Are Bt toxins killing aquatic insects?

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Contents

1. THE INCIDENT:

In an article of PNAS (Proceedings of the National Academy of Sciences, USA) it is reported that aquatic organisms are potentially harmed by residues and toxins of Bt maize, the paper does not convince in many aspects, as detailed below in a letter to the editor.

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 of the National Academy of Sciences of the United States of America, 104, pp 16204-16208
<Go to ISI>://WOS:000250128800043 10.1073/pnas.0707177104 AND
http://www.pnas.org/cgi/content/abstract/0707177104v2
AND http://www.botanischergarten.ch/Bt/Rosi-Marschall-Bt-Aquatic-2007.pdf

The abstract:

> *"Corn (Zea mays L.) that has been genetically engineered to produce the Cry1Ab protein (Bt corn) is resistant to lepidopteran pests. Bt corn is widely planted in the mid-western United States, often adjacent to headwater streams. We show that corn byproducts, such as pollen and detritus, enter headwater streams and are subject to storage, consumption, and transport to downstream water bodies. Laboratory feeding trials showed that consumption of Bt corn byproducts reduced growth and increased mortality of non-target stream insects. Stream insects are important prey for aquatic and riparian predators, and widespread planting of Bt crops has unexpected ecosystem-scale consequences."* (Rosi-Marshall et al., 2007)

In chapter 6 read about the most recent publications of an author group, lead previously by Prof. Galen Dively (Jensen et al., 2010; Swan et al., 2009d) and others, they contain rebuttals of the Rosi-Marshalls claims, mainly on grounds of a better baseline comparison of complex factors influencing aquatic organisms.

2. Summary

The news of potential harm of Bt crops to aquatic organisms has spread rapidly on many websites, Greenpeace also supports the arguments of the authors, and the former EU commissioner for the environment Stavros Dimas opposed the new maize traits from Pioneer and Syngenta on grounds of the precautionary principle and referring to a comment by Greenpeace on the above study: . http://uk.reuters.com/articlePrint?articleId=UKL2524238420071025

The critique can be summarized as follows (more details and literature below):

- There is extensive literature demonstrating that in the realistic concentrations Bt toxins do not harm aquatic organism.
- The authors extrapolated in a problematic way from a laboratory test to a field system based on a single study.
- The Bt source of the study is not specified, there is a possibility that Dipel, a Bt spray is also involved and there are several different Bt toxins which could be involved and even the natural Bt toxin of soil (bacteria) could have influenced the results.
- And in addition, the Bt maize involved in the study is not disclosed and later publications have shown that differences in various hybrid traits might influence the results *more* than the presence or absence of Bt-toxins in the litter. A clear baseline is lacking and even other pesticides might be involved.
- In two recent publications of (Swan et al., 2009d) and (Jensen et al., 2010) the paper of Rosi-Marshall has been contradicted in several major statements.

Summarized, it can be said that the study of Rosi-Marshall lacks the quality which is needed for such far reaching conclusions. Despite this, the study has been heavily promoted by groups opposing GMO crops without the necessary scientific justification.

3. Comments and letters to the editors of PNAS by panel of scientists

3.1. Letter of scientist panel to the editor of PNAS

A consortium of scientists signing this comment in a letter to the editors below has analyzed the paper and came to critical conclusions, which seriously question the conclusions of the paper.

*“We are deeply concerned by the appearance in PNAS of a recent article, "Toxins in transgenic crop byproducts may affect headwater stream ecosystems," (**10**,1073 (2007)), by (Rosi-Marshall et al., 2007) apparently funded by NSF. We recognize that it is not unusual for papers to be published with minor flaws or infelicities, even after peer review and revision, but the article by (Rosi-Marshall et al., 2007) contains egregious methodological flaws and omissions, and presents conclusions not supported by the data.*
We call your attention, in particular, to the following:

1) There is extensive evidence in the literature that corn pollen produced by currently available Bt corn varieties contain extremely low amounts of Bt toxin. This was shown in a series of six papers by top scientists published in PNAS after the Losey Bt corn pollen-Monarch debacle, an intensive and time-consuming effort to try to set the science straight (Hellmich et al., 2001; Oberhauser et al., 2001; Pleasants et al., 2001; Scriber, 2001; Sears et al., 2001; Stanley-Horn et al., 2001; Zangerl et al., 2001). How many busy scientists and how much scarce money will we need to divert to calm this new scare?

2) The authors extrapolated from a laboratory test to a field system based on a single study. Such extrapolation is problematic to begin with; not only did the authors lack the statistical confidence necessary for a valid extrapolation, in another venue (Pokelsek et al., 2007) they reported they did not find these effects in the field [including also Hydropsyche borealis], a salient fact not mentioned in the PNAS paper. This discrepancy should have been disclosed and discussed. In addition, earlier relevant studies concluded that Bacillus thuringiensis (Bt) endotoxin concentrations in aquatic systems are extremely low and are metabolized rapidly in water (Douville et al., 2007; Douville et al., 2005).

3) The title implies transgenic crops are the only source of Bt toxins, but endotoxins in commercial Bt insecticides such as Dipel, Xentari, Foray, and Thuricide are also used by farmers, including organic farmers, to control insects, and in some areas intensively. If the authors are measuring the effect of Bt toxin at all, how do they know the toxin comes from the transgenic Bt crops rather than from these organic Bt insecticides? If they lack data to distinguish the sources, isn't the term `transgenic' in the title simply gratuitous and sensationalistic?

4) The authors seem unaware that there are several variant forms of Bt endotoxin, as they failed to disclose which one(s) they were seeking and measuring. Toxicological studies use known quantities of known toxins, and look for a dose response. If their study included specific assays, they were not reported. If they were not conducted, the report was, at best, premature.

5) The authors do not disclose which Bt-corn isolines were tested. Different hybrids can differ significantly in both secondary metabolites and in antinutrient quantity (as well as in kind and amount of Bt toxin expressed). By not using isolines, they could have been seeing the effect of different concentrations in different hybrids of antinutrients or of other factors unrelated to Bt toxin. Similarly, the authors do not disclose quantitative measurements of tissue sampled, e.g., "Leaves were added… as needed." This lack of detail precludes others from replicating their study.

6) The authors conclude that growing Bt-corn may cause downstream adverse effects in waterways, but they fail to consider alternative explanations. Moreover, they analyze their results in a vacuum. In the real world, the choices are not `Bt-corn' versus `no intervention', and to imply that that is the case displays a remarkable ignorance of agriculture. Farmers grow more than one species and cultivar, and often use more than one pesticide strategy. For example, if a farmer were to control insects using conventional pesticides (that is, absent Bt corn plants), how would those pesticidal treatments affect caddisflies? For all we know, Bt corn may be environmentally preferable to traditional pesticides or other strategies to control insects. The authors imply otherwise without providing the comparative evidence.

The points above illustrate sloppy experimental design and interpretation that should have been detected by even a cursory peer review. Where were the crucial qualitative and quantitative data on source tissue, distinction of diverse types of Bt toxins, and discussion of alternate explanations for their results? We are at a loss to explain how qualified reviewers and editors could be unaware of flaws of this magnitude. Publication of this flawed paper has seriously jeopardized the credibility of PNAS as a high quality, scientific forum.

Sincerely,

Alan McHughen, Professor, University of California, Riverside.
Brian Federici, Professor, University of California, Riverside.
Henry Miller, M.D., The Hoover Institution, Stanford University.
Klaus Ammann, Prof. emerit. Delft University of Technology, the Netherlands
C. Kameswara Rao, Professor. Foundation for Biotechnology Awareness and Education, Bangalore, India.
Prof. Dr. Ingo Potrykus, Chairman, Humanitarian Golden Rice Board & Network
Dr. Piero Morandini, Dept. of Biology, University of Milan, Italy

C. J. Leaver, CBE, FRS, FRSE, Sibthorpian Professor of Plant Science,
University of Oxford, UK
S. Shantharam, Director, Biotechnology Education Programs, Asian Institute of Technology, Bangkok, Thailand
Mark Sears, University of Guelph, Ontario, Canada.
C. S. Prakash, Professor, Plant Molecular Genetics, Tuskegee University, USA"

Citations within the letter are moved to the end of the full text of the ASK-FORCE contribution.

Two of the undersigned have also written to the journal with similar contents, (Beachy et al., 2008; Parrott, 2008) and an answer has been published by (Rosi-Marshall et al., 2008), where the authors admitted that due to methodological flaws the results cannot be addressed properly to the environmental impact of Bt maize.

3.2. Letter of Beachy et al. published in PNAS

Two of the undersigned have also written to the journal with similar contents, (Beachy et al., 2008; Parrott, 2008) and an answer has been published by (Rosi-Marshall et al., 2008), where the authors admitted that due to methodological flaws the results cannot be addressed properly to the environmental impact of Bt maize.

Beachy, R.N., Fedoroff, N.V., Goldberg, R.B., & McHughen, A. (2008)
The burden of proof: A response to Rosi-Marshall et al. Proceedings of the National Academy of Sciences, pp 0711431105
openlink: http://www.pnas.org AND http://www.botanischergarten.ch/Bt/Beachy-Rosi-Marshall-Burden-2008.pdf

"To the Editor: A recent paper in PNAS (1) purports to show that insect-resistant crops have unexpected effects on nontarget insects in streams. A sentence in the Abstract reads ''Stream insects are important prey for aquatic and riparian predators, and widespread planting of Bt crops has unexpected ecosystems-scale consequences.'' The data presented in the paper do not support this statement. Because previous studies reported no significant effects on caddisflies (Glare & O'Callaghan, 2000), the topic of the present study leads the reader to reconsider the issue. However, the authors of the recent paper made fundamental errors in experimental design that make it impossible to draw the conclusion that Bt crops have impacts on aquatic insects: (i) They failed to use proper control materials, which would have to have been isogenic, nontransgenic tissues. It is well known that the chemical composition of leaves varies widely between different maize genotypes. It is possible that the claimed negative impacts on larval growth were attributable to chemical components in the tissue and not to the Bt protein. (ii) They failed to identify and to quantify the Bt protein, other leaf chemicals, and agricultural chemicals in stream waters, making it impossible to repeat the study or to draw conclusions from the data. Publications that report studies lacking appropriate controls and include unfounded summary statements on a topic such as this can cause significant damage. It is unfortunate that this paper, like the previous claim of effects on Monarch butterflies (Hellmich et al., 2001; Losey, 1999) is being used to fuel the contentious debate over the safety of genetically modified crops." (Beachy et al., 2008)

3.3. Letter of Wayne Parrott in PNAS

Parrott, W. (2008)
Study of Bt impact on caddisflies overstates its conclusions: Response to Rosi-Marshall et al. Proceedings of the National Academy of Sciences, pp. E10.
http://www.pnas.org AND http://www.botanischergarten.ch/Bt/Parrott-Rosi-Marshall-2008.pdf

"To the Editor: Ecological studies can help ensure that new biotechnologies provide maximum benefit while minimizing detrimental effects. Accordingly, a recent study (1) published in PNAS is appropriate but lacks the genetic and toxicological components necessary for proper execution and interpretation. The study used different maize hybrids. Because all maize hybrids differ in many traits, any trait that differs between the hybrids, e.g., the level of trypsin inhibitors present, could easily explain the results. Because isogenic lines were not used, it is impossible to attribute the observed effect to Bt as opposed to any other factor that differed.
The study assumed that pollen from currently grown Bt maize contains toxic levels of Bt when the levels in pollen are negligible (Mendelsohn et al., 2003) and innocuous (Hellmich et al., 2001). The presence and type of Bt toxin was never verified or quantified. If any Bt was present, the level administered to the larvae is unknown. Yet, dose–response measurements are key to establishing toxicity. Even if their results were really due to Bt, it is impossible to extrapolate with any confidence from an aquarium to a whole ecosystem where many more variables come into play. Given these limitations, the conclusion that ''widespread planting of Bt crops has unexpected ecosystem-scale consequences'' is untenable. The data cannot even support the more tentative conclusion that ''Bt corn byproducts may have negative effects,'' because no cause and effect was shown specific to Bt." (Parrott, 2008).

3.4. The answer of E. Rosi-Marshall: (Rosi-Marshall et al., 2008) in PNAS

Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., & Whiles, M.R. (2008)
Reply to Beachy et al. and Parrott: Study indicates Bt corn may affect caddisflies. Proceedings of the National Academy of Sciences, 105, 7, pp E11-E11
10.1073/pnas.0712192105 AND http://www.pnas.org/content/105/7/E11.short AND
http://www.botanischergarten.ch/Bt/Rosi-Marschall-Bt-Aquatic-reply-2008.pdf

"Beachy et al. (Beachy et al., 2008) and Parrott (Parrott, 2008) have questioned some findings reported in our recent paper (Rosi-Marshall et al., 2007); here, we respond to issues raised by these authors. All tissues identified as ''Bt'' in our paper (Rosi-Marshall et al., 2007) were verified to contain Cry1Ab protein by using Bt Cry1Ab protein Immuno-Strips (Agdia materials identified as ''non-Bt'' were similarly confirmed to lack Cry1Ab protein.
The quantity of Cry1Ab protein actually consumed (in pollen or leaf tissue) by an individual insect could not be determined because of variation in feeding rates among individuals in any particular experiment. Our goals for the research did not include developing a traditional dose–response relationship because (i) the dose depended on individual feeding rates, and (ii) a dose–response relationship would have little relevance in assessing the effect of Cry1Ab containing materials on actual stream ecosystems in which organisms select among multiple food resources, not all of which would contain Cry1Ab protein. The goal of our feeding experiments was to determine whether trichopterans were at all susceptible to the effects of Cry1Ab protein, not to determine a safe level of exposure in a toxicological context. Growth of trichopterans can be affected by many factors, including nutritional quality of food resources. As we stated (Rosi-Marshall et al., 2007) we paired ''Bt'' and ''non-Bt'' materials on the basis of nutritional quality (carbon: nitrogen ratios and lignin content). The use of isogenic hybrids would have resulted in food resources of different nutritional quality (Saxena & Stotzky, 2001b) and Cry1Ab content, and this would have confounded the experiments. We cannot fully disregard the unlikely possibility that some other leaf constituent was responsible for observed differences between the ''Bt'' and ''non-Bt'' treatments. However, we argue that the presence or absence of Cry1Ab protein is the most likely explanation for observed differences in trichopteran growth and mortality. We encourage others to pursue further research to develop a broader body of knowledge on the effects of Cry1Ab protein on aquatic insects.
We agree that extrapolation from laboratory experiments to ecosystems is unjustified without supporting evidence from field measurements. We (Rosi-Marshall et al., 2007) presented several lines of evidence suggesting that Cry1Ab-containing materials could potentially affect headwater stream ecosystems: (i) inputs of corn pollen and detritus to streams were documented and quantified, (ii) trichopterans collected from streams contained pollen in their guts or often were found associated with decaying corn detritus, and (iii) laboratory feeding trials indicated trichopterans are susceptible to the effects of Cry1Ab. Further study may reveal that the potential for detrimental effects is not realized in situ in streams or that effects are limited spatially or temporally and thus may not outweigh the benefits associated with the planting of Bt corn—only further study will reveal whether this is the case. Regarding the concern of (Beachy et al., 2008) and (Parrott, 2008) that the final sentence of our abstract overstated the conclusions of the paper, we agree that the sentence should

have articulated the potential for ecosystem-scale consequences within streams, rather than suggesting that such consequences were observed in situ.
Lastly, (Beachy et al., 2008). imply that our publication (Rosi-Marshall et al., 2007) and statements therein could ''cause significant damage.'' We are unsure what (Beachy et al., 2008) believe to have been significantly damaged. We argue that the wise use of any new technology requires a full understanding of both the benefits and the potential costs. In the case of corn genetically modified to express the Bt-endotoxin, the environmental costs appeared not to have been fully assessed, and we believe the studies we reported (Rosi-Marshall et al., 2007) contribute to a better understanding of potential effects on aquatic ecosystems. (Rosi-Marshall et al., 2008).

3.5. Comment on the reply of Rosi-Marshall

In their reply (Rosi-Marshall et al., 2008) admitted a few critical points and call for more research. However, their generalizations at the end are still not justified, since they do not take into account recent literature about Bt toxin impact on aquatic systems, and the *potential* effects described in this article merit further research *from the point of view of basic research*, but they clearly belong into the category of the "nice-to-knows", not really relevant to modern agriculture, since the real problem of the original paper is its *lack of a true baseline comparison*, namely a comparison to the situation *in reality* that one has to compare Bt maize fields and non-Bt maize fields with all its implications, and this means insofar as that a truly comparative toxicological research should include many other factors, such as fertilizer, pesticides and other cultivation factors, which would then lead to a more holistic view of the whole issue. This is certainly not the case in the paper of (Rosi-Marshall et al., 2007) - this is also acknowledged by the authors themselves. Regarding the argument, that the nutritional quality of leaves with a higher lignin content could have blurred the comparison – derived from a paper by (Saxena & Stotzky, 2001b) does not really convince, since the agronomic view would demand a full reality check, even if the nice-to-knows basic agricultural ecology insight could have demanded another procedural decision.

3.6. General comment

There is no space here for an extensive literature review on comparative experiments related to the ecological impact of Bt toxins, here just one typical example with abstract, it relates to soil biota:

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Andersen, M.N., Cortet, J., Messean, 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 thuringiens is CryIAb toxin. Plant and Soil, 275, 1-2, pp 135-146
<Go to ISI>://000233381600013 AND http://www.botanischergarten.ch/Bt/Griffiths-Comparison-protozoa-2005.pdf

"Field trials were established at three European sites (Denmark, Eastern France, South-West France) of genetically modified maize (Zea mays L.) expressing the CryIAb Bacillus thuringiensis toxin (Bt), the near isogenic non-Bt cultivar, another conventional maize cultivar and grass. Soil from Denmark was sampled at sowing (May) and harvest (October) over two years (2002, 2003); from E France at harvest 2002, sowing and harvest 2003; and from SW France at sowing and harvest 2003. Samples were analysed for microbial community structure (2003 samples only) by community-level physiological-profiling (CLPP) and phospholipid fatty acid analysis (PLFA), and protozoa and nematodes in all samples. Individual differences within a site resulted from: greater nematode numbers under grass than maize on three occasions; different nematode populations under the conventional maize cultivars once; and two occasions when there was a reduced protozoan population under Bt maize compared to non-Bt maize. Microbial community structure within the sites only varied with grass compared to maize, with one occurrence of CLPP varying between maize cultivars (Bt versus a conventional cultivar). ***An overall comparison of Bt versus non-Bt maize across all three sites only revealed differences for nematodes,***

with a smaller population under the Bt maize. Nematode community structure was different at each site and the Bt effect was not confined to specific nematode taxa. The effect of the Bt maize was small and within the normal variation expected in these agricultural systems*." (Griffiths et al., 2005).*

In essence this paper demonstrates the considerable effort which is necessary to come to true comparison within agricultural reality, the paper (Griffiths et al., 2005) works with randomized field plots, is extended over two seasons with preparations already starting the year before, and makes sure that every possible effort is made to take into account as many relevant agricultural and environmental factors as possible, thus assuring that the results have maximum agricultural and ecological relevance. Also the extensive field work of the ecology school of Swane demonstrates clearly the complexity of the task and a demand for prudent interpretation, omitting assumptions for the sake of maybe justified whistle blowing: (Ball et al., 2008; Cardinale et al., 2002; Swan et al., 2009a; Swan et al., 2009b; Swan et al., 2008; Swan et al., 2009c; Swan & Palmer, 2000, 2004, 2006a, b). (More comments see chapter 6)

3.7. Additional comment to fig. 3 in original paper: clear omission of the authors to comment on positive or at least neutral effects in their own paper

Figure 3 in (Rosi-Marshall et al., 2007) needs an additional comment about the potential ecological effects of *L. liba*: The mortality of *L. liba* when fed with mean ambient concentrations is *lower* for Bt maize compared to non-Bt maize – a fact which is hardly mentioned in the paper:

"In laboratory feeding trials, we found that the leafshredding trichopteran, Lepidostoma liba, had 50% lower growth rates when they were fed Bt corn litter compared with non-Bt corn litter (P<0.008; Fig. 3A), ***although mortality of L. liba among litter types did not differ."***

This statement is then clearly missing in the conclusions, because they do not fit into the picture of the main message the authors want to give, their own results documented in fig.3 are just ignored:

*"****Our results indicate that Bt corn byproducts may have negative effects on the biota of streams in agricultural areas****. Based on these findings, we suggest that the assessment of potential nontarget effects from transgenic crops should be expanded to include relevant aquatic organisms, such as stream insects"*

See below fig 3 B, in the caption just neutrally mentioned as "ecological effects" and if you do not read precisely that the authors are dealing with *mortality rates* you just do not get the message properly.

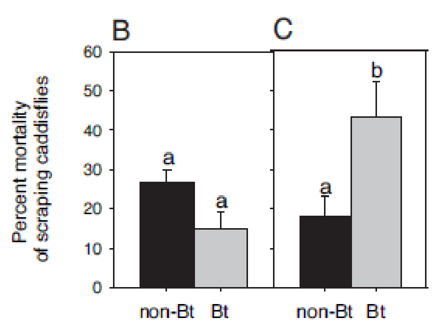

Fig. 1 Potential ecological effects of Bt corn. (*A*) Growth rates of the shredding caddisfly *L. liba* fed non-Bt and Bt corn leaves (*P* _ 0.008, Student's *t* test). (*B* and *C*) Mortality rates of the scraping caddisfly *H. borealis* when fed non-Bt and Bt corn pollen at mean ambient concentrations (0.055 gm_2) (*P*_ 0.42, Tukey's post hoc test) (*B*) and high concentrations (2.75 gm_2) (*P*_0.059; Tukey's post hoc test) (*C*). Error bars represent standard errors, and significant differences are indicated by different letters. From (Rosi-Marshall et al., 2007).

3.8. Omission of positive to neutral field data from a previous field experiment

Our comment in the letter to the editors of PNAS:

> *"The authors extrapolated from a laboratory test to a field system based on a single study. Such extrapolation is problematic to begin with; not only did the authors lack the statistical confidence necessary for a valid extrapolation, in another venue (Pokelsek et al., 2007) they reported they did not find these effects in the field [including also Hydropsyche borealis], a salient fact not mentioned in the PNAS paper."*

The answer given by Rosi-Marshall in the Nature feature of Rosi-Marshall (Waltz, 2009) (which the accused authors were not able to read before publication):

> *The paper was also accused of omitting contrary findings. In June 2007, four months before Rosi-Marshall's PNAS paper was published, Jillian Pokelsek, a master's student at Loyola University Chicago working with Rosi-Marshall, presented results from a preliminary field experiment at the annual meeting of the North American Benthological Society in Columbia, South Carolina. The work showed that Bt maize pollen did not influence the growth or mortality of filter-feeding caddis flies. The society posted an abstract of the presentation on its website attributing the work to Pokelsek, Rosi-Marshall, Tank, Royer and four other scientists who also authored the PNAS paper.*
>
> *It was not mentioning this study that prompted Miller and Ammann's accusation of misconduct. The authors defend the omission on the grounds that the data in the meeting presentation were not published or peer-reviewed, and were less reliable than those in the PNAS paper. "Field experiments are inherently difficult to control and have lower statistical power to detect significant differences compared with controlled laboratory experiments, thus we included the more controlled and statistically rigorous lab experiments in our paper," Tank and Rosi-Marshall told Nature.* ***Also, the caddis flies in the student presentation belonged to a different family, with different feeding mechanisms to those in the PNAS study.***
>
> *Miller's response: "I don't want to split hairs," he says. "If you don't do appropriate controls or if you draw conclusions that are erroneous, I think that's misconduct." But Ammann says he has a "bad feeling" about the accusation. "Maybe we should have been more careful with the wording."*

A careful checking of the published abstract of Pokelsek et al. (Pokelsek et al., 2007) reveals, that actually at least one species, *Hydropsyche borealis* was the same as the caddisflies analyzed by the study published in PNAS. Again, Rosi-Marshall et al. produce erroneous and inexact statements even in the reply to our letter to the PNAS-editors. Consequently, Ammanns (my!) feeling "is a bit less bad", considering all the facts. The results of the Pokelsek field study should have at least be mentioned, and if necessary, critically discussed and dismissed. Overall, it remains at least a suspicion, that unwelcome facts should not be mentioned in the PNAS original article. This suspicion is underpinned by comments in chapter 3.7 above.

4. Undue influence of the paper on the European GMO debate

4.1. The German Governmental decision about the Bt maize MON810 influenced by the paper

Also Minister of Agriculture of Germany, Ilse Aigner, did not hesitate to use this paper in her scientific justification of the rejection of the MON810 Bt Maize in Germany.

This premature decision is full of errors in its argumentation and does not really reflect the status of scientific research on the biosafety of Bt Maize,

http://www.botanischergarten.ch/Bt/BLV-BUND-mon_810_bescheid-20090417.pdf

Therein the comments on the impact of Bt toxins on aquatic ecosystems are likewise premature and they are based also on a superficial analysis of the original paper of Rosi-Marshall. German:

> *„Rosi-Marshall et al. (2007) wiesen nach [sic], dass beim Anbau von transgenem Mais Köcherfliegenlarven Bt ausgesetzt sein können. Es wurde auch gezeigt, dass bei Bt-Pollen-Dichten, die in der gleichen Größenordnung lagen wie sie im Feld*

vorliegen [sic], Köcherfliegenlarven auf Cry1Ab empfindlich reagierten (höhere Sterblichkeit und bis zu 50% längere Entwicklungszeiten). Trichoptera (Köcherfliegen), die Teil der meisten aquatischen Ökosysteme sind, spielen eine Hauptrolle in aquatischen Nahrungsnetzen und können in den meisten Binnengewässern gefunden werden.“
English:
“Rosi-Marshall et al. (2007) demonstrated [sic] that due to the growing of transgenic maize larvae of caddisflies can be exposed to Bt. It was also demonstrated, that the caddisfly larvae reacted sensitively to the Cry1Ab Bt toxin concentrations similar to those in the field [sic] (higher mortality and prolonged developing times up to 50%. Trichoptera (caddisflies) are part of most aquatic ecosystems and can be found in most headwater streams.”

4.2. Contradiction by the experts on biotechnology of the German government

The argument about aquatic organisms allegedly harmed by Bt toxins is clearly contradicted (among many other false, pseudoscientific arguments in this decision) by a report published beginning of July: http://www.botanischergarten.ch/Bt/BLV-ZKBS-MON810-20090707.pdf
The paragraph referring to the Rosi-Marshall paper is fully cited in German and English:

„(Rosi-Marshall et al., 2007):
Die Autoren haben Eintrag und Transport von Pollen und Ernterückständen aus Bt- und Nicht-Bt-Maisbeständen in feldnahe Gewässer gemessen. Zusätzlich wurden Pollen oder Rückstände von Mais, der das Bt-Protein exprimiert, in Laborversuchen an die sich detritivor ernährenden Larven der Köcherfliegenart Lepidostoma liba sowie an die sich filtrierend ernährenden Larven der Köcherfliegenart Hydropsyche borealis verfüttert. Im Ergebnis führte die Aufnahme von Bt-Mais-Pflanzenmaterial zu einer geringeren Wachstumsrate bei L. liba und die Aufnahme von Bt-Mais-Pollen zu einer erhöhten Mortalität bei H. borealis.
Die Verwendung von Köcherfliegenlarven als Testorganismen für das lepidopteren-spezifische Cry1Ab-Protein ist nachvollziehbar, da Köcherfliegen in relativ enger Verwandtschaft zu Schmetterlingen stehen. Effekte des Cry1Ab-Proteins auf Köcherfliegen wären somit denkbar.
Allerdings weist die Arbeit von (Rosi-Marshall et al., 2007) erhebliche methodische Schwächen auf. So wird die Quelle des Bt-Mais-Pollens und des Nicht-Bt-Mais-Pollens nicht angegeben. Unklar bleiben auch Sorte, Linie bzw. Isogenität der Bt-Mais-Einträge. Dosis-Wirkungsbeziehungen, wie für toxikologische Untersuchungen üblich, werden für das Bt-Protein nicht erhoben.
Außerdem wurde bei den Untersuchungen zum Eintrag von Pollen und Maisrückständen in die feldnahen Gewässer versäumt, deren Bt-Protein-Gehalte zu messen. Es wurden keine eindeutigen Unterschiede zwischen den Zersetzungsraten von Bt- und Nicht-Bt-Maisabfällen festgestellt. Es fehlen weiterhin Informationen zur potenziellen Exposition der Köcherfliegenlarven gegenüber dem Bt-Protein im Gewässer. Lediglich aus den in einer Abbildung gemachten Angaben zum Eintrag von Bt-Pollen in die Gewässer und den bekannten Gehalten von Bt-Protein in Maispollen lässt sich überschlägig kalkulieren, dass ein sehr geringer jährlicher Eintrag (9 – 90 ng/m² Wasseroberfläche) besteht. Auch die jährliche Menge an eingetragenem Pflanzenmaterial ist mit max. 8 g/m2 als gering einzuschätzen. In beiden Fällen sind die entsprechenden Mengen an Bt-Protein, auch bei kurzfristigem zeitlichen Auftreten der Expositionsquellen (Blühphase), angesichts des sogleich einsetzenden Proteinabbaues als vernachlässigbar für Köcherfliegenlarven BVL Stellungnahme der ZKBS zur Risikobewertung von MON810
einzustufen (Douville et al., 2007; Douville et al., 2005).
Fragen werfen auch die Fütterungsversuche mit der Köcherfliegenart L. liba auf. Die Herkunft der Bt-Maisblätter und der Nicht-Bt-Maisblätter wurde ebenfalls nicht angegeben. Es ist jedoch sicher, dass als Nicht-Bt-Variante nicht die Blätter einer isogenen Maislinie verwendet wurden. Begründet wird dies mit der Studie von (Saxena & Stotzky, 2001b), die bei ihren Versuchen in den Blättern der isogenen Bt-Linie einen zwischen 33 bis 97% höheren Ligningehalt fanden. Nach Auffassung der Autoren verschlechtert der höhere Ligningehalt die nutritive Qualität der Blätter. (Rosi-Marshall et al., 2007) wählten zum Vergleich daher Blätter einer anderen Maislinie mit einem im Vergleich zum Bt-Mais vermeintlich ähnlichem Ligningehalt und C/N-Verhältnis aus. Allerdings werden keine quantitativen Angaben über die Inhaltsstoffe (Lignin, C/N oder weitere) der in den Labortest verwendeten Maispflanzen gemacht. Aufgrund der nicht angegebenen Herkunft und der fehlenden Charakterisierung des verwendeten Pflanzenmaterials ist nicht auszuschließen, dass sich die in den

Fütterungsversuchen verwendeten Bt- und Nicht-Bt-Pollen bzw. Bt- und Nicht-Bt-Blätter nicht nur im Hinblick auf die Anwesenheit von Bt- Protein unterschieden. Zudem wurde die Konzentration von Bt-Protein im Blattmaterial nicht bestimmt. Auch die in den Fütterungsversuchen verabreichten Blattmengen wurden nicht angegeben (Zitat: „Leaves were added to aquaria as needed").
Die fehlende Standardisierung ist ein kardinaler Mangel, der vor allem für die von den Autoren durchgeführten Laboruntersuchungen gilt. Unabhängig davon misst die ZKBS den beobachteten in vitro Effekten auf Köcherfliegenlarven trotz u.U. großer Mengen in Gewässer eingetragenen Bt-Maismaterials geringe Bedeutung zu. Gründe für die Einschätzung sind folgende: Die natürliche Exposition von Köcherfliegenlarven gegenüber dem Bt-Protein in Gewässern, die an Bt-Maisfelder grenzen, ist sowohl räumlich (Abstand zu Gewässern und Verteilung von Maisfeldern in der Landschaft) als auch zeitlich (kurze Blütezeit) begrenzt. Darüber hinaus wird die potenzielle Exposition der Wasserorganismen erheblich eingeschränkt durch die geringen Mengen und Konzentrationen des Bt-Proteins im Pflanzenmaterial sowie dessen vergleichsweise raschem Abbau in Gewässern. Die im Labor beobachteten Mortalitätseffekte wurden nur bei unnatürlich hoher Exposition und nur einer Spezies gefunden. Zwar wiesen die Autoren im Falle von der Köcherfliegenart H. borealis eine signifikant erhöhte Mortalität bei Verfütterung von Bt-Maispollen im Vergleich zu Nicht-Bt-Maispollen nach, doch lag die Pollenmenge zwei- bis dreimal höher als der maximal gemessene jährliche Polleneintrag in ein Gewässer. In den Versuchen mit der Köcherfliegenart L. liba wurde keine erhöhte Mortalität bei Fütterung mit Pflanzenmaterial von Bt-Mais festgestellt, jedoch eine verminderte Wachstumsrate. Angesichts der in der Natur zeitlich beschränkten Exposition (Blühzeit des Mais) bei gleichzeitig meist niedrigerer Bt-Konzentration am Wirkort ist auch die im Laborexperiment gezeigte verminderte Wachstumsrate als nicht relevante Umweltwirkung einzustufen.
Fazit:
Die ZKBS stellt fest, dass in den Untersuchungen an Köcherfliegenlarven von Rosi-Marshall et al. (2007), nicht eindeutig der kausale Zusammenhang zwischen Bt-Protein oder der gentechnischen Veränderung und negativen Wirkungen hergeleitet wurde. Die Studie wurde auch von anderen Autoren hinsichtlich ihrer Durchführung und der getroffenen Schlussfolgerungen kritisiert ((Beachy et al., 2008); (Parrott, 2008). Die Autoren räumen selbst ein, dass sie nicht ausschließen können, dass Unterschiede zwischen den verwendeten Maissorten und nicht das Bt-Protein Ursache der beobachteten Wirkungen sind (Rosi-Marshall et al., 2008). Darüber hinaus stellt die ZKBS fest, dass die von (Rosi-Marshall et al., 2007) in Laborexperimenten erhaltenen Ergebnisse unter Berücksichtigung der anzunehmenden Exposition unter Freilandbedingungen nicht relevant sein dürften. Diese Schlussfolgerung wurde von den Autoren in ihrer Replik ebenfalls erwogen (Rosi-Marshall et al., 2008).

September 2, 2009: official English translation (ZKBS, 2009), the paragraphs regarding aquatic organisms:

"1. (Rosi-Marshall et al., 2007)
The authors measured the dispersion and transport of pollen and crop residues of Bt and non-Bt maize fields near headwater stream ecosystems. In addition, pollen and maize residues expressing Bt protein were fed in laboratory tests to detritivore larvae of the Trichoptera species Lepidostoma liba as well as to filtering larvae of the Trichoptera species Hydropsyche borealis. The authors concluded that the intake of Bt maize plant material led to a lower growth rate of the L. liba and the intake of Bt maize pollen to an increased mortality rate of the H. borealis.

The use of Trichoptera larvae as test organisms for the Lepidoptera-specific Cry1Ab protein is understandable, as Trichopterae are relatively closely related to butterflies. Thus, effects of the Cry1Ab protein on Trichoptera could be possible.

The work of (Rosi-Marshall et al., 2007) however, exhibits substantial methodological shortcomings. For instance, the source of the Bt maize pollen and the non-Bt maize pollen is not indicated. The type, line and isogenity of the Bt maize inputs remain unclear as well. Dose-effect relations, as usual for toxicological examinations, are not recorded for the Bt protein.

In addition, the authors failed to measure the Bt protein content of pollen and maize residues during the examination of the

dispersion into surface water near fields. No clear difference between the decomposition rates of Bt and non-Bt maize waste was observed. Furthermore, information on the possible exposure of the Trichoptera larvae to the Bt protein contained in headwater stream ecosystems is missing. However, from the authors data contained in a figure on the dispersion of Bt pollen into waters and the known contents of Bt proteins in maize pollen one can estimate that the annual dispersion is extremely low (9 - 90 ng/m2 water surface). With a maximum of 8 g/m2, the annual amount of dispersed plant material can also be regarded as low. In both cases, the respective Bt protein amounts can be regarded as negligible for Trichoptera larvae even in case of short-term occurrence of the sources of exposure (blooming period) and in view of the protein degradation that starts immediately (Douville et al., 2007; Douville et al., 2005).
Feeding tests with the Trichoptera species L. liba also raise questions. The origin of the Bt maize leaves and the non-Bt maize leaves is not indicated either. It is certain, however, that not the leaves of an isogenic maize line have been used as non-Bt variant. This was substantiated by the study of (Saxena & Stotzky, 2001b) who found a higher lignin content of between 33 and 97% in the leaves of the isogenic Bt line. In the authors' opinion, the higher lignin content deteriorates the nutritional quality of the leaves. Thus, (Rosi-Marshall et al., 2007) selected another maize line with an alleged lignin content and C/N ratio similar to that of Bt maize. Quantitative data on the constituents (lignin, C/N or others) of the maize plants tested in the laboratory, however, are not specified. Due to the missing data on the origin and the characteristics of the plant material used, it cannot be excluded that the Bt and non-Bt pollen and the Bt and non-Bt leaves did not only differ with respect to the presence of Bt protein. In addition, the concentration of the Bt protein in the leave material has not been determined. The leave amounts administered during the feeding tests have not been indicated either (Quote: "Leaves were added to aquaria as needed.").

The missing standardisation is a crucial deficit, which above all applies to the laboratory tests conducted by the authors. Irrespective of this fact, the ZKBS attaches little importance to the observed in vitro effects on Trichoptera, although possibly large amounts of Bt material might be dispersed into waters. This assessment is based on the following reasons: The natural exposure of Trichoptera larvae to Bt protein in headwater stream ecosystems adjacent to fields is limited both with regard to space (distance to waters and distribution of maize fields in the landscape) and time (short blooming period). Furthermore, the potential exposure of water organisms is substantially limited by the small amount and the low concentration of Bt protein in the plant material as well as its comparatively rapid degradation in waters.

The mortality effects observed in the laboratory have only been found in case of unnaturally high exposure and for one species only. Although the authors verified a significantly higher mortality rate of the Trichoptera species H. borealis when fed with Bt maize pollen compared to non-Bt maize pollen, the pollen amount was two to three times higher than the maximum annual pollen input into waters measured. During the experiments with the Trichoptera species L. liba, no increased mortality rate could be observed when fed with Bt maize plant material, but a reduced growth rate.

In view of the temporarily limited exposure period in nature (blooming period of maize) in line with the mostly low Bt concentration in natural habitats the reduced growth rate demonstrated in the laboratory can also be classified as non-relevant environmental effect.
Conclusion
The ZKBS states that the causal correlation between the Bt protein or the genetic alteration and the negative effects on Trichoptera larvae has not been sufficiently demonstrated in the publication of (Rosi-Marshall et al., 2007). The study has also been criticized by other authors with regards to its experimental execution and the conclusions drawn ((Beachy et al., 2008; Parrott, 2008). The authors themselves admit that they cannot exclude that the difference between the maize varieties used and not the Bt protein may have caused the observed effects (Rosi-Marshall et al., 2008). In addition, the ZKBS states that the results obtained by (Rosi-Marshall et al., 2007) within the scope of laboratory experiments cannot be regarded as relevant, when considering the estimated exposure under field conditions. This conclusion has also been considered by the authors in their reply to critical comments (Rosi-Marshall et al., 2008)

Also the EFSA (EFSA, 2007) commented and refuted the paper of (Rosi-Marshall et al., 2007) with clear words:

"In summary, the conclusions of the paper (Rosi-Marshall et al., 2007) are not supported by the data presented in this paper. The GMO Panel is of the opinion that based on the available information such a low level of exposure to Trichoptera in aquatic ecosystems is unlikely to cause a toxic effect."

4.3. Comments of Bruce Chassy December 2009

December 15 Bruce Chassy comments on the paper of Marvier et al. (Marvier et al., 2007) and also the publication of Rosi-Marshall et al. (Rosi-Marshall et al., 2007) in a blog of food technology [1], the e perspective: The paragraph referring to Rosi-Marshall:

> *"Santa Clara University Biology Professor Michelle Marvier and her colleagues have recently published a meta-analysis of field studies that concluded that Bt crops are generally more benign for non-target invertebrates than chemical insecticides. A second meta-analysis of lab studies found no harmful effects of Bt proteins on honeybees. Although these reports will probably fail to convince skeptics, they raise an important question: Can meta-analysis be used to tease meaningful results out of a series of studies that, taken individually, are inconclusive? Given the cost and methodological complexity of ecological studies, it's an important question.*
>
> *The answer is affirmative only if the studies analyzed are sufficiently well designed and conducted to yield useful data. The need to discard or discount flawed studies presents a constant challenge to meta-analysts. Even when carefully selected criteria for inclusion or exclusion of a data set are stated a priori, researchers are accused of bias when they exclude a study that seems to favor one point of view over another.*
>
> *If we are to avoid the GIGO—garbage-in garbage-out—effect in meta-analysis, incomplete and/or flawed studies must be excluded. A perfect example is the study reported in PNAS by Rosi-Marshall et al. (2007) which claimed to show that pollen from Bt-maize was injurious to caddisflies in a laboratory aquatic ecosystem, but was flawed in numerous ways. For example, pollen produced by currently available varieties of Bt-maize contains very low concentrations of Bt toxin. In addition, the authors extrapolated from a laboratory experiment to a field system based on a single study, an extrapolation that is problematic, especially given that they used pollen in doses higher than the maximum encountered under field conditions. Possibly the most damning of all is that they reported elsewhere that they had failed to find these effects in the field"*

4.4. Comments on a paper by Griffiths 2009 on the influence of aquatic microbial respiration possibly influenced by nearby grown Bt maize

In an extensive study, (Griffiths et al., 2009) reported Bt maize leafes having a faster decomposition rate in the studied headwater streams than non-Bt maize. Microbial decomposition rates did not differ between non-Bt and Bt maize, and decomposition rates were not negatively affected by genetic engineering, most likely because the Bt toxin does not adversely affect the aquatic microbial assemblage involved in maize decomposition. They also found that the caddisflies studied by (Rosi-Marshall et al., 2007) were depauperate in these agricultural streams, and most likely did not play a role in maize decomposition.

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[1] Bruce Chassy: Can Meta-analysis Help Biosafety Research? http://foodtecheperspective.wordpress.com/2009/12/15/can-meta-analysis-help-biosafety-research/

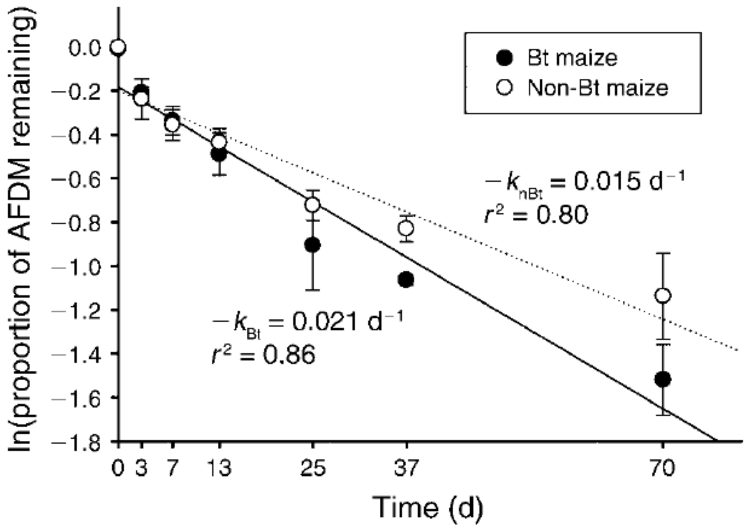

Fig. 2 Decomposition of Bt maize and non-Bt maize in three agricultural streams over a period of 70 days. The decomposition coefficients (_kBt ¼ 0.021 d_1 and _knBt ¼ 0.015 d_1) differed significantly between Bt and non-Bt maize (nested ANCOVA: F1, 108 ¼ 7.95, P ¼ 0.006). Data are means 6 SE from replicate streams (n¼3 streams per data point); AFDM is ash-free dry mass.

4.5. Paper by Ricroh 2010 demonstrating the undue influence of flawed papers on the regulatory processes in Europe

(Ricroch et al., 2010) published an analysis summarizing the undue influence of questionable publications and statements by NGOs on the regulatory process in Europe:

> *"We have examined the justifications invoked by the German government in April 2009 to suspend the cultivation of the genetically modified maize varieties containing the Bt insect-resistance trait MON810. We have carried out a critical examination of the alleged new data on a potential environmental impact of these varieties, namely two scientific papers describing laboratory force-feeding trials on ladybirds and daphnia, and previous data on Lepidoptera, aquatic and soil organisms. We demonstrate that the suspension is based on an incomplete list of references, ignores the widely admitted case-by-case approach, and confuses potential hazard and proven risk in the scientific procedure of risk assessment. Furthermore, we did not find any justification for this suspension in our extensive survey of the scientific literature regarding possible effects under natural field conditions on non-target animals. The vast majority of the 41 articles published in 2008 and 2009 indicate no impact on these organisms and only two articles indicate a minor effect, which is either inconsistent during the planting season or represents an indirect effect. Publications from 1996 to 2008 (376 publications) and recent meta-analyses do not allow to conclude on consistent effects either. The lower abundance of some insects concerns mainly specialized enemies of the target pest (an expected consequence of its control by Bt maize). On the contrary, Bt maize have generally a lower impact than insecticide treatment.* ***The present review demonstrates that the available meta-knowledge on Cry1Ab expressing maize was ignored by the German government which instead used selected individual studie****. (Ricroch et al., 2010).*

4.6. Comments on activities of NABU (Naturschutzbund Deutschlands e.V.)

It is not a coincidence, that NABUS as an anti-GMO-activist group invited Rosi-Marshall and her colleague to a heavily publicized event for the presentation of their PNAS results. They had hopes that this would influence negatively the regulatory situation even more on Bt maize in Germany (which is hardly possible…).
The announcement of Steffi Ober, the biotechnology expert of NABUS, announces this with clear words:

4.6.1. Announcement of NABUS event on impact of Bt toxins on aquatic systems in Germany, January 14, 2010

Translation to English:

"The insecticide Bt toxin from genetically modified corn can reach over pollen distribution, or also by littering of harvested corn in streams, rivers and lakes. This hotly debated research results by the American research group of Rosi-Marshall will now for the first time be presented in the German Republic during a NABU-session. "Waters are often directly situated as head streams in the agricultural landscape. In Brandenburg we will also find water holes in the middle of corn fields. Water-rich states such as Brandenburg are interested directly to know the size of risk exposure to Bt corn, " said NABU President Olaf Tschimpke. "So far, the issue of water in biological safety has been ignored. The need to correct this omission by the responsible regulators is now urgent," demanded Tschimpke.

Emma Rosi-Marshall of the Institute of Ecosystems at Millbrook and Jennifer Tank of the University of Notre Dame in Indiana are so far the only people in the world who have researched the topic of Bt toxin impacting water ecosystems [sic!]. Their investigation focused on twelve typical head streams in the midwestern United States. They found out among other things, that caddis fly larvae have been harmed by the Bt toxin [sic!]. Also Germany will now investigate this summer on how much pollen and maize litter at harvest in the fall will impact the water systems. This research project is for the first time presented publicly by the Federal Office for Nature Conservation and also by the Land Brandenburg at the NABU-session. What role the impact of Bt toxin plays in the ecosystem will be further discussed although based on a thin factual basis due to lack of research data.
European and U.S. authorities have persistently ignored open questions presented by Nature protection and consumer organizations about the risks of genetically modified maize, even after alarming studies such as those of Rosi-Marshall and Tank have been published. "This behavior is irresponsible and outrageous," said gene expert from NABU Steffi Ober. She continued: "Instead of conducting product research for the industry to optimize the gene transfer, biosafety research should finally run research based on the facts of real experience in order to protect nature and consumers."
Similar text still on the NABU website under
http://www.nabu.de/modules/presseservice/index.php?popup=true&show=1604&db=presseservice

A more detailed text on the impact of Bt toxins on aquatic systems on the NABU website:

4.6.2. NABUS: Is transgenic maize a threat to our waters?

New knowledge on the impact of genetically modified maize
The US-American researchers Rosi Marshall (now Cary Institute) and Jennifer Tanks (University of Notre Dame, Indiana) are studying 12 headline streams for several years in the corn belt of the Middle West of the USA. On 90% of the surface maize is grown, nearly 50 percent of them are BT varieties (Rosi-Marshall et al., 2007). The BT toxin enters the waters via maize pollen and maize leafs, decomposed by aquatic organisms such as caddis flies larvae. By feeding they are shredding small maize fragments or scrap deposits. Both US scientists suspect the digestion of BT toxin by the larvae of caddis flies and other

organisms in the field causes a significantly reduced performance. As additional stressor the toxin could affect the regeneration of already pre-loaded water ecosystems, so their thesis.

Retarded growth in caddis fly larvae

Butterfly larvae stressed by ingesting BT pollen develop more slowly.

BT pollen has been detected in field in the stomach 50 percent of the surveyed caddis fly larvae. This is evidence that caddis fly larvae actively take in maize pollen. In order to explore the effect of Bt toxin Bt pollen have been fed on Lepistoma liba larvae. The result is food for thought: growth was significantly slowed. Another attempt with an algae feeding experiment with Trichoptera species Helicopsyche borealis showed increased mortality if the larvae took up BT toxin in elevated concentrations. This shows the same pattern as other investigations on butterflies, which were made by German scientists (including (Felke & Langenbruch, 2005) and (Lang & Vojtech, 2006). The researchers pointed to butterfly larvae stressed by including even low doses of BT pollen develop on a slower rate and thus are vulnerable to feeding enemies and diseases on a higher level.

Emma Rosi-Marshall's and Jennifer Tank's balance sheet:

- *That in the lab with the slightly higher BT concentrations than the open a reduced growth and increased mortality of caddis fly larvae can be seen.*
- *That significant input of maize pollen and litter has been shown in the field, as well as high concentrations of BT toxin in the headstreams have been demonstrated. This can be explained insofar as the toxin is contained in the whole plant and significant amounts of pollen and litter are blown after flowering and washed after harvest in the soil and the nearby waters. The toxin is then released by decomposition.*
- *A comprehensive examination of the headwater streams showed: all waters were burdened in the BT maize growing region with the BT toxin. Already after a decade of BT maize cultivation, the BT toxin was spread all over the places.*
- *In contrast to the spray application of BT toxin, which leads to short-term pressures, the waters of the Bt corn growing region are fed with the toxin throughout the year.*
- *That field testing carried out showed no significant effects, which may be because of the strong load of water with pesticides superimposed on possible effects. Also reference waters without toxin exposure are missing, which means that there are no niches for sensitive species and individuals in the water ecosystems in the region.*

BT toxin in all waters present

In the USA only the cobs are harvested.
A major difference in the cultivation of maize here is, that only the cobs are harvested in the United States, the remaining plants are left in the field. In Germany most of the remaining corn plant is chaffed for silage, keeping only a small remnant of the corn plant in the field. In addition, our own headwaters near maize fields are in a better ecological condition compared to the surveyed areas in the USA.
The typical cultivation system of BT maize and genetically modified soya leads to a high input of pesticides and fertilizers in soil and water. Interestingly enough Rosi Marshall and Jennifer Tank can demonstrate in their still unpublished study that BT toxin can be detected in all headwaters.

Risk assessment so far ignored
The two women researchers stressed that water ecosystems has been previously systematically ignored in the risk assessment studies of BT maize, therefore important relevant data are missing and more studies are required. This means that one should quantify the impact of Bt Toxin from maize pollen and litter on so-called non-target organisms, which should not intentionally be damaged such as the European corn borer.

New research project in Brandenburg

The Lakes of Brandenburg are the habitat of the water lily borer (Seerosen-Zünsler), closely related with the maize borer. In order to learn more, a research project of the Federal Office for nature conservation runs from 2009 to 2011 with the State of Brandenburg, which examines the risks of BT maize cultivation to the aquatic environment in Brandenburg. To estimate on how much BT toxin reaches the waters it is necessary quantify how much litter is reaching the pre-flooders in autumn. Other factors include the drift of litter from the field into the adjacent waters during the winter, and transport dynamics in the streams and subsequent deposition and accumulation in the lakes. There will also be additional input through Bt maize pollen drift. There are also expert workshops dealing with the question of what effects the BT toxin will have on water ecosystems.
In the lakes of Brandenburg, the brown china mark (Seerosen-Zünsler) exists, a close relative to the European corn borer who is the target of Bt endotoxin strategies. The project is coordinated by NABU expert Steffi Ober.
Translation by Klaus Ammann of German text on NABU website see
http://www.nabu.de/themen/gentechnik/biologischeVielfalt/11966.html

4.6.3. Comments by Klaus Ammann:

Although I wrote a detailed including a previous but detailed version of the ASK-FORCE blog on the PNAS-publication of Rosi-Marshall et al. 2007 to Steffi Ober, there seems to have been no reaction and most probably no reading of my texts and the critic (Steffi Ober actually remained silent, not reacting to my objections). Reading the comments of NABU, the political stance to avoid planting of Bt maize in Germany is quite obvious.
This is why I repeat here the most blatant mistakes in the NAUB website texts, which should be corrected:

- There has been an extensive literature, even before the team of Galen Dively went public with statements that the impact of Bt toxins on aquatic systems must be minimal.
- Risk assessment consequently did not ignore the impact of Bt toxins on aquatic systems.
- It is, even by measures of the PNAS publication of Rosi-Marshall clear that under realistic concentrations, the feeding experiments did not show any detrimental effects of Bt toxin on caddis flies.
- With the data produced by the authors, it is not possible to distinguish between the impact of Bt sprays and Bt endotoxins, the source of Bt toxins in the published study is still in the dark.

4.7. Comments on the complexity of Bt food webs

There is no doubt, that the regulatory debate on Bt toxicity requires a set of research measures, which is best described by papers like (Raybould, 2007; Raybould et al., 2007; Romeis et al., 2008). They describe with precision on how such field work needs to be organized, but they also distinguish between the necessary questions for regulatory clarification required by the law and further detailed studies, which are of high interest for agricultural ecology, but with little relevance to environmental safety in real agricultural systems. Papers like the ones mentioned for aquatic ecology (Jensen et al., 2010), (Griffiths et al., 2009), (Jensen et al., 2010) and (Swan et al., 2009d) here depict the complexity, without indulging into negative assumptions exploiting an exaggerated principle of precaution. Still, it is difficult to draw the line between nice-to-knows and need-to-knows. Alone the long neglected influence of bacterial activity on leaf surfaces adds to a multitude of possible factors regulating the speed of leaf decomposition, as has been shown already by (Findlay & Arsuffi, 1989). As a further example it is good to take into account the meticulous ecological analysis and modeling of (Mulder & Lotz, 2009). They explored the energy flow of any (terrestrial) food web under stress, which incorporates several factors and pooled information on ecosystem services and on the different responses of soil invertebrates to induced perturbations in other trophic levels. This kind of dynamic complexity invites clearly for introducing bias in research methodology, as has been clearly shown by (Romeis et al., 2004) and (Shelton et al., 2009a).

5. Discussion of the Nature article “Battle Fields”

The News Feature was published September 3 with the headline:

> *“Papers suggesting that biotech crops might harm the environment attract a hail of abuse from other scientists. Emily Waltz asks if the critics fight fair.” (Waltz, 2009)*

Waltz, E. (2009)
Battle Field, News Feature. Nature, 461, pp 27-32
Open source: http://www.nature.com/news/2009/090902/pdf/461027a.pdf AND
http://www.botanischergarten.ch/Discourse/Waltz-Batte-Fields-2009.pdf

It is only between 2005 and 2009 that a certain fatigue of new negative arguments against GM crops is developing, and it is interesting to note that opponents now shift their emphasis on negative arguments in socio-economics. But this might also be the reason why in a desperate routine of repetitious 'negative' GM crop stories get into journals, often also on rehashed events which have been clearly rebutted scientifically many years before. Those 'news stories' often pass uncontested and get printed in "news"-media due to a mix of short memory effects of uninformed people of all kind, or worse: they are purposefully repeated by activists counting on short memory of press and public. And the major journals cannot be excepted here as has been shown (and up to now not contradicted) by (Miller et al., 2008). There seem also to exist economic incentives to publish a text which create economic incentives, a tendency which can lead to boulevardization of scientific journals. A strange side effect should be mentioned here: scientists, who defend good science in biosafety research, sometimes get blamed because they use straightforward language when criticizing flawed papers.
The most recent example is this news feature published by Nature (Waltz, 2009), it will be discussed below in some major elements:
There are several controversial hints in this Nature story discussed here put forward by a science journalist not specialized, nor experienced in the hot scientific regulatory debate on GM crops, suggesting that to criticize flawed papers with strong language is detrimental to the progress of scientific research.
Emily Waltz, the author of the news feature in Nature, contacted all the mentioned scientists (Henry Miller, Robert Wager, Bruce Chassy, Wanye p about 3 weeks before she went to print, asking them all about their comments, and she, by requesting the comments, promised to come back with her interviews. All the scientists blamed for strong language have given Waltz the requested comments about her preliminary statements, many of them with several extensive mails including lots of citations and factual details, and the answers were not coordinated among them. Emily Waltz had some two weeks to reply, but there was silence after those suggestions altogether. It was therefore very disappointing to all of us to see her comments unchanged and a feedback to many of our remarks was totally lacking.

5.1. On omissions by Rosi-Marshall of their own fieldwork showing no detrimental effects of Bt toxins to aquatic organisms

Our original comment in the letter to the editors of PNAS cited above:

> *"The authors extrapolated from a laboratory test to a field system based on a single study. Such extrapolation is problematic to begin with; not only did the authors lack the statistical confidence necessary for a valid extrapolation, in another venue (Pokelsek et al., 2007) they reported they did not find these effects in the field [including also Hydropsyche borealis], a salient fact not mentioned in the PNAS paper."*

The answer given by Rosi-Marshall in the Nature feature of Rosi-Marshall (Waltz, 2009) (which the accused authors were not able to read before publication):

> *"The paper was also accused of omitting contrary findings. In June 2007, four months before Rosi-Marshall's PNAS paper was published, Jillian Pokelsek, a master's student at Loyola University Chicago working with Rosi-Marshall, presented results from a preliminary field experiment at the annual meeting of the North American Benthological Society in Columbia, South Carolina. The work showed that Bt maize pollen did not influence the growth or mortality of filter-feeding caddis flies.*

The society posted an abstract of the presentation on its website attributing the work to Pokelsek, Rosi-Marshall, Tank, Royer and four other scientists who also authored the PNAS paper.
It was not mentioning this study that prompted Miller and Ammann's accusation of misconduct. The authors defend the omission on the grounds that the data in the meeting presentation were not published or peer-reviewed, and were less reliable than those in the PNAS paper. "Field experiments are inherently difficult to control and have lower statistical power to detect significant differences compared with controlled laboratory experiments, thus we included the more controlled and statistically rigorous lab experiments in our paper," Tank and Rosi-Marshall told Nature. ***Also, the caddis flies in the student presentation belonged to a different family, with different feeding mechanisms to those in the PNAS study.***
Miller's response: "I don't want to split hairs," he says. "If you don't do appropriate controls or if you draw conclusions that are erroneous, I think that's misconduct." But Ammann says he has a "bad feeling" about the accusation. "Maybe we should have been more careful with the wording."

A careful checking of the published abstract of Pokelsek et al. (Pokelsek et al., 2007) reveals, that actually at least one species, *Hydropsyche borealis* was the same as the caddisflies analyzed by the study published in PNAS. It is amazing: Again, Rosi-Marshall et al. produce erroneous and inexact statements even in the reply to our letter to the PNAS-editors and the editing journalist Emily Waltz did not carefully check her statements: Consequently, Ammanns (my!) feeling "is a bit less bad", considering all the facts. The results of the Pokelsek field study should have at least be mentioned, and if necessary, critically discussed and dismissed. Overall, it remains more than a suspicion, that unwelcome (positive!) facts should not be mentioned in the PNAS original article. This suspicion is underpinned by comments in chapter 3.7 above about fig. 3 in Rosi-Marshalls own publication.

5.2. Calling on experts with a pronounced negative attitude towards GM crops and discrediting strong language

It is only between 2005 and 2009 that a certain fatigue of new negative arguments against GM crops is developing, and it is interesting to note that opponents now shift their emphasis on negative arguments in socio-economic realms. But this might also be the reason why in a desperate routine of repetitious 'negative' GM crop stories get into journals, often also on rehashed events which have been clearly rebutted scientifically many years before. Those 'news stories' often pass uncontested and get printed in "news"-media due to a mix of short memory effects of uninformed people of all kind, or worse: they are purposefully repeated by activists counting on short memory of press and public. A strange effect should be mentioned here also, related to the Nature battlefield article: scientists, who defend good science in biosafety research, sometimes get blamed because they use straightforward language when criticizing flawed papers, see a typical example in (Shelton et al., 2009b).
There are several controversial hints in this Nature story put forward by a science journalist not specialized, nor experienced in the hot scientific regulatory debate on GM crops, suggesting that to criticize flawed papers with strong language is detrimental to the progress of scientific research. This statement was supported by interviewed writers such as Chapela[2] and Schubert[3] who defend independent scientific whistle blowers, but who themselves have a proven negative agenda about GM

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[2] See the blog of Rowell and comments at the end of K.Ammann: http://www.botanischergarten.ch/Mexico/Rowell-Immoral-Maize-20090427.pdf

[3] GMO Pundit Schubert and Answer by David Tribe: http://gmopundit.blogspot.com/2006/02/david-schubert-alleges-systematic.html

crops, and in the case of Schubert often do not really dig into the rich and available food safety literature (Schubert is a pharmacist); this is well documented by some additional publications on the controversy in Nature Biotechnology: (Bradford et al., 2005a; Bradford et al., 2005b; Schubert, 2005), most probably unknown to Emily Waltz. The real blow about Schubert's credibility as a critic of GM crop breeding is coming from another two rebuttals published in Nature Biotechnology (Avery, 2002; Beachy et al., 2002), the authors dismantle Schubert as spreading flawed scientific arguments and also accuse him of ignoring peer reviewed literature contradicting diametrically his statements. Schubert tries to apply regulatory regimes developed in the pharmaceutical industry to the regulation of GM food, which is scientifically questionable. It is detrimental to the reputation of Nature to let pass such questionable calls on "experts", see about this problem also in (Durodie, 2003).

Related to Chapela a few remarks: The latest controversy on gene flow of Mexican maize starts a renewed debate on the reliability of PCR texts for the detection of GM maize (E. BITOCCHI, 2009; Marris, 2005; Mercer & Wainwright, 2008; Mijangos-Cortés et al., 2007; Ortiz-Garcia et al., 2005a; Ortiz-Garcia et al., 2005b; Ortiz-Garcia et al., 2006; Pineyro-Nelson et al., 2009; Rowell & Comments Ammann K., 2009; SCHOEL & FAGAN, 2009; Snow, 2009; Soleri et al., 2005) and Chapela does not really make there a convincing figure. His strong anti-GM-crop agenda is there well documented.

In the meanwhile, several letters to the editor have been written commenting the feature of Emily Waltz in Nature [4], the majority not supporting her thesis.

Incidentally: Strong language has been used before in the history of science, remember some really bitter and hefty disputes about the history of discovery of the double helix structure of DNA between Watson and Crick (Friedberg, 2007), who both later made their peace again.

Other numerous examples of a fight out in the open are documented about evolution when Darwin proposed his revolutionary ideas. Two citations of strong language may suffice: In a debate on natural selection (Punnett, 1928) writes on a dispute with William Bateson:

"By these admission almost the last shred of that teleological fustian with which Victorian philosophy loved to clothe the theory of evolution is destroyed. Those who would proclaim that whatever is right will be wise henceforth to base this faith frankly on the impregnable rock of superstition and to abstain from direct appeals to natural fact."

Another clear example of sharp and relentless scientific controversy with strong language has been described in detail by Strick (Strick, 1999), among the numerous juicy examples:

"His [Bastian's] tone was sharp in response to Huxley's public accusations that his technique was sloppy (a much more high-powered attack than Huxley ever adopted in private when attempting to correct young scientists). Huxley replied with an equally sharp tone, now saying sweepingly that "what Bastian got out of his tubes was exactly what he put into them," i.e. contaminants".

And one last word about strong language: The word "abuse" has been printed by Nature in the Battlefield paper (Waltz, 2009) very prominently in the subtitle, when attacking a group of authors included me who criticize flawed papers in the GM crop debate with harsh words – what an irony! – And to be quite clear: no complaints from my side….

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[4] Letters to the editor about the featue in Nature of Emily Waltz: http://www.botanischergarten.ch/Discourse/Waltz-Battle-Field-Letters-2009.pdf

5.3. Some letters to Nature on the subject

5.3.1. Piero Morandini, University of Milano, Italy

"I am one of the people who published a critical commentary of the Rosi-Marshall paper (see ref. 9 above (Miller et al., 2008)). Here are my further comments about the piece above.

The main reason for the strong criticism of papers like Rosi-Marshall is exactly about bad science with major policy implications.

If I publish a paper about sex determination in asparagus that has no interest beyond the scientific circles, then it does not damage anybody beyond these circles if it is wrong and the damage is mild. On the contrary, when a paper claiming problems with transgenic plants is published, it may be used by people opposed ideologically to ag-biotech.

We have seen this in the past with Loosey, Chapela and others. These papers have been used or are used to prevent introduction of the technology in several European countries and, as a consequence, in several other countries in the third world. Even if the content of the papers is later dismissed as irrelevant or wrong, the claims are still propagated for years. Papers that could have an influence on policy and regulation for years must be screened by editors and referees with the utmost care first and then by the scientific community at large once they have been published.

The scientific community has the moral obligation of looking more carefully into matters which could impact many more people, both positively or negatively.

A truly constructive criticism in science must have one aim: seek the truth.

The role of peers (referees and later colleagues who read the paper) is to help authors to do this. It is a matter of humility to submit your own research results to the scientific world. If you can't stand this sort of criticism, you are loving your results more than the truth. Sometimes the wording of the criticism may be more or less pleasing (whoever has received referees comments on a manuscript knows it very well). Things lived with passion bring often excesses in feelings. But what matters more is the end result.

These papers do not "alert us to possible reasons to look into this more carefully". A research badly designed, for instance with no proper controls or with unreasonable doses completely out of real life range or situation is simply irrelevant and does not advance our understanding and may even be misleading further research. A wonderful example of this is the data accumulated on synthetic substances with test for carcinogens made at high dose in the 70s-80s. They turned out to be deadly wrong and said nothing about the effect at the real doses we are exposed to. Rather than alert about possible reasons to look more into this, they concealed the reality for many years because people did not do the proper control with natural substances (which had the same frequency, 60%, of carcinogens as synthetic substances).

Bad science caused bad regulation and unnecessary spending as well as useless research. Try interview Bruce Ames (professor of Biochemistry at Berkeley) on this or read some of his pieces:

http://potency.berkeley.edu/pdfs/Paracelsus.pdf or a video: http://www.bruceames.org/bnalect.cancer1.php

Looking at a transgenic corn causing a reduction on non target organisms without comparing the effect of a conventional corn treated with insecticides is a non real world situation. In Italy this year we are experiencing a strong outbreak of root corn borer. They are treating with insecticides but nobody measures the effect on biodiversity.

I see much more depressing for scientists spending years on developing a new product to see thir research fields destroyed or their products not brought to the market because of insane regulatory burden.

These regulations, especially in the EU, are fuelled by bad science and ideological opposition.

Golden rice is a prime example of unjustifiable delay. I know of several otherproducts with real benefits which never made it to the market."

P. Morandini, Dept. of Biology, University of Milan (Italy), 04 Sep, 2009

5.3.2. Robert Wager

"It was with surprise that I read Emily Waltz 's article "Battlefield". Waltz used the word "attack" four times in the article yet never once put forward evidence of anything other than challenges to the science methodology, interpretation, incomplete citations, over reaching conclusions etc. Attempting to claim victim status is a very successful method used to avoid the real issue, in this case the BAD Science. I was left wondering if the intent of the article was to quiet the scientific criticisms. As far as I can determine the scientific criticisms were right on the mark. It is too bad Emily Waltz and Nature did not appear to understand this point. When poor quality science goes unchallenged we all lose."

Robert Wager Vancouver Island University, Nanaimo BC Canada robert.wager@viu.ca, 07 Sep, 2009

5.3.2. Jens Katzek

SIR, You misidentified the victims in your News Feature on conflicts among scientist over genetically modified crops (Nature 461, 27-32, 2009). The real victims on this battlefield are not the handful of people critizised for their research, but those scientists who want to realize the potential of plant biotechnology and the farmer who apply authorized products. These people have to endure bomb threats, insulting letters and telephone calls, destruction of their fields (almost no UK field experiment has survived since 2000) and harassment of their children at school. As author of a UK Food Standards Agency report concluding that organic food provides no additional nutritional or health benefit, Alan Dangour was bombarded with hate mail from activists.

The whole biotech debate is an emotionalized mess fuelled by lobbyists and societys zero risk mentality. Scientists should not be wary of publishing their results just because they could be deliberately misinterpreted. But they must be vigilant. As Kai Diekmann, chief editor of BILD, the largest newspaper in Germany, said in a recent television broadcast: More than 10 Million readers is a huge responsibility. I have to consider every single word before it is printed.
Why are some scientists so sensitive if weak data are published? When I first met Ingo Potrykus, the inventor of the famous golden rice (so called because of its extra beta-carotene content), I was still Germanys top anti-GM campaigner with Friends of the Earth. Some 15 years after our public debate, I now understand his frustration. As a humanitarian and Roman Catholic he has worked hard to develop rice varieties he believes could improve the lives of millions of poor children likely to become blind. But Greenpeace and other activists are sabotaging his efforts with false claims, initially that children could be poisoned by excess vitamin A [5] and later that 4 kilograms of rice is the daily requirement for a therapeutic effect [6]. Scientists should think more carefully about the impact their words might have on the future of society and their responsibility towards it.
Dr. Jens A. Katzek (Germany), Bio Mitteldeutschland GmbH, Halle, Germany, posted Sept, 6 2009, published in Nature Vol. 461 (15.10.2009), p. 875.

Summary of the extensive comments on the Nature feature "Battle Fields": The question, whether the opponents to GM crops fight in a fair way, would have been more justified.

6. Papers on the impact of Bt toxins contradicting Rosi-Marshall by the research group lead by Prof. em. Galen Dively

A first scientific paper addressing the errors of Rosi-Marshall was published on a similar subject by (Swan et al., 2009c), compare also some previous papers of the same authors (Ball et al., 2008; Kaushal et al., 2008; Swan et al., 2009a; Swan et al., 2009b; Swan et al., 2008; Swan & Palmer, 2006a, b). The research group paints a more differentiated picture: no correlation in all researched data have been found between Bt- and non-Bt effects.

6.1. Paper of Swan et al. 2009 on processing transgenic crop residues in stream ecosystems

The abstract of (Swan et al., 2009d):

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[5] See Dennis T. Avery in Center for Global Food issues: http://www.cgfi.org/2000/03/07/what-do-environmentalists-have-against-golden-rice/

[6] See Greenpeace website http://www.greenpeace.org/international/news/failures-of-golden-rice

"Research in agricultural ecosystems is uncovering how the management of crop fields leads to the delivery of transgenic crop residues to adjacent waterways. Aquatic consumers encountering this material may be reduced in abundance and ⁄ or limit their feeding activity, subsequently altering organic matter breakdown rate, which is a key ecosystem process in streams.

We investigated the effect of the transgenic nature of senesced corn (Zea mays L.), tissue on breakdown rates, invertebrate abundance and invertebrate community composition in nine streams draining agricultural fields over 2 years (2004–2006). We studied corn tissue modified to express protein toxins from the bacterium Bacillus thuringiensis (Bt) from four hybrid families, each with its single, stacked and non-Bt near-isoline.

In 2004, we identified two instances whereby Bt leaf litter degraded slower (67–68%) than corresponding near isolines. At one site this was associated with significantly fewer individuals of Pycnopsyche sp., a leaf-chewing caddisfly. In 2005–2006, ***no differences in breakdown were found between Bt and non-Bt near isolines. Multivariate analysis of invertebrate communities found no difference associated with Bt treatment****.*

Principle components analysis identified important abiotic factors as explanatory variables influencing breakdown, but no interaction was found between these and Bt treatment. Breakdown was strongly related to total invertebrate abundance occurring on experimental litter bags, but this did not interact with Bt treatment across all hybrid x isolines x site combinations.

Synthesis and applications. Ecological interactions facilitate breakdown of allochthonous detritus, and understanding the potential disruption of these interactions is important to the management of ecosystem processes. ***The results from our study suggests that corn tissue breakdown is unlikely to be altered by Bt, but more so by hybrid- and site-specific factors such as nutrients.*** *Management of agricultural streams will need to consider multiple sources of stress at larger scales, such as nutrient loading and temperature, which probably overwhelm the potential for consumer mediation of ecosystem processes in these ecosystems." (Swan et al., 2009c).*

Left: **breakdown rate** right: **invertebrate abundance**

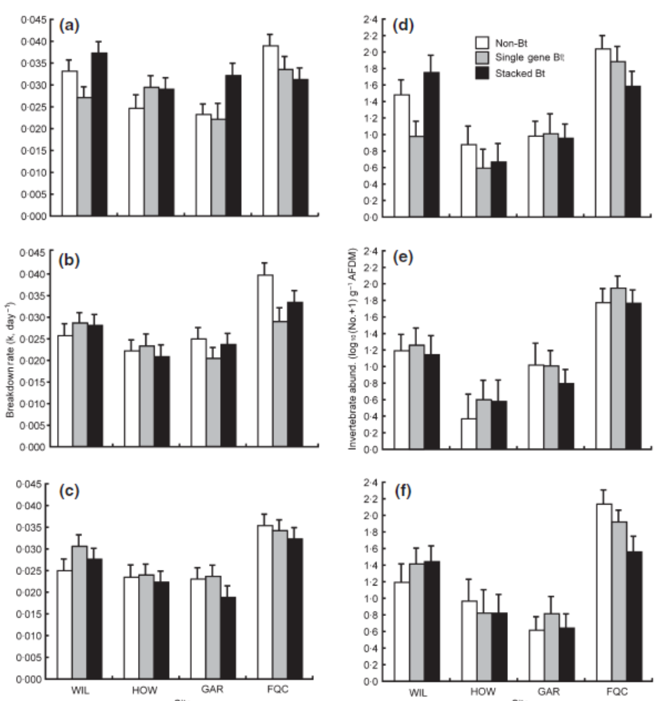

Fig. 3 Results of the 2005–2006 study. Panel (a,b) refers to hybrid A, (c,d) hybrid B and (e,f) hybrid C. For each site ▪ isoline treatment combination, breakdown rates are left, invertebrate abundance on the right. Bars are the least-squares means + 1 SE. From (Swan et al., 2009c).

6.2. Paper of Jensen et al. 2010

In a long awaited paper (Jensen et al., 2010) come to the conclusion, that Bt toxins in various combinations do not show directly any bioactivity in the streams studied: (see also the comments in the introduction under (Swan et al., 2009d). Actually, adverse effects to aquatic nontarget shredders involve complex interactions arising from plant genetics and environment **that cannot be ascribed to the presence of Cry1Ab proteins.**

The abstract is given here in extenso:

"Corn (Zea mays L.) transformed with a gene from the bacterium Bacillus thuringiensis (Bt) comprises 49% of all corn in the United States. The input of senesced corn tissue expressing the Bt gene may impact stream-inhabiting invertebrates that process plant debris, especially trichopteran species related to the target group of lepidopteran pests. Our goal was to assess risk associated with transgenic corn debris entering streams. First, we show the input of corn tissue after harvest was extended over months in a stream. Second, using laboratory bioassays based on European corn borer [Ostrinia nubilalis (Hübner)], we found no bioactivity of Cry1Ab protein in senesced corn tissue after 2 wk of exposure to terrestrial or aquatic environments. Third, we show that Bt near-isolines modify growth and survivorship of some species of invertebrates. Of the four nontarget invertebrate species fed Bt near-isolines, growth of two closely related trichopterans was not negatively affected, whereas a tipulid crane fly exhibited reduced growth rates, and an isopod exhibited reduced growth and survivorship on the Cry1Ab near-isoline but not on the stacked Cry1Ab + Cry3Bb1 near-isoline. ***Because of lack of evidence of bioactivity of Bt after 2 wk and because of lack of nontarget effects on the stacked near-isoline, we suggest that tissue-mediated differences, and not the presence of the Cry1Ab protein, caused the different responses among the species****. Overall, our results provide evidence that adverse effects to aquatic nontarget shredders involve complex interactions arising from plant genetics and environment* ***that cannot be ascribed to the presence of Cry1Ab proteins****."* *(Jensen et al., 2010). See the figures and captions below for more explanations.*

Fig. 4 shows the dynamics of the movement of corn tissue in the river systems studied:

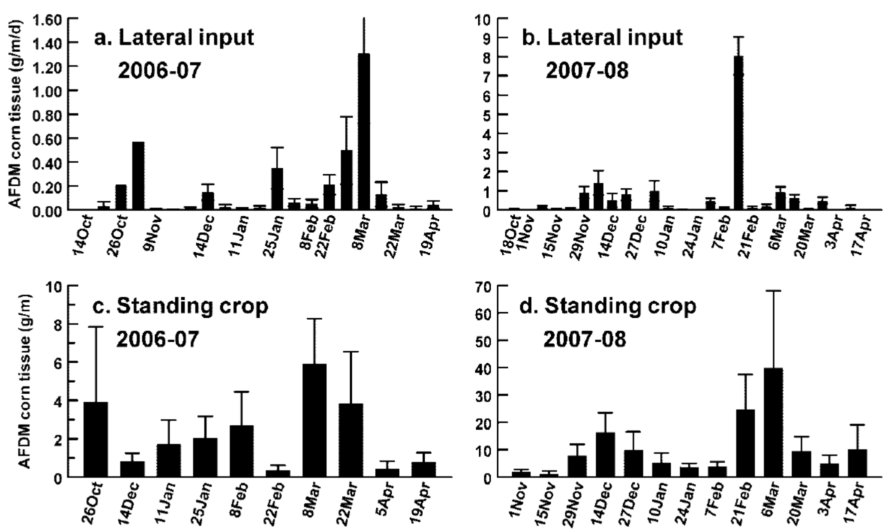

Fig. 4 Movement of corn tissue subsequent to harvest from a field to an adjacent stream expressed as means _ SE. (a) Lateral input during 2006-2007. (b) Lateral input during 2007-2008. (c) Standing stock biomass of corn during 2006-2007. (d) Standing stock biomass of corn during 2007-2008. From (Jensen et al., 2003).

In a final comment, the authors point to the ecological complexity and also to the fact, that some questions remain open for future research:

> *"The increasing rarity of true non-Bt isolines will make it difficult to untangle any existing causal relationships in similar studies in the future. Our results indicate a need for an aquatic artificial diet that would allow the partitioning and testing of individual tissue components, as well as the direct delivery of the Cry proteins to aquatic nontarget organisms in a tier 1 test consistent with Rose (2007).*
> *Two caveats should be considered in the extrapolation of our results to existing populations in agricultural headwater streams. The presence of riparian buffers or Þlter strips in many production areas would likely decrease the input of corn tissue into headwater streams bordering agricultural production. USDA conservation programs and scientific evidence regarding water quality and nitrogen removal are encouraging the implementation of conservation buffers adjacent to streams to mitigate agricultural impact on waterways (Mayer et al., 2007). Second, the lack of food choice in our nontarget organism bioassays could overestimate exposure risks, because tissue containing bioactive Cry proteins may be actively avoided by the shredders in the stream. For example, (Swan et al., 2009d) found significantly fewer P. scabripennis larvae colonizing Bt corn litter in the same stream during a controlled litter breakdown study.* ***To our knowledge, however, no choice studies have been performed with genetically engineered corn tissue and aquatic consumers."*** *From (Jensen et al., 2010).*

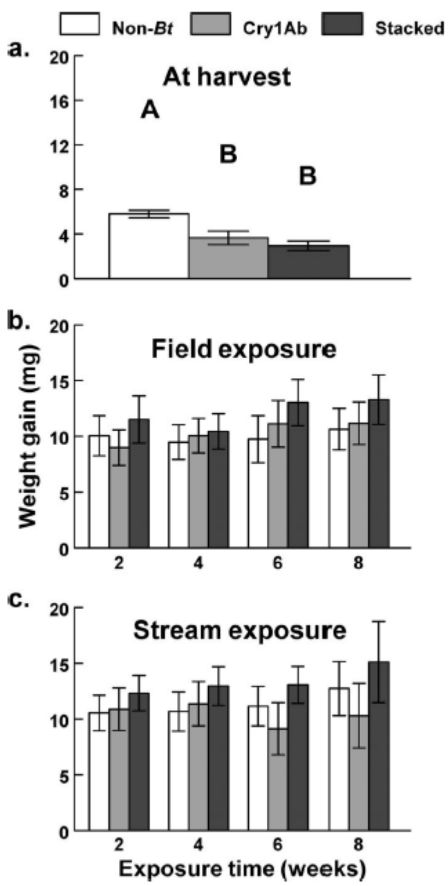

Fig. 5 Bioactivity of *Bt* in senesced corn tissue as determined by growth change (mean weight gain _ SE) of target European corn borer larvae after 7 d on diet incorporating tissue from three near-isolines. (a) Corn tissue at 0 wk (_time of harvest). (b) Corn tissue after exposure to the terrestrial environment in a riparian area between corn field and stream. (c) Corn tissue after exposure to the aquatic environment of an agricultural stream. From (Jensen et al., 2010).

7. Conclusion and Summary

As a summary, one can conclude: the paper published by Rosi-Marshall et al. is conveying unfounded concerns about the accumulation of Bt protoxins from GM crops in Water and soil in headwater streams near Bt maize fields.

7.1. Aquatic systems

(Douville et al., 2007) tested the short time persistence of Bt proteins in aquatic systems. The cry1Ab gene persisted for more than 21 and 40 days in surface water and sediment, respectively. The removal of bacteria by filtration of surface water samples did not significantly increase the half-life of the transgene, but the levels were fivefold more abundant than those in unfiltered water at the end of the exposure period. In sediments, the cry1Ab gene from Bt corn was still detected after 40 days in clay- and sand-rich sediments. Field surveys revealed that the cry1Ab gene from transgenic corn and from naturally occurring Bt was more abundant in the sediment than in the surface water. The cry1Ab transgene was detected as far away as the Richelieu and St. Lawrence rivers (82 km downstream from the corn cultivation plot), suggesting that there were *multiple sources* of this gene and/or that it undergoes transport by the water column. Sediment-associated cry1Ab gene from Bt corn tended to decrease with distance from the Bt cornfield. Sediment concentrations of the cry1Ab gene were significantly correlated with those of the cry1Ab gene in surface water ($R = 0.83$; $P = 0.04$). The data indicate that DNA from Bt corn and Bt were persistent in aquatic environments and were detected in rivers draining farming areas. However, the authors also refer to their own previous study (Douville et al., 2005), where the results showed that Bt corn endotoxin is degraded more rapidly in water than in soils ($t_{1/2}$: 4 and 9 days, respectively), while crystals appeared to be more resilient, as expected. The isotopic patterns of 13C and 15N in Bt-corn endotoxin differed markedly from Bt, making it possible to track the source of Cry1Ab in the environment. Preliminary field surveys indicate that Cry1Ab is fairly *uncommon* in aquatic environments, *being found only at trace concentrations when it is detected*. This will say that Bt protoxins are highly unlikely to cause any environmental problems in aquatic systems. As a whole, the publications of Douville et al. are anyway not convincing, because they lack an important scientific quality: the baseline comparison is totally lacking. As an example: There are several publications from the same river system, such as (Tall et al., 2008) and many others which clearly point to metal and phosphorus contamination of the river sediments, causing negative effects to the fauna and flora.

Critical reference is given to the paper of (Rosi-Marshall et al., 2007) on the occurrence of Bt protein in headwater stream ecosystems, written in an unnecessary alarming style and not even confirmed with hard field data in the chapter on non-target insects of this report. There is not even a hint on the nature of the Bt toxin (it could well be at least partially of natural origin), and when you compare her own (!) figure 3 B the graph with realistic concentrations, then you see that Bt shows a clearly lower mortality of the scraping caddisflies experiment – so what?? And again it shows, like the work of (Douville et al., 2007), the unfortunately widespread sin in agricultural science of a lacking a proper baseline comparison.

In the latest publications (Swan et al., 2009d) and (Jensen et al., 2010), the major statements of the negative impact of Bt toxins on aquatic organisms by the research group of Rosi-Marshall have been clearly contradicted. (Rosi-Marshall et al., 2008; Rosi-Marshall et al., 2007). During an invited talk at the anti-GMcrop group at the NABU symposium on 17. December 2009 http://www.gmo-safety.eu/en/news/730.docu.html
Where they somehow defused their own first strong statements about the detrimental effects of Bt toxins on aquatic organisms:

> *"At the NABU symposium in Berlin, the ecologists Emma Rosi-Marshall and Jennifer Tank presented initial, not-yet-published results of these current investigations. They have expanded their experiment protocol to also include a third variety of caddis fly. Their original findings were confirmed: feeding with Bt-maize increased the mortality rate in one strain of caddis fly, and reduced growth rates were seen in another type.*
> ***However, again, these laboratory findings were not confirmed in the field trials, according to Rosi-Marshall and Tank in Berlin****. They have examined 12 streams and drainage channels in an American maize-growing area over several years. In contrast to central Europe, 'sweet corn' is the primary maize crop in the USA. During harvesting all plant material except the maize corns themselves are left on the field. When cultivating Bt-maize, considerable amounts of harvest residues can end up, in particular, in the drainage channels.*
> ***Despite the high proportion of Bt-maize cultivated, Rosi-Marshall and Tank have up to now found no clear evidence that would indicate that the caddis flies are affected by the Bt-maize. Both scientists suspect that these drainage channels near the maize fields may be strongly polluted as a result of the intensive agricultural cultivation that this might hide the effects of the Bt-maize****.*
> *Rosi-Marshal and Tank want to publish their current finding in spring at the latest."*

7.2. Soil systems

The whole question on persistence of Bt toxins in soil will be treated in a separate ASK-FORCE contribution. There are again, after a first whistle blower phase (Saxena et al., 1999), enough long term studies to demonstrate that accumulation does not take place to a degree that it could harm soil organisms, here just as an example two papers: (Head et al., 2002; Saxena & Stotzky, 2001a)

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