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LETTER TO THE EDITOR

# A case of "pseudo science"? A study claiming effects of the Cry1Ab protein on larvae of the two-spotted ladybird is reminiscent of the case of the green lacewing

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A recent report on the potential negative impact in a laboratory setting of the Cry1Ab protein on larvae of the two spotted ladybird *Adalia bipunctata* (Schmidt et al. 2009) has gained notoriety. It was used in Germany, along with some other studies supposedly showing a negative impact of the transgenic MON810 maize on non-target organisms, to temporarily ban the cultivation of this Bt-maize under a safeguard clause conforming with Article 23 of the EU directive 2001/18/EC. This decision, although officially communicated as based on new evidence, was in fact based on flawed science and has been recognized to be politically motivated by a number of the stakeholders involved (Sinha 2009). The present temporary ban of MON810 by the German Government will now be considered by the EU commission, which will consult the European Food Safety Authority (EFSA), and then decide on the merits of the scientific data. Whether the ban will then be lifted or not lies within the commitological decision making process within the European Union. The data on *A. bipunctata* was first published in the Proceedings of the German Society for General and Applied Entomology half a decade ago (Schmidt et al. 2004). Thus, it does not even constitute "new evidence".

In their experiment, Schmidt et al. fed *A. bipunctata* larvae with eggs of the flour moth *Ephestia kuehniella* that had been sprayed with solutions of Cry1Ab and Cry3Bb of different concentrations. Mortality in the treatments was higher than in the control groups. Larval developmental time and adult biomass were also measured, but there were no differences between the experimental groups. The authors concluded that the Cry proteins, and especially Cry1Ab, had a negative impact on *A. bipunctata* larvae, based on some unknown mode of action.

There are some obvious methodological flaws and inconsistencies in the Schmidt et al. (2009) paper, however:

Firstly, the quantity of stock solutions applied and the actual quantity of test proteins taken up by the ladybird larvae is not reported. It is only written that the eggs of the flour moth *E. kuehniella* used as food were "sprayed" with the different Cry protein dilutions. There is also no information given on the food consumption by the larvae during their development. The authors thus did not know, at least they did not state, the actual dose that the tested individuals took up during the experiment. Thus, the first important criterion defining risk, i.e., the degree of exposure, was not properly quantified.

Secondly, the mortality rates in identical control groups for the three separate treatments varied greatly between 7.5 and 20.8% for the 1st larval instars. These differences are neither explained nor addressed in the statistical analysis and the interpretation of the data, but strongly suggest methodological problems.

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Thirdly, the treatment groups with the highest Bt-protein concentrations did not show an increase in mortality rates over the groups treated with lower concentrations. This was the case for both Cry1Ab and Cry3Bb, and is a very astonishing result that contradicts classical dose-response relationship models. It is also interesting to note in this context that the Coleopteran-specific Cry3Bb protein had a smaller effect than the Lepidopteran-specific Cry1Ab, whereas one might expect the opposite to be the case for this test organism.

Fourthly, none of the treatments had any impact on either larval developmental time, or on the body-weight of adult beetles. This means that the larvae either died, or seemed to be totally unaffected by the treatments with Cry proteins. This is also surprising, since it is well established that susceptible organisms suffer from sub-lethal effects long before direct toxic effects (i.e. mortality) can be observed (e.g. Sears et al. 2001).

Schmidt et al. also totally neglect relevant recent literature. For example, Bai et al. (2005) reported the absence of any effects of rice pollen containing Cry1Ab on *Propylea japonica*. Similarly, a study by Álvarez-Alfageme et al. (2008) did not report any effects of Cry1Ab on another coccinellid, *Stethorus punctillum*. The discussion in the paper by Schmidt et al. would also have benefited from published results from a large number of field studies with Bt-maize reporting on a variety of beetle families encompassing Coccinellidae, Carabidae and Lathridiidae (de la Poza et al. 2005; Ahmad et al. 2006; Eckert et al. 2006; Toschki et al. 2006; Floate et al. 2007; Leslie et al. 2007). A recent report by Dhillon and Sharma (2009) that did show effects of Cry1Ab on larvae of *Cheilomenes sexmaculatus* in a laboratory setting, but using an exposure regime more relevant to the field situation, showed that "direct exposure to *Bt* toxins expressed in transgenic plants […] will have little effect on the activity and abundance of the ladybird". The claims and interpretations by Schmidt et al. need to be put into the context of the vast body of evidence showing that genetically modified plants expressing Cry1Ab do not have a negative impact on ladybirds.

An absence of ecological considerations in the interpretation of data is another striking flaw of the Schmidt et al. paper:

*Adalia bipunctata* are aphid predators and it is unclear to what extent these beetles would take up Bt-proteins in the field. For it is well documented that Bt-maize varieties do not carry Cry proteins in their phloem sap, and that aphid predators are unlikely to be exposed to Bt-proteins via their prey (Head et al. 2001; Raps et al. 2001; Dutton et al. 2002; Lundgren and Wiedenmann 2005). Therefore, exposure will only occur if these beetles consume maize pollen (see Cottrell and Yeargan 1998; Lundgren et al. 2005). This consumption of plant materials in comparison to that of aphid prey needs to be quantified in the field to assess the potential risk. Schmidt et al. touch these issues only superficially, without reaching reasonable conclusions on the relevance of their own data. In this instance, it is important to note that the expression level for Cry1Ab in pollen from MON810 (the only Bt-maize cultivated in the European Union) is extremely low, ranging to a maximum of 97 ng Cry1Ab/g fresh weight (Nguyen and Jehle 2007). Interestingly, Schmidt et al. give false expression levels of 7.93–10.34 μg Cry1Ab/g fresh weight (which is the expression level in leaves according to their source, AGBIOS), that exaggerate the potential exposure in the field and thus give a false impression on the relevance of this exposure pathway.

*Adalia bipunctata* cannibalise their own eggs and suffer from intra-guild predation by other Coccinellids (Schellhorn and Andow 1999; Burgio et al. 2002). The authors fail to give a perspective on the quantitative relevance of a potential additional mortality based on the effects of Bt-proteins in relation to these two alternative common mortality factors.

Moreover, *A. bipunctata* is in fact not a very common ladybird in maize, at least not in Germany (Rauschen et al. 2009). The significance of a potential negative impact on the limited populations occurring in maize cropping systems for the population as a whole on a larger regional scale remains doubtful.

Overall, the findings and interpretations in Schmidt et al. and the consideration of this paper for the justification of the ban of MON810 in Germany appear erroneous.

In the past, similar results on potential negative impacts of Cry1Ab expressing Bt-maize varieties on another beneficial insect, the green lacewing *Chrysoperla carnea*, had been published. After about 10 years of research, we now recognize that these early claims were flawed, and that effects measured in the original investigations were based on poor prey quality, and not on direct toxic effects of Cry1Ab (Shelton et al. 2009; for a detailed account see Romeis et al. 2009). The green lacewing case has been very prevalent in discussions concerning the potential risks of Bt-plants for the environment and is still often cited by non-governmental organisations and certain ill-informed regulatory bodies, although scientifically has been thoroughly refuted. Sadly, it appears that results from the flawed experiments utilizing the two-spotted ladybird might be used to justify an indefensible political position and misinform the lay public. In the overall context of non-target risk assessment (Romeis et al. 2008), the Schmidt et al. paper at best indicates a potential hazard of an insecticidal compound to larvae of *A. bipunctata* under certain artificial conditions. The next step must be to assess the relative importance of different exposure pathways, and to assess whether the effects are still visible under more realistic exposure regimes. If they do, it remains to be assessed whether these effects have wider implications for the exposed population of this species and for its function as a biological control agent. Only if these questions are thoroughly and reasonably addressed, in a scientifically sound manner, can reliable conclusions on real potential risks be drawn. And only then can political decision makers reach judgments that are not clouded by the results of ill-conceived and shoddy research.

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