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

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Although scores of experiments have examined the ecological consequences of transgenic *Bacillus thuringiensis* (Bt) crops, debates continue regarding the nontarget impacts of this technology. Quantitative reviews of existing studies are crucial for better gauging risks and improving future risk assessments. To encourage evidence-based risk analyses, we constructed a searchable database for nontarget effects of Bt crops. A meta-analysis of 42 field experiments indicates that nontarget invertebrates are generally more abundant in Bt cotton and Bt maize fields than in nontransgenic fields managed with insecticides. However, in comparison with insecticide-free control fields, certain nontarget taxa are less abundant in Bt fields.

Public debate regarding risks and benefits of genetically modified (GM) crops continues unabated (*1*–*5*). One reason for the unrelenting controversy is that disagreements about new technologies often have little to do with scientific uncertainty but instead arise from differing personal values and differing levels of trust in public institutions (*6*, *7*). However, in the case of GM crops, scientific analyses have also been deficient (*4*). In particular, many experiments used to test the environmental safety of GM crops were poorly replicated, were of short duration, and/or assessed only a few of the possible response variables (*8*). Much could be learned and perhaps some debates settled if there were credible quantitative analyses of the numerous experiments that have contrasted the ecological impact of GM crops with those of control treatments involving non-GM varieties.

Here, we describe a meta-analysis of field studies involving *Bacillus thuringiensis* (Bt) crops, which represent the predominant modification entailing the novel production of pesticidal substances (Cry proteins) in crop plants. The incorporation of bacterial-derived *cry* genes into plants means that a wide variety of species are exposed, on a relatively continuous basis, to pesticidal Cry proteins. We restricted our analyses to lepidopteran-resistant cotton expressing Cry1Ac protein, lepidopteran-resistant maize expressing Cry1Ab protein, and coleopteran-resistant maize expressing Cry3Bb protein, because the aggregate collection of field experiments assessing these Bt crops is large enough to draw some compelling conclusions (*9*–*11*).

The standard approach to assessing nontarget effects entails measurements of abundance, survival, or growth of nontarget species when exposed to a GM variety versus when exposed to the same or similar variety lacking the genetic modification. We focused on field studies, and the response variable we analyzed is the abundance of nontarget invertebrates, sampled in a variety of ways. For each experiment, we recorded many attributes, including locations, durations, plot sizes, and sample sizes (*12*) (table S2). Experiments relied on two different types of control treatments, each reflecting a different philosophy of risk assessment: (i) controls entailing non-GM varieties grown under identical conditions but treated with insecticides and (ii) controls entailing non-GM varieties grown under identical conditions and with no insecticides applied. A third type of comparison, in which both Bt and control plants were treated with insecticides, was occasionally used.

We report a weighted mean effect size, Hedges' *d*, calculated as the difference between the means of the Bt and the control treatments divided by the pooled standard deviation and weighted by the reciprocal of sampling variance. Negative values indicate lower abundance (whereas positive values indicate higher abundance) in Bt plots compared with abundance in control plots.

The mean abundance of all nontarget invertebrate groups lumped together is significantly reduced in Cry1Ac cotton fields compared with mean abundance in non-GM, insecticide-free fields [Fig. 1A, white bars; 95% confidence intervals (CI) do not overlap with $d = 0$]. However, the abundance of nontarget invertebrates is significantly higher in Bt cotton compared with that of control fields sprayed with insecticides (Fig. 1A, hatched bars). There was no significant difference in the abundance of nontarget invertebrates for studies where both the Bt and the control fields were treated with insecticides (Fig. 1A; gray bars). Thus, the different types of experimental comparison revealed significantly different effects of Bt crops [Fig. 1A, left; between-groups heterogeneity (Q_b) = 49.96; degrees of freedom (df) = 2; $P < 0.001$]. Results were qualitatively similar when analyses were restricted to the related transgenic events MON531 and MON757 (Fig. 1A, right).

For all Cry1Ab maize events, the overall mean abundance of nontarget invertebrates was significantly lower in Bt compared with that in control fields that lacked insecticide applications (Fig. 1B; leftmost white bar). However, the mean abundance of nontarget invertebrates was greater in Cry1Ab maize than in non-GM maize sprayed with pyrethroid insecticides (Fig. 1B; leftmost hatched bar).

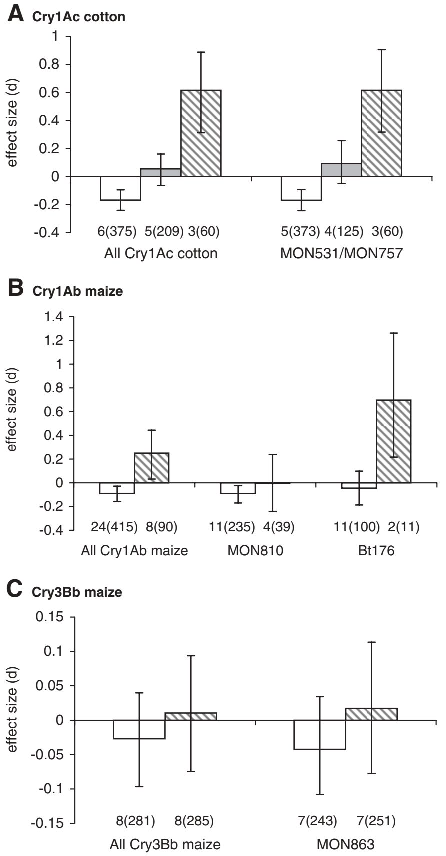

Fig. 1. Meta-analysis of field studies assessing abundance of nontarget invertebrate species for (**A**) lepidopteran-resistant Cry1Ac cotton, (**B**) lepidopteran-resistant Cry1Ab maize, and (**C**) coleopteran-resistant Cry3Bb maize. Effect size is Hedges' *d*, and error bars represent bias-corrected 95% CI. Values below each bar indicate the number of different papers or reports and, in parentheses, the number of lines of data summarized (each line of data represents a comparison of a group's average abundance in a Bt versus control treatment). White bars compare the abundance of nontarget invertebrates in Bt and non-GM varieties, without insecticide applications. Gray bars compare the abundance of nontarget invertebrates in Bt and non-GM varieties, both treated with insecticides. Hatched bars compare the abundance of nontarget invertebrates in insecticide-free Bt varieties versus non-GM varieties managed with applications of [(A)] any chemical insecticide and [(B) and (C)] pyrethroids.

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Effects measured by using these two different types of control treatments differed significantly (e.g., Fig. 1B, left pair of bars; $Q_b = 19.36$; df = 2; $P < 0.001$). Qualitatively different patterns emerged when analyses were restricted to single transgenic events. For MON810, effect sizes measured using controls with versus without insecticides did not significantly differ (Fig. 1B, middle bars; $Q_b = 0.71$; df = 2; $P = 0.39$). For Bt176, the two control types yielded significantly different effects (Fig. 1B, right bars; $Q_b = 9.41$; df = 2; $P = 0.012$), but there was no significant reduction in abundance observed with the insecticide-free controls (white bar, 95% CI overlaps with $d = 0$).

For Cry3Bb maize, the mean abundance of nontarget invertebrates was not significantly different in Bt fields compared to abundance in non-GM maize either with or without pyrethroid applications (Fig. 1C; for the left pair of bars, $Q_b = 0.37$ and $P = 0.51$). This same pattern held when analyses were restricted to event MON863.

The general indication of our analyses is that if agriculture with insecticide applications is the standard of comparison and if adoption of Bt crops truly reduces insecticide applications, then Bt crops may increase the abundance of nontarget invertebrates overall. Alternatively, if the comparison is made to farming systems without insecticides, some nontarget groups are significantly less abundant in Bt than in control fields (Fig. 2). Not surprisingly, the mean abundance of nontarget lepidopterans is significantly reduced in Cry1Ac cotton (Fig. 2A), which targets related lepidopteran pests. There were insufficient data to test this question in Cry1Ab maize. However, the mean abundance of nontarget coleopterans does not appear to be reduced in coleopteran-resistant Cry3Bb maize (Fig. 2C).

Coleopterans and hemipterans appear to be slightly less common in Cry1Ac cotton than non-GM, insecticide-free cotton (Fig. 2A). Although these groups both include a wide variety of functional groups (herbivores, predators, detritivores, etc.), we found no indication that some functional groups exhibit stronger effect sizes than others [see Supporting Online Material (SOM) text for additional details]. Lastly, hymenopterans are less common on average in Cry1Ab and Cry3Bb maize compared with hymenopterans in non-GM, insecticide-free controls (Fig. 2, B and C, respectively). For the Cry1Ab comparison, data on hymenopterans mostly comprised parasitic wasps of the braconidae and ichneumonidae. For Cry3Bb maize, data included parasitic wasps and ants. It is unclear whether the reduced abundance of these groups (coleopterans, hemipterans, and hymenopterans) is due to direct toxicity or is a response to reduced availability of prey in Bt crops. A significant reduction of collembolans in Cry1Ab maize is based on too few observations to be credible at this point (Fig. 2B).

To investigate the sensitivity of our findings to how we designed our comparisons, we performed additional analyses with use of several different subsets of experimental comparisons as the foundation (*12*). For example, we used only studies in peer-reviewed journals or only studies that identified invertebrate taxa to at least the level of family. With only minor exceptions, results using these alternative queries were qualitatively similar to those reported here (SOM text).

To facilitate additional syntheses, we have created a publicly accessible, searchable database, detailing methods and results of lab and field studies examining nontarget invertebrates and Bt crops (http://delphi.nceas.ucsb.edu/btcrops/). While assembling this database, we found numerous studies that did not report measures of variance to accompany treatment means (40% of 64 reports of field studies), did not clearly present the sample size (20%), or improperly used subsamples to calculate measures of variance (22%). By corresponding with authors, we were often able to resolve these issues. If regulatory agencies were to require researchers to enter details regarding their study methods and results into a similar database, it would be easy to spot omitted information and postpone approval of pesticidal crops until complete records were submitted.

Our analyses provide some support to the claim that GM plants can reduce environmentally undesirable aspects of agriculture, particularly the nontarget impacts of insecticides. However, we examined only one type of genetic modification, and most of the underlying studies entailed controlled field experiments with small spatial scales as opposed to actual farming systems, where continued insecticide use sometimes occurs with Bt crops. Secondly, the conclusion that adoption of Bt cotton or maize may entail ecological benefits assumes a baseline condition of insecticide applications. In reality, both types of control treatment reflect farming practices: in 2005, insecticides were applied to 23% of maize acreage cultivated in 19 states surveyed by the U.S. Department of Agriculture (USDA) (*13*). Moreover, the vast majority of Bt maize acreage comprises varieties used for silage or processed foods (e.g., corn syrup) for which insecticide use has typically been limited. Insecticides are more commonly used in cotton production, with 71% of surveyed cotton acreage treated in 2005 (*13*).

Studies such as those synthesized here investigate whether changes in invertebrate abundance are statistically significant. Whereas the lack of a difference is generally considered a signal of environmental safety, it is harder to interpret whether statistically sig-

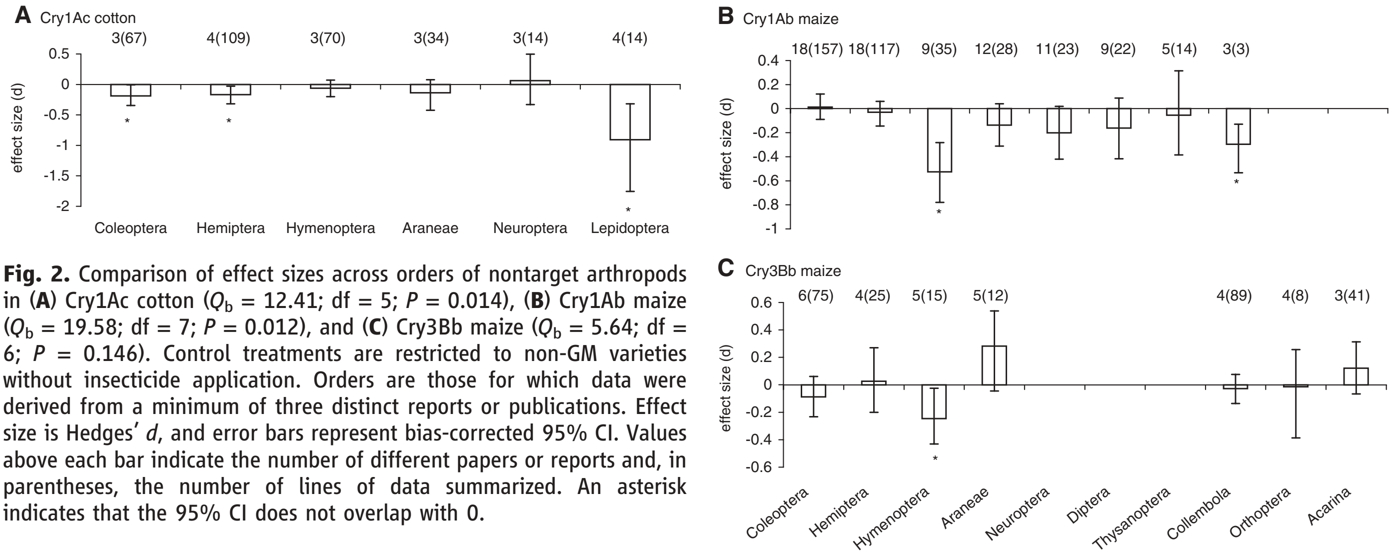

Fig. 2. Comparison of effect sizes across orders of nontarget arthropods in (**A**) Cry1Ac cotton ($Q_b = 12.41$; df = 5; $P = 0.014$), (**B**) Cry1Ab maize ($Q_b = 19.58$; df = 7; $P = 0.012$), and (**C**) Cry3Bb maize ($Q_b = 5.64$; df = 6; $P = 0.146$). Control treatments are restricted to non-GM varieties without insecticide application. Orders are those for which data were derived from a minimum of three distinct reports or publications. Effect size is Hedges' *d*, and error bars represent bias-corrected 95% CI. Values above each bar indicate the number of different papers or reports and, in parentheses, the number of lines of data summarized. An asterisk indicates that the 95% CI does not overlap with 0.

nificant differences in abundance translate into ecologically important changes. Regardless of one's philosophical perspective on risk assessment for GM crops, enough experimental data has accumulated to begin drawing empirically based conclusions, as opposed to arguing on the basis of anecdote or handpicked examples.

References and Notes

1. B. Breckling, R. Verhoeven, Eds., *Risk Hazard Damage: Specification of Criteria to Assess Environmental Impact of Genetically Modified Organisms* (Naturschutz und Biologische Vielfalt, Bonn, Germany, 2004), vol. 1, p. 256.
2. L. L. Wolfenbarger, P. R. Phifer, *Science* **290**, 2088 (2000).
3. H. Torgersen, *EMBO Rep.* **5**, S17 (2004).
4. A. A. Snow *et al.*, *Ecol. Appl.* **15**, 377 (2005).
5. D. A. Andow, C. Zwahlen, *Ecol. Lett.* **9**, 196 (2006).
6. P. Slovic, *Risk Anal.* **19**, 689 (1999).
7. P. Sturgis, H. Cooper, C. Fife-schaw, *New Genet. Soc.* **24**, 31 (2005).
8. M. A. Marvier, *Ecol. Appl.* **12**, 1119 (2002).
9. G. L. Lövei, S. Arpaia, *Entomol. Exp. Appl.* **114**, 1 (2005).
10. J. Romeis, M. Meissle, F. Bigler, *Nat. Biotechnol.* **24**, 63 (2006).
11. B. W. Clark, T. A. Phillips, J. R. Coats, *J. Agric. Food Chem.* **53**, 4643 (2005).
12. Materials and methods are available as supporting material on *Science* Online.
13. USDA, *Agricultural Chemical Usage 2005 Field Crops Summary* (National Agricultural Statistics Service, USDA, Washington, DC, 2006).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5830/1475/DC1
Materials and Methods
SOM Text
Tables S1 and S2
References
Data

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An Ancient Mechanism Controls the Development of Cells with a Rooting Function in Land Plants

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Root hairs and rhizoids are cells with rooting functions in land plants. We describe two basic helix-loop-helix transcription factors that control root hair development in the sporophyte (2*n*) of the angiosperm *Arabidopsis thaliana* and rhizoid development in the gametophytes (*n*) of the bryophyte *Physcomitrella patens*. The phylogeny of land plants supports the hypothesis that early land plants were bryophyte-like and possessed a dominant gametophyte and later the sporophyte rose to dominance. If this hypothesis is correct, our data suggest that the increase in morphological complexity of the sporophyte body in the Paleozoic resulted at least in part from the recruitment of regulatory genes from gametophyte to sporophyte.

The invasion of land by plants in the Paleozoic was accompanied by marked changes in plant structure and life cycle and resulted in diversification of terrestrial ecosystems and pronounced climate change (*1*–*3*). One of the most important transformations that occurred during the first 100 million years after plants colonized the land was the rise to dominance of the diploid phase (sporophyte) of the life cycle (the land-plant life cycle comprises independent haploid and diploid organisms). The phylogenetic relationship among green algae and land plants suggests that the haploid phase (gametophyte) was morphologically more complex than the smaller diploid phase (sporophyte) in the earliest land plants (*4*). This changed over a period of ~100 million years to a situation in which the diploid phase became larger and more morphologically complex (*4*). This rise to dominance of the diploid phase of the life cycle was accompanied by an enormous increase in morphological diversity evident in Devonian floras and has persisted to the present day, when the land floras are largely dominated by diploid plants (*3*). To date, we have little understanding of the genetic basis of such a metamorphosis of the land plant body. The characterization of the function of regulatory genes such as *LEAFY* (*LFY*) in both bryophytes and angiosperms suggests that the increase in sporophyte diversity was brought about through the modification of the activities of sporophyte-specific genes with sporophyte-specific functions (*5*). Here we show that genes that specifically promote the development of root hairs in diploid sporophytes of angiosperms also control the development of cells with similar functions in the haploid gametophytes of mosses. This suggests that genes with gametophyte functions in ancestral land plants were recruited to function in the sporophyte during the metamorphosis of the land plant body.

Root hairs are highly polarized cells that increase the surface area of the plant that is in contact with the growth substrate. They play important roles in nutrient acquisition and anchorage in those land plants that have roots (*6*, *7*). The *Arabidopsis thaliana* root epidermis is organized in alternate rows of hair-forming cells (H cells) that produce a tip-growing protuberance (root hairs) and rows of non–hair cells (N cells) that remain hairless. *AtRHD6* (*ROOT HAIR DEFECTIVE 6*) positively regulates the development of H cells–*Atrhd6* mutants develop few root hairs (Fig. 1A) (*8*). We cloned *AtRHD6* using an enhancer trap line (*Atrhd6-2*) in which the *GUS* reporter gene is expressed in H cells but not in N cells (Fig. 1, C and D, and fig. S1). *AtRHD6* encodes the basic-helix-loop-helix (bHLH) transcription factor At1g66470 (*9*). The identification of another independent allele (*Atrhd6-3*) with a similar phenotype and the complementation of the *Atrhd6-3* mutation with a whole gene *AtRHD6p::GFP:AtRHD6* translational fusion with the GREEN FLUORESCENT PROTEIN (GFP) confirmed that the defect in root hair development observed in this mutant is due to mutation of *At1g66470* (Fig. 1A). This complementing *AtRHD6p::GFP:AtRHD6* fusion indicates that AtRHD6 protein accumulates in H-cell nuclei in the meristem and elongation zones (Fig. 1B) but disappears before the emergence of the root hair (data not shown). The spatial pattern of N cells and H cells in the *A. thaliana* root epidermis is controlled by a transcriptional network including the posi-

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