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A simple question in a complex environment: How much Bt toxin do genetically engineered MON810 maize plants actually produce?

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Genetically engineered (GE) MON810 maize was authorised for cultivation in the European Union in 1998 (EU Commission 1998). This maize event was developed by Monsanto by introducing a modified DNA coding for a bacterial toxin derived from *Bacillus thuringiensis* (Cry1Ab). The bacterial gene sequence is used in a truncated form, representing a more activated form of the protein (Hilbeck & Schmidt, 2006).

Monsanto published some data in 2002 on the Bt concentration in MON810 (Monsanto 2002). Data was compiled from field trials in Europe and the U.S. from 1994 to 1996. Means and ranges were given for leaves and other plant material. Leaves, for example, showed a Bt concentration ranging (for all data) from 5.21 to 15.06 µg/g fresh weight. The means of Bt concentrations in leaves from different trials were reported to range from 8.60 to 12.15 µg/g fresh weight.

Monsanto came to the conclusion that: “The Cry1Ab protein levels in tissues collected from plants of YieldGard corn event MON 810 have been consistent across several years of evaluation in the U.S. and Europe […]. The consistency of Cry1Ab protein levels through years of breeding supports the stability of the insert, an important component of product performance.” (Monsanto 2002).

But as Nguyen and Jehle (2007) write, “there is an almost complete lack of peer-reviewed literature on the Cry1Ab expression of MON810 at different plant growth stages [...].” Recent data and publications submitted by Monsanto (2007) to the EU authorities also do not offer much detailed information about Bt concentrations in MON 810 plants. The available data from these submissions is integrated in Table 1. Further a written answer by the EU Commission (2008) related to Bt concentration in MON810 mainly offered information about stacked events. This was left aside here since in stacked events additional interactions between the traits might emerge in the plants.

In this short review, the authors look into the question of whether the data and methods available are sufficient to show that the level of Bt protein in the plants is generally consistent. Related issues such as environmental risk assessment will be discussed shortly.

Recent data on Bt content in MON810 question the consistency of Bt toxin levels

Two recent research projects in Germany studied the Bt toxin concentrations in MON810 plants. Nguyen and Jehle (2007) studied the Bt concentration in MON810 and Bt176 plants for three years at two sites in Germany and found the highest Bt concentrations in leaves. Across seasons, the average Bt concentration for MON810 ranged from 2.4 to 6.4 µg/g fresh weight in top leaves, and, from 3.8 to 5.7 µg/g fresh weight in bottom leaves, during the four development stages tested. The authors (Nguyen & Jehle, 2007) concluded that: "Although our studies corroborate the tendencies of reported Cry1Ab contents of MON810, a considerable variation in the expression levels of Cry1Ab was observed. The observed variation exceeds variation levels reported previously and may be due to the large number of analysed samples and different growing years. They suggest a certain plant to plant variation in Cry1Ab expression."

Besides Nguyen and Jehle (2007) only Greenpeace published findings on Bt concentrations in leaves from MON810 plants based on systematic investigations during different plant growth stages (Lorch & Then 2007). The analysis of 619 leaf samples from Germany and Spain taken during the 2006 growing season showed levels ranging from below the detection limit to 15 µg/g fresh weight. The report (Lorch & Then 2007): "The Bt concentrations in the plants that we sampled and analysed do not tally with the Bt content stated in the documentation used for the approval for commercial growing of MON810. They are much lower then the means given by Monsanto as part of the summarised data, and they show a very high variation in the field. The majority of MON810 plants only produce low amounts of Bt toxin."

Factors which can influence the level of Bt protein in transgenic plants

Both research projects (Nguyen & Jehle 2007, Lorch & Then 2007) show greater variability in their data than that published by Monsanto. This variability can be caused by several factors. In this context, one should distinguish factors affecting the method of measuring Bt proteins (see Table 1) from other factors, such as interactions between environment and genotype over time and location. We are not aware of any published research that systematically explores these impact factors.

Tab 1: Factors influencing or correlating with the Bt content in transgenic plants MON810.

Author	Factor: Impact
Abel & Adamczyk (2004)	- photosynthesis: Bt content and photosynthesis are positively correlated
Bruns & Abel (2007)	- nitrogen fertiliser: Bt content and nitrogen fertiliser are positively correlated
Griffiths et al. (2006)	- soil quality: can increase or decrease Bt content
	- pesticide use: spraying of insecticide increases Bt content in leaves and roots (pyretroid, Decis)
	- growing process: Bt content increases towards flowering
Nguyen & Jehle (2007)	- significant difference between two field sites in different climatic regions
	- growing process: Bt content in leaves increases during growing season

Table 1 gives an overview of factors thought to have some influence on or correlate with the Bt level in transgenic plants, as discussed in published literature. Several factors, such as climate, soil and chemicals applied (fertilizers and pesticides), are described as relevant in the existing literature.

Test methods used and their influence on determining Bt toxin levels

The use of different protocols may explain a significant part of the observed variability in Bt concentrations, certainly between the different studies (see Crespo et al. 2008). In a research project commissioned by Greenpeace (Greenpeace 2007), a laboratory was asked to compare different, commercially available ELISA methods for the quantification of Bt proteins. While findings were consistent within different protocols, the comparison between protocols showed surprisingly high variation. In relation to different parts of plants such as kernels, stems and leaves, findings varied between the different protocols by 5 to 100 percent. While the comparison of Bt concentrations in stems showed very similar results, kernels showed mean variations of 28 percent and leaves between 11 to 40 percent (related to upper or lower leaves). In this light, it is remarkable that no official ring testing has been done in the European Union so far – at least to our knowledge.

General discussion

Some experts (biosicherheit 2007a) believe that the findings published so far on Bt content in transgenic plants "are within a biologically explainable range." While this is likely to explain some of the reported variability, it does not address the most relevant questions. First of all, the actual range of variation is not yet known since the research needed to establish this has not been carried out to date – at least not in the public domain. Secondly, the findings known so far raise questions on resistance management, efficacy (very low levels of Bt in plants is eventually a quality control issue) and risk assessment (high levels of Bt in plants) (Lorch & Then 2007). In this context, the toxicological properties of the (truncated) Bt proteins expressed in plants should also be studied further (Hilbeck & Schmid 2006). This in turn also touches on the question of the general mode of action of the Bt toxin and how important co-factors are for its efficacy (Broderick 2006). Thirdly, given the number of possible factors listed in Table 1, it seems to be a matter of principle to understand more about the underlying mechanisms likely to influence the protein concentrations in transgenic plants before any further commercialisation of Bt plants is authorised. It might also allow us to learn more about transgene functioning in other GE plants.

In a broader context, these effects and mechanisms are also relevant regarding ongoing climate change, which can have an impact on GE plants in several direct or indirect ways. Changing soil and water conditions, especially in regions like Germany's State of

Brandenburg, for example, known to suffer dry and hot weather conditions in many seasons, can affect Bt content.

However, the importance of determining the actual range of Bt concentration in transgenic plants under different environmental conditions, and the factors which influence this, do seem to be accepted and understood by some experts and authorities. Commenting on the Nguyen and Jehle findings (2007), biosicherheit (2007b) wrote: "This finding highlights the importance of carrying out such experiments under local climatic conditions and with local varieties." Its regulatory importance was recently highlighted by the EU Commission draft decision on Bt11, where the issue was mentioned as being relevant for the market authorisation of this Bt maize (EU Commission 2007).

Conclusion

It is urgent to conduct further research in this area, especially to pursue the following:

- development of internationally accepted standard protocols to determine the Bt content in transgenic plants, evaluated in ring testing;
- systematic investigation of the impact of environmental factors on Bt expression levels in transgenic plants;
- systematic research on the impact of genetic/epigenetic factors on Bt expression levels in transgenic plants;
- review of strategies for resistance management in the light of very low and highly variable doses of Bt toxin in transgenic plants;
- review of environmental risk assessments for non-target organisms in light of the actual Bt content in transgenic plants and the possible impact of (co-)factors that can influence the toxicological qualities of Bt toxins and their metabolites.

The lessons learned and an increased understanding gained from such needed research would also help raise the standards of risk assessment applied by the European Food Safety Authority (EFSA).

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