

Is the impact of Bt maize on non-target insects significantly negative?

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1. The claim:

Lovei, G. L., D. A. Andow and S. Arpaia (2009). "Transgenic Insecticidal Crops and Natural Enemies: A Detailed Review of Laboratory Studies." *Environmental Entomology* **38**: 293-306.

<http://www.ingentaconnect.com/content/esa/envent/2009/00000038/00000002/art00001>

*“This review uses a data-driven, quantitative method to summarize the published, peer-reviewed literature about the impact of genetically modified (GM) plants on arthropod natural enemies in laboratory experiments. The method is similar to meta-analysis, and, in contrast to a simple author-vote counting method used by several earlier reviews, gives an objective, data-driven summary of existing knowledge about these effects. Significantly more non-neutral responses were observed than expected at random in 75% of the comparisons of natural enemy groups and response classes. These observations indicate that Cry toxins and proteinase inhibitors often have non-neutral effects on natural enemies. This synthesis identifies a continued bias toward studies on a few predator species, especially the green lacewing, *Chrysoperla carnea* Stephens, which may be more sensitive to GM insecticidal plants (16.8% of the quantified parameter responses were significantly negative) than predators in general (10.9% significantly negative effects without *C. carnea*). Parasitoids were more susceptible than predators to the effects of both Cry toxins and proteinase inhibitors, with fewer positive effects (18.0%, significant and non-significant positive effects combined) than negative ones (66.1%, significant and non-significant negative effects combined). GM plants can have a positive effect on natural enemies (4.8% of responses were significantly positive), although significant negative (21.2%) effects were more common. Although there are data on 48 natural enemy species, the database is still far from adequate to predict the effect of a Bt toxin or proteinase inhibitor on natural enemies”. (Lovei et al., 2009).*

2. Comments by Shelton et al.

Shelton, A., Naranjo, S., Romeis, J., Hellmich, R., Wolt, J., Federici, B., Albajes, R., Bigler, F., Burgess, E., Dively, G., Gatehouse, A., Malone, L., Roush, R., Sears, M., & Sehnael, F. (2009)

Setting the record straight: a rebuttal to an erroneous analysis on transgenic insecticidal crops and natural enemies. *Transgenic Research*, 18, 3, pp 317-322

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AND <http://www.botanischergarten.ch/Bt/Shelton-Setting-Record-Straight-Lovei-2009.pdf>

In a nearly unprecedented speed (Lovei et al., 2009) has been rebutted sharply by (Shelton et al., 2009), a worldwide consortium of specialists on research of non-target insects related to Bt crops: In an unusual straightforward text they criticize heavily the previous publication, and the author of this study agrees fully: it is time to recognize that such studies, even when published in peer reviewed journals, do not help to progress scientific risk assessment of GM crops, on the contrary, they hinder future developments by providing to highly questionable arguments a scientific aura. The pitfalls are not easy to see, due to the complexity of the matter (food webs of parasitoid insects and their prey over development and time). For lay persons, politicians and even regulators it is difficult to develop a critical view and to distinguish junk science from high quality papers. The extended abstract of (Lovei et al., 2009) reads as follows (important lines are in bold)Extended abstract of first part (Shelton et al., 2009)

*“As scientists involved in risk assessment of transgenic insecticidal plants, we are greatly concerned about the publication by (Lovei et al., 2009) implying that insect-protected crops based on the Cry proteins of Bacillus thuringiensis may have substantial negative impacts on non-target organisms. We believe that (Lovei et al., 2009) use inappropriate and unsound methods for risk assessment that have led them to reach conclusions that are in conflict with those of several recent comprehensive reviews and metaanalyses (e.g., (Marvier et al., 2007; Naranjo, 2009; O’Callaghan et al., 2005; Romeis et al., 2006; Wolfenbarger et al., 2008). (Lovei et al., 2009) base their findings on an analysis of 55 laboratory studies of Cry proteins and 27 studies of proteinase inhibitors (PIs; including lectins) that were published through mid-2007 and conclude that these proteins “often have non-neutral effects on natural enemies”. They further conclude that “parasitoids were more susceptible than predators to the effects of both (toxins)” and that “conclusions that Bt...gene products have no harm to natural enemies are currently over-generalized and premature”. **We are deeply concerned about the inappropriate methods used in their paper, the lack of ecological context, and the authors’ advocacy of how laboratory studies on non-target arthropods should be conducted and interpreted.** Essentially, the authors have conducted a data-mining exercise without prior elaboration of a risk hypothesis framework (Romeis et al., 2008) that can provide context to their findings and interpretations. Therefore, we believe it is very important that readers consider the following points as they read (Lovei et al., 2009).”*

2.1. Data selection and analyses

*“**We have a major concern with the authors’ selection and use of multiple non-independent measures of various life history and behavioral traits in the analysis.** As an example, they justify the use of development times and survival rates on individual instars as independent measures of effect by testing whether there is evidence of “matching” among the total development time or survivorship and individual times and rates for each of multiple stadia. Based on various criteria, the percentage matching was [50-84% but the test statistic for independence was significant, so all stadia measurements were used in the analyses. Their justification for using this method is that there might be “...complex instar-specific mortality schedules and patterns of development time”. The fundamental effect of such an approach is that it inflates purported effects in the data, and the authors acknowledge this. However, their dismissal of the potential effect that the instar analysis may have on their conclusions is confusing and unjustified. Although they state “data-driven reading of the quantitative data...provided a more accurate picture of the literature...than (the reviews) by (O’Callaghan et al., 2005; Romeis et al., 2006)”, they provide no evidence to support this statement. They cite (Bai et al., 2005) as an example of the need to use their analytical methods to tease out negative results, but neglect to note that in the study total larval development and survivorship of Propylea japonica (Thunberg) (Coleoptera: Coccinellidae) were unaffected by exposure to Cry proteins. These are the quantities that ultimately affect population growth and are of primary importance as measurement endpoints in risk assessment studies (including those conducted for pesticide assessment; (Romeis et al., 2008). For this reason, (Bai et al., 2005) correctly concluded “Bt rice pollen had no negative impacts on P. japonica fitness...”. (Lovei et al., 2009) also fail to justify the many other instances of non-independence in their data set where multiple, correlated life history and behavioral traits were measured on the same cohort of subject organisms. For*

example, many studies measured ovi-position per day and total adult ovi-position which are clearly correlated. The quality of independence in a meta-analysis is essential to obtain accurate and unbiased results, and authors need to go to great lengths to ensure independence, even if it means omitting hard-earned data (e.g., (Marvier et al., 2007); (Wolfenbarger et al., 2008) (Naranjo, 2009). The use of non-independent data is analogous to pseudo-replication, a well-understood problem in the scientific literature.

Secondly, (Lovei et al., 2009) claim to use a weighted effect size estimator “similar to (but not the same as)... Hedge’s *g*” to quantify experimental effects but do not provide sufficient methodological detail to permit others to repeat their analyses. They cite their prior work (Lovei & Arpaia, 2005) for methods on effect size calculation but no details are provided there either. Various effect size estimators have been developed (Hedges L.V & Olkin, 1985) and it is important to understand their strengths and limitations when interpreting results derived from these. Finally, (Lovei et al., 2009) describe an arbitrary and inappropriate classification of responses as positive or negative, but not statistically so, and then go on to ascribe importance to such non-statistically valid conclusions. By their own admission, the *P* values of these comparisons would be roughly 0.30, which would be considered non-significant and devoid of further meaning and interpretation, even if the goal was to increase the statistical power of the test. We believe it is incorrect to draw conclusions or implications based on results that are not statistically valid.”

Another difficult question of prey/host-quality effects were erroneously treated in their paper (Lovei et al., 2009). By not addressing the basic factor of ‘study quality’ they produce flawed statistics: **Studies seemingly showing detrimental effects of Bt toxins to lacewings are caused by the poor quality of the prey fed to the larvae.** This ends in another major blow to the publication they contradicted the statements of (Lovei et al., 2009) regarding **parasitoids:**

“Parasitoids in general were more susceptible to the effects of both Cry toxins and proteinase inhibitors, with fewer positive effects (always >26%, significant and non-significant combined) and more negative ones (between 42.1 and 75.0% significant and non-significant combined). There was no marked difference in the effect of Cry toxins versus PIs, although PIs were more likely to have positive effects and less likely to have negative ones (Table 4). Two species, *C. chloridae* and *E. pennicornis*, accounted for 30.3% of all of the observations, including 45.2% of all observation on proteinase inhibitors. *C. chloridae* may be more sensitive to Cry1A/Cry2A than the other parasitoids studied, and *E. pennicornis* may be less sensitive to proteinase inhibitors than the other parasitoids studied (Table 4).

Considering the sensitivity of the response classes studied (Tables 5 and 6), for predators, 22 of 35 comparisons were significantly non-random ($P < 0.05$; Table 5) with fewer neutral responses than expected. The number of observations in the 12 non-significant classes averaged only 16.8 (range, 11-34) compared with 51.0 (range, 20-156) for the significantly non-random classes, which suggests that some of the non-significant classes are type 2 errors (non-significant only because of a small sample size). When comparing classes with similar sample sizes, none of the classes appeared to be more sensitive at detecting non-neutral responses. For 31 of the 35 classes, positive responses were similar to negative responses. Only Cry1A/Cry2A survival had more negative responses than positive ones, and this was because of the response of *C. carnea*, which accounted for most of the negative responses by

predators to Cry1A/Cry2a (Table 5). Survival and reproduction responses to PIs also had more negative responses than positive ones (Table 5), suggesting that PIs have more negative effects on predators than Cry toxins.

For parasitoids (Table 6), 25 of 31 class responses were significantly non-random ($P < 0.05$), with fewer neutral responses than expected. The number of observations in the six non-significant classes was 16.7 (range, 12-19) compared with 52.0 (range, 18-136) for the significantly non-random classes, which suggests that some of the non-significant classes are type 2 errors. For 12 of the 31 response classes, there were more negative responses than positive ones (Table 6). This was particularly true for parasitoids tested with Cry1A/Cry2A (including Cry1A + CpTI), for which 12 of 15 class responses were significantly more negative than positive (Table 6). Growth seemed to be a more sensitive response than development. A greater proportion of the responses for growth were negative than for development within toxins and parasitoid species (Table 6)."

These statements of (Lovei et al., 2009) are refuted by (Shelton et al., 2009), the paragraphs are cited extensively including the cited literature:

2.2. Prey/host-quality mediated effects, comments from (Shelton et al., 2009):

*"Experimental studies must have properly formulated hypotheses, experimental designs and testing methods; otherwise the interpretation of the outcomes of such tests is unreliable. This basic factor of 'study quality' is not addressed in the analysis by (Lovei et al., 2009) rather their methodology implies that all studies are equally valid and should be given equal weight, providing each study has adequate statistical properties. **We believe their approach is fundamentally flawed and does a disservice to environmental risk assessment.***

*One example of this problem can be seen in reports on *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), a lacewing species that has been the subject of several studies. (Hilbeck et al., 1998a) observed reduced fitness of *C. carnea* larvae when fed on Bt maize-reared lepidopteran larvae and claimed it was associated with the Cry1Ab protein and that Cry1Ab is toxic to *C. carnea* (Hilbeck et al., 1998b). However, subsequent studies clearly demonstrated that Cry1A proteins are not toxic to *C. carnea* larvae (Romeis et al., 2004), (Rodrigo-Simon et al., 2006), (Lawo & Romeis, 2008) and that these proteins do not bind to the midgut of *C. carnea*, a prerequisite for toxicity (Rodrigo-Simon et al., 2006). These results strongly indicate that the effects observed by (Hilbeck et al., 1998a) were due to *C. carnea* feeding on poor quality (sick or dying) lepidopteran prey. Additional studies with aphids (which do not ingest Cry1Ab) and spider mites [which contain high concentrations of biologically active Cry1Ab (Obrist et al., 2006), neither of which is affected when feeding on Bt maize, demonstrated that, when these herbivores fed on Bt maize and were in turn consumed by *C. carnea*, the predator was not harmed (Dutton et al., 2002). These results emphasize that care must be taken when designing laboratory studies to assess the potential effects of Cry proteins and other insecticidal factors on predators; otherwise the results can easily be misinterpreted. We believe that this is certainly the situation that caused (Lovei et al., 2009) to conclude incorrectly that "significant negative effects of Cry1A/Cry2A on *C. carnea* were 6.2 times more likely to occur than positive ones".*

*“Teasing out the effects of insecticidal factors on parasitoids is potentially even more difficult due to their close relationship with the host; if the host dies, the parasitoid dies. If Bt-susceptible hosts are fed on a Cry protein source and then parasitized, **impacts of host quality on parasitoid fitness are expected and could be confused with toxic effects of the Cry protein** (Romeis et al., 2006) (Chen et al., 2008b). Using the diamondback moth (DBM) and its major parasitoid, *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), it was clearly shown that when DBM resistant to Cry1C were parasitized, there were no effects on parasitoids that fed internally on DBM (Chen et al., 2008a). **This study overcame any host-mediated effects to show the complete lack of toxicity of Cry1C to the parasitoid.** This result is consistent with previous reports about the lack of toxicity of Cry1 to hymenopteran parasitoids (Schuler et al., 2004) (Schuler et al., 2003).*

*Overall, it is critical to account for prey- or host mediated effects in such toxicological studies. A recent meta-analysis using Hedge’s *d*, a weighted effect size estimator with a sample size bias-corrector, and based on comparative laboratory studies of Bt Cry toxicity published through November 2008 (Naranjo, 2009) clearly shows a negative effect of low quality hosts (susceptible hosts compromised by feeding on Bt plant tissues or purified Cry proteins) on survival, development and reproduction of parasitoids (Fig. 1). In contrast, the overall effects are neutral or even positive when high quality, uncompromised hosts are provided (Bt-resistant hosts or hosts not susceptible to Cry proteins). The effect of prey quality on predators is less pronounced, compared to parasitoids, but even a small negative effect of low prey quality on survival is neutralized when they are provided high quality prey containing Cry proteins.*

*These examples demonstrate that just using a “quantitative” summary of previous laboratory studies can lead to spurious results; studies must be properly designed to tease out the effects of the insecticidal factor versus the quality of the prey or host. However, (Lovei et al., 2009) **did not assess the quality of the studies they used in their analyses nor did they properly partition the data so that issues of prey/host quality could be separately examined. As a consequence, their conclusion that “parasitoids were more susceptible than predators to the effects of ... Cry toxins...” is due to the fact that a large majority of tritrophic studies on parasitoids have been conducted with susceptible, sub-lethally affected lepidopteran larvae as hosts** (Romeis et al., 2006) (Naranjo, 2009). (OECD Consensus Documents, 2007). These indirect and potentially adverse effects are common for any method of pest control and are of minor concern within an environmental risk assessment context (OECD Consensus Documents, 2007), they should be differentiated from direct effects of a toxin (EFSA, 2008).”*

3. Final comments

It is recommended to read in full the rebuttal of Shelton and the impressive consortium of specialists on non-target insect risk assessment research related to Bt maize. Basically, there is nothing to add.

The authors cite and comment an impressive array of literature published in peer reviewed literature, which leaves only some minor questions about the regulation of Bt maize open, which should and can no more influence the positive decisions of the regulatory authorities.

The author of this ASK-FORCE piece has re-cited all literature included in the text pieces given above of (Shelton et al., 2009), included the full text links for easier understanding, and in a few cases the citations are enhanced and renewed with more recent publications and official reports.

4. Some thoughts about peer review in science

The literature on non-target organism impact of Bt maize is huge, there are some 190 publications in peer reviewed literature, which are commented in a chapter on a book on Bt maize in work by the author, and all those papers have a direct baseline comparison, which makes them scientifically valid. It is amazing to see, that still some research groups like the one of (Lovei et al., 2009) can publish today on the impact of non-target insects without giving a shread of baseline comparison. But the Lovei group is not alone, the author of this ASK-FORCE piece has singled out in a separate chapter of his coming Bt maize report dozens of publications without baseline comparison. The author of this study hopes that we will develop very soon some scientific code of conduct on how such impact studies should be conducted. Such a code will have to be adapted to the needs of the special field of environmental and health impact of GM crops. In a thoughtful publication of peer review, (Scott, 2007) give a broad minded summary of many aspects of peer review, demonstrating the high complexity of the matter. He draws prudent conclusions, which shall be cited here:

“In conclusion, peer review potentially includes a range of criteria—scientific, organizational, and relating to social relevance—all of which are largely concealed in the current policy of treating peer review as a ‘black box’—a tool for the exclusive use of the scientific community that is thought best left to work autonomously. It would be premature to suggest a definitive list of criteria for use in peer review, but the above discussion summarizes various contributions to suggest a range of criteria. The employment of a more structured approach to the criteria used in peer review might offer the chance to increase the levels of transparency and openness in peer review. A more plural approach to the criteria used in peer review would be an outcome that might lead to significant advantages for those involved in conducting, and being the subjects of, peer review. It might also help to ensure that peer review is not seen as an opaque affair through which scientists escape scrutiny and accountability.”

But hopes are not so high (as (Scott, 2007) demonstrated (see also (Reale et al., 2007), when you realize how difficult the situation is, when it comes to a hot topic such as the biotechnology of crops – just to give you one example, where we criticize major peer reviewed journals for not

following simple rules of proper peer review: (Miller et al., 2008). This situation makes it all the more important to rebut consequently all publications which hurt some basic rules of science. The author would be happy, if we could avoid in this debate the dangerous waters of “democratization of science” on cost of crystal clear criteria established specifically for a variety of scientific disciplines. One of the foremost criteria should be in our case to *first* establish a framework of a risk hypothesis as proposed by a comprehensive consortium of specialists in this field of research (Romeis et al., 2008).

5. Negative influence of papers like (Lovei et al., 2009) on the regulatory process in Europe

This rebuttal of (Lovei et al., 2009) alone makes it difficult to understand the German ban on Bt maize MON810 (BVL, 2009), again it is dismantled by scientific assessments as a purely political and populist decision (in which German populist politicians like minister Ilse Aigner and Bavarian prime minister Horst Seehofer) have explicitly (ab)used this and other studies) in order to provide to their purely politically motivated rejection a scientific aura.

There are signs of hope, since it seems that this time Minister Ilse Aigner has overdone: Apart from Monsanto which has decided to sue about the German decision in court (which Monsanto lost in the mean while...), there are now many scientists alarmed by the negative decision:

In a letter to the German government more than 1600 scientists from Germany protested against the decision:

<http://www.botanischergarten.ch/Bt/Gruene-Gentechnik-Petition-Aigner-germ-engl-20090417.pdf>

This protest was supported by some international experts:

<http://www.botanischergarten.ch/Bt/Moses-FAZ-Merkel-letter-20090417.pdf>

Excerpt from the letter of Prof. Vivian Moses and colleagues to the Frankfurter Allgemeine and Chancellor Merkel:

“We hope that you will yet exercise your authority to dismiss this ill-intentioned decision by Minister Aigner and save the reputation of Germany, German science and German leadership. We can understand a minister making an unfortunate mistake if that error is acknowledged and reversed. We will not understand if Frau Aigner receives the support of her colleagues at the highest levels of government.”

The scientists got support by the governmental science committee ZKBS (Zentrale Kommission für die Biologische Sicherheit) with a report refuting practically all arguments of the ministry as not valid and accused the ministry of selective use of scientific papers of highly questionable quality: (ZKBS-BLV, 2009). Also an article in Nature Biotechnology is clearly taking position against this decision (Ricroch et al., 2009), and one member of the ZKBS clarifies the regulatory position in an European perspective: (Bartsch, 2009), which will make it very difficult

for some European countries like Italy, Greece, France, and Luxemburg to maintain a negative position regarding the regulation of transgenic crops. Another comment is again coming from Nature Biotechnology (Sinha, 2009) with comments on the use and abuse of science in the political battles on GM crops.

The case of the rejection of the Bt maize MON810 will be treated in another contribution in detail, the German decision will shortly be available in an English translation.

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http://www.bvl.bund.de/cln_027/nn_1208608/DE/06_Gentechnik/093_ZKBS/01_Allg_Stellungnahmen/04_pflanzen/Mon810-Neubewertung-Juli09.html AND <http://www.botanischergarten.ch/Bt/BLV-ZKBS-MON810-20090707.pdf>