GM insect-resistant (Bt) maize in Europe: a growing threat to wildlife and agriculture

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Cultivation of GM Bt maize will harm European wildlife and jeopardise conventional and organic farming. Despite this, there is a threat that genetically modified (GM) insect-resistant (Bt) maize could be grown in Europe on a large scale as companies are submitting new GM Bt maize varieties to the EU approval process. Potential effects on the natural and agricultural environments include toxic effects on beneficial and non-target organisms, such as butterflies; long-term effects on soil health and rivers and impacts on sustainable farming practices. Contamination of non-GM crops is already happening from the small acreage of GM Bt maize that is grown in Europe: coexistence with non-GM crops is impossible. Decision-makers at all levels in the EU and national governments need to take into account the evidence contained in this briefing, put a halt to the expansion of risky GM crops in the EU and, instead, invest in ecological agriculture that creates jobs and stimulates rural development, whilst promoting biodiversity by protecting soil, water and the climate.

1) Different types of GM Bt maize

Maize has been genetically modified (GM, also called genetically engineered, GE) in a number of ways to produce several different types of GM maize. However, virtually all the GM maize grown around the world exhibit only two major traits, herbicide tolerance (e.g. Monsanto's Roundup Ready) and insect resistance (e.g. Monsanto's MON810, Syngenta's Bt11 and Pioneer's 1507). MON810, Bt11 and 1507 maize varieties have been genetically modified through the insertion of a gene from the *Bacillus thuringiensis* (Bt) soil bacterium, to produce a pesticide, the Bt protein or toxin. MON810 has already been approved for cultivation in the EU but is subject to national bans and, in 2008, only grown on less than 0.2% of EU land used for cereal production, mainly in Spain and, to a lesser extent, the Czech Republic, Germany, Slovakia, Portugal, Romania and Poland[1]. Bt11 and 1507 are currently in the final stages of the EU authorisation process.

This briefing summarises the main environmental and agricultural impacts of these types of GM insect-resistant maize varieties. Not all these maize varieties produce the same type of Bt toxin. MON810 and Bt11 produce the Cry1Ab type of toxin, whilst 1507 produces a different type of Bt toxin, known as Cry1F. Both types of Bt toxin are proven to be toxic to larvae of certain moths and butterflies so many of the environmental concerns regarding impacts on non-target organisms are shared. However, since the two Bt toxins differ in their activities, they must be evaluated separately. Whilst there have now been a considerable number of studies on Bt crops producing Cry1Ab, there are hardly any for Cry1F.

2) GM Bt crops: environmental concerns

In its natural form, farmers practising organic and other sustainable growing methods have used Bt formulations since the 1950s as a spray to kill pests without damaging beneficial and non-target insects or other wildlife. However, both the Cry1Ab and Cry1F Bt toxins produced by GM insect-resistant maize are significantly different: they are a shorter, or truncated, form of the protein. This truncated (or shortened) form is less selective than Bt sprays and therefore has potential to harm non-target insects in addition to the pests for which it is intended[2].

The environmental effects of growing Bt maize in Europe include:

a) Toxic effects on non-target organisms such as butterflies

Current Bt maize crops have been genetically modified to be toxic to certain species of moths and butterflies (Lepidoptera), e.g. the European corn borer (*Ostrinia nubilalis*), which are pests of maize. However, larvae of non-target moths and butterflies, e.g the European peacock butterfly (*Inachis io*) may inadvertently ingest the Bt toxin whilst feeding on plants growing near Bt maize field. The effects of pollen from Bt maize on larvae of the monarch butterfly in North America is the most well known example of this phenomenon[3]. Monarch butterfly larvae are thought to be particularly sensitive to Cry1Ab and less so to Cry1F[4]. Long-term exposure to Bt pollen from two Bt (Cry1Ab) maize types (MON810 and Bt11) caused reduced survival of monarch butterfly larvae to adulthood[5]. Exposure to anthers (the part of the flower that carries pollen) of Bt (Cry1Ab) maize has also been shown to affect the behaviour of monarch butterfly larvae, possibly attempting to avoid the Bt toxin[6]. These studies show that long-term and subtle effects are important. Many species of butterflies in Europe are already facing multiple threats, such as climate change and loss of habitat[7]. Additional stress from exposure to Bt pollen could further threaten certain species of butterflies and moths. Thus, t**here is a very real possibility that non-target organisms such as butterflies will be harmed by cultivation of Bt maize.**

In 2006, a review of the ecological effects of GM maize[8] concluded: *"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."* The same is still true today.

Long-term laboratory studies of the effects of Bt maize on non-target organisms are essential[9]. In the case of the monarch butterfly no short-term adverse effects (4-5 days) were noted[10], it was only when longer-term studies (2 years) were carried out that the adverse effects became clear.

b) Toxic effects on beneficial insects

GM Bt maize could adversely affect beneficial insects that are important in the natural control of maize pests, for example those that eat the maize pests[11]. This has been shown for green lacewings[12]. The toxic effects of GM Bt crops on lacewings were via the prey the lacewings ate, which in turn ingest the GM Bt crop, i.e. indirect toxicity. Thus, the Bt toxins from GM maize can kill non-target species and be passed higher up the food chain, an effect that has never been observed with the Bt toxin in its natural form.

The indirect toxicity of the Bt toxin to non-target beneficial insects makes evaluation of adverse effects difficult. There have been several different laboratory studies on beneficial insects, often with different methodologies. However, a recent review of these studies concluded that "*Collectively [the experiments] indicate that the use of genetically modified crops may result in negative effects on the natural enemies of crop pests.*"[13]

The exact mechanism of how the Bt toxin causes indirect toxicity is not yet well understood, and further research is necessary to fully understand how Bt crops could affect beneficial insects. "*Key experiments explaining the mode of action not only in this particular affected non-target species [lacewings] but also in most other affected non-target species are still missing.*" [14]

There are also concerns that Bt (Cry1Ab) maize may affect the learning performance of honey bees[15], which are important pollinators. Although the study used much higher concentrations than those found in Bt maize plants, it raises concerns that the short-term direct toxicity testing normally employed in risk assessments is not sufficient to determine any possible sub lethal effects (i.e. any effects that impair health or function, but do not kill) on beneficial insects. Such sub-lethal effects (e.g. effects on learning ability) are crucial as they may affect the functionality of beneficial insects.

For non-specific effects of Bt crops on non-target organisms, the type of farming practice used as a comparator is also important. Average abundance of non-target invertebrates were lower in fields of Bt (Cry1Ab) maize, compared to fields were no pesticides were sprayed. However, this effect was reversed in fields where broad-spectrum pesticides were used[16]. The only useful comparisons are to ecological farming methods.

The EU environmental risk assessment for Bt crops include only short-term single species mortality studies. These studies would not detect any effects on organisms higher up the food web, such as the effects on lacewings, nor any subtle effects on behaviour. This approach has been highly criticised and many scientists have suggested that **the effects of Bt crops, including maize, need to be studied at multiple levels of the food web[17].**

c) Possible long term harm to soil ecosystems

The Bt toxin exuded by GM Bt (Cry1Ab) maize has been shown to persist in the soil whilst remaining biologically active[18]. The long-term, cumulative effects from the continued growth of GM Bt maize over several years have not been adequately considered in a European context, even though they are thought to be highly important in terms of the risk assessment[19] and could affect yields of future non-Bt crops[20]. The persistence of Cry1F in soils under European climates is unknown, as there have been virtually no studies on the behaviour of Cry1F and its potential effects on soil organisms[21].

GM Bt maize varieties generally contain higher level of lignin than their non-GM counterparts[22]. Lignin is well known for its capability to influence digestibility of plant material to herbivores and could slow the decomposition of Bt maize residues in the soil. Indeed, GM Bt maize decomposes less in soil than non Bt maize and this may be related to their higher lignin content[23].

Soils are vital ecosystems to agriculture and biodiversity. However, soil ecology is poorly understood. Growing Bt crops may be problematic for long-term soil health, as Bt crops contain proteins that are known to be toxic to certain insects, and are suspected of being toxic to a range of non-target organisms such as earthworms and nematodes[24]. However, results from studies on soil organisms are not consistent. Whilst several studies reveal differences, especially on soil microorganisms[25], it is not clear if these are transitory or long-term effects, nor whether they are adverse effects. **The long-term effects of Bt maize on soil health remain a area of high concern.**

A comprehensive review of studies to date on the fate and effects of Bt crops on soil ecosystems[26] concluded that *"The lack of evidence of negative effects of Bt crops does not mean that other GM plants are without risk. Moreover, the possibility of long-term effects of Bt crops cannot be excluded and must be examined on a case-by-case basis, especially as a number of issues related to the interpretation of the scientific data on the effects of Bt crops on the environment are still controversially debated."*

d) Persistence in aquatic ecosystems

The Bt toxin from maize (either exuded by roots during growth or as crop residues left in the field) can enter streams where it might be toxic to aquatic (insect) life, possibly resulting in ecosystem level effects. In the United States, agricultural waste from Bt maize has been shown to enter streams[27]. Initial ecotoxicity tests on the standard test organisms for water quality, the water flea (*Daphnia magna*), showed a significantly reduced fitness performance when fed with MON810, indicating a toxic effect[28]. Although aquatic microbes were unaffected, there are concerns that caddisflies, who are close relatives of moths and butterflies, could be affected[29]. This exposure pathway for Bt toxin has not been previously considered and is omitted from the current environmental risk assessments of Bt crops, although this could be significant to the aquatic food web and ultimately the health of aquatic ecosystems.

The Cry1Ab gene is persistent in aquatic environments[30] and has been found in the tissues of fresh water mussels in areas where Bt (Cry1Ab) maize is cultivated, accumulated via microorganisms ingested by the mussels[31]. The consequences of the presence of the Cry1Ab GM gene are not clear, for example, it is not known if the gene is expressed (active).

Interactions in the natural environment are complex and not fully understood. This means that it is impossible to fully predict how an introduced element may interact with the natural environment and this can lead to surprises. Commercial scale cultivation of Bt crops could, for example, lead to an accumulation of Bt toxins in aquatic environments, with potentially harmful effects. However, this possibility is not taken into consideration in the current EU risk assessment for Bt maize. **Thus, the risk assessment process for GM Bt plants, e.g. maize, is not capable of evaluating potential harm to the environment.**

e) Increased pest resistance to Bt

In the US there are complex requirements to establish areas (called refugia) planted with non-Bt crops within a Bt maize field, in order to slow down the development of insect resistance to the Bt toxin. However, refugia may not be practical on small farm holdings in Europe and elsewhere, which are very different from the large field sizes in the US.

Cross pollination will cause contamination of non-Bt maize in refugia and could undermine the effectiveness of refugia, as pests will still be exposed to the Bt toxin in the refugia[32]. Variability in expression of the Bt toxin will also affect the speed at which resistance develops. If the levels become too low, the toxin may not kill the pest but instead let it survive on the Bt maize, assisting the development of resistance. MON810 has highly variable expression levels of Bt and levels also increase with the increased use of nitrogen fertilizer[33].

There are overwhelming scientific data to support concerns of insect pest resistance[34]. If widespread resistance were to occur, the insect-resistant properties of GM crops would become ineffective. The application of new and even more toxic chemical pesticides would, therefore, be inevitable. There is evidence that insect resistance is now appearing for cotton pests in the US (although the use of refugia in this case has slowed down resistance). However, this resistance is reported not to have caused crop failures because farmers are still using insecticides to control the target pest[35]. **Thus increased resistance poses a serious threat to sustainable and ecological agricultural methods.**

f) Bt maize: swapping one pest for another?

Several studies have shown that other pest insects are filling the void left by the absence of the one (or very few) insect pests that Bt crops target. Most of the evidence comes from Bt cotton, but similar effects could be seen if Bt maize is cultivated.

- For the Bt (Cry3a) potato, 42% of field tests revealed a significant, positive effect on the abundance of a variety of sucking insects, which are secondary pests that also attack potatoes[36]. The authors concluded that this may have hampered the development of this Bt potato, which is no longer commercially grown.

- For Bt (Cry1Ac) cotton, after a few years of cultivation, farmers in China and elsewhere have to spray more pesticides for secondary pests – those not controlled by the Bt toxin[37]. Bt cotton was first introduced and promoted to farmers as a crop that would reduce the use of pesticides. However, it was soon evident that some insects, which were not an important pest before the introduction of Bt cotton, were becoming a problem. These secondary pests had previously been controlled by the broad spectrum pesticides used in conventional cotton. Hence, the use of Bt cotton may lead to initial reductions in pesticide usage but allows secondary pests to thrive. As a consequence, the level of pesticide spraying for these pests has increased several fold.

- For Bt maize, larvae of the western bean cutworm (another pest insect for maize) are starting to fill the niche of the pest insect controlled by Bt (Cry1Ab) maize in the US[38]. Western bean cutworm larvae are not susceptible to Cry1Ab and their numbers have increased in certain years on various types of GM Bt maize, compared to conventional maize. However, this effect was not noted for Cry1F, suggesting the western bean cutworm larvae is also susceptible to the Cry1F toxin. Similarly, several varieties of Bt (Cry1Ab) maize are more susceptible to a corn leaf aphid than their conventional equivalents[39]. Aphids are damaging to maize crops but the increase in aphid numbers also enhanced the performance of parasitic wasps that feed on aphid honeydew. Therefore, the overall effect is not clear. Analyses of the plant sap (liquid) the aphids fed on, revealed significantly higher levels of certain amino acids in Bt maize, which might partially explain the observed increased aphid numbers. It is not known, however, what causes the changes in plant sap chemistry. It could well be an unexpected effect caused by the genetic engineering process.

Plant-insect interactions are complex; where one pest is controlled, another may thrive and take its place. Thus, any initial reductions in pesticide usage arising from the use of Bt crops are expected to be only temporary.

3) Coexistence is impossible

There are many studies confirming long distance pollination from GM maize up to 1 000 m away[40]. In all of the EU reports published on gene flow and coexistence (e.g. EEA, 2002; IPTS/JRC, 2002, IPTS/JRC/ESTO, 2006[41]) maize has been shown to be amongst the most difficult GM crops to contain (alongside oilseed rape), due to the high cross pollination rate and the large distances that viable maize pollen can travel. GM maize is described as presenting a "medium to high risk" for cross-pollination with other crops[42].

The small acreage of Bt corn grown in Spain is reported to be creating conflicts within society. "*The liability scheme is perceived as transferring the problem to the organic farmers. As a result, many farmers are reluctant to publicly report cases of contamination in a context where there is a need for social cohesion, as in small villages. One organic farmer said:* "as a consequence of social pressure, when farmers suffer contamination, they do not want to say so. Last year there were four contamination cases and two made it public but two did not. For fear of confronting the people in the town ... so they have to assume the economic cost, the environmental cost, and the cost of losing the organic certification but they do not say so" *(interview). Consequently, data on admixture cases are not systematically registered, although the organic certification is withdrawn in these cases*"[43].

In addition, organic farming is diminishing as a result of GM contamination. "*As a result [of these cases], from 2004 (when the first analyses were done) to 2007, the area devoted to organic maize was reduced by 75% in Aragon [where* GM *Bt maize is concentrated].*"[44]

There is a possibility that GM maize plants could survive the winter in Mediterranean Europe to contaminate future non-GM maize. Maize plants have been shown to survive over a growing season, even in the UK, a comparatively cold part of Europe[45]. Occasionally, maize volunteers (plants that have not been intentionally planted) have been noted from spilled seed in uncultivated fields and roadsides in the year following GM maize production[46]. Should any volunteer GM maize plants inadvertently grow near a maize crop, the resulting pollen could cross-pollinate, resulting in genetic contamination.

Co-existence is impossible. Non-GM maize (i.e. conventional and organic) will inevitably become contaminated in Europe. There is no liability legislation in place that would award compensation for farmers whose crops are contaminated and therefore devalued by GM maize in Europe.

Conclusion

All types of GM Bt maize have the potential to cause serious harm to Europe's natural and agricultural environment and wildlife. These potential effects include:

- effects on non-target organisms, including indirect toxicity and long-term effects;
- effects on soil health, especially long-term effects;
- accumulation and persistence of the Bt toxin in aquatic environments;
- build up of insect resistance;
- increases in other pests, and;
- impacts on sustainable farming practices.

The current EU risk assessment process is wholly inadequate to assess these potential threats as it does not assess sub-lethal or long-term effects; indirect toxicity; unexpected effects (e.g. increases in secondary pests); nor unanticipated pathways of the Bt toxin into the environment. The lack of data on the behaviour of Cry1F in the agro-environment and its potential toxic effects on non-target species is particularly problematic for the risk assessment of Bt crops. Several unexpected effects and pathways have been discovered in connection with Cry1Ab maize (MON7810 and Bt11), it is highly likely that such unexpected effects may yet be linked to Cry1F maize (1507).

Releases of GM organisms are irreversible. In particular, GM maize is uncontrollable because of the high cross-pollination rate and the large distances that maize pollen travels. Therefore, in Europe and elsewhere, co-existence of conventional and organic maize with GM maize is impossible. Hence, the cultivation of GM maize will erode farmer's choice to say no to GM crops and consumer choice to avoid GM food.

Decision-makers at all levels in the EU and national governments need to put a halt to the expansion of risky GM crops in the EU. The future of agriculture lies in ecological farming that creates jobs, stimulates rural development, and promotes biodiversity by protecting soil, water and the climate. Ecological systems ensure healthy farming and healthy food today and in the future, and do not contaminate the environment with chemical inputs or genetic engineering.

References

[1] Area under GM cultivation (insect resistant Bt maize MON810 is the only GM crop that is commercially grown in the EU) from: GMO-Compass. 2008. Commercial GM Crops in the EU in 2008. http://www.gmo-compass.org/eng/agri_biotechnology/gmo_planting/392.gm_maize_cultivation_europe_2008.html Area of arable land under cereal production taken from: EUROSTAT. 2008. Eurostat crops products (excluding fruits and vegetables) (annual data): http://nui.epp.eurostat.ec.europa.eu/nui/show.do?dataset=apro_cpp_crop&lang=en

[2] Hillbeck, A. 2001. Transgenic host plant resistance and non-target effects. In: Genetically engineered organisms: assessing environmental and human health effects. Letourneau, D.K. and B.E. Burrows [eds.] Boca Raton, FL: CRC Press. Hilbeck, A., M.S. Meier and A. Raps. 2000. Review on non-target organisms and Bt plants. Report prepared for Greenpeace International, Amsterdam, EcoStrat GmbH, Ecological Technology Assessment & Environmental Consulting, Zurich, Switzerland

[3] Losey, J.E., L.S. Raynor and M.E. Carter. 1999. Transgenic pollen harms monarch larvae. Nature 399: 214; Sears, M.K., R.L. Hellmich, D.E. Stanley-Horn, K.S. Oberhauser, J.M. Pleasants, H.R.

Mattila, B.D. Siegfried, and G.P. Dively. 2001. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. Proceedings of the National Academy of Sciences 98: 11937-11942.

[4] 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. Proceedings of the National Academy of Sciences 98: 11925–11930.

[5] Dively, G.P., Rose, R., Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Calvin, D.D. Russo, J.M. & P.L. Anderson. 2004. Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab expressing corn during anthesis. Environmental Entomology 33: 1116-1125.

[6] Prasifka, P.L., Hellmich, R.L., Prasifka, J.R. & Lewis, L.C. 2007. Effects of Cry1Ab-expressing corn anthers on the movement of monarch butterfly larvae. Environmental Entomology 36: 228-33.

[7] Thomas, J.A., M.G. Telfer, D.B. Roy, C.D. Preston, J.J.D. Greenwood, J. Asher, R. Fox, R.T. Clarke and J.H. Lawton. 2004. Comparative losses of British butterflies, birds and plants and the global extinction crisis. Science 303: 1879-1881.

[8] Dolezel, M., Heissenberger, A. & Gaugitsch, H. 2006. Ecological effects of genetically modified maize with insect resistance and/or herbicide tolerance. Bundesministerium für Gesundheit und Frauen, Sektion IV Radetzkystraße 2, 1031 Wien. http://www.bmgf.gv.at/cms/site/detail.htm?thema=CH0255&doc=CMS1134457515326

[9] Andow, D.A. & Hilbeck, A.. 2004. Science-based risk assessment for non-target effects of transgenic crops. Bioscience, 54: 637-649. Snow, A. A., Andow, D.A., Gepts, P., Hallerman, E. M., Power, A., Tiedje, J. M. & Wolfenbarger, L.L. 2005. Genetically engineered organisms and the environment: current status and recommendations. Ecological Applications, 15: 377–404.

[10] Stanley-Horn, D.E., G.P. Dively, R.L. Hellmich, H.R. Mattila, M.K. Sears, R. Rose, L.C.H. Jesse, J.E. Losey, J.J. Obrycki and L. Lewis. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. Proceedings of the National Academy of Sciences 98: 11931-11936.

[11] Harwood, J.D., Wallin, W.G. & Obrycki, J.J. 2005. Uptake of Bt endotoxins by non-target herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Molecular Ecology 14: 2815-2823.
Lövei, G.L. & Arpaia, S. 2005.The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomologia Experimentalis et Applicata 114: 1–14, 2005.

[12] Andow, D.A. and A. Hilbeck. 2004. Science-based risk assessment for non-target effects of transgenic crops. Bioscience 54: 637-649.
Obrist, L.B., Dutton, A., Romeis, J. & Bigler, F. 2006. Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. BioControl 51: 31-48.
Hilbeck, A. & Schmidt, J.E.U. 2006. Another view on Bt proteins – how specific are they and what else might they do? Biopesticides International. 2: 1-50.

[13] Lövei, G.L. & Arpaia, S. 2005. The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomologia Experimentalis et Applicata 114: 1–14.

[14] Lövei, G.L. & Arpaia, S. 2005. The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomologia Experimentalis et Applicata 114: 1–14.

[15] Ramirez-Romero, R., Desneux, N., Decourtye, A. Chaffiol, A., Pham-Delègue, M.H. 2008. Does Cry1Ab protein affect learning performances of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae)? Ecotoxicology and Environmental Safety 70: 327–333.

[16] Marvier, M., McCreedy, C., Regetz, J. & Kareiva, P. 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316: 1475-1477.

[17] Knols, B.G.J. and M. Dicke. 2003. Bt crop assessment in the Netherlands. Nature Biotechnology 21: 973-974. Andow, D.A. & A. Hilbeck. 2004. Science-based risk assessment for non-target effects of transgenic crops. Bioscience, 54: 637-649.
Snow, A. A., Andow, D.A., Gepts, P., Hallerman, E. M., Power, A., Tiedje, J. M. & Wolfenbarger, L.L. 2005. Genetically engineered organisms and the environment: current status and recommendations. Ecological Applications, 15: 377–404. Andow, D.A. & Zwahlen, C. 2006. Assessing environmental risks of transgenic plants. Ecology Letters 9: 196-214.

[18] 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. Molecular Ecology 14: 2539–2551
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.

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

[19] Marvier, M. 2002. Improving risk assessment for non-target safety of transgenic crops. Ecological Applications 12: 1119-1124.

Clark, B.W., Phillips, T.A. & Coats, J.R. 2005. Environmental fate and effects of *Bacillus thuringiensis* (Bt) proteins from transgenic crops: a review. Journal of Agricultural and Food Chemistry 53: 4643-4653.

[20] Icoz, I. & Stotzky, G. 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology & Biochemistry 40: 559–586.

[21] Icoz, I. & Stotzky, G. 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology & Biochemistry 40: 559–586.

[22] Saxena, D and Stotzky, G. 2001. Bt corn has a higher lignin content than non-Bt corn. American Journal of Botany 88: 1704-1706.

Poerschmann, J., Gathmann, A., Augustin, J., Langer, U. & Górecki, T. 2005. Molecular composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize – Characterization of lignin patterns. Journal of Environmental Quality 34: 1508-1518.

[23] Flores, S., Saxena, D., Stotzky, G. 2005. Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biology & Biochemistry 37 1073–1082.

[24] Zwahlen, C. A. Hilbeck, R. Howald and W. Nentwig. 2003, Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris*. Molecular Ecology 12:1077 –1086.

Griffiths, B.S., Caul, S. Thompson, J., Birch, A.N.E., Scrimgeour, C., Andersen, M.N., Cortet, J., Messéan A., Sausse, C., Lacroix, B. & Krogh, P.H. 2005. A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* CryIAb toxin. Plant and Soil 275: 135-146.

Hössa, S., Arndt, M., Baumgarte, S., Tebbe, C.C., Nguyend, H.T. & Jehled, J.A. 2008. Effects of transgenic corn and Cry1Ab protein on the nematode, *Caenorhabditis elegans*. Ecotoxicology and Environmental Safety 70: 334–340.

[25] Mulder, C., Wouterse, M., Rutgers, M. & Posthuma, L. 2007. Transgenic maize containing the Cry1Ab protein ephemerally enhances soil microbial communities. Ambio 36: 359 – 361.

Mulder, C., Wouterse, M., Raubuch M., Roelofs, W. & Rutgers, M. 2006. Can transgenic maize affect soil microbial communities? PLoS Computational Biology 2: 1165 – 1172.

Baumgarte, S. & Tebbe, 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. Molecular Ecology 14: 2539 – 2551.

[26] Icoz, I. & Stotzky, G. 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology & Biochemistry 40: 559–586.

[27] Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C., Griffiths, N.A., Pokelsek, J. & Stephen, M.L. 2007. Toxins in transgenic crop byproducts may affect headwater stream ecosystems. Proceedings National Academy of Sciences 41: 16204–16208.

Griffiths, N.A., Tank, J.L., Royer, T.V., Rosi-Marshall, E.J., Whiles, M.R., Chambers, C.P., Frauendorf, T.C. & Evans-White, M.A. 2009. Rapid decomposition of maize detritus in agricultural headwater streams. Ecological Applications 19: 133-142.

[28] Bøhn, T., Primicerio, R., Hessen, D.O. & Traavik, T. 2008. Reduced fitness of *Daphnia magna* fed a Bt-transgenic maize variety. Archives of Environmental Contamination and Toxicology DOI 10.1007/s00244-008-9150-5.

[29] Griffiths, N.A., Tank, J.L., Royer, T.V., Rosi-Marshall, E.J., Whiles, M.R., Chambers, C.P., Frauendorf, T.C. & Evans-White, M.A. 2009. Rapid decomposition of maize detritus in agricultural headwater streams. Ecological Applications 19: 133-142.

[30] Douville, M., Gagne, F, Blaise, C. & André, C. 2007. Occurrence and persistence of *Bacillus thuringiensis* (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. Ecotoxicology and Environmental Safety 66: 195–203.

[31] Douville, M., Gagne, F., André, C. & Blaise, C. 2009. Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (*Elliptio complanata*) near cornfields: evidence of exposure by bacterial ingestion. Ecotoxicology and Environmental Safety 72: 17–25.

[32] Chilcutt, C.H. and B.E.Tabashnik. 2004. Contamination of refuges by *Bacilus thuringensis* toxin genes from transgenic maize. Proceedings of the National Academy of Sciences 101:7526-7529.

[33] Bruns, H.A. & Abel, C.A. 2007. Effects of nitrogen fertility on Bt endotoxin levels in corn. Journal of Entomological Science 42: 35 – 34.

Nguyen, H.T. & Jehle, J.A. 2007. Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize MON810. Journal of Plant Diseases and Protection 114: 82 – 87.

[34] See for example Andow, D.A. 2001. Resisting resistance to Bt corn. In: Genetically engineered organisms: assessing environmental and human health effects. Letourneau, D.K. and B.E. Burrows [eds.] Boca Raton, FL: CRC Press.

[35] Tabashnik, B.E., Gassmann, A.J., Crowder, D.W. & Carrière, Y. 2008. Insect resistance to Bt crops: evidence versus theory. Nature Biotechnology 26: 199-202.

[36] Cloutier, C.; Boudreault, S.; Michaud, D. 2008. Impact of Colorado potato beetle-resistant potatoes on non-target arthropods: a meta-analysis of factors potentially involved in the failure of a Bt transgenic plant. Cahiers Agricultures 17: 388-394.

[37] Men, X., Ge, F., Edwards, C.A. & Yardim, E.N. 2005. The influence of pesticide applications on *Helicoverpa armigera* Hübner and sucking pests in transgenic Bt cotton and non-transgenic cotton in China. Crop Protection 24 319–324.

Deguine, J-P., Ferron, P. & Russell, D. 2008. Sustainable pest management for cotton production. A review. Agronomy for Sustainable Development 28: 113–137.

Wang, S., Just, D.R. & Pinstrup-Andersen, P. 2008. Bt-cotton and secondary pests. International Journal of Biotechnology 10: 113–121.

[38] Catangui M.A. & Berg R.K. 2006.Western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera : Noctuidae), as a potential pest of transgenic Cry1Ab *Bacillus thuringiensis* corn hybrids in South Dakota. Environmental Entomology 35 1439-1452.

[39] Faria, C.A., Wäckers, F.L., Pritchard, J., Barrett, D.A. & Turlings, T.C.J. 2007. High susceptibility of Bt maize to aphids enhances the performance of parasitoids of lepidopteran pests. PLoS ONE 2: e600. doi:10.1371/journal.pone.0000600.

[40] See, for example, Jarosz, N., Loubet, B., Durand, B., Foueillassar, X. and Huber, L. 2005. Variations in maize pollen emission and deposition in relation to microclimate. Environmental Science and Technology 39: 4377 – 4384.

Halsey, M.E., Remund, K.M., Davis, C.A., Qualls, M., Eppard, P.J. & Berberich, S.A. 2005. Isolation of maize from pollen-mediated gene flow by time and distance. Crop Science 45: 2172-2185.

[41] EEA 2002: Eastham, K. & Sweet, J. 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Expert's Corner Series, European Environment Agency, Copenhagen. http://www.eea.eu.int/

IPTS/JRC 2002: Anne-Katrin Bock, A-K, Lheureux, K., Libeau-Dulos, M., Nilsagård, H., Rodriguez-Cerezo, E. 2002. Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture. Joint Research Centre (DG JRC), Institute for Prospective Technological Studies http://www.jrc.cec.eu.int/download/GMCrops_coexistence.pdf

IPTS/JRC/ESTO 2006: Messean, A., Angevin, F., Gómez-Barbero, M., Menrad, K. & Rodríguez-Cerezo, E. 2006. New case studies on the coexistence of GM and non-GM crops in European agriculture. Joint Research Centre (DG JRC), Institute for Prospective Technological Studies and European Science and Technology Observatory Technical Report EUR 22102 EN. http://www.jrc.es

[42] Treu, R. & Emberlin, J. 2000. Pollen dispersal in the crops maize *(Zea may*s), oil seed rape *(Brassica napus* ssp *oleifer*a), potatoes *(Solanum tuberosum*), sugar beet *(Beta vulgaris* ssp *vulgari*s) and wheat *(Triticum aestivum*). A report for the Soil Association from the National Pollen Research Unit. Available at http://www.soilassociation.org.

[43] Binimelis, R. 2008. Coexistence of plants and coexistence of farmers: is an individual choice possible? Journal of Agricultural and Environmental Ethics doi 10.1007/s10806-008-9099-4

[44] Binimelis, R. 2008. Coexistence of plants and coexistence of farmers: is an individual choice possible? Journal of Agricultural and Environmental Ethics doi 10.1007/s10806-008-9099-4

[45] Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D. & Rees, M. 2001. Transgenic crops in natural habitats. Nature 409: 682-683.

[46] Eastham, K. & Sweet, J. 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Expert's Corner Series, European Environment Agency, Copenhagen. http://www.eea.eu.int/