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A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae)

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Summary. Honey bees (*Apis mellifera* L.) are the most important pollinators of agricultural crops worldwide and are a key test animal used in the tiered safety assessment of genetically engineered insect resistant crops. We conducted a meta-analysis of 22 independent studies that assessed potential effects of Bt Cry proteins on honey bee survival (or mortality). Our results show that Bt Cry proteins used in commercialized GM crops for control of lepidopteran and coleopteran pests do not negatively affect either honey bee larvae or adults. These findings support safety assessments that conclude minimal or low risk of Bt crops to this vital insect pollinator.

Key words: ecological risk assessment, meta-analysis, non-target organisms

The value of honey bees (Hymenoptera: *Apis mellifera* L.) to U.S. agriculture exceeds $14 billion annually, and nearly a third of the U.S. diet depends on the activity of honey bees (Johnson 2007; Morse & Calderone 2001). In late 2006, U.S. commercial beekeepers reported a rapid decline in adult bee populations, with many hives showing symptoms that were inconsistent with mite damage or other known causes of colony loss (Stokstad 2007). This phenomenon, also observed in Europe, has been termed Colony Collapse Disorder (CCD) because it was characterized by a sudden loss in foraging bees that failed to return to the hive. Typically, affected colonies had healthy, capped brood; a laying queen with attendants; food reserves; and minimal evidence of parasitic mites and other pests. The cause of CCD is yet unknown, but mites, pathogens, management stress, weather effects, and pesticides have been suggested (Stokstad 2007). Some have suggested that genetically engineered insect resistant crops might be involved (Latsch 2007; McDonald 2007).

Currently, all commercialized genetically engineered insect resistant crops are based on crystalline (Cry) proteins encoded by genes derived from the soil dwelling bacterium *Bacillus thuringiensis* (Bt). Studies on the mode of action and toxicology of Bt Cry proteins have established that these proteins are toxic to select groups of insects. Cry proteins currently produced in commercialized Bt crops target insects in the orders Lepidoptera (moths) and Coleoptera (beetles). Because of this specificity, most experts feel it is unlikely that Bt crops would impact honey bee populations. Nevertheless, because of their importance to agriculture, honey bees have been a key test species used in environmental safety assessments of Bt crops (OECD 1998; U. S. EPA. 1996). These assessments have involved purified Cry proteins assayed against both honey bee larvae and adults, as well as tissue studies using pollen collected from Bt crops.

To date, no individual tests involving Bt crops or Cry proteins have shown significant impacts on honeybees (Mendelhsohn et al. 2003; Romeis et al. 2006). However, there is always the possibility that small sample sizes and high variability undermine the power of individual risk assessment experiments (Marvier 2002). Given the continued speculation about adverse impacts of Bt crops on honeybees, there is a need for a formal meta-analysis. Such a meta-analysis can combine all of the existing experiments and provide a more compelling analysis of Bt effects. A recent meta-analysis, synthesizing results from 42 field studies involving Bt cotton and maize (Marvier et al. 2007), was not able to examine effects on honey bees because few studies report field data for this group (but see Rose et al. 2007).

Here we report a meta-analysis of 22 independent laboratory studies (Table 1) that focused on the chronic and/or acute toxicity of Bt Cry proteins or Bt plant tissues (pollen) on honey bee larvae and adults. Data associated with these studies were extracted from the

Nontarget Effects of Bt Crops Database (http://delphi.nceas.ucsb.edu/btcrops/; Marvier et al. 2007). Available studies reported a range of response variables including survival, growth, development, and abundance. Most studies reported survival (or mortality), and to maximize consistency among studies and reduce issues of non-independence, we focused only on survival data. Studies involved two categories of Bt Cry proteins either expressed in Bt plant tissues or produced by genetically modified *Bacillus thuringiensis* or *Escherichia coli* strains: lepidopteran-active (Cry1, Cry2, or Cry9 class) and coleopteran-active (Cry3 class) proteins. Several studies included multiple independent experiments or measures of survival for multiple stages. All studies included a comparison to a negative (no Cry protein) control, and presented treatment means accompanied by standard deviations (s) and sample sizes (n) necessary to calculate the metric of effect size, Hedges' d (Marvier et al. 2007).

Hedges' d was calculated for each study as the difference between the means of the Bt Cry protein and control treatments divided by the pooled standard deviation and weighted by the sampling variance. Negative values indicate lower survival (whereas positive values indicate higher survival) in Bt Cry protein treatments compared to non-Bt control treatments. Bias-corrected bootstrap 95% confidence intervals (CIs) were used to determine if specific effect sizes differed significantly from zero.

When all studies were combined, no statistically significant effect of Bt Cry protein treatments on survival of honey bees was detected (N = 38, d = 0.012, 95% CI = -0.039 to 0.069). In fact, the mean d-values were never negative, which is resounding evidence of negligible impact on honeybees. When data for lepidopteran-active and coleopteran-active Bt Cry proteins were compared using a fixed categorical meta-analysis model, the above pattern of no significant effects held true for each class of protein (Fig. 1). No significant difference in effect sizes was detected between lepidopteran-active and coleopteran-active proteins (Q = 0.321, df = 1, P = 0.41); nor was any significant within-group heterogeneity detected for effect sizes calculated for either lepidopteran-active (Q_w = 11.641, df = 30, P > 0.99) or coleopteran-active proteins (Qw = 3.432, df = 6, P = 0.75).

No significant effects occurred with either larval or adult stages. This pattern was consistent when data from studies using lepidopteran-active and coleopteran-active Bt Cry proteins were analyzed either together (Fig. 2a) or separately (Fig. 2b), except that there was a significantly positive effect of coleopterative-active Bt Cry proteins on honey bee adults (d = 0.449; 95% CI = 0.270 to 0.700; Fig. 2c; result based on only two studies). No significant differences in effect sizes were detected between larvae and adults in any of the above analyses (Fig. 2a: Q = 0.361, df = 1, P = 0.36; Fig. 2b: Q = 0.150, df = 1, P = 0.58; Fig. 2c: Q = 0.354, df = 1, P = 0.38), nor were any significant within-group heterogeneities detected for the effect sizes calculated for either larvae (Fig. 2a: Q_w = 8.831, df = 24, P > 0.99; Fig. 2b: Qw = 5.881, df = 19, P > 0.99; Fig. 2c: Q_w = 2.926, df = 4, P = 0.55) or adults (Fig. 2a: Q_w = 6.202, df = 12, P = 0.91; Fig. 2b: Qw = 5.611, df = 10, P = 0.85; Fig. 2c: Qw = 0.153, df = 1, P = 0.70).

The lack of adverse effects of Bt Cry proteins on both larval and adult honey bees is consistent with our knowledge of the activity-spectrum and mode of action of different classes of Bt Cry proteins. To date, no class of Bt Cry protein has been found to be directly toxic to hymenopteran insects. Our findings strongly support the conclusion that the Cry proteins expressed in the current generation of Bt crops are unlikely to have any adverse effects on the survival of honey bees.

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Figure 1. Meta-analysis of studies that report survival of honey bees exposed to Bt Cry proteins or plant tissues (pollen): (a) lepidopteran-active proteins and (b) coleopteran-active proteins. Effect size is Hedge's *d,* and error bars represent bias-corrected bootstrap 95% confidence intervals. Positive mean effect sizes indicate improved survival when exposed to Cry proteins compared to water or sugar-water control treatments. N = number of lines of independent data summarized by each bar.

Figure 2. Comparison of effect sizes for larval and adult honey bees exposed to different Bt Cry proteins or plant tissues: (a) lepidopteran-active and coleopteran-active proteins combined, (b) lepidopteran-active Bt Cry proteins only, and (c) coleopteran-active protein only. Error bars and N are as described for Figure 1.

Short title: Bt crop effects on honey bees

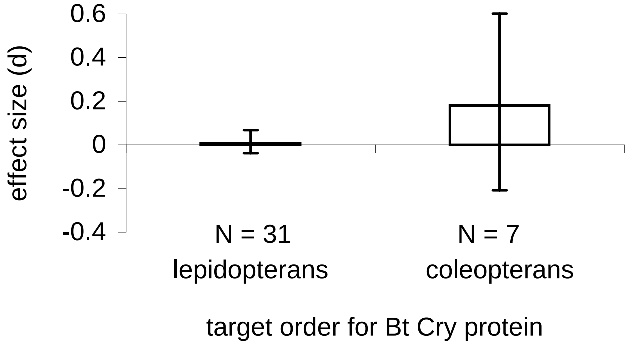
Table 1. Major characteristics of the laboratory studies included in the meta-analysis.

reference	**Cry protein class**	**Cry protein sources**	**target insect order**	**sample size (n)**	**stages exposed**	**endpoints measured**	**peer-reviewed**
Arpaia 1996	Cry3B	GM *E. coli*	Coleopteran	2	Larvae	Survival & growth	Yes
Hanley et al. 2003	Cry1F/Cry1Ab	Corn pollen	Lepidoptera	3 - 150	Larvae	Mortality & growth	Yes
Maggi 1993a	Cry1Ac	B.t.k.	Lepidoptera	3	Adults	Mortality	No
Maggi 1993b	Cry1Ac	B.t.k	Lepidoptera	4	Larvae	Survival	No
Maggi 1993c	Cry3A	B.t.t.	Coleoptera	3	Adults	Mortality	No
Maggi 1993d	Cry3A	B.t.t.	Coleoptera	4	Larvae	Mortality & development	No
Maggi 1996	Cry3A	B.t.t.	Coleoptera	4	Larvae	Mortality	No
Maggi 1999a	Cry1F	Corn pollen	Lepidoptera	4	Larvae	Mortality	No
Maggi 1999b	Cry3Bb1	GM *E. coli*	Coleoptera	4	Adults	Mortality	No
Maggi 1999c	Cry3Bb1	GM *E. coli*	Coleoptera	4	Larvae	Mortality	No

Maggi 2000a	Cry2Ab2	Bt	Lepidoptera	4	Adults	Mortality	No
Maggi 2000b	Cry2Ab2	Bt	Lepidoptera	4	Larvae	Mortality	No
Maggi 2000c	Cry2Ab2	Bt	Lepidoptera	4	Larvae	Mortality & development	No
Maggi & Sims 1994a	Cry1Ab	B.t.k.	Lepidoptera	3	Adults	Mortality	No
Maggi & Sims 1994b	Cry1Ab	B.t.k	Lepidoptera	4	Larvae	Survival	No
Malone et al. 1999	Cry1Ba	Bt strains	Lepidoptera	3	Newly-emerged adults	Survival	Yes
Malone et al. 2001	Cry1Ba	Bt strains	Lepidoptera	20	Newly-emerged adults	Survival	Yes
Malone et al. 2004	Cry1Ba	Bt strains	Lepidoptera	9	Newly-emerged adults	Survival	Yes
Palmer & Krueger 1997	Cry9C	Corn pollen	Lepidoptera	6	Adult	Mortality	No
Rose et al. 2007	Cry1Ab	Corn pollen & plants	Lepidoptera	4 - 10	All stages	Mortality, growth, and abundance	Yes

Sims 1995	Cry1Ac	Bt strains	Lepidoptera	2 - 3	Larvae & newly-emerged adults	Mortality	Yes
Sims 1997	Cry2A	Bt strains	Lepidoptera	2	Larvae & newly-emerged adults	Mortality	Yes

1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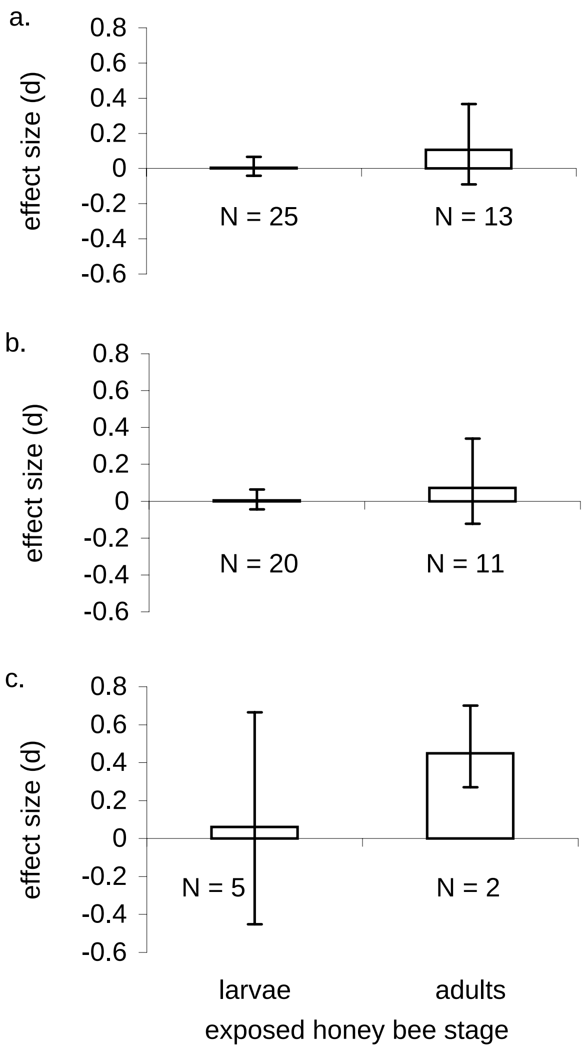
N = 31
lepidopterans
N = 7
coleopterans
target order for Bt Cry protein


a.
effect size (d)
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0.6
0.4
0.2
0
-0.2
-0.4
-0.6
N = 25
N = 13
b.
effect size (d)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
N = 20
N = 11
c.
effect size (d)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
N = 5
N = 2
larvae
adults
exposed honey bee stage