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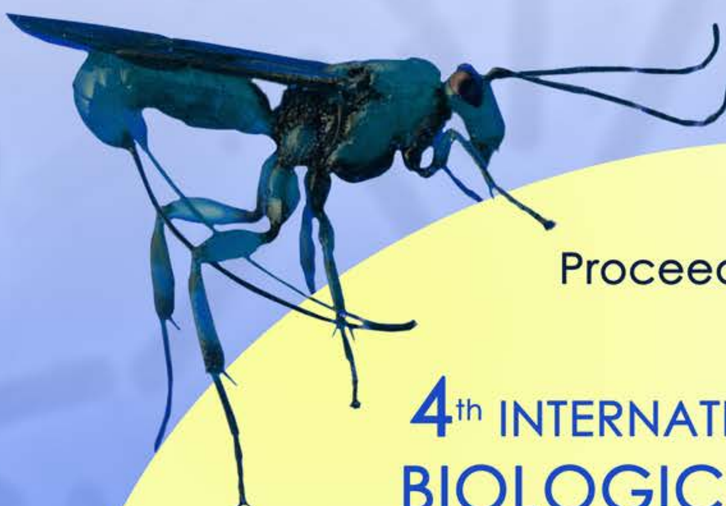

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Effects of *Bt* crops on arthropod natural enemies: A global synthesis

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Introduction: The global adoption of transgenic crops producing the insecticidal proteins from *Bacillus thuringiensis* Berliner (Bacillaceae), (*Bt*) continues to grow with 66 M hectares of *Bt* crops grown in a total of 25 countries in 2011 (James 2011). Unintended environmental effects from the technology continue to be of concern, with one of the key issues being effects on valued non-target organisms. A large number of non-target studies have been completed over the past 15-20 years in support of risk assessment in *Bt* crops. In late 2008 Naranjo (2009) cataloged over 360 original research articles that have studied the effects of *Bt* crops on non-target invertebrates. These include studies of 9 crop plants and dozens of *Bt* proteins in a total of 15 countries. Since 2008 the pace of research in this area has diminished only slightly, with a total of over 520 studies as of mid 2012, and the number of participating countries has expanded. A number of reviews and meta-analyses have been conducted on portions of this literature in an attempt to summarize and synthesize general and specific patterns (e.g. Romeis et al. 2006, Marvier et al. 2007, Wolfenbarger et al. 2009, Naranjo 2009). In general, these summaries and analyses have demonstrated that *Bt* crops have negligible or no effect on non-target arthropod abundance in the field or on various measures of their biology in the laboratory. However, many additional studies have been published since the latest reviews and synthetic studies were completed. Here I re-assess the world literature on arthropod natural enemies and quantify non-target effects of *Bt* crops via meta-analyses of both laboratory and field studies. The primary question is: do we now have enough data to conclusively determine the effects of current *Bt* crops on non-target arthropod natural enemies?

Background and Approach: Marvier et al. (2007) assembled the original non-target invertebrate database and published the first general meta-analysis based on field studies examining *Bt* cotton and maize. A year later Wolfenbarger et al. (2008) modified the database and performed meta-analyses to examine the effects of *Bt* cotton, maize and potato on the field abundance of ecological functional guilds. Naranjo (2009) then updated the full database with a number of new field and laboratory studies, performed additional meta-analysis of the field studies and also completed the first meta-analyses of laboratory studies. Finally, Duan et al. (2010) performed meta-analyses to examine the relationship between laboratory and field results based on a subset of the database focused on survival. Here, I have again updated the database, but only for natural enemy guilds. This latest database now includes 92 laboratory and 67 field studies on arthropod natural enemies. Many potential studies could not be included due to incomplete data reporting by study authors. This new database covers 1 Phylum, 2 Classes, 13 Orders and 46 Families of predators and 1 Phylum, 1 Class, 3 Orders and 25 Families of parasitoids. For meta-analyses I used Hedge's d, a weighted effect size estimator that is calculated as the difference between an experimental (*Bt*) and control (non-*Bt*) mean response (abundance, survival rate, development time, fecundity, etc.) divided by a pooled standard deviation and corrected for small sample size bias. Fixed, weighted categorical analyses were performed with MetaWin 2.1 (Rosenberg et al. 2000).

Results: I first conducted a cumulative meta-analysis on the field studies for two functional guilds, predator and parasitoids (Figure 1). This analysis provides a running snapshot of overall results as new studies became available over time. It clearly shows that while results have become less variable over time, with increased sample size, the conclusion that *Bt* crops do not significantly affect the abundance of non-target natural enemies has been clear from even the earliest studies. The addition of new studies since 2008 has not altered that conclusion.

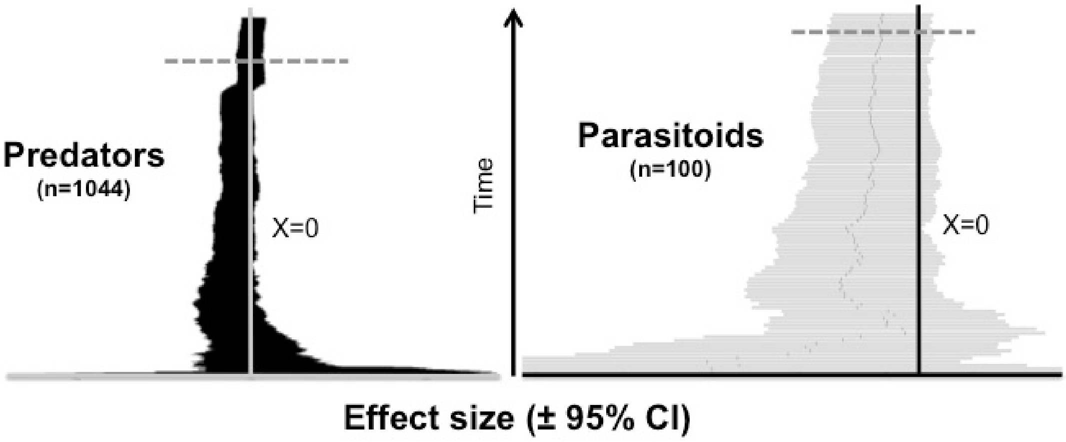

Figure 1. Cumulative meta-analysis of field studies examining abundance of arthropod predators and parasitoids. The analyses show the effect of adding more studies over time on the effect size and its associated 95% confidence interval. The dotted gray line indicates new studies that were added since 2008. Results show that the effect size was never significantly different from zero (=no difference between *Bt* and non-*Bt* treatments) and that conclusion has not changed with the addition of recent studies.

The study database was then parsed by country of origin for these two functional groups. For 11 countries, effect sizes were not significantly different from zero for either group. Studies for predators in the USA actually show a significant positive effect size indicating that abundance was on average slightly higher in *Bt* crops compared with the non-*Bt* control. Parsing by five *Bt* crops (cotton, maize, potato, eggplant, rice), again effects on predators and parasitoids were largely neutral, the only difference being a higher abundance of predators on *Bt* potato. Neutral effects were also seen for predators and parasitoids regardless of the pest target of the *Bt* crop (Lepidoptera or Coleoptera) or the number or type of insecticidal proteins produced by the crop (single or pyramided, Cry or VIP). These results are all consistent with prior meta-analyses (Wolfenbarger et al. 2008, Naranjo 2009) as are the results that the alternative use of insecticides to control the target pest led to large reductions in abundance of natural enemies in non-*Bt* crops.

Laboratory studies have focused on either direct exposure of natural enemies to *Bt* proteins through plant material or spiked artificial diets, or more commonly, through tri-trophic exposures where the prey is fed on *Bt* plants or diets and then provided to the predator or parasitoid. The former exposure pathway has provided unequivocal results: *Bt* proteins do not affect life history characteristics such as development, reproduction or survival. Results from tri-trophic exposure studies have been variable (Figure 2), but careful examination of these studies show that the nature of the prey provided to the natural enemy is critical to interpreting *Bt* effects. Prey that are susceptible to the *Bt* proteins (target pests or related species) are negatively affected by these proteins and this reduces their quality as prey for natural enemies. Results clearly show, especially for parasitoids, that attacking these compromised, or low quality, prey negatively affects life history traits. However, if low quality issues are removed by using non-susceptible prey or prey that are resistant to *Bt* proteins then effects of the *Bt* proteins are either neutral or sometimes even positive (Figure 2). This provides conclusive evidence that it is prey quality and not *Bt* proteins that are associated with negative effects. This also explains why recent results from an analysis by Lövei et al. (2009, but see rebuttal by Shelton et al. 2009) deviate from all other synthetic analyses and reviews – they did not account for prey quality mediated effects.

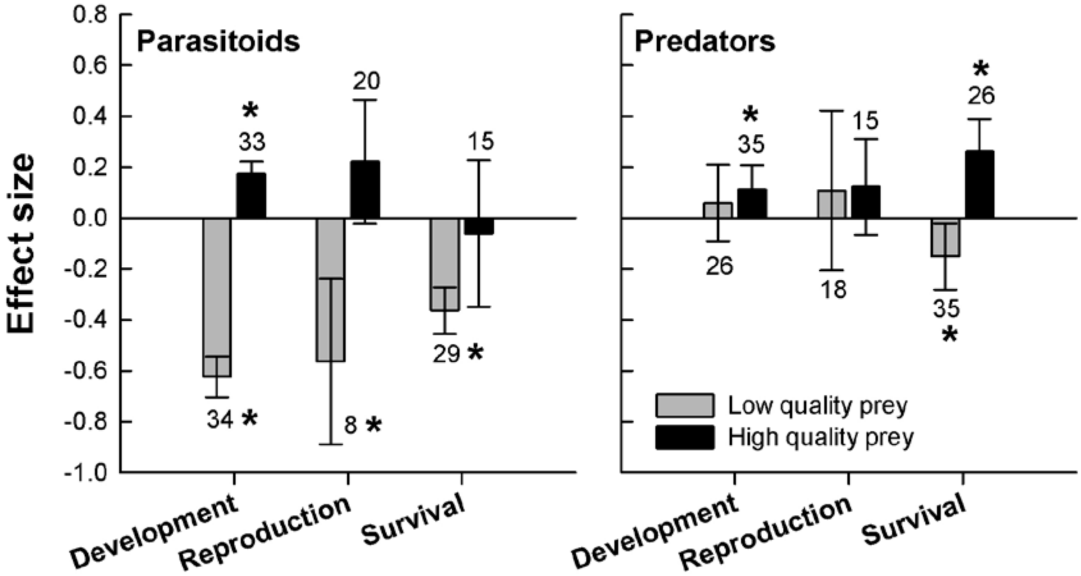

Figure 2. Meta-analyses of studies examining effects of *Bt* proteins on arthropod predators and parasitoids when the natural enemy was exposed to the *Bt* proteins indirectly through their prey. Prey are either susceptible to the *Bt* proteins, and thus compromised (low quality), or unaffected by *Bt* proteins (high quality). Mean effect sizes are plotted so that negative values indicate poorer performance from indirect *Bt* exposure. Error bars are 95% confidence intervals and asterisks indicate the mean effect size is significantly different from zero; numbers are sample size. Modified from Naranjo (2009) to include new studies since 2008.

Conclusion: A large number of laboratory and field studies have been conducted to measure the non-target effects of *Bt* crops and several reviews and meta-analyses have synthesizes these data. Here I show that the addition of new studies since 2008 have not changed the conclusion that *Bt* proteins are highly selective and do not negatively affect non-target arthropod natural enemies. For field studies, origin of the study, crop species, or the type or pyramiding of proteins does not influence these results. Laboratory studies clearly show no direct effects of *Bt* protein on life history characteristics of arthropod predators or parasitoids and further show that any indirect negative effects of exposure of natural enemies through their prey are due to prey quality and not to *Bt* proteins. Overall, the analyses support the conclusion that available data indicate a lack of effect of *Bt* proteins on arthropod natural enemies.

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