they speculate that the higher nitrogen and energy content in Bt11 might explain the lower consumption observed. They could also show that the Bt toxin concentration of the faeces was 1/10 of the food plant material. Thus the authors conclude that the Bt toxin from the plant can reach the soil via excretion of organisms thus making it available to other non-target soil organisms not ingesting the plant itself.

PONT & NENTWIG (2005) examined Bt toxin dissipation in leaves of Bt11 and Bt176 varieties and feeding of *P. scaber*. The isopods fed and excreted less plant biomass when feeding on Bt176 than when feeding on Bt11 leaves. Consequently *P. scaber* excreted significantly more Bt protein when feeding on Bt11. Although the isopods fed less on Bt176 maize the digestion of the Bt toxin was significantly higher for this maize variety (80,5 %) than for Bt11 (61,1 %) indicating that Bt11 leaves were less sufficiently digested. As an explanation of these effects the authors suggest the higher Bt toxin and lignin content in Bt11 leaves compared to Bt176 leaves. They also showed in herbivore bioassays that the faeces of the isopods fed on both Bt-varieties were still insecticidal, although less insecticidal than leaves of Bt11 and similar to leaves of Bt176.

Effects on nematodes

Regarding effects of the Cry toxins on nematodes very few published studies are currently available. Only the preliminary results of MANACHINI & LOZZIA (2004) and the EU funded project ECOGEN give some indications about the effects of Bt corn on the nematofauna which are also controversial. MANACHINI & LOZZIA (2004) found no significant differences in abundance, biodiversity, trophic structure or similarity index of the nematofauna between Bt and non-Bt maize fields. However, there was a tendency of more individuals in Bt maize. In one location, however, they found more fungal feeders in the Bt corn while in non-Bt corn more bacterial feeders were found. In contrast, in one other study a reduction of nematode numbers under Bt maize was observed (CAUL et al. 2004). Another study comparing Bt versus non-Bt maize expressing the Cry1Ab toxin found differences in nematode numbers, with a smaller population under the Bt maize (GRIFFITHS et al. 2005b, submitted). Generally, effects on microfaunal communities due to Bt maize (Cry1Ab) seem to be small and less than due to effects of different cultivars and crops (GRIFFITHS et al. 2005a, 2005b, both submitted).

Effects on collembola

The preliminary results of the EU-funded project ECOGEN (www.ecogen.dk) also seem to confirm previous indications that effects of Bt maize containing the Cry1Ab toxin are negligible to soil collembola (HECKMANN et al., 2005 submitted).

Bt toxin uptake by higher plants

The concern about Bt toxin uptake by conventional, non genetically modified plants when grown in soil previously planted with Bt crops could so far not be confirmed. Several non-

Bt plants were shown not to take up Bt-toxins released via Bt biomass or root exudates into the soil (SAXENA & STOTZKY 2002).

Summary: Effects on soil organisms and processes

Previous results regarding **Bt toxin release** via root exudates were confirmed by SAXENA et al. (2002, 2004) showing that Bt toxin release and toxicity is dependent on the Bt plant species. In contrast, the concern of **Bt toxin uptake** by non-Bt plants from the soil previously planted with Bt maize plants could not be confirmed (SAXENA & STOTZKY 2002).

Estimates on the decomposition, persistence and larvicidal activity of Cry toxins in the studies cited here are to some extent controversial. The earlier studies which showed that the Bt toxin **persists in soil and retains its larvicidal activity** especially when bound on surface-active soil particles thus being resistant to degradation by micro-organisms were confirmed by studies of SAXENA & STOTZKY (2002), LEE et al. (2003), ZWAHLEN et al. (2003a, 2003b) and BAUMGARTE & TEBBE (2005) while others (HOPKINS & GREGORICH 2003, DUBELMAN et al. 2005) do not confirm such prolonged persistence of Cry toxins and report that Bt toxins are rapidly degraded and non-bioactive in the field soon after harvest. One possible explanation for the inconsistent results in decomposition rates and persistence between authors could be that estimates on the persistence and larvicidal activity vary depending on the Bt biomass used. Apparently the roots of the maize plant are a much larger reservoir for the Bt toxin which is better protected from degradation than the leaves. HOPKINS & GREGORICH (2003) used only Bt leaves in their experiments and persistence of the Bt toxin in the leaves is much lower than that of the roots as confirmed by BAUMGARTE & TEBBE (2005). Also different methodological approaches may account for the differences observed. The degree of physical pre-destruction prior to the microbial degradation process may explain some of the contrasting results. Ground leaf samples decompose faster than leaf litter cut into centimetre large pieces as smaller particles provide a larger surface for microbial colonisation and thus degradation. Correspondingly, the Bt toxin will be degraded faster as well. Also leaves on top of the soil surface decomposed faster than when incorporated into the soil (ZWAHLEN et al. 2003a). Differences in decomposition of Bt toxins may be also due to different environmental conditions. Temperature is an important determinant of biological degradation activity and therefore will influence degradation of Cry toxins under natural conditions. ZWAHLEN et al. (2003b) clearly showed that higher temperatures in the laboratory led to faster microbial degradation than temperatures under field conditions which may also explain the much shorter decomposition time reported by HOPKINS & GREGORICH (2004). Their incubation of leaf litter at 20°C in the laboratory resulted in no detection of Cry toxin after 14 days while the average soil temperature in the field trials of ZWAHLEN et al. (2003a, 2003b) was 8,5 °C ranging from 1-19 °C depending on the season. The degradation of proteins in soils and therefore the insecticidal activity depends also largely on the availability of free and bound toxins to microbes as sources of carbon and nitrogen. Therefore the rate of decay of these toxins is varying between free and bound toxins (STOTZKY 2004). There is some evidence that the extent of clay in a soil determines the ability of the Cry toxins to persist in that specific soil and to have a higher larvicidal activity at least for short time periods (40 days) with high clay soils apparently leading to higher toxin absorption (SAXENA & STOTZKY 2002). Moreover, no indication for a saturation effect of Bt toxin soil adsorption has been reported (PAGEL-WIEDER et al. 2004, LEE et al. 2003).

Reports on the **decomposition rate of Bt maize compared to non-Bt maize** largely confirm the previous results provided by STOTZKY (2000, 2002). Decomposition of Bt maize plants seems to be significantly lower than decomposition of non-Bt maize plants even for different Bt corn hybrids and different Bt plant species (FLORES et al. 2005, DINEL et al. 2003, POERSCHMANN et al. 2005) although some authors do not confirm this slower decomposition (HOPKINS & GREGORICH 2003). The reason for the slower decomposition rate of Bt maize is still unknown. STOTZKY (2004) argues that neither differences in C:N ratios of the biomass nor changes or inhibition of the soil microbiota

could explain the effect observed. There is evidence that different Bt plants and especially Bt corn, even different transformation events, have consistently higher lignin contents in the stems than non-Bt maize, as determined by different methods and different authors (see references in STOTZKY 2004) although some authors do not find any consistent effects of the Bt trait on lignification (JUNG & SHEAFFER 2004). However, it is so far unclear if this higher lignin content is mainly related to the stems or to all parts of the plant. There is some evidence that lignin content is even lower in Bt maize leaves which would also explain the 2-5% faster decomposition of Bt maize leaves compared to the isogenic maize line (ESCHER 2000). POERSCHMANN et al. (2005) showed that the G-type lignin was mainly responsible for the higher lignin content of Bt maize stems. This structural component is assumed to be more resistant to chemical and biological breakdown than other lignin-types. The conclusions of DINEL et al. (2003) are that Bt maize significantly increases the ratio of saturated to unsaturated lipids in soils thus affecting microbial activity.

The results of the examination of effects of Bt biomass on **microbial communities** depend highly on the method used. Bacterial culturing techniques such as bacterial plate counts do not result in any measurable effect of Cry toxins on the soil microbial community (MUCHAONYERWA et al. 2004, BRUSETTI et al. 2004) confirming earlier results of SAXENA & STOTZKY (2001). The analysis of culturable bacteria seems to be less sensitive to small shifts within a bacterial community than molecular fingerprinting methods based on total soil DNA (BRUSETTI et al. 2004, STOTZKY 2004). However, the use of molecular fingerprinting methods such as rRNA sequence analysis as used by BRUSETTI et al. (2004) and BAUMGARTE & TEBBE (2005) were able to detect only slight differences in bacterial communities when exposed to the Cry1Ab toxin. Community-level physiological profiling (CLPP) as done by BLACKWOOD & BUYER (2004) showed significant effects on the bacterial community only for the high clay soil type. However, CLPP seems to be limited by its reliance on bacterial cultivation and has been shown to be less sensitive than PCR fingerprinting in distinguishing between microbial communities associated with different plant genotypes (see BRUINSMA et al. 2003 and references therein). As BRUINSMA et al. (2003) note, the most striking conclusion on the research of effects of the Cry toxin on soil micro-organisms is the wide variety of parameters studied and techniques employed. Most of the studies have been able to detect some sort of effect but the wide variety of techniques used makes a comparison of results difficult.

Negative effects of Bt maize on **mycorrhizal fungi** have been confirmed by one study (TURRINI et al. 2004) while indications of adverse effects of Bt toxins in soil on higher soil organisms are rare. Two studies which evaluated effects of Bt toxins on **earthworms** (ZWAHLEN et al. 2003b, VERCESI et al., in press) find no consistent effects but some indications for adverse effects are given. However, further studies are needed to exclude the possibility of long-term adverse effects of the Cry1Ab toxin on earthworms. Effects of Bt toxins on **isopods** were examined in three studies (ESCHER et al. 2000, WANDELER et al. 2002, PONT & NENTWIG 2005). Reduced consumption of Bt leaves was observed for both Bt11 and Bt176 although it is unclear whether this effect is explained by differences in plant quality or direct toxic effects. There are also indications of less sufficient digestion of Bt11 leaves and it has been shown that the Bt toxin is still contained in faeces of isopods and is still insecticidal thus representing another source of exposition to other non-target soil organisms which do not themselves ingest the Bt maize plant or parts of it. Regarding effects of Bt maize on the **nematofauna** and **collembola** no clear conclusions can be made. No consistent negative effects have so far been reported on soil nematofauna although some differences in nematode density or certain feeding groups of nematodes between Bt and non-Bt maize fields were reported (MANACHINI & LOZZIA 2003, CAUL et al. 2004, GRIFFITHS et al. 2005b, submitted). Correspondingly, preliminary results of Bt maize impacts on collembola showed no negative effects so far.

Bt maize containing other than the Cry1Ab toxin

Effects on non-target Lepidoptera

Reports on ecological effects of other than Cry1Ab toxin are still rare. Effects of Cry toxins other than Cry1Ab on non-target Lepidoptera were studied by HELLMICH et al. (2001) and MATTILA et al. (2005).

The relative toxicity of Bt pollen with the Cry1Ac toxin (hybrid DBT418) and the Cry1F toxin (hybrid Tc1507) to monarch larvae was evaluated by HELLMICH et al. (2001) in laboratory bioassays. Using the purified toxin for 1st instar monarch larvae it was shown that both Bt toxins were less toxic than those of Cry1Ab. Concentrations that produce 50% mortality (LC50) for the Cry1Ac toxin were approximately 10fold compared to those of Cry1Ab although the EC50 (concentration that produces 50% growth inhibition) was similar to that of Cry1Ab for the first instar monarch larvae. For Cry1F toxin the EC50 was approximately 5000fold the EC50 of the Cry1Ab toxin, even the highest concentration tested did not produce any mortality of the larvae. Both events showed no significant effects on the weight of the larvae which consumed pollen for 4 days (HELLMICH et al. 2001).

MATTILA et al. (2005) used the stacked hybrid MON810 x MON48006 (Cry1Ab x Cry2Ab2) as well as MON863 (Cry3Bb1) to feed first instar monarch larvae with Bt-pollen dusted milkweed leaves for 4 days in laboratory bioassays. They found that pollen of the stacked event had significant lethal and sublethal effects on monarch larvae while such an effect was not observed for the Cry3Bb1 toxin containing hybrid. These adverse effects translated into reduced survival at high pollen densities (3200 grains/cm²), reduced weight gain after 10 days as well as reduced consumption after 2 and 4 days (up to 29% less) compared to the MON863 hybrid. Although pollen density had no significant effect on leaf consumption, consumption was highest for larvae feeding on the non-pollen control and lowest for larvae feeding 3200 grains/cm², the highest pollen density tested. In contrast, when using the hybrid MON863 they found no adverse effects of Bt pollen regarding the parameters measured, even at the highest density tested.

Effects on pollinators

In feeding studies HANLEY et al. (2003) evaluated the effect of Bt maize TC1507 containing the Cry1F toxin in pollen on worker larvae of the honey bee. They also evaluated if Bt corn pollen could be used to control the greater wax moth larvae, an important pest of honey bees attacking beeswax combs in weak or dead colonies or combs placed in storage. No differences in larval mortality, pupal mortality or pupal weight between TC1507 Bt pollen fed and non-Bt pollen fed worker larvae was observed. However, in their feeding trials with the wax moth the mortality of larvae was significantly higher for Cry1F pollen fed larvae than for non Bt pollen fed larvae thus indicating the susceptibility of the toxin to lepidopteran larvae but not to honey bee worker larvae.

Effects on other non-target organisms

Effects on other non-target organisms were reported by AVILLA et al. (2005) who used different Cry toxins (Cry1Ab, Cry1Ac, Cry2Ab) in artificial diets in order to feed neonate lepidopteran larvae of *Helicoverpa armigera* which were subsequently fed to the predator *Chrysoperla carnea* and found no effects on larval mortality, development or adult emergence or fecundity of *Chrysoperla carnea*. RODRIGO-SIMON & FERRE (2005) suggest that the binding of the Cry-protein to the epithelial cells of the midgut is necessary for a toxic action and performed binding experiments by feeding *Chrysoperla carnea* and *Helicoverpa armigera* with concentrated purified Cry1Ac toxin. While specific binding and cell damage in epithelial midgut cells of the susceptible target species *Helicoverpa armigera* was found, no such effect was found for *Chrysoperla carnea*. Effects of the Cry1F toxin on non-target species (herbivores, predators, parasitoids) are so far still lacking. Regarding non-target effects of the Cry3Bb1 toxin, several studies are

available (AHMAD et al. 2005, AL-DEEB & WILDE 2003, BHATTI et al. 2005a, BHATTI et al. 2005b, BITZER et al. 2005, CARTER et al. 2004, DUAN et al. 2002, LUNDGREN & WIEDENMANN 2002).

Both AHMAD et al. (2005) and AL-DEEB & WILDE (2003) monitored in field experiments ground and foliage dwelling non-target arthropods in Bt maize containing the Cry3Bb1 toxin. While AHMAD et al. (2005) found generally no significant differences in the numbers of arthropods between Bt and non-Bt fields, some significant effects were found for some groups and years (e.g. 2002: significantly more spiders in non-Bt fields than other treatments). The visual inspections of AL-DEEB & WILDE (2003) showed significant effects on abundance of two species, a coccinellid *Hippodamia convergens* and a bug nymph *Orius insidiosus*, with significantly more individuals of adult *Hippodamia convergens* in Bt maize than in non-Bt maize in one location in one year and significantly less nymphs of *Orius insidiosus* in Bt than in non-Bt fields in the other year, but not consistently over both years. Pitfall trapping showed significant differences for Elateridae and Formicidae in Bt fields but only in single locations in different years and therefore not consistently.

BHATTI et al. (2005a, 2005b) evaluated the effect of Bt maize MON 863 on foliage and ground dwelling arthropods in field experiments over three years comparing Bt maize with the isogenic line with either no insecticide treatment or the application of a seed insecticide before planting or a soil insecticide at planting or a foliar insecticide during the growth of the corn. Regarding the ground dwelling arthropods they found significant effects of the Bt treatment only for the target organism *Diabrotica virgifera virgifera* which was lower in abundance in the Bt maize plots and for one dipluran family which were significantly less in abundance in the Bt plots in two of the three years when sampled by pan traps. Also Chilopoda sampled by pitfall trapping were more abundant in non-Bt fields than in Bt fields in all three years but this effect was significant in one year only. However, when using pan traps Chilopoda abundance was slightly higher in Bt fields and significantly more abundant in Bt fields with seed treatment compared to the non-Bt field with seed treatment in all three years. No differences between Bt and non-Bt plots was observed for Carabidae, Staphylinidae, Araneae and other taxa. Significant effects of insecticides were observed for some groups and some years. Regarding the foliage dwelling arthropods sampled by sticky traps the target pest species *Diabrotica virgifera virgifera* and the corn flea beetle *Chaetocnema pulicaria* were significantly less abundant in Bt maize compared to non-Bt maize. For the aphid *Rhopalosiphum maidis* no difference in abundance between Bt and non-Bt plots was observed. When considering predator species significant differences between Bt and non-Bt maize were observed for the coccinellid *Coleomegilla maculata* which were significantly more abundant in non-Bt maize in one year. This effect was not significant in the other two years although a tendency for reduced abundances in Bt maize was also given. One hymenoptera species, the braconid wasp *Macrocentris cingulum*, was significantly less abundant in Bt maize in one year but more abundant in Bt maize in another year. For other taxa of the Coccinellidae, Hemiptera, Neuroptera (*Chrysoperla carnea*), syrphid flies or Araneae no differences between Bt and non-Bt plots could be observed. Effects of the foliar applied insecticide were found for the pest species *Diabrotica virgifera virgifera* and *Chaetocnema pulicaria* and the predators *Coleomegilla maculata*, some other coccinellids and some hemipteran species. Aphids were more abundant in sprayed fields and there was also a trend for Nabidae (Hemiptera) to be slightly more abundant in insecticide treated fields.

Adverse effects of Cry3Bb1 on the coccinellid *Coleomegilla maculata* were studied by DUAN et al. (2002) and LUNDGREN & WIEDENMANN (2002). DUAN et al. (2002) used laboratory bioassays in order to test the effect of Cry3Bb1 pollen on larvae and adult ladybird beetle *Coleomegilla maculata*. They observed no differences in the percentage survival to adults, days to pupation, days to adult emergence or adult mass between Bt, non-Bt or bee pollen fed larvae. Also adult ladybird beetles showed no difference in percentage survival at the end of the trial when fed either Bt or non-Bt pollen, however adult beetles fed a bee-pollen diet had a significantly higher survival rate. No differences in reproductive parameters, as measured by the number of eggs laid per female, could be observed. However, although the authors measured the Cry-concentration of the pollen

over the experiment, the final concentration of the Cry toxin in the diets used was not measured. This and the fact that the range of Cry toxin concentration in the pollen was highly variable (37-101 µg/g fresh weight at the start of the trial and 49-135 µg/g fresh weight when pollen was stored in freezer) gives no indications on the effective exposure of the ladybird beetle larvae and adults to the Cry-toxin.

Also LUNDGREN & WIEDENMANN (2002) evaluated effects of Cry3Bb1 pollen on the pollen-feeding coleopteran predator *Coleomegilla maculata*. As reported by Duan et al. (2002) no effects of any Bt pollen concentration on developmental parameter (larval duration) of 1st-4th instar larvae as well as no effects on survival, weight of pupae or pupal duration and reproductive parameters of adult beetles were found. Strangely, they did not detect the toxin in adult beetles and also not in larvae fed transgenic pollen. They speculate that the toxin is not activated or does not bind to the gut, therefore having a short gut retention period or the toxin may be altered in the gut such that it could not be detected by the assay. However, this might also be due to the fact that they tested the body but not the foregut of the beetles. HARWOOD et al. (2005) have shown that the Cry toxin was only present in the foregut of coccinellid species tested but not in the body itself.

In contrast, the results of the EU funded project Bt-BioNoTa (SCHMIDT et al. 2004) show that the Cry3Bb1 toxin, when contained in a transgenic aubergine, resulted in a significantly higher mortality of the coccinellid *Adalia bipunctata* at a concentration of 25µg/ml of the activated toxin compared to the non-Bt isolate. Although developmental time for this species increased with higher concentrations this effect was not significant. In their tritrophic feeding assays with *Stethorus punctillum* (Coleoptera: Coccinellidae) feeding on the mite *Tetranychus urticae* (Acarina: Tetranychidae) which were reared on either Bt or non-Bt aubergine plants the same authors report no significant effect on mortality or developmental time, although mortality was slightly higher in the Bt group. CARTER et al. (2004) found no adverse effect of Cry3Bb1 toxin containing maize on the mite *Rhizoglyphus robini* (Acari: Acaridae). In laboratory studies as well as in field surveys they found no significant differences between Bt and non-Bt maize plants. In the laboratory assay the mites even preferred Bt corn roots over non-Bt corn roots although it is unclear if this could be due to the two different fungicides that were used for seed treatment in the non-Bt control plants compared to Bt maize plants or due to slight differences of the maize lines used.

In Germany a project is currently aiming at the evaluation of potential environmental impacts of corn rootworm resistant maize (EBER & SCHUPHAN 2005, presented at the EIGMO meeting, June 2005 in Lleida, Spain). Above and below soil surface insects as well as the environmental fate of this kind of Bt toxin in the soil will be evaluated. The work is funded for 2005 until 2008 from the German Ministry of Research with 10 research groups involved across Germany. Also BABENDREIER et al. (2005, EIGMO meeting) are currently evaluating non-target effects of Western Corn Rootworm resistant maize varieties.

Effects on soil organisms and processes

Regarding effects of other toxins than the Cry1Ab toxin on soil organisms and processes five studies used the Cry3Bb1 toxin (AL-DEEB et al. 2003, AHMAD et al. 2005, BITZER et al. 2005, DEVARE et al. 2004 and possibly CHEVALLIER et al. 2003), while the Cry1F toxin was used in one study only (HERMAN et al. 2002a) and the binary insecticidal toxins Cry34Ab1 and Cry35Ab1 were also used in another study of HERMAN et al. (2002b).

CHEVALLIER et al. (2003) used Bovine Serum Albumin (BSA) protein and a coleopteran specific Cry toxin, possibly Cry3Bb1, to study the distribution between the solution and the surface of clay particles from soils and to investigate its microbial biodegradation. They found that more than 90% of the proteins were irreversibly bound to the clay particles and did not desorb which they explain by the presence of organic matter in the clay fraction. The higher the clay concentration was the lower was the percentage of free protein. The proteins were microbially used but microbial growth decreased as clay

concentration increased which the authors explain by the increasing unavailability of the clay-adsorbed proteins to microorganisms.

DEVARE et al. (2004) evaluated corn rootworm resistant Bt maize (Cry3Bb1) for effects on the microbial community and found no effect of the Bt corn on either microbial biomass, microbial activity as measured by nitrogen-mineralization, short-term nitrification and soil respiration and bacterial community analysis. Differences in bacterial community were only shown between years but not between treatments.

AL-DEEB et al. (2003) examined soil microarthropods (mites and collembola) and nematoda (phytoparasitic, fungivorous, bacteriovorous, omnivorous) in different locations and of different Bt events (MON853, MON863, MON862) and different treatments (Bt, Bt with seed treatment, isoline, isoline with seed treatment, isoline with insecticide) in different combinations. They found no consistent effects of the abundance of soil microarthropods in the Bt plots although some statistically significant effects were evident. However, from this study it is not clear which plant varieties were used (e.g. RX670) and treatments often consisted of only a few rows (e.g. Manhattan: 6 rows 9,14m long) which might be too small to detect effects. Additionally, the statistical significances given do not seem to be reasonable in some cases as the abundance of some species was twice as high in the Bt treatment compared to that of the isoline (e.g. nematode population of *Helicotylenchus*, 2000, Table 3) with a low standard deviation or even no standard deviation is given (Table 5).

AHMAD et al. (2005) analysed soil microarthropods (acarina and collembolans) from Bt (Cry3Bb1) and non-Bt maize fields. Numbers of soil mites were significantly higher in Bt maize plots with seed treatment compared to all other treatments and numbers of one collembolan family (Isotomidae) was significantly lower in non-Bt maize with seed treatment compared to other treatments in one year and one location. In this location and year no Bt toxin was detected by ELISA (detection limit 1ng/ml). However, extraction efficiency of Bt toxin in this location was very low, especially for low amounts of Bt toxin (mean 25% for 5ng/g). In contrast, in the location and year where the toxin was detected from soil samples (3,4 to 6,9 ng/g dry soil) no differences were detected in the number of soil mites or Collembola. However, on this location Roundup Ready Bt maize and Roundup Ready non-Bt maize were used and no non-transgenic maize was used as control. Bt toxin concentration in this location was different between sampling times being highest in the early season (6,9 ng/g soil) and lowest in the late season (3,4 ng/g soil). Only sampling near the maize plants gave detectable results, while sampling between rows did not result in detectable amounts of the Bt toxin.

Another recent study evaluated the effects of MON863 on springtails in field experiments at two sites over several years (BITZER et al. 2005). Abundance of both soil surface or epedaphic and subsurface or euedaphic springtails was principally not different between Bt and non-Bt plots in both locations and all years although in both cases one species of epedaphic collembolan was more abundant in non-Bt fields in one location and year and another species was more abundant in non-Bt fields in one location and year. Community structure and diversity was also not affected by the Bt treatment for both collembolan groups in both locations and years. In contrast, epedaphic collembola were consistently higher in soil insecticide treated fields than in seed treated fields and showed increased abundance in some years for some species for soil insecticides. Similarly, the euedaphic species were more abundant in soil insecticide treatments in one year. The authors conclude that effects of the Bt corn on collembolan are probably small due to the fact that this management type of corn does not affect springtail predators.

HERMAN et al. (2002a) examined the growth inhibition of *Heliothis virescens* neonate larvae in bioassays when fed the Cry1F toxin from a recombinant bacterial strain by feeding a soil suspension added to the insect diet at five different concentrations. Growth inhibition of the larvae declined consistently with time. Time until 50% of decay (DT50) and 90% of decay (DT90) of the toxin occurred was 0,6 days and 6,9 days, respectively. Less than 4% of the initial activity was still present after 28 days. Similarly, the one study that examined the binary insecticidal Crystal proteins Cry34Ab1/Cry35Ab1 was conducted by the same authors (HERMAN et al. 2002b). They used the microbially produced binary insecticidal crystal proteins and found less than 5% mortality for highest concentration used at day 0. They established several models for the degradation of this

protein and calculated 1,7-2,4 days until 50% of decay of these toxins depending on the degradation model used.

Summary: Effects of Bt maize containing other than the Cry1Ab toxin

Effects of other toxins than the Cry1Ab toxin on **non-target Lepidoptera** have been studied for the toxins Cry1F, Cry1Ac, Cry3Bb1 and one stacked hybrid containing the Cry1Ab and the Cry2Ab2 toxin. In both laboratory feeding studies (HELLMICH et al. 2001, MATTILA et al. 2005) first instar monarch larvae were used as younger larvae generally show a higher sensitivity towards Cry toxins. It was shown that the Cry1Ac and the Cry1F toxins were less toxic to the larvae than the Cry1Ab toxin. Mainly the Cry1F toxin did not result in any lethal or sublethal effects even when fed in high concentrations to the larvae. This was also observed for the Cry3Bb1 toxin which showed no adverse effects on the monarch larvae even when exposure to this toxin was high.

In total eight studies were found considering effects of other toxins than the Cry1Ab toxin on **non-target organisms** other than non-target Lepidoptera (AHMAD et al. 2005, AL-DEEB & WILDE 2003, BHATTI et al. 2005a, BHATTI et al. 2005b, BITZER et al. 2005, CARTER et al. 2004, DUAN et al. 2002, LUNDGREN & WIEDENMANN 2002) which consider only the Cry3Bb1 toxin. Laboratory studies evaluating effects of the Cry3Bb1 toxin on coccinellids showed generally no lethal or sublethal effects of the Cry3Bb1 toxin on this predator species. However, in some studies the effective exposure of the beetles to the toxin is unclear. In contrast, the same toxin showed adverse effects when expressed in aubergine plants resulting in higher mortalities when fed directly to the coccinellid but not in tritrophic assays. Also no adverse effects were reported for spider mites when fed with Cry3Bb1 toxins. Studies which evaluated the abundance of foliar and ground dwelling arthropods under field conditions report no consistent effects of the Cry3Bb1 toxin between Bt (MON863) and non-Bt maize. Although two pest species were negatively affected by the Bt maize, abundances of arthropod predators were not affected or the significant effects were restricted to some predator groups or years. In some cases the effects were significant but contrasting in different years (e.g. braconids in BHATTI et al. 2005b) or when different sampling methods were used (e.g. Chilopoda in BHATTI et al. 2005a). Consistent effects over years and locations of the Bt treatment for the sampled arthropod groups were not reported while they were obvious for some insecticidal treatments. However, trends or significant effects were found in one study on the predator *Coleomegilla maculata* and one dipluran family which were both less abundant in Bt maize compared to the non-Bt isolate.

Effects of other toxins than Cry1Ab on **soil organisms and processes** were studied by some authors for the Cry3Bb1 toxin while only one study is available considering the Cry1F toxin and the binary toxins Cry34Ab1/Cry35Ab1, respectively. It has been shown that in the soil the majority of Cry3Bb1 toxins bind to clay particles and do not desorb depending on the clay concentration of the soil type and thus being protected from microbial degradation, similarly to the Cry1Ab toxin. No effects of the Cry3Bb1 toxin either on microbial biomass and activity or on soil microarthropods and nematodes could be shown so far, although some methodological flaws of these studies are evident such as inaccurate description of plant varieties used, use of herbicide tolerant maize as control, few maize rows per treatment or no indication of standard deviations and irreproducible statistical evaluation (see e.g. AHMAD et al. 2005, AL-DEEB et al. 2003). A recent, more detailed study finds no significant and consistent effects of the Cry3Bb1 toxin on Collembola abundance, community structure and diversity while this was true for some insecticidal treatments. Studies on effects of the Cry1F toxin or the binary toxin Cry34Ab1/Cry35Ab1 on soil organisms and processes are still rare. Those studies using these Cry toxins report a very short time until 50% of decay of these toxins is observed (0,6-2,4 days).

Regarding non-target effects of other toxins than Cry1Ab toxins to **pollinators** only one study evaluated the effects of the Cry1F toxin on honey bee larvae, indicating no significant adverse effects on several life table parameters.

In conclusion, most studies of ecological effects of other toxins than the Cry1Ab toxin relate to the Cry3Bb1 toxin contained in the maize event MON863. Generally, there are no consistent adverse impacts of this event reported, either in laboratory or in field studies. However, some studies suffered from severe methodological problems and detailed evaluations of certain non-target groups are still lacking.

Effects of herbicide tolerant maize

Effects on weeds

The results of the British Farm Scale Evaluations reported larger weed abundance under herbicide tolerant management for herbicide tolerant fodder maize compared to conventional maize. In the herbicide tolerant maize fields more arable weeds were found than in conventional maize crops (HEARD et al. 2003), particularly biomass of dicotyledonous weeds and their seed-rain was higher. However, the conventionally managed maize fields were treated with atrazine, a herbicide that is being withdrawn from the approved lists of EU chemicals. Re-analysis of the data separating sites where atrazine was used in the conventional treatments lead to the prediction that weed abundance for atrazine applied pre-emergence in conventional crops was significantly less than of other conventional herbicide regimes where atrazine was used post-emergence, other triazines pre-emergence or non-triazines post-emergence (PERRY et al. 2004). Weed abundance in the few sites that were treated with non-triazines only was slightly but consistently greater than those where triazines were used. The comparison of the herbicide tolerant and conventional treatments (excluding atrazine as pre-emergence) showed that final plant density was still significantly higher in herbicide tolerant treatments than in other treatments (atrazine post-emergence or non-atrazines pre- or post-emergence). However, no significant differences were found for biomass, seed rain or seedbank change of weeds. The larger weed abundances previously reported for herbicide tolerant management were caused partly by the effectiveness of atrazine used pre-emergence. The favourable effect of the herbicide tolerant management compared to the conventional treatment was reduced by approximately 2/3 if pre-emergence atrazine sites were excluded from the analysis. The authors expect final weed numbers still to be larger in herbicide tolerant than in conventional maize with weed biomass of dicotyledonous weeds being larger and monocotyledonous weed biomass being smaller under herbicide tolerant than under conventional management.

PURICELLI & TUESCA (2005) evaluated the effect of exclusive applications (one application pre-planting, one application 40 days after planting) of glyphosate under two different tillage systems (no tillage and conventional) in different crop rotations of herbicide tolerant soybean and maize. They report that regular and continuous glyphosate application led to quantitative and qualitative changes in weed communities. While weed richness increased over years in post-application counts it decreased in pre-application counts under different crop rotations. PURICELLI & TUESCA (2005) observed a decrease in density of early emergence annual broad-leaved weeds, wind dispersed weeds as well as grassy annuals while the density of late emergence annual broad-leaved weeds increased over a time period of five years. The effects of the glyphosate application on species diversity were inconsistent. The authors conclude that the effect of glyphosate application was more important than crop sequence to explain weed community changes.

Another major issue that has been discussed with the use of herbicide tolerant plants is the occurrence of weeds which develop resistance to the herbicide applied, glyphosate or glufosinate. Currently seven species of weeds are reported to be resistant against herbicides such as glyphosate or glyphosate-trimesium, with the most frequent reports of resistance for horseweed (*Conyza canadensis*), which is termed to be one of the most important herbicide resistant weeds, just recently found to be resistant in the US state of California (www.weedscience.com). Glyphosate resistance was identified in horseweed three years after the adoption of glyphosate-resistant soybean (see reference in OWEN & ZELAYA 2005). Resistance has also been reported for goosegrass, *Eleusine indica*, and the rigid ryegrass, *Lolium rigidum*, which was the first weed to be found resistant in 1996. Additionally, resistance in common waterhemp, *Amaranthus tuberculatus*, has also been recently documented (OWEN & ZELAYA 2005). Beside these species with evolved resistance, at least seven species are naturally resistant to glyphosate without documented selection by the herbicide (OWEN & ZELAYA 2005, NANDULA et al. 2005).

Interestingly, as reported in OWEN & ZELAYA (2005) growers in the mid-western USA are experiencing problems when managing volunteer maize with glyphosate, even if glyphosate-resistant maize was not planted the previous year. Apparently the glyphosate resistance transgene has moved widely in pollen and resulted in volunteer maize with glyphosate resistance. Therefore growers now often include graminicides to control this maize weed problem. This is consistent with the field results of PURICELLI & TUESCA (2005) who predict higher abundances of herbicide tolerant weeds with regular and continual glyphosate application.

Other effects on plants (non-weeds)

BLACKBURN & BOUTIN (2003) showed significant effects of glyphosate treatment on seedling germination and growth or health characteristics of 19 species of 7 families of non-crop and crop plants. They conclude that spray drift and deposition of glyphosate on non-target plants could have serious ecological consequences due to reduced germination and consequently reduced abundance or competitive ability of that species in non-target drift zones.

Effects on the bacterial community

SCHMALENBERGER & TEBBE (2002) found no effects of the inserted herbicide tolerant trait (PAT gene) on the composition of the bacterial community in rhizospheres of herbicide tolerant maize (BASTA resistant). By the use of single-stranded community profiles of partial sequences of the 16S rRNA gene they showed that the plant species is more important in the selection of bacterial communities than other factors such as soil origin or agricultural treatment. However, from their study the amount of active ingredient of the herbicides applied on the maize plots it is unknown. In contrast to SCHMALENBERGER & TEBBE (2002) the results of SESSITSCH et al. (2004) detected some bands specifically in transgenic herbicide tolerant oilseed rape when using 16S rRNA and DGGE. At each plant growth stage they observed similar community profiles and plant growth stage had most impact on microbial populations. At late flowering and senescence the microflora was more affected by herbicide application than by the genetic transformation. When they used dot-blot hybridization they found higher amounts of rRNA in the rhizosphere of transgenic plants at the late flowering stage. The BASTA application (0,03mg/cm²) lead to increased abundances of high GC gram-positive bacteria and reduced low GC gram-positive bacteria. At the senescent stage of the plants more microbial biomass was registered in transgenic plants without BASTA application and the activity of several enzymes such as invertase, urease and alkaline phosphatase was significantly enhanced in transgenic plants without BASTA application. BASTA application significantly decreased enzyme activity. The authors suggest that altered root exudate composition and/or concentration of the transgenic plants could be responsible for the effects observed but toxicity of late breakdown product of BASTA can also not be excluded.

Effects on non-target arthropods

The result of the British Farm Scale Evaluations showed that herbicide management in herbicide tolerant maize had no effect on the total number of carabids, collembolan, staphylinid beetles or spiders (DEWAR et al. 2005) and had even positive effects on invertebrates, attributable to changes in weed abundance. More seed-eating carabids, Staphylinidae and some linyphiid spiders were observed. In August, more detritus-feeding collembola were found in herbicide tolerant maize, probably as a response to the greater quantity of food in the crops sprayed later with glyphosate or glufosinate ammonium. Some spiders (*Erigone* sp.) were more abundant in herbicide tolerant maize compared to conventionally managed maize. When using vortis samples more collembolan species were present in herbicide tolerant maize. Neither the abundance of bees nor of butterflies was significantly reduced in herbicide tolerant maize. Similarly to PERRY et al. (2004) who re-evaluated the Farm Scale Evaluations data of weeds, BROOKS et al. (2005) predict trends for invertebrates (Collembola, Carabidae, Staphylinidae, Araneae, earthworms) after withdrawal of triazines using similar methods as PERRY et al. (2004). The results can be summarized as follows:

- When atrazine was used pre-emergence, invertebrate abundance was usually decreased compared to herbicide tolerant crops.
- When atrazine (post-emergence), other triazines (pre-emergence) and non-triazines (post-emergence) were compared to invertebrate communities in herbicide tolerant maize fields a similar trend was observed. A significant difference between the two treatments (herbicide tolerant versus others) was observed for Collembolan families Isotomidae (sampled by pitfall traps) and Entomobryidae (sampled by Vortis) and Collembolan total numbers, for the carabid species *Loricera pilicornis* and *Harpalus rufipes* as well as for Araneae (*Erigone* agg., *Oedothorax* spp.) and earthworms, mirroring the results for weeds, although this decrease in abundance was not so strong as in the original evaluation of the FSE including atrazine pre-emergence. Although for most indicators a higher abundance was recorded in the herbicide tolerant crop for other indicators (Carabidae: *Bembidion* spp., *Trechus quadristriatus*, Araneae: *Oedothorax* spp.) abundance was reduced under herbicide tolerant management regime.
- The effect of atrazine alone applied pre-emergence on invertebrate abundance was similar to the effect of atrazines post-emergence and other triazines and non-triazines either pre- or post-emergence (e.g. some Collembolan families).
- For some species such as the carabid *Loricera pilicornis*, the atrazine treatment post-emergence had the strongest effect on abundance.
- There is no indication that non-triazine treatment differs from other treatments (other triazine pre-emergence or atrazine post-emergence).

In summary, excluding pre-emergence atrazines resulted in consistent reductions of the treatment effects similarly to those reported by PERRY et al. (2004), although the extent of these reductions for invertebrates is less than those reported for weeds.

Also ROSCA (2004) evaluated community similarity of epigeic and plant dwelling arthropods in herbicide resistant maize fields (NK603) and isogenic fields. Consistently with the results of the Farm Scale Evaluations the authors report on no significant difference in community similarity between these two types of treatments as evaluated by the use of the Sørensen index based on the number of species existing in each community.

HOUGH-GOLDSTEIN et al. (2004) evaluated the impact of varying weed communities on arthropod populations in herbicide resistant maize using different herbicide treatments: two post-emergence applications of glyphosate at different times (21, 31 and 41 days after planting plus 10 days later), a pre-emergence treatment with acetochlor plus atrazine and a weed-free treatment with acetochlor plus atrazine at planting plus glyphosate post-emergence as needed and no weed treatment. Weed biomass in late August in one year was highest in the pre-emergence treatment while it was lowest in the weed free treatment and the application of glyphosate at 41 days after planting plus 10 days later. They also showed that for these herbicide treatments corn yields were not significantly different compared to the weed-free treatment. Increased weediness clearly increased the densities of the most common carabid *Harpalus pensylvanicus* and *Gryllus* sp., two omnivorous species that are supposed to feed – at least in part – on weed seeds. The carabid *H. pensylvanicus* was also significantly more abundant in pre-emergence treatments than in the other treatments in two years. In contrast, Tiger beetles (Cicindelidae) were more abundant in plots with less vegetation. Wolf spiders (Lycosidae) were variable in their response with a short-term increase in abundance after glyphosate treatment. Other taxa such as Staphylinidae or Formicidae were not affected in their abundance by the different herbicide treatment.

Other effects

TERMORSHUIZEN & LOTZ (2002) predict that well-known side effects of herbicides on non herbicide-tolerant crops such as susceptibility to pests and diseases will also occur in herbicide-tolerant crops and review the literature regarding side effects of glyphosate and glufosinate ammonium on soil-borne pathogens in non herbicide tolerant crops. In

conclusion they hypothesize that the delay of the herbicide use in herbicide tolerant crops result in increased infections by root pathogens.

THOMAS et al. (2004) determined the effect of glyphosate on pollen viability and seed set in glyphosate-resistant corn GA21 and NK603 various growth stages. Pollen viability was reduced regardless of hybrid with glyphosate treatments applied at the V6 growth stage or later, as indicated by morphological alterations such as large vacuoles and lower starch accumulations. In contrast, no effects on kernel set or weight were found. Expression analysis of the NK603 hybrid at anthesis showed that the highest expression of EPSPS occurred in pollen with progressively less in other tissues.

Other studies regarding herbicide tolerant maize refer to the toxicology profile of glyphosate on entomopathogenic fungi (MORJAN et al. 2002) or non-target organisms such as amphibians or aquatic communities (RELYEA 2005a, 2005b, 2005c) or spiders (HAUGTHON et al. 2001a, 2001b). Moreover, distribution and metabolism of the herbicide glufosinate in cell cultures or in herbicide tolerant plants (RUHLAND et al. 2002, RUHLAND et al. 2004) has been studied. Other studies report on the environmental fate of glyphosate (MAMY et al. 2005) or leaching of glyphosate and its metabolites from agricultural fields (KJAER et al. 2005).

Summary: Effects of herbicide tolerant maize

Recent reports on ecological effects of herbicide tolerant maize are generally rare. The studies cited most frequently are related to the British Farm Scale Evaluations (FSE) which evaluated the ecological effects of herbicide tolerant maize on weeds and weed-related invertebrates over a variety of locations and several years. Due to heavy criticism the results of the Farm Scale Evaluations were re-analysed separating the fields in which the herbicide atrazine was used pre-emergence (PERRY et al. 2004, BROOKS et al. 2005). This re-analysis showed that the favourable effect of the herbicide tolerant maize fields which had previously shown a higher biodiversity in both weeds and arthropods was less prominent when the pre-emergence atrazine treatment in the comparison group was eliminated. Both weed and invertebrate abundance was significantly reduced by atrazine pre-emergence use. Moreover, the favourable effects of the herbicide tolerant treatments were only relevant for final plant density (weed abundance) but not for weed biomass, seed rain or seedbank change. The results of the British FSE are somehow contradicted by another study (PURICELLI & TUESCA 2005) which showed reduced richness and density especially for early emergence annual broad-leaved weeds and grassy annual weeds while only some later emergences annual weeds increased in density.

Other studies of herbicide tolerant maize mostly refer to the building up of resistance to the herbicides used in herbicide tolerant plants. Since the introduction of herbicide tolerant plants herbicide resistant weeds have developed and are also predicted to increase in abundance and frequency. The spread of volunteer herbicide tolerant maize has been reported and seems already to pose an agronomic problem under some agricultural conditions.

Other effects of herbicide tolerant maize relate mainly to direct toxicological effects of the herbicide such as the possible spray drift effects on non-weed plants or direct effects on non-target organisms and the environmental fate of these herbicides.

Sequence of Bt176 maize

Bt176 maize has been approved under Directive 90/220/EEC in 1997. Since it was placed on the market the sequence of this GM crop has been discussed controversially.

Corn line CG00526 (*Zea mays* L.) was transformed with two different constructs conferring resistance to European Corn Borer via expression of the Cry1Ab toxin (construct pCIB4431) and herbicides tolerance to glufosinate-ammonium via expression of the bar gene (construct pCIB3064). Both plasmids contain a copy of the bla gene, conferring resistance to ampicillin, under the control of a bacterial promoter.

A report of the Belgium Biosafety Advisory Council in 2003 (DE SCHRIJVER & MOENS 2003) summarised some of the discrepancies between the molecular data originally presented in the notification C/F/94/11-03 and the data provided by several scientific institutions namely CLO (Centrum Landbouwkundig Onderzoek, Melle, Belgium), JRC (Joint Research Center, Ispra, Italy) or INRA (Institut National de la Recherche Agronomique, Versailles, France)

There are still uncertainties about the copy number of the different inserts present in event Bt176. KOZIEL et al. (1993) indicated that there may be at least five copies of the cry1Ab genes present in Bt176 which is consistent with other sources. For the gene conferring herbicide resistance (bar) the original dossier indicates a copy number of at least two whereas a different study by INRA provides results which suggest the presence of at least four truncated inserts of the bar gene. This clearly correlates with a quantitative analysis of the 35S promoter which suggests that 4 copies of the p35S sequence are present in event Bt176. A more detailed study (CLO) of one of the bar cassettes revealed that beside the trait-specific DNA sequences of the pUC18-Vector adjacent to the 35S promoter were co-transferred into the maize genome. The literature search revealed no published studies in the scientific literature regarding changes of the sequence of Bt176 since its commercialisation.

Table 1: Comparison of molecular data from Dossier C/F/94/11-03 with data from other sources (DE SCHRIJVER & MOENS 2003)

	Dossier C/F/94/11-03	Findings by other sources (CLO, JRC, INRA)
<i>bar</i> -construct	At least 2 copies No information about 5' and 3' junctions	At least 4 copies pUC18 DNA at the 5' junction 35S-terminator is missing in at least one of the copies <i>bar</i> gene is followed by maize DNA at the 3'end
<i>Cry1Ab</i> construct	2 to 5 copies	At least 5 copies

References

- ABEL, C. A. & ADAMCZYK, J. J., JR. (2004): Relative concentration of Cry1A in maize leaves and cotton bolls with diverse chlorophyll content and corresponding larval development of fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) on maize whorl leaf profiles. *J Econ Entomol* 97: 1737-1744.
- ACCINELLI, C.; SCREPANTI, C.; VICARI, A. & CATIZONE, P. (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.; WILDE, G. E. & ZHU, K. Y. (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. & WILDE, G. E. (2003): Effect of Bt corn expressing the Cry3Bb1 toxin for corn rootworm control on aboveground nontarget arthropods. *Environmental Entomology* 32: 1164-1170.
- AL-DEEB, M. A.; WILDE, G. E.; BLAIR, J. M. & TODD, T. C. (2003): Effect of Bt corn for corn rootworm control on nontarget soil microarthropods and nematodes. *Environmental Entomology* 32: 859-865.
- ANDERSON, P. L.; HELLMICH, R. L.; PRASIFKA, J. R. & LEWIS, L. C. (2005): Effects on fitness and behaviour of monarch butterfly larvae exposed to a combination of cry1Ab expressing corn anthers and pollen. *Environmental Entomology* 34: 944-952.
- ANDERSON, P. L.; HELLMICH, R. L.; SEARS, M. K.; SUMERFORD, D. V. & LEWIS, L. C. (2004): Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. *Environmental Entomology* 33: 1109-1115.
- ARPAS, K.; TOTH, F. & KISS, J. (2004): Analysis of web content of *Theridion impressum* L. Koch (Araneae: Theridiidae) in BT (DK440BTY, MON810, Cry1Ab) and isogenic (DK440) maize. IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 23-29.
- AVILLA, C.; PEREZ-CASTILLO, S.; CAMUNEZ, S. & GONZALEZ-ZAMORA, J. E. (2005): Prey-mediated effects of B.t. toxins (cry1Ac, cry2Ab and cry1Ab) on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). EIGMO Meeting Abstracts IOBC/WPRS Working Group on GMOs in Integrated Plant Production, Meeting on Ecological Impact of GMOs, Lleida, Catalonia, Spain, June 1-3, 2005: 59.
- BABENDREIER, D.; KELLER, S.; KUHLMANN, U.; ROMEIS, J.; TÖPFER, S.; TURLINGS, T. & BIGLER, F. (2005): Biosafety research of transgenic *Diabrotica* resistant Bt-maize: Development and evaluation of monitoring methods. EIGMO Meeting Abstracts IOBC/WPRS Working Group on GMOs in Integrated Plant Production, Meeting on Ecological Impact of GMOs, Lleida, Catalonia, Spain, June 1-3, 2005: 77.
- BAUMGARTE, S. & TEBBE, C. C. (2005): Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. *Mol. Ecol.* 14: 2539-2551.
- BHATTI, M. A.; DUAN, J.; HEAD, G.; JIANG, CH.; MCKEE, M. J.; NICKSON, T. E.; PILCHER, C. L. & PILCHER, C. D. (2005a): Field Evaluation of the Impact of Corn

- Rootworm (Coleoptera: Chrysomelidae)-Protected *Bt* Corn on Ground-Dwelling Invertebrates. *Environmental Entomology* 34: 1325-1335.
- BHATTI, M. A.; DUAN, J.; HEAD, G.; JIANG, CH.; MCKEE, M. J.; NICKSON, T. E.; PILCHER, C. L. & PILCHER, C. D. (2005b): Field Evaluation of the Impact of Corn Rootworm (Coleoptera: Chrysomelidae)-Protected *Bt* Corn on Foliage-Dwelling Arthropods. *Environmental Entomology* 34: 1336-1345.
- BITZER, R. J.; RICE, M. E.; PILCHER, C. D.; PILCHER, C. L. & LAM, W. F. (2005): Biodiversity and Community Structure of Epedaphic and Euedaphic Springtails (Collembola) in Transgenic Rootworm *Bt* Corn. *Environmental Entomology* 34: 1346-1376.
- BLACKBURN, L. G. & BOUTIN, C. (2003): Subtle effects of herbicide use in the context of genetically modified crops: a case study with glyphosate (Roundup). *Ecotoxicology* 12: 271-285.
- BLACKWOOD, C. B. & BUYER, J. S. (2004): Soil microbial communities associated with Bt and non-Bt corn in three soils. *J Environ Qual.* 33: 832-836.
- BOURGUET, D.; CHAUFaux, J.; MICOUD, A.; DELOS, M.; NAIBO, B.; BOMBARDE, F.; MARQUE, G.; EYCHENNE, N. & PAGLIARI, C. (2002): *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). *Environ. Biosafety Res.* 1: 49-60.
- BRINK, A.; PRUTZ, G. & DETTNER, K. (2004): Bioassays on the effects of insect-resistant *Bacillus thuringiensis*-maize on the pupal hyperparasitoid *Tetrastichus howardi* (Hymenoptera: Eulophidae). [Original Title: Biotests zur Wirkung von insektenresistentem *Bacillus thuringiensis*-Mais auf den pupalen Hyperparasitoiden *Tetrastichus howardi* (Hymenoptera: Eulophidae)]. *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie* 14: 411-414.
- BROOKS, D. R.; CLARK, S. J.; PERRY, J. N.; BOHAN, D. A.; CHAMPION, G. T.; FIRBANK, L. G.; HAUGHTON, A. J.; HAWES, C.; HEARD, M. S. & WOIWOD, I. P. (2005): Invertebrate biodiversity in maize following withdrawal of triazine herbicides. *Proc. Biol. Sci.* 272: 1497-1502.
- BRUINSMA, M.; KOWALCHUK, G. A. & VAN VEEN, J. A. (2003): Effects of genetically modified plants on microbial communities and processes in soil. *Biol. Fertil. Soils* 37: 329-337.
- BRUSETTI, L.; FRANCA, P.; BERTOLINI, C.; PAGLIUCA, A.; BORIN, S.; SORLINI, C.; ABRUZZESE, A.; SACCHI, G.; VITI, C.; GIOVANNETTI, L.; GIUNTINI, E.; BAZZICALUPO, M. & DAFFONCHIO, D. (2004): Bacterial communities associated with the rhizosphere of transgenic Bt 176 maize (*Zea mays*) and its non transgenic counterpart. *Plant and Soil* 166: 11-21.
- CANDOLFI, M. P.; BROWN, K.; GRIMM, C.; REBER, B. & SCHMIDLI, H. (2004): A faunistic approach to assess potential side-effects of genetically modified Bt-corn on non-target arthropods under field conditions. *Biocontrol, Science and Technology* 14: 129-170.
- CARTER, M. E.; VILLANI, M. G.; ALLEE, L. L. & LOSEY, J. E. (2004): Absence of non-target effects of two *Bacillus thuringiensis* coleopteran active delta-endotoxins on the bulb mite, *Rhizoglyphus robini* (Claparede) (Acari, Acaridae). *Journal of Applied Entomology* 128: 56-63.

- CAUL, S.; CORTET, J.; GRIFFITHS, B. S.; THOMPSON, J.; HOLMSTRUP, M.; KROGH, P. H.; ANDERSEN, M. N. & MESSEAN, A. (2004): Effects of Bt maize on soil organisms: results of a two year study on three European sites. XIVth International Colloquium on Soil Zoology and Ecology, Session 7 poster contribution.
- CHEVALLIER, T.; MUCHAONYERWA, P. & CHENU, C. (2003): Microbial utilisation of two proteins adsorbed to a vertisol clay fraction: Toxin from *Bacillus thuringiensis* subsp. *tenebrionis* and bovine serum albumin. *Soil Biology and Biochemistry* 35: 1211-1218.
- CORTET, J.; ANDERSEN, M. N.; CAUL, S.; GRIFFITHS, B. S.; JOFFRE, R.; LACROIX, B.; SAUSSE, CH.; THOMPSON, J. & KROGH, P. H. (2005): Decomposition processes under Bt (*Bacillus thuringiensis*) maize: results of a multi-site experiment. *Soil Biology and Biochemistry* xx: xxx-xxx.
- COSTA, S. D.; BARBERCHECK, M. E. & KENNEDY, G. G. (2000): Sublethal acute and chronic exposure of Colorado potato beetle (Coleoptera: Chrysomelidae) to the delta-endotoxin of *Bacillus thuringiensis*. *J. Econ. Entomol.* 93: 680-689.
- DALY, T. & BUNTIN, D. G. (2005): Effect of *Bacillus thuringiensis* Transgenic Corn for Lepidopteran Control on Nontarget Arthropods. *Environmental Entomology* 34: 1292-1301.
- DE SCHRIJVER, A. & MOENS, W. (2003): Report on the molecular characterisation of the genetic map of event Bt176. Belgian Biosafety Advisory Council. Scientific Institute of Public Health. Division of Biosafety and Biotechnology (SBB) http://www.biosafety.be/TP/MGC_reports/Report_Bt176.pdf.
- DE LA POZA, M.; PONS, X.; FARINOS, G. P.; LOPEZ, C.; ORTEGO, F.; EIZAGUIRRE, M.; CASTANERA, P. & ALBAJES, R. (2005): Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain. *Crop Protection* 24: 677-684.
- DELRIO, G.; VERDINELLI, M. & SERRA, G. (2004): Monitoring of pest and beneficial insect populations in summer sown Bt maize. IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 43-48.
- DEVARE, M. H.; JONES, C. M. & THIES, J. E. (2004): Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity, and diversity. *Journal of Environmental Quality* 33: 837-843.
- DEWAR, A. J.; CHAMPION, G. T.; MAY, M. J. & PIDGEON, J. D. (2005): The UK farm scale evaluations of GM crops - a post script. *Outlooks on Pest Management* 16: 164-173.
- DINEL, H.; SCHNITZER, M.; SAHARINEN, M.; MELOCHE, F.; PARE, T.; DUMONTET, S.; LEMEE, L. & AMBLES, A. (2003): Extractable soil lipids and microbial activity as affected by Bt and non Bt maize grown on a silty clay loam soil. *Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes* B38: 211-219.
- DIVELY, G. P. & ROSE, R. (2002): Effects of Bt transgenic and conventional insecticide control on the non-target natural enemy community in sweet corn. 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii, USA, 14-18 June 2002 USDA Forest Service FHTET: 265-274.
- DIVELY, G. P.; ROSE, R.; SEARS, M. K.; HELLMICH, R. L.; STANLEY-HORN, D. E.; CALVIN, D. D.; RUSSO, J. M. & ANDERSON, P. L. (2004): Effects on Monarch Butterfly Larvae (Lepidoptera: Danaidae) after continuous exposure to cry1Ab expressing corn during anthesis. *Environmental Entomology* 33: 1116-1125.

- DUAN, J. J.; HEAD, G.; MCKEE, M. J.; NICKSON, T. E.; MARTIN, J. W. & SAYEGH, F. S. (2002): Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata*. *Entomologia Experimentalis et Applicata* 104: 271-280.
- DUBELMAN, S.; AYDEN, B. R.; BADER, B. M.; BROWN, C. R.; JIANG, CH. & VLACHOS, D. (2005): Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. *Environmental Entomology* 34: 915-921.
- DUTTON, A.; KLEIN, H.; ROMEIS, J. & BIGLER, F. (2002): Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecological Entomology* 27: 441-447.
- DUTTON, A.; ROMEIS, J. & BIGLER F. (2003a): Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt maize expressing cry1Ab as a case study. *Bio Control* 48: 611-636.
- DUTTON, A.; KLEIN, H.; ROMEIS, J. & BIGLER F. (2003b): Prey-mediated effects of B.t. spray on the predator *Chrysoperla carnea* in maize. *Biological Control* 26: 215
- DUTTON, A.; OBRIST, L.; D`ALESSANDRO, M.; DIENER, L.; MÜLLER, M.; ROMEIS, J. & BIGLER, F. (2004a): Tracking Bt-toxin in transgenic maize to assess the risks on non-target arthropods. IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 57-63.
- DUTTON, A.; D`ALESSANDRO, M.; ROMEIS, J. & BIGLER F. (2004b): Assessing expression of Bt-toxin (cry1Ab) in transgenic maize under different environmental conditions. IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 49-55.
- DUTTON, A.; ROMEIS, J. & BIGLER F. (2005): Effects of Bt maize expressing Cry1Ab and Bt spray on *Spodoptera littoralis*. *Entomologia Experimentalis et Applicata* 114: 161-169.
- EBER, S. & SCHUPHAN, I. (2005): Biosafety research of transgenic *Diabrotica* resistant Bt-maize: Development and evaluation of monitoring methods. EIGMO Meeting Abstracts IOBC/WPRS Working Group on GMOs in Integrated Plant Production, Meeting on Ecological Impact of GMOs, Lleida, Catalonia, Spain, June 1-3, 2005: 76
- ECKERT, J.; GATHMANN, A. & SCHUPHAN, I. (2004): Impact of growing Bt-maize on non target organisms: thrips and their antagonists. [Original Title: Auswirkungen des Anbaus von Bt-Mais auf Nichtzielorganismen: Thripse und ihre Gegenspieler.]. *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie* 14: 439-442.
- ESCHER, N.; KÄCH, B. & NENTWIG, W. (2000): Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber* (Crustacea: Isopoda). *Basic and Applied Ecology* 1: 161-169.
- FELKE, M.; LORENZ, N. & LANGENBRUCH, G. A. (2002): Laboratory studies on the effects of pollen from Bt-maize on larvae of some butterfly species. *Journal of Applied Entomology* 126: 320-325.
- FELKE, M. & LANGENBRUCH, G. A. (2005): Wirkung von Bt-Mais-Pollen auf Raupen des Tagpfauenauges im Laborversuch. *Gesunde Pflanzen* (xx): xxx-xxx.
- FLORES, S.; SAXENA, D. & STOTZKY, G. (2005): Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biology and Biochemistry* 1-10.

- FREIER, B.; SCHORLING, M.; TRAUGOTT, M.; JUEN, A. & VOLKMAR, CH. (2004): Results of a 4-year plant survey and pitfall trapping in Bt maize and conventional maize fields regarding the occurrence of selected arthropod taxa. IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 79-84.
- GRIFFITHS, B. S.; CAUL, S.; THOMPSON, J.; BIRCH, N. E.; SCRIMGEOUR, CH.; CORTET, J.; FOGGO, A. & KROGH, P. H. (2005a): Bt and non-Bt maize, with and without insecticide. Journal of Environmental Quality xxx: xxx-xxx.
- GRIFFITHS, B. S.; CAUL, S.; THOMPSON, J.; BIRCH, N. E.; SCRIMGEOUR, CH.; ANDERSEN, M. N.; CORTET, J.; MESSEAN, A.; SAUSSE, CH.; LACROIX, B. & KROGH, P. H. (2005b): A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* cry1Ab toxin. Plant and Soil xx: xxx-xxx.
- GUERRIERI, E.; LINGUA, G.; DIGILIO, M. C.; MASSA, N. & BERTA, G. (2004): Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? Ecological Entomology 29: 753-756.
- HANLEY, A. V.; HUANG, Z. Y. & PETT, W. L. (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. & OBRYCKI, J. J. (2000): Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. Oecologia 125: 241-248.
- HARWOOD, J. D.; WALLIN, W. G. & OBRYCKI, J. J. (2005): Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Mol.Ecol. 14: 2815-2823
- HAUGHTON, A. J.; BELL, J. R.; BOATMAN, N. D. & WILCOX, A. (2001a): The effect of the herbicide glyphosate on non-target spiders: Part II. Indirect effects on *Lepthyphantes tenuis* in field margins. Pest. Manag. Sci 57: 1037-1042.
- HAUGHTON, A. J.; BELL, J. R.; WILCOX, A. & BOATMAN, N. D. (2001b): The effect of the herbicide glyphosate on non-target spiders: Part I. Direct effects on *Lepthyphantes tenuis* under laboratory conditions. Pest. Manag. Sci 57: 1033-1036.
- HEAD, G.; BROWN, C. R.; GROTH, M. E. & DUAN, J. J. (2001): Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomologia Experimentalis et Applicata 99: 37-45.
- HEARD, M. S.; HAWES, C.; CHAMPION, G. T.; CLARK, S. J.; FIRBANK, L. G.; HAUGHTON, A. J.; PARISH, A. M.; PERRY, J. N.; ROTHERY, P.; ROY, D. B.; SCOTT, R. J.; SKELLERN, M. P.; SQUIRE, G. R. & HILL, M. O. (2003): Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. II. Effects on individual species. Philos. Trans. R. Soc. Lond B Biol Sci 358: 1833-1846.
- HECKMANN, L.-H.; GRIFFITHS, B. S.; CAUL, S.; THOMPSON, J.; PUSZTAI-CAREY, M.; MOAR, W. J.; ANDERSEN, M. N. & KROGH, P. H. (2005): Consequences for *Protaphorura armata* (Insecta: Collembola) following exposure to genetically modified *Bacillus thuringiensis* (Bt) maize and non-Bt maize. Environ Pollut xx: xxxx-xxxx.
- HELLMICH, R. L.; SIEGFRIED, B. D.; SEARS, M. K.; STANLEY-HORN, D. E.; DANIELS, M. J.; MATTILA, H. R.; SPENCER, T.; BIDNE, K. G. & LEWIS, L. C. (2001): Monarch

- larvae sensitivity to *Bacillus thuringiensis* - purified proteins and pollen. Proc. Natl. Acad. Sci U.S.A 98: 11925-11930.
- HERMAN, R. A.; WOLT, J. D. & HALLIDAY, W. R. (2002a): Rapid degradation of the Cry1F insecticidal crystal protein in soil. J. Agric. Food Chem. 50: 7076-7078.
- HERMAN, R. A.; SCHERER, P. N. & WOLT, J. D. (2002b): Rapid degradation of a binary, PS149B1, delta-Endotoxin of *Bacillus thuringiensis* in soil, and a novel mathematical model for fitting curve-linear decay. Environmental Entomology 31: 208-214.
- HILBECK, A.; BAUMGARTNER, M.; FRIED, P.M. & BIGLER, F. (1998a): Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27 (2), 480-487.
- HILBECK, A.; MOAR, W.J.; PUSZTAI-CAREY, M.; FILIPPINI, A. & BIGLER, F. (1998b): Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chysopidae). Environ. Entomol. 27 (5), 1255-1263.
- HILBECK, A.; MOAR, W.J.; PUSZTAI-CAREY, M.; FILIPPINI, A. & BIGLER, F. (1999): Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*. Entomologia Experimentalis et Applicata 91: 305-316.
- HOPKINS, D. W. & GREGORICH, E. G. (2003): Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize. European Journal of Soil Science 54: 793-800.
- HOUGH-GOLDSTEIN, J. A. & VANGESSEL, M. J. W. A. P. (2004): Manipulation of weed communities to enhance ground-dwelling arthropod populations in herbicide-resistant field corn. Environmental Entomology 33: 577-586.
- JASINSKI, J. B.; EISLEY, J.B.; YOUNG, C.E.; KOVACH, J. & WILLSON, H. (2003): Selected Nontarget Arthropod Abundance in Transgenic and Nontransgenic Field Crops in Ohio. Environmental Entomology 32: 407-411.
- JUNG, H. G. & SHEAFFER, C. C. (2004): Influence of Bt transgenes on cell wall lignification and digestibility of maize stover for silage. Crop Science 44: 1781-1789.
- KJAER, J.; OLSEN, P.; ULLUM, M. & GRANT, R. (2005): Leaching of glyphosate and amino-methylphosphonic acid from Danish agricultural field sites. J Environ Qual. 34: 608-620.
- KOZIEL, M.G.; BELAND, G.L.; BOWMAN, C.; CAROZZI, B.; CRENSHAWM, R.; CROSSLAND, L.; DAWSON, J.; DESAI, N.; HILL, M.; KADWELL, S.; LAUNIS, K.; LEWIS, K.; MADDOX, D.; MCPHERSON, K.; MMEGHJI, M.R.; MERLIN, E.; RHOES, R.; WARREN, G.W.; WRIGHT, M.; EVOLA, S.V (1993): Field Performance of Elite Transgenic Maize Plants Expressing an Insecticidal Protein Derived from *Bacillus thuringiensis*. Biotechnology 11, 194-200
- LADNER, D. T. & ALTIZER S. (2005): Oviposition preference and larval performance of North American monarch butterflies on four *Asclepias* species. Entomologia Experimentalis et Applicata 116: 9-20.
- LANG, A.; LUDY, C. & VOJTECH, E. (2004): Dispersion and deposition of Bt maize pollen in field margins. Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz 111: 417-428.

- LEE, L.; SAXENA, D. & STOTZKY, G. (2003): Activity of free and clay-bound insecticidal proteins from *Bacillus thuringiensis* subsp. *israelensis* against the mosquito *Culex pipiens*. Appl. Environ. Microbiol. 69: 4111-4115.
- LOPEZ, M. D.; PRASIFKA, J. R.; BRUCK, D. J. & LEWIS, L. C. (2005): Utility of Ground Beetle Species in Field Tests of Potential Nontarget Effects of Bt Crops. Environmental Entomology 34: 1317-1324.
- LOSEY, J. E., RAYOR, L. S. & CARTER, M. E. (1999): Transgenic pollen harms monarch larvae. Nature 399: 214.
- LUMBIERRES, B.; ALBAJES, R. & PONS, X. (2004): Transgenic Bt maize and *Rhopalosiphum padi* (Hom., Aphididae) performance. Ecological Entomology 29: 309-317.
- LUNDGREN, J. G. & WIEDENMANN, R. N. (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.
- MALONE, L. A.; TODD, J. H.; BURGESS, E. P. J. & PHILIP, B. A. (2005): Will GM crops expressing insecticidal proteins harm honey bees? Aspects of Applied Biology 74: 115-118.
- MAMY, L.; BARRIUSO, E. & GABRIELLE, B. (2005): Environmental fate of herbicides trifluralin, metazachlor, metamitron and sulcotrione compared with that of glyphosate, a substitute broad spectrum herbicide for different glyphosate-resistant crops. Pest. Manag. Sci 61: 905-916.
- MANACHINI, B. & LOZZIA, G. C. (2004): Studies on the effects of Bt corn expressing cry1Ab on two parasitoids of *Ostrinia nubilalis* Hb. (Lepidoptera: Crambidae). IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 109-116.
- MATTILA, H. R.; SEARS, M. K. & DUAN, J. J. (2005): Response of *Danaus plexippus* to pollen of two new Bt corn events via laboratory bioassay. Entomologia Experimentalis et Applicata 116: 31-41.
- MEIER, M. S. & HILBECK, A. (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.
- MEISSLE, M.; VOJTECH, E. & POPPY, G. M. (2003): Implications for the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) when developing in Bt maize-fed *Spodoptera littoralis* larvae (Lepidoptera: Noctuidae). IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 117-123.
- MEISSLE, M.; VOJTECH, E. & POPPY, G. M. (2005): Effects of Bt maize-fed prey on the generalist predator *Poecilus cupreus* L. (Coleoptera: Carabidae). Transgenic Res. 14: 123-132.
- MEISSLE, M. & LANG, A. (2005): Comparing methods to evaluate the effects of Bt maize and insecticide on spider assemblages. Agriculture, Ecosystems & Environment 107: 359-370.
- MINORSKY, P. V. (2001): The hot and the classic. Plant Physiol. 127: 709-710.
- MUCHAONYERWA, P.; WALADDE, S.; NYAMUGAFATA, P.; MPEPEREKI, S. & RISTORI, G. G. (2004): Persistence and impact on micro-organisms of *Bacillus thuringiensis*

- proteins in some Zimbabwean soils. Plant and Soil [Special Issue: Impact of genetically modified organisms] 266: 41-46.
- MUSSER, F. R. & SHELTON, A. M. (2003): Bt sweet corn and selective insecticides: impacts on pests and predators. J. Econ. Entomol. 96: 71-80.
- NANDULA, V. K.; REDDY, K. N.; DUKE, S. O. & POSTON, D. H. (2005): Glyphosate-resistant weeds: current status and future outlook. Outlooks on Pest Management 16: 183-187.
- OBERHAUSER, K. S.; PRYSBY, M. D.; MATTILA, H. R.; STANLEY-HORN, D. E.; SEARS, M. K.; DIVELY, G.; OLSON, E.; PLEASANTS, J.M.; LAM, W.-K. F. & HELLMICH, R. L. (2001): Temporal and spatial overlap between monarch larvae and corn pollen. Proceedings of the National Academy of Sciences 98: 11913-11918.
- OBRIST, L.; KLEIN, H.; DUTTON, A. & BIGLER F. (2005a): Effects of Bt maize on *Frankliniella tenuicornis* and exposure of thrips predators to prey-mediated Bt toxin. Entomologia Experimentalis et Applicata 115: 409-416.
- OBRIST, L.; DUTTON, A.; ALBAJES, R.; BIGLER F. (2005b): Exposure of arthropod predators to cry1Ab toxin in Bt maize fields in Spain. IOBC/WPRS Working Group on GMOs in Integrated Plant Production, Meeting on Ecological Impact of GMOs, Lleida, Catalonia, Spain, June 1-3, 2005, p 20.
- OWEN, M. D. & ZELAYA, I. A. (2005): Herbicide-resistant crops and weed resistance to herbicides. Pest. Manag. Sci 61: 301-311.
- PAGEL-WIEDER, S.; GESSLER, F.; NIEMEYER, J. & SCHRODER, D. (2004): Adsorption of the *Bacillus thuringiensis* toxin (CryI Ab) on Na-montmorillonite and on the clay fractions of different soils. Journal of Plant Nutrition and Soil Science 167: 184-188.
- PERRY, J. N.; FIRBANK, L. G.; CHAMPION, G. T.; CLARK, S. J.; HEARD, M. S.; MAY, M. J.; HAWES, C.; SQUIRE, G. R.; ROTHERY, P.; WOIWOD, I. P. & PIDGEON, J. D. (2004): Ban on triazine herbicides likely to reduce but not negate relative benefits of GMHT maize cropping. Nature 428: 313-316.
- PILCHER, C. D.; RICE, M. E. & OBRYCKI, J. J. (2005): Impact of Transgenic *Bacillus thuringiensis* Corn and Crop Phenology on Five Nontarget Arthropods. Environmental Entomology 34: 1302-1316.
- PLEASANTS, J. M.; HELLMICH, R. L.; DIVELY, G. P.; SEARS, M. K.; STANLEY-HORN, D. E.; MATTILA, H. R.; FOSTER, J. E.; CLARK, P. & JONES, G. D. (2001): Corn pollen deposition on milkweeds in and near cornfields. Proceedings of the National Academy of Sciences 98: 11919-11924.
- POERSCHMANN, J.; GATHMANN, A.; AUGUSTIN, J.; LANGER, U. & GORECKI, T. (2005): Molecular Composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize - characterization of lignin patterns. Journal Environmental Quality 34: 1508-1518.
- PONS, X. & STARY, P. (2003): Spring aphid-parasitoid (Hom., Aphididae, Hym., Braconidae) associations and interactions in a Mediterranean arable crop ecosystem, including Bt maize. Anzeiger für Schädlingskunde 76: 133-138.
- PONS, X.; LUMBIERRES, B.; LOPEZ, C. & ALBAJES, R. (2004): No effects of Bt maize on the development of *Orius majusculus*. IOBC/WPRS Bull 27: 131-136.

- PONS, X.; LUMBIERRES, B.; LOPEZ, C. & ALBAJES, R. (2005): Abundance of non-target pests in transgenic Bt-maize: A farm scale study. *European Journal of Entomology* 102: 73-79.
- PONT, B. & NENTWIG, W. (2005): Quantification of Bt-protein digestion and excretion by the primary decomposer *Porcellio scaber*, fed with two Bt-corn varieties. *Biocontrol Science and Technology* 15: 341-352.
- PRASIFKA, J. R.; HELLMICH, R. L.; DIVELY, G. P. & LEWIS, L. C. (2005): Assessing the Effects of Pest Management on Nontarget Arthropods: The Influence of Plot Size and Isolation. *Environmental Entomology* 34: 1181-1192.
- PRUETZ, G. & DETTNER, K. (2004): Effect of Bt corn leaf suspension on food consumption by *Chilo partellus* and life history parameters of its parasitoid *Cotesia flavipes* under laboratory conditions. *Entomologia Experimentalis et Applicata* 111: 179-187.
- PRUETZ, G.; BRINK, A. & DETTNER, K. (2004): Transgenic insect-resistant corn affects the fourth trophic level: effects of *Bacillus thuringiensis*-corn on the facultative hyperparasitoid *Tetrastichus howardi*. *Naturwissenschaften* 91: 451-454.
- PURICELLI, M. & TUESCA, D. (2005): Weed density and diversity under glyphosate-resistant crop sequences. *Crop Protection* 24: 533-542.
- RAHMAN, M. M.; ROBERTS, H. L. S. & SCHMIDT, O. (2004): The development of the endoparasitoid *Venturia canescens* in Bt-tolerant, immune induced larvae of the flour moth *Ephestia kuehniella*. *Journal of Invertebrate Pathology* 87: 129-131.
- RAMIREZ-ROMERO, R.; BERNAL, J. S.; CHAUF AUX, J.; GILLOGLY, P. & KAISER, L. (2004): Cry1Ab protoxin effects on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). *Commun. Agric. Appl. Biol. Sci.* 69: 183-184.
- RAPS, A.; KEHR, J.; GUGERLI, P.; MOAR, W. J.; BIGLER, F. & HILBECK, A. (2001): Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. *Mol. Ecol.* 10: 525-533.
- RAUSCHEN, S.; ECKERT, J.; GATHMANN, A. & SCHUPHAN, I. (2004): Impact of growing Bt-maize on cicadas: diversity, abundance and methods. *Bulletin OILB/SROP* 27: 137-142.
- RELYEA, R. A. (2005a): The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. *Arch. Environ. Contam. Toxicol.* 48: 351-357.
- RELYEA, R. A. (2005b): The lethal impact of Roundup on aquatic and terrestrial amphibians. *Ecological Applications* 15: 1118-1124.
- RELYEA, R. A. (2005c): The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications* 15: 618-627.
- RODRIGO-SIMON, A. & FERRE, J. (2005): A microscopic approach to determine the impact of B.t. cry proteins in non-target organisms: lack of cry1Ac binding to *Chrysoperla carnea* (Stephens) midgut epithelial cells. EIGMO Meeting Abstracts IOBC/WPRS Working Group on GMOs in Integrated Plant Production, Meeting on Ecological Impact of GMOs, Lleida, Catalonia, Spain, June 1-3, 2005: 58.

- ROMEIS, J.; DUTTON, A. & BIGLER, F. (2004): *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). J. Insect Physiol. 50: 175-183.
- ROSCA, I. I. (2004): Impact of genetically modified herbicide resistant maize on the arthropod fauna. IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 143-146.
- RUHLAND, M.; ENGELHARDT, G. & PAWLIZKI, K. (2002): A comparative investigation of the metabolism of the herbicide glufosinate in cell cultures of transgenic glufosinate-resistant and non-transgenic oilseed rape (*Brassica napus*) and corn (*Zea mays*). Environ. Biosafety Res. 1: 29-37.
- RUHLAND, M.; ENGELHARDT, G. & PAWLIZKI, K. (2004): Distribution and metabolism of D/L-, L- and D-glufosinate in transgenic, glufosinate-tolerant crops of maize (*Zea mays* L ssp *mays*) and oilseed rape (*Brassica napus* L var *napus*). Pest. Manag. Sci 60: 691-696.
- SAXENA, D.; FLORES, S. & STOTZKY, G. (1999): Transgenic plants: insecticidal toxin in root exudates from Bt corn. Nature 402: 480.
- SAXENA, D. & STOTZKY, G. (2000): Insecticidal toxin from *Bacillus thuringiensis* is released from roots of transgenic Bt corn in vitro and in situ. FEMS Microbiology Ecology 33: 35-39.
- SAXENA, D. & STOTZKY, G. (2001): *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria and fungi in soil. Soil Biology and Biochemistry 33: 1225-1230.
- SAXENA, D. & STOTZKY, G. (2001): Bt corn has a higher lignin content than non-Bt corn. American Journal of Botany 88: 1704-1706.
- SAXENA, D.; FLORES, S. & STOTZKY, G. (2002): Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biology and Biochemistry 34: 133-137.
- SAXENA, D.; STEWART, C. N.; ALTOSAAR, I.; SHU, Q. & STOTZKY, G. (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.
- SAXENA, D. & STOTZKY, G. (2002): Bt toxin is not taken up from soil or hydroponic culture by corn, carrot, radish, or turnip. Plant and Soil 239: 165-172.
- SCHMALENBERGER, A. & TEBBE, C. C. (2002): Bacterial community composition in the rhizosphere of a transgenic, herbicide-resistant maize (*Zea mays*) and comparison to its non-transgenic cultivar Bosphore. FEMS Microbiology Ecology 40: 29-37.
- SCHMIDT, J. E. U.; BRAUN, C. U.; L'ABATE, C.; WHITEHOUSE, L. P. & HILBECK, A. (2004): Studies on effects of *Bacillus thuringiensis*-toxins from transgenic insect-resistant plants on predaceous lady beetles (Coleoptera: Coccinellidae). [Original Title: Untersuchungen zu Effekten von *Bacillus thuringiensis*-Toxinen aus transgenen insektenresistenten Pflanzen auf rauberische Marienkafer (Coleoptera: Coccinellidae).]. Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie 14: 419-422.

- SCHULER, T. H.; POTTING, R. P.; DENHOLM, I. & POPPY, G. M. (1999): Parasitoid behaviour and Bt plants. *Nature* 400: 825-826.
- SCHULER, T. H.; DENHOLM, I.; JOUANIN, L.; CLARK, S. J.; CLARK, A. J. & POPPY, G. M. (2001): Population-scale laboratory studies of the effect of transgenic plants on nontarget insects. *Mol. Ecol.* 10: 1845-1853.
- SCHULER, T. H.; POTTING, R. P.; DENHOLM, I.; CLARK, S. J.; CLARK, A. J.; STEWART, C. N. & POPPY, G. M. (2003): Tritrophic choice experiments with Bt plants, the diamondback moth (*Plutella xylostella*) and the parasitoid *Cotesia plutellae*. *Transgenic Res.* 12: 351-361.
- SCHULER, T. H.; DENHOLM, I.; CLARK, S. J.; STEWART, C. N. & POPPY, G. M. (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). *J. Insect Physiol.* 50: 435-443.
- SCHULER, T. H.; CLARK, A. J.; CLARK, S. J.; POPPY, G. M.; STEWART, C. N., JR. & DENHOLM, I. (2005): Laboratory studies of the effects of reduced prey choice caused by Bt plants on a predatory insect. *Bull. Entomol. Res.* 95: 243-247.
- SEARS, M. K.; HELLMICH, R. L.; STANLEY-HORN, D. E.; OBERHAUSER, K. S.; PLEASANTS, J. M.; MATTILA, H. R.; SIEGFRIED, B. D. & DIVELY, G. (2001): Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. *Proceedings of the National Academy of Sciences* 98: 11937-11942.
- SEHNAL, F.; HABUSTOVA, O.; SPITZER, L.; HUSSEIN, H. M. & RUZICKA, V. (2004): A biannual study on the environmental impact of Bt maize. IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 147-160.
- SESSITSCH, A.; GYAMFI, ST.; TSCHERKO, D.; GERZABEK, M. H. & KANDELER, E. (2005): Activity of microorganisms in the rhizosphere of herbicide treated and untreated transgenic glufosinate-tolerant and wildtype oilseed rape grown in containment. *Plant and Soil* 266: 105-116.
- SHIRAI, Y. & TAKAHASHI, M. (2005): Effects of transgenic Bt corn pollen on a non-target lycaenid butterfly, *Pseudozizeeria maha*. *Applied Entomology and Zoology* 40: 151-159.
- SISTERSON, M. & TABASHNIK, B. E. (2005): Simulated effects of transgenic Bt crops on specialist parasitoids of target pests. *Environmental Entomology* 34: 733-742.
- STANLEY-HORN, D. E.; DIVELY, G. P.; HELLMICH, R. L.; MATTILA, H. R.; SEARS, M. K.; ROSE, R.; JESSE, L. C.; LOSEY, J. E.; OBRYCKI, J. J. & LEWIS, L. (2001): Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proc. Natl. Acad. Sci. U.S.A* 98: 11931-11936.
- STOTZKY, G. (2000): Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality* 29: 691-705.
- 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.
- SZEKERES, D.; KADAR, F. & SZENTKIRALYI, F. K. J. (2005): Seasonal dynamics of important predatory beetles (Coleoptera: Carabidae, Coccinellidae) on Bt-(cry1Ab, MON810) and on isogenic maize plots in a three year field experiment in

- Hungary. EIGMO Meeting Abstracts IOBC/WPRS Working Group on GMOs in Integrated Plant Production, Meeting on Ecological Impact of GMOs, Lleida, Catalonia, Spain, June 1-3, 2005: 70.
- TAPP, H. & STOTZKY, G. (1998): Persistence of the insecticidal toxins from *Bacillus thuringiensis* subsp. *kurstaki* in soil. *Soil Biology and Biochemistry* 30: 471-476.
- TERMORSHUIZEN, A. J. & LOTZ, L. A. P. (2002): Does large-scale cropping of herbicide-resistant cultivars increase the incidence of polyphagous soil-borne plant pathogens? *Outlook on Agriculture* 31: 51-54.
- THOMAS, W. E.; PLINE-SRNIC, W.; THOMAS, J. F.; EDMISTEN, K. L.; WELLS, R. & WILCUT, J. W. (2004): Glyphosate negatively affects pollen viability but not pollination and seed set in glyphosate-resistant corn. *Weed Science* 52: 725-734.
- TURRINI, A.; SBRANA, C.; NUTI, M.-P.; PIETRANGELI, B.-M. & GIOVANNETTI, M. (2004): Development of a model system to assess the impact of genetically modified corn and aubergine plants on arbuscular mycorrhizal fungi. *Plant and Soil* 266: 69-75.
- VENDITTI, M. E. & STEFFEY, K. L. (2002): Field effects of Bt corn on the impact of parasitoids and pathogens on European corn borer in Illinois. 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii, USA, 14-18 June 2002 USDA Forest Service FHTET: 278-283.
- VERCESI, M. L.; KROGH, P. H. & HOLMSTRUP, M. (2005): Can *Bacillus thuringiensis* (Bt) corn residues and Bt-corn plants affect life-history traits in the earthworm *Aporrectodea caliginosa*? *Applied Soil Ecology*, in press.
- VOJTECH, E.; MEISSE, M. & POPPY, G. M. (2005): Effects of Bt maize on the herbivore *Spodoptera littoralis* (Lepidoptera: Noctuidae) and the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). *Transgenic Res.* 14: 133-144.
- VOLKMAR, C. & FREIER, B. (2003): Spinnenzoenosen in Bt Mais und nicht gentechnisch veraenderten Maisfeldern. *Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz* 110: 572-582.
- VOLKMAR, CH.; TRAUGOTT, M.; JUEN, A.; SCHORLING, M. & FREIER, B. (2003): Spider communities in Bt maize and conventional maize fields. IOBC/WPRS Working Group "GMOs in Integrated Production" 27 (3): 165-170.
- WANDELER, H.; BAHYLOVA, J. & NENTWIG, W. (2002): Consumption of two Bt and six non-Bt corn varieties by the woodlouse *Porcellio scaber*. *Basic and Applied Ecology* 3: 357-365.
- WENDONG, L.; KONGMING, W.; XIAOQI, W.; GUIRONG, W. & YUYUAN, G. (2005): Impact of pollen grains from Bt transgenic corn on the growth and development of chinese Tussah Silkworm, *Antheraea pernyi* (Lepidoptera: Saturniidae). *Environmental Entomology* 34: 922-928.
- WRAIGHT, C. L.; ZANGERL, A. R.; CARROLL, M. J. & BERENBAUM, M. R. (2000): Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proceedings of the National Academy of Sciences* 97: 7700-7703.
- ZANGERL, A. R.; MCKENNA, D.; WRAIGHT, C. L.; CARROLL, M.; FICARELLO, P.; WARNER, R. & BERENBAUM, M. R. (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* 98: 11908-11912.

- ZWAHLEN, C.; NENTWIG, W.; BIGLER F. & HILBECK, A. (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.; HILBECK, A.; GUGERLI, P. & NENTWIG, W. (2003a): Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Molecular Ecology* 12: 765-775.
- ZWAHLEN, C.; HILBECK, A.; HOWALD, R. & NENTWIG, W. (2003b): Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris*. *Mol.Ecol.* 12: 1077-1086.

In der vorliegenden Arbeit werden Studien zusammengefasst und evaluiert, die in wissenschaftlichen Journalen innerhalb der letzten 3-4 Jahre veröffentlicht wurden und ökologische Effekte von insektenresistentem (Bt) bzw. herbizidtolerantem Mais berücksichtigen. Der Großteil dieser Studien behandelt ökologische Effekte von Bt Mais, der das Cry1Ab Toxin enthält. Nur wenige Studien sind verfügbar, die andere Cry Toxine berücksichtigen, wie z. B. Cry3Bb1 oder Cry1F. Einige der Studien beziehen sich auf ökologische Auswirkungen von Bt Mais, der das Cry1Ab Toxin enthält, auf Tagfalter, jene Gruppe von Nichtzielorganismen, für die erstmals negative Effekte durch den gentechnisch veränderten Mais Bt176 beschrieben wurden.

**Bestelltelefon:
01/711 00-4700**