Effects of *Bacillus thuringiensis* on non-target arthropods

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Assessment of the ecological risks of plants expressing toxins

Assessing the ecological risks of insect resistant genetically modified (GM) plants is one of the challenges with regard to this new technology. Although insecticidal toxins expressed in GM plants are most often targeted against specific insect pests, the possible risks that these new plants pose to the environment cannot be ignored. Much attention has been directed towards pollen dispersal and out-crossing of GM plants with wild related plants or non-GM crops. The development of resistance of the target pest towards the insecticidal protein is also a major concern which is very often addressed in risk assessments. Relatively less attention has been drawn towards investigating the risks that GM plants pose to non-target arthropods and the consequences for biological control agents. Natural regulation of pest insects through biological control is one major objective in any integrated pest management system and it is therefore of relevance to maintain and to further natural enemies of pests. Insect-resistant GM plants could negatively affect non-target arthropods and could have a direct impact on pest populations. Interactions between pests, non-target herbivore arthropods and natural enemies need to be investigated in GM crops in order to understand the mechanisms and key components of sustainable food production.

Relevance of the laboratory results for the field situation

To date 11.4 million hectares are planted world wide with maize and cotton expressing toxins of *Bacillus thuringiensis* Berliner (*Bt*). These proteins confer effective protection to the crop from damage by certain phytophagous insect pests. For example *Bt*-maize expressing Cry1Ab toxin is resistant to attack of the European corn borer, *Ostrinia nubilalis*. *Bt*-proteins are also the primary active components of *Bt*-based microbial insecticides (*Bt*-sprays). *Bt*-sprays are widely used in agriculture, forest and non-crop habitats (e.g., swamps) and are in general considered safe due to their specificity. Although *Bt* has been considered safe, laboratory studies have shown that *Bt*-maize can affect non-target arthropods such as lepidopteran insects (i.e. Monarch Butterfly) as well as beneficial insects (i.e. the green lacewing). The ecological relevance of these results in the field situation have recently been evaluated for both the Monarch butterfly and the green lacewing. The data show that neither of these non-target insects are at risk from commercial grown *Bt*-maize. The results of the green lacewing *Chrysoperla carnea* will be presented.

Evaluation of the impact of Bt-maize on the green lacewing predator

An ecological evaluation on the impact of *Bt*-maize on the green lacewing predator was performed in our institute. A sequential procedure to determine exposure of the lacewing to the toxin and tests to establish hazard were conducted. Exposure of lacewing larvae to the *Bt*-toxin depends on the feeding behaviour of the predator in the field and the expression of the toxin in the plant. The relevant herbivores fed upon in the field by the lacewing are aphids, spider mites and to a lower extent lepidopteran larvae. Aphids which are the predominant prey for lacewings in the field were shown not to ingest the toxin, meaning that upon feeding on aphids, lacewings are not exposed to the toxin. Spider mites which can be an important prey for lacewings, do ingest the toxin when reared on *Bt*-maize. However, when lacewings were fed with spider mites containing the *Bt*-toxin negative effects were not observed. Only when lacewings were fed 'sick' lepidopteran larvae reared on *Bt*-maize, an effect on green lacewing larvae was noted. Interestingly, a similar result was observed when the lacewing was fed with 'sick' lepidopteran larvae which were fed plants sprayed with the commercial *Bt*-spray Dipel. The evidence from our work leads to the conclusion that *Bt*-maize and *Bt*-spray can both affect lacewings when these are fed with contaminated 'sick' larvae. However, the possibility in the field, that lacewings feed only on lepidopteran larvae would never occur. Hence, it is important to put the GM debate into proper perspective by asking the relevant question. For example, whether the new technology poses more, less or a similar risk compared to current pest control practices. From our results we can establish that *Bt*-maize is not more risky than *Bt*-spray to the lacewing predator.

Risk assessment testing procedure for non-target natural enemy arthropods
As more insect resistant transgenic plants are developed and going into commercialisation there is an increasing need for a risk assessment testing procedure for non-target natural enemy arthropods. We have developed a sequential procedure which, together with a tiered testing scheme, can be adopted as a guide for risk assessment of insect-resistant transgenic crops. As a first step, relevant herbivores and natural enemies in the cropping system need to be defined. As a second step, exposure of natural enemies to the insecticidal protein needs to be determined. Exposure will depend on the feeding behaviour of the herbivores and natural enemies together with the expression of insecticidal protein in the plant. For natural enemies which are exposed to the insecticidal protein a tiered testing procedure from laboratory to field tests is recommended. Our proposed sequential testing scheme has yielded conclusive results for a number of non-target beneficial insects on *Bt*-maize. Although, we have not verified these results in the field, field studies conducted in other countries have confirmed out laboratory and greenhouse experiments.

Consequences for the future
Advances in plant molecular biology and biochemistry have further allowed for the development of GM plants expressing several *Bt*-toxins (stacking or pyramid genes) or plants expressing lectins or proteinase inhibitors which have other modes of activity and which target other pests. In the future, field tests with these plants will be required and our sequential risk assessment procedure can be implemented.

In addition to field testing of GM plants, there is a need to know more of sub-lethal and lethal effects on population dynamics of non-target arthropods and the consequences for pest control. This includes, a better understanding on effects of GM plants on insect behaviour. Future research is also needed to evaluate the risk of GM plants in relation to other pest control methods, such as chemical control. This has to be performed in an overall risk assessment which combines ecotoxicological data and relevant laboratory and field data as proposed in our tiered approach.