



Supporting Online Material for

A Meta-Analysis of Effects of Bt Cotton and Maize on Nontarget Invertebrates

Michelle Marvier,* Chanel McCreedy, James Regetz, Peter Kareiva

*To whom correspondence should be addressed. E-mail: mmarvier@scu.edu

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Other Supporting Online Material for this manuscript includes the following:
available at www.sciencemag.org/cgi/content/full/316/5830/1475/DC1

Data file (1139208s2.csv)

Materials and Methods

To locate studies of the nontarget effects of *Bt* crops, we used multiple search criteria (e.g. nontarget* and *Bt* or *Bacillus thuringiensis*) in the online databases Agricola, BioAbstracts, PubMed, and ISI Web of Science. Additional studies were found by searching the reference lists of empirical and review papers, performing general internet searches, and sending a list of references accompanied by a request for additional suggestions to over 100 researchers who work on this subject.

Once a study was located, it had to meet a series of criteria in order to be included in the full database of *Bt* nontarget effect studies (<http://delphi.nceas.ucsb.edu/btcrops/>). Specifically, studies had to: (i) involve a field crop species genetically transformed to express one or more *cry* genes derived from *Bacillus thuringiensis*; (ii) measure effects of the transformed crop for one or more groups of non-target invertebrate; (iii) include a comparison to a non-transgenic control or a range of exposure levels to the transgenic plant or plant products (e.g. pollen); and (iv) be written in English.

For each study, we recorded the authors' affiliations (academic, government, or corporate), details about the *Bt* crop (e.g., species, Cry protein, transgenic event and variety), the nontarget organism (e.g., taxonomy, functional group, age or stage), and the control treatment (e.g., crop variety, whether sprayed with insecticides or not). If authors did not specify the functional role of the nontarget group, we used functional roles as described by (SI) or one of several credible internet resources. We also recorded study location, plot sizes, study duration, sampling method, and other methodological details. For many studies, we scanned data figures and used Adobe Photoshop software to extract means and measures of within treatment variance. In these cases we

contacted authors directly to verify scanned data values. We also contacted authors, as needed, to obtain other pieces of information.

The full database includes laboratory and field studies, a wide range of response variables (e.g., abundance, growth, survival, reproduction), and data for *Bt* cotton, eggplant, maize, oilseed rape, potato, rice, and tobacco. For the present set of analyses, we queried this database for studies that: (i) involved one of three categories of *Bt* crops (lepidopteran resistant Cry1Ac cotton, lepidopteran resistant Cry1Ab maize, and coleopteran resistant Cry3Bb maize) for which we had suitable data to calculate effect sizes derived from a minimum of five distinct publications or reports (Table S1); (ii) occurred in the field or in a cage in the field; (iii) reported abundance as a response variable for a nontarget group; (iv) included a comparison to a non-transgenic control (either sprayed with an insecticide or not); and (v) presented treatment means, accompanied by standard deviations (s) and sample sizes (n) (or the author directly provided these values to us) necessary to calculate our metric of effects size, Hedges' d ($S2$) (i.e., we required $n_1 > 0$, $n_2 > 0$, $n_1 + n_2 > 2$, and $s_1(n_1 - 1) + s_2(n_2 - 1) > 0$). Measures of standard error, $s_{\bar{x}}$, were transformed to standard deviations ($s = s_{\bar{x}} \sqrt{n}$) as needed. Although we could have included data from additional studies if we had used an unweighted response ratio, the statistical power of the meta-analysis may have been compromised by using such an approach ($S3$). Application of these criteria yielded data from a suite of 42 suitable publications or reports (Table S2).

Many studies reported data for multiple nontarget groups, sampling dates, sampling methods, and/or control treatments (e.g., treated with different insecticides). To balance concerns regarding non-independence with our desire to retain as much valuable information as possible, we applied the following additional rules in our query:

1. If a *Bt* crop was compared to multiple non-transgenic varieties, we selected the non-transgenic variety that was either isogenic to the *Bt* variety or as close as possible. However, when multiple *Bt* treatments (different events or hybrids) were compared to a single control treatment, we allowed the data for the control treatment to be reused multiple times in our analyses. If a *Bt* crop was compared to multiple control treatments (e.g., the non-GM variety sprayed with one of three different pesticides), we used different approaches for maize and cotton. For maize, we limited the insecticide treatments to those using pyrethroids. For cotton, because there were not enough data for any one class of insecticides, we included all insecticide types and allowed the data for the *Bt* treatment to be reused multiple times in our analyses.

2. If taxonomic groups were lumped in different ways within the same paper (e.g., by individual species and then lumped by family or functional group), we used data separated out to the finest possible taxonomic resolution. We did, however, allow data from multiple distinct taxonomic groups; for some experiments, there might be data on scores of different species.

3. For studies reporting repeated measures on the same plots, we used two approaches: (i) if authors were willing to provide raw data, we took means across the repeated samples, and (ii) we recorded values from the time point with the peak abundance for each treatment. Note that the peak abundance in the control treatment may have occurred at a different time than the peak abundance in the *Bt* treatment. If both seasonal mean and peak abundance data were available for a particular taxonomic group within a particular experiment, we selected the seasonal mean data for our analyses.

4. When distinct experiments were reported within a single paper (e.g., a replicated experiment performed at multiple sites or a replicated experiment performed in distinct years with treatments randomly re-assigned to plots each year), we allowed one entry per species (or the next finest

taxonomic grouping) per experiment. There were two primary reasons why multiple entries might exist for a taxonomic group within an experiment: data were reported for 1.) multiple lifestages (e.g., eggs, larvae, and adults) of a nontarget group and/or 2.) multiple sampling methods (e.g., pitfall traps, visual counts, and sweepnets). In such cases, we selected the lifestage or sampling method that detected the highest abundance of nontarget organisms in the control treatment. If the abundances in the control treatments were tied, we selected the lifestage or method that resulted in the lowest standard deviation for the control group. In the few cases where both the mean and standard deviation of the control groups were tied, we selected the method that sampled the most mature lifestage (i.e. we selected data for adults rather than larvae when all else was equal).

We applied the above rules to query our full database and generated a data file used in all subsequent analyses. This data file is available as part of the supplemental online material.

The field studies included in our analyses occurred in North America, Europe, Asia, and Australia (Table S2). Although not specifically mentioned in most studies, the climatic conditions and soil types likely varied substantially across the collection of studies. The insecticide treatments also encompassed a range of approaches – some researchers reported using insecticides on an as-needed basis, whereas others used them according to standard practices for the region, without any indication of whether they were needed. Sampling methods also varied across studies and included visual counts, suction sampling, sweep nets, pitfall traps, and parasitism rates. Although the units for abundance differed from study to study, or even among taxa within a study, our metric of effect size (Hedges' *d*) converts the difference in mean abundance between the two treatments to standard deviation units, allowing somewhat disparate measures to be combined in any one analysis.

Supplemental Results

Coleopterans and hemipterans were slightly less common in Cry1Ac cotton than non-GM, insecticide-free cotton (Fig. 2a). There were no striking patterns when comparisons were made across functional groups within these orders (coleopterans by functional group: $Q_b = 1.44$, $df = 5$, $P = 0.848$; hemipterans by functional group: $Q_b = 3.90$, $df = 4$, $P = 0.322$). There were also no differences among families within the coleopterans (coleopterans by family: $Q_b = 4.76$, $df = 12$, $P = 0.883$). However, the taxonomic families of hemipterans did differ significantly (hemipterans by family $Q_b = 20.21$, $df = 11$, $P = 0.011$), with significant negative effects observed in the Pentatomidae, Miridae, Nabidae, Aleyroididae, and Scutelleridae.

Sensitivity Analyses

To examine the robustness of our conclusions to plausible alternative modeling choices, we examined how results presented in Figure 1 differ when we excluded:

- a. studies that were not peer-reviewed;
 - b. data that represented “peak days” measures (as opposed to single measures or seasonal means);
- and
- c. data that were not recorded for invertebrate groups segregated at least to the level of taxonomic family.

Our results were generally robust to these modifications of our data query. Excluding data from studies that were not peer-reviewed (or where it was not clear) resulted in no qualitative changes to our findings, with the exception that the lower abundance of non-target invertebrates in Cry1Ab maize compared to insecticide-free controls was diminished. Excluding data taken from peak days (rather than point estimates or seasonal means) had no qualitative effect on our

conclusions. Excluding data that were not recorded for invertebrate groups segregated at least to the level of taxonomic family, our findings were again qualitatively unchanged.

Table S1. Three categories of *Bt* crops included in our meta-analyses.

<i>Bt</i> crop	Typical target pest species	Target pest order
Cry1Ac cotton	<i>Helicoverpa zea</i> or <i>H. armigera</i> (cotton bollworm), <i>Heliothis virescens</i> (tobacco budworm), <i>Pectinophora gossypiella</i> (pink bollworm)	Lepidoptera
Cry1Ab maize	<i>Ostrinia nubilalis</i> (European corn borer), <i>Helicoverpa zea</i> (corn earworm)	Lepidoptera
Cry3Bb maize	<i>Diabrotica</i> spp. (corn rootworm)	Coleoptera

Table S2. Summary of all studies included in analyses of the effects of *Bt* crops upon non-target invertebrates. *n* is the number of replicate plots or fields (the physical unit assigned by the researcher to a treatment or treatment combination) per treatment. NTC: non-transgenic control without insecticide sprays; CWI: non-transgenic control with insecticide applications; IOB: insecticides on both *Bt* and control treatments; n.s.: not specified; *: experiments described in this reference overlap at least partially with those of the previous reference. Values shown here reflect only the data that were included in our analyses; reports may have included additional experiments or comparisons with different values. The full dataset for our analyses is available as part of the supplemental online material.

Citation	Location (continent)	<i>Bt</i> crop	Cry protein	<i>Bt</i> event	Plot size (range; ha)	<i>n</i> (range)	Type of control	Peer reviewed
(S4)	North America	maize	Cry3Bb	MON863, n.s.	0.003-0.023	4	NTC & CWI	Yes
(S5)	North America	maize	Cry3Bb	n.s.	0.003-0.023	4	NTC & CWI	Yes
(S6)	North America	maize	Cry3Bb	MON853, MON862, MON863	0.002-0.023	2-4	NTC & CWI	Yes
(S7)*	North America	maize	Cry3Bb	MON862, MON863	0.002-0.023	2-4	NTC & CWI	Yes
(S8)	North America	cotton	Cry1Ac	MON531 x MON1445	24.28-32.37	5	NTC	n.s.
(S9)	Europe	maize	Cry1Ab	MON810	0.09	6	NTC	n.s.
(S10)	Asia	cotton	Cry1Ac	MON531/757/1076	1.44-5.6	7	IOB	Yes
(S11)	North America	maize	Cry3Bb	MON863	0.033	4	NTC & CWI	No

(S12)	North America	maize	Cry3Bb	MON863	0.033	4	NTC & CWI	Yes
(S13)*	North America	maize	Cry3Bb	MON863	0.033	4	NTC & CWI	Yes
(S14)	North America	maize	Cry3Bb	MON863	0.033	4	NTC & CWI	Yes
(S15)	Europe	maize	Cry1Ab	MON810, Bt176	0.09	4	NTC	Yes
(S16)	North America	maize	Cry1Ab	MON810	0.053	4	NTC	Yes
(S17)	Europe	maize	Cry1Ab	Bt176	0.4-0.7	3-4	NTC	Yes
(S18)	Europe	maize	Cry1Ab	Bt176	0.006	5	NTC	n.s.
(S19)	North America	maize	Cry1Ab	Pacha/Bt11	0.4	3	NTC & CWI	Yes
(S20)	North America	maize	Cry1Ab	Bt11	0.044-0.066	4	NTC & CWI	n.s.
(S21)	North America	maize	Cry1Ab	n.s.(Rogers 'Empire' Bt)	0.059	4-9	NTC	Yes
(S22)	Europe	maize	Cry1Ab	MON810	0.25	8	NTC & CWI	Yes
(S23)	North America	cotton	Cry1Ac	MON531/757/1076	0.021-0.106	4	NTC & CWI	Yes
(S24)	North America	cotton	Cry1Ac	MON531/757/1076	5-30	3-4	IOB	Yes
(S25)	Europe	maize	Cry1Ab	Bt176, MON810	0.15	3-5	NTC & CWI	n.s.
(S26)	North America	maize	Cry1Ab	MON810	0.39	3	NTC & CWI	Yes
(S27)	Europe	maize	Cry1Ab	Bt176	2	3	NTC	Yes
(S28)	Europe	maize	Cry1Ab	Bt176	n.s.	9	NTC	n.s.
(S29)	Europe	maize	Cry1Ab	Bt176	0.15	4	NTC & CWI	Yes

(S30)	Asia	cotton	Cry1Ac	MON531	0.4	3	NTC & CWI	Yes
(S31)	North America	maize	Cry1Ab	Bt11	0.003	3-16	NTC & CWI	Yes
(S32)	North America	cotton	Cry1Ac	MON531	0.085-2	3-4	NTC, CWI, & IOB	Yes
(S33)	North America	maize	Cry1Ab	Bt176	0.405	3	NTC	Yes
(S34)	North America	maize	Cry1Ab	Bt176, MON810	0.002-0.005	3	NTC	Yes
(S35)	North America	maize	Cry1Ab	Bt11, Bt176	0.047	4-36	NTC	Yes
(S36)	Europe	maize	Cry1Ab	Bt176	0.4-1	12	NTC	Yes
(S37)	Europe	maize	Cry1Ab	MON810	0.25	8	NTC & CWI	n.s.
(S38)	Europe	maize	Cry1Ab	MON810	0.5	5	NTC	n.s.
(S39)	North America	cotton	Cry1Ac	MON531	1.04	7	IOB	Yes
(S40)	North America	cotton	Cry1Ac	MON531	0.008	7	NTC	Yes
(S41)	Europe	maize	Cry1Ab	MON810	0.09	6	NTC	Yes
(S42)	North America	cotton	Cry1Ac	n.s. (DPL458, DPL555)	5.95-9.08	3	IOB	Yes
(S43)	Europe	maize	Cry1Ab	MON810	0.09	6	NTC	Yes
(S44)	Australia	cotton	Cry1Ac	MON531, MON757	1.2-3.675	2-3	NTC	Yes
(S45)	North America	maize	Cry1Ab	Bt11	0.003-0.056	4	NTC	Yes

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