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D. A. Andow
Department of Entomology and Center for
Community Genetics
University of Minnesota
St. Paul, MN

G. L. Lövei
Department of Agroecology
Aarhus University
Flakkebjerg Research Centre
Slagelse, Denmark

Errors in Logic and Statistics Plague a Meta-Analysis (response to Andow and Lövei 2012)

TO THE EDITOR:

As we noted previously (Shelton et al. 2009a,b), we strongly believe in the power of meta-analyses to help advance our collective understanding of the potential risks of Bt crops for nontarget organisms by identifying negative, neutral, and positive effects of the technology in both laboratory and field studies. Although we agree on this point, it is equally important that such studies do not contain errors in logic or statistics. We acknowledge that Andow and Lövei (2012) have corrected a statistical error in their previous publications (Lövei et al. 2009), but point out another statistical error (see below) in their latest letter. However, more important than these statistical errors, we question their continued attribution of hazard to a protein rather than, more accurately, to poor prey or host quality. We believe this is an error in logic. Therefore, we strongly oppose the latest statement by Andow and Lövei (2012) that their conclusions of detectable non-zero effects of Cry proteins on nontarget organisms were "... criticized by Shelton et al. (2009a,b) on statistical grounds." They missed the point again. Our primary criticism then and now is that they continue to ignore prey and host-quality effects and the ecological context in their effort to inform risk assessment.

Besides the fault in logic of ignoring prey or host quality, we also take issue with the statement in their latest letter (Andow and Lövei 2012): "We conclude not that there *could have been* type II error, but that there *has been* type II error." Given the nature of statistical hypothesis testing, such certainty is simply not possible. The only thing they can conclude is that they rejected the null hypothesis. This could just as likely represent a type I error on their part as it could a type II error by every other meta-analysis conducted to date—an unlikely conclusion implied by their statement. Whether their statement is an error in statistics,

logic, or both is a matter of debate, but it is an error. We also would like to point out that unlike other published meta-analyses that have made the underlying database accessible to the scientific community (e.g., Wolfenbarger et al. 2008, Duan et al. 2010), the database supporting the report by Lövei et al. (2009) was not made accessible and therefore cannot be measured for statistical veracity or other important criteria by interested parties. However, in the end, the statistical issues debated exhaustively by both parties are irrelevant if there are errors in the logic of attributing hazard to a protein rather than poor prey or host quality, as they did in their study (Lövei et al. 2009). We strongly believe that the analysis and conclusions stated by Lövei et al. (2009) do not provide evidence for toxic effects of Bt Cry proteins on natural enemies.

We agree that tritrophic laboratory studies have reported adverse effects of Bt-transgenic plants on natural enemies. However, these effects must be regarded as prey-quality effects rather than toxic effects of the plant-expressed Cry proteins, because these tritrophic studies have used Bt susceptible insects as hosts and prey for the natural enemy. When a host is susceptible and ingests a Bt protein, its quality is reduced and when it is fed to a natural enemy this might result in a negative effect on the natural enemy. But it is not the protein itself that has the effect! A careful reading of the literature shows that when effects have been observed, it was the poor quality of the Bt-susceptible host that was responsible for the observed findings (Romeis et al. 2006, Naranjo 2009). This has been verified in tritrophic studies conducted with Bt-resistant or nonsusceptible herbivores. The literature has shown that allowing Bt-resistant hosts to ingest Bt proteins and then feeding the hosts to natural enemies (both predators and parasitoids) has revealed no effects on the natural enemies (Table 1). Likewise, the literature has shown that exposing natural enemies to nonsusceptible prey that have fed on Bt proteins has revealed a lack of effect (Table 1). Meta-analyses (Naranjo 2009) have further demonstrated that, with removal of prey and host quality as a confounding factor, the effects of Bt proteins are either neutral or

Table 1. Phenomena and selected citations that illustrate the safety of Bt proteins to natural enemies

Phenomenon	Citations
Allowing Bt resistant hosts to ingest Bt proteins and then feeding the hosts to natural enemies (both predators and parasitoids) has revealed no effects on the natural enemies	Chen, M., J.-Z. Zhao, H. L. Collins, E. D. Earle, J. Cao, and A. M. Shelton. 2008. A critical assessment of the effects of Bt transgenic plants on parasitoids. <i>PLoS ONE</i> 3: e2284. doi:10.1371/journal.pone.0002284. Lawo, N. C., F. L. Wäckers, and J. Romeis. 2010. Characterizing indirect prey-quality mediated effects of a <i>Bt</i> crop on predatory larvae of the green lacewing, <i>Chrysoperla carnea</i> . <i>J. Insect Physiol.</i> 56: 1702–1710. Li, Y., J. Romeis, P. Wang, Y. Peng, and A. M. Shelton. 2011. A comprehensive assessment of the potential effects of Bt cotton on <i>Coleomegilla maculata</i> demonstrates no detrimental effects by Cry1Ac and Cry2Ab. <i>PLoS ONE</i> 6: e22185. doi:10.1371/journal.pone.0022185. Tian, J., H. L. Collins, J. Romeis, S. E. Naranjo, R. L. Hellmich, and A. M. Shelton. 2012. Using field-evolved resistance to Cry1F maize in a lepidopteran pest to demonstrate no adverse effects of Cry1F on one of its major predators. <i>Transgen. Res.</i> doi:10.1007/s11248-012-9604-4.
Exposing natural enemies to non-susceptible prey that have fed on Bt proteins has revealed a lack of effect	Zahlen, C., W. Nentwig, F. Bigler, and A. Hilbeck. 2000. Tritrophic interactions of transgenic <i>Bacillus thuringiensis</i> corn, <i>Anaphothrips obscurus</i> (Thysanoptera: Thripidae), and the predator <i>Orius majusculus</i> (Heteroptera: Anthocoridae). <i>Environ. Entomol.</i> 29: 846–850. Bernal, C. C., R. M. Aguda, and M. B. Cohen. 2002. Effect of rice lines transformed with <i>Bacillus thuringiensis</i> toxin genes on the brown planthopper and its predator <i>Cyrtorhinus lividipennis</i> . <i>Entomol. Exp. Appl.</i> 102: 21–28. Dutton, A., H. Klein, J. Romeis, and F. Bigler. 2002. Uptake of Bt toxin by herbivores feeding on transgenic maize and consequences for the predator <i>Chrysoperla carnea</i> . <i>Ecol. Entomol.</i> 27: 441–447. Bai, Y. Y., M. X. Jiang, J. A. Cheng, and D. Wang. 2006. Effects of Cry1Ab toxin on <i>Propylea japonica</i> (Thunberg) (Coleoptera: Coccinellidae) through its prey, <i>Nilaparvata lugens</i> (Homoptera: Delphacidae), feeding on transgenic Bt rice. <i>Environ. Entomol.</i> 35: 1130–1136. Álvarez-Alfageme, F., N. Ferry, P. Castañera, F. Ortego, and A.M.R. Gatehouse. 2008. Prey mediated effects of <i>Bt</i> maize on fitness and digestive physiology of the red spider mite predator <i>Stethorus punctillum</i> Weise (Coleoptera: Coccinellidae). <i>Transgen. Res.</i> 17: 943–954. Meissle, M., and J. Romeis. 2009. The web-building spider <i>Theridion impressum</i> (Araneae: Theridiidae) is not adversely affected by <i>Bt</i> maize resistant to corn rootworms. <i>Plant Biotechnol. J.</i> 7: 645–656. García, M., F. Ortego, P. Castañera, and G. P. Farinós. 2010. Effects of exposure to the toxin Cry1Ab through Bt maize fed-prey on the performance and digestive physiology of the predatory rove beetle <i>Atheta coriaria</i> . <i>Biol. Control</i> 55: 225–233. Li, Y., and J. Romeis. 2010. Bt maize expressing Cry3Bb1 does not harm the spider mite, <i>Tetranychus urticae</i> , or its ladybird beetle predator, <i>Stethorus punctillum</i> . <i>Biol. Control</i> 53: 337–344. Álvarez-Alfageme, F., F. Bigler, and J. Romeis. 2011. Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of <i>Adalia bipunctata</i> (Coleoptera: Coccinellidae): the importance of study design. <i>Transgen. Res.</i> 20: 467–479.
Assessing the effects of Bt proteins on natural enemies and their function has demonstrated the safety of Bt proteins	Musser, F. R., and A. M. Shelton. 2003. Bt sweet corn and selective insecticides: their impacts on sweet corn pests and predators. <i>J. Econ. Entomol.</i> 96: 71–80. Candolfi, M. P., K. Brown, C. Grimm, B. Reber, and H. Schmidli. 2004. A faunistic approach to assess potential side-effects of genetically modified <i>Bt</i> -corn on non-target arthropods under field conditions. <i>Biocontrol Sci. Technol.</i> 14: 129–170. Head, G., W. Moar, M. Eubanks, B. Freeman, J. Ruberson, A. Hagerty, and S. Turnipseed. 2005. A multi-year, large-scale comparison of arthropod populations on commercially managed <i>Bt</i> and non- <i>Bt</i> cotton fields. <i>Environ. Entomol.</i> 34: 1257–1266. Naranjo, SE 2005. Long-term assessment of the effects of transgenic <i>Bt</i> cotton on the abundance of non-target arthropod natural enemies. <i>Environ. Entomol.</i> 34: 1193–1210. Chen, M., J.-Z. Zhao, H. L. Collins, E. D. Earle, J. Cao, and A. M. Shelton. 2008. A critical assessment of the effects of Bt transgenic plants on parasitoids. <i>PLoS ONE</i> 3: e2284. doi:10.1371/journal.pone.0002284. Wolfenbarger, L. L., S. E. Naranjo, J. G. Lundgren, R. J. Bitzer, and L. S. Watrud. 2008. Bt crops effects on functional guilds of non-target arthropods: A meta-analysis. <i>PLoS ONE</i> 3: e2118. doi:10.1371/journal.pone.0002118. Naranjo, S. E. 2009. Impacts of <i>Bt</i> crops on non-target organisms and insecticide use patterns. <i>CAB Reviews: Perspect. Agric., Vet. Sci., Nutrit. Nat. Resour.</i> 4: 1–23. doi: http://dx.doi.org/10.1079/PAVSNNR20094011. Lu, Y., K. Wu, Y. Jiang, Y. Guo, and N. Desneux. 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. <i>Nature</i> . doi:10.1038/nature11153.

positive. In our previous rebuttal (Shelton et al. 2009b), we estimated that 73% of the observations in the Lövei et al. (2009) Bt analysis were based on tritrophic exposures and that nearly half of all their

data could not distinguish between the effects of prey and host quality and Bt toxicity. Statistical issues aside, the faulty logic of the Lövei et al. (2009) analyses, in which they attribute hazard to a protein rather than

poor prey or host quality, cannot and should not inform risk assessment.

Beyond the incorrect conclusion of Lövei et al. (2009) of direct toxic effects of Bt proteins on natural enemies, one might also hypothesize that the Bt protein has an indirect or secondary effect on a natural enemy and therefore should be addressed in a risk assessment. In reality, any integrated pest management practice, be it an insecticide, parasitoid, or host plant resistance factor, might affect host quality similar to what occurs when a susceptible larva feeds on a Bt protein. The challenge to entomologists is to find a tactic that is the least disruptive to the functioning of natural enemies. To this point, the literature is clear that Bt proteins are far safer to natural enemies and their function than most, if not all, traditional insecticides (Table 1). This point was reinforced most recently by Lu et al. (2012) in their 20-yr study in China that showed that there was a marked increase in abundance of generalist arthropod predators, decreased aphid abundance and reduced insecticide use with the widespread adoption of Bt cotton. Their long-term studies, and many others, indicate that Bt plants promote biological services in agricultural landscapes, a point not acknowledged by the authors in their original paper (Lövei et al. 2009) or any subsequent rebuttal.

The arguments by Lövei et al. (2009), Andow et al. (2009) and Andow and Lövei (2012) do not provide any basis for their suggestion that Tier one laboratory toxicity tests are not appropriate for the nontarget arthropod risk assessment of insecticidal genetically engineered crops. A recent meta-analysis of published studies on nontarget effects of Bt crops has confirmed that laboratory studies "...predicted effects that were on average either more conservative than or consistent with effects measured in the field" (Duan et al. 2010). What is critically important is that the laboratory studies should follow a set of study design criteria to reveal robust and interpretable results to support the environmental risk assessment (Romeis et al. 2011). One of these criteria is that nonsusceptible herbivores be used as hosts or prey in tritrophic studies.

There are bigger issues in this exchange of correspondence. It is important that readers of this and other journals understand the implications of the purported effects of Bt proteins suggested by Lövei et al. (2009) and Andow and Lövei (2012). Regulatory agencies need to assure the safety of Bt crops before and during their commercial release. Therefore, they and the scientific community need to pay critical attention to how studies are conducted and analyzed. Studies that have errors in logic or errors in statistics should be dismissed so they do not influence regulatory decisions, public policy, or perceptions about agricultural biotechnology.

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Anthony M. Shelton
Cornell University/NYSAES
630 W. North St.
Geneva, NY 11456

Steven E. Naranjo
USDA–ARS
Arid-Land Agricultural Research Center
21881 N. Cardon Lane
Maricopa, AZ 85238

Jörg Romeis
Agroscope Reckenholz-Tänikon Research Station
ART
Reckenholzstr 191
8046 Zurich, Switzerland

Richard L. Hellmich
USDA–ARS
Corn Insects and Crop Genetics Research Unit
Iowa State University
Genetics Laboratory c/o Insectary
Ames, Iowa 50011