Field Studies Assessing Arthropod Nontarget Effects in *Bt* Transgenic Crops: Introduction

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TEN YEARS AGO THE first crops modified through genetic engineering to produce novel in-plant protectants became commercially available in the United States. Since that time, the adoption and use of crops with built-in resistance to lepidopteran and coleopteran insect pests and tolerance to certain herbicides has grown rapidly in both industrial and developing countries worldwide. By 2004, 17 countries had adopted transgenic crops ranging from corn and cotton producing insecticidal proteins of *Bacillus thuringiensis* Berliner (*Bt*) to corn, cotton, soybean, and canola-containing genetic constructs conferring tolerance to the herbicides glyphosate or glufosinate. It is estimated that in 2004, ≈81 million hectares of transgenic crops were cultivated in these 17 countries, the United States leading the way with 47.6 million hectares (James 2004). In 2004, 32% of the field corn and 46% of the upland cotton acreage in the United States was planted with genetically modified cultivars producing one or more *Bt* toxins (USDA 2004).

The rapid and widespread adoption of transgenic crops in the United States and elsewhere has prompted extensive debate over multiple issues related to human safety and environmental risk. The putative environmental risks that have been articulated include outcrossing of nontransgenic plants through pollen drift, horizontal transfer of transgenes to unrelated organisms, loss of susceptibility to *Bt* toxins in target pests, disruption of ecosystem processes, and direct or indirect effects on nontarget organisms and biodiversity. There has been equal discourse expounding the potential benefits associated with the use of transgenic crops in agricultural production systems, including significant reductions in use of conventional, broad-spectrum insecticides, improved suppression of target pests, improved yields, reductions in production costs leading to increased profitability, and increased opportunities for biological control.

Governmental agencies such as the U.S. Environmental Protection Agency (EPA) in the United States require toxicological testing of a limited number of representative nontarget organisms as one component of the commercial registration process for transgenic crops producing insecticidal proteins. However, there have been calls by research advisory groups (e.g., National Research Council, EPA-FIFRA Scientific Advisory Panels) and private advocacy groups for long-term field studies to examine potential ecological impacts of transgenic crops as a critical component of the postcommercialization testing process. Chief among these ecological concerns is the potential impact of transgenic crops on nontarget organisms and biodiversity.

In response to the rapidly growing importance and interest in transgenic technologies as new tools for modern pest management, this issue of *Environmental Entomology* introduces a new subject area entitled "Transgenic Plants and Insects." To inaugurate this new section of the journal, we present the results of 11 field studies (presented in 13 papers) conducted in the United States and Australia that focus on the longer-term assessment of potential nontarget effects of transgenic *Bt* cotton and corn active against lepidopteran and coleopteran pests (Table 1). These studies encompass two crop plants (upland cotton and hybrid corn) producing five insecticidal proteins and involve evaluation of a wide taxonomic breadth of nontarget arthropods. With one exception, studies were conducted over a minimum of three site-years (seven studies were ≥3 yr in duration) in either controlled, moderate-sized research plots or in commercial fields subject to typical grower production practices. A variety of sampling methods was employed, and analytical methods included univariate, multivariate, and community level approaches.

Prasifka et al. lead off with a 2-yr evaluation of the effects of experimental plot size and isolation on the interpretation of changes in nontarget arthropod abundance, using hybrid corn and conventional insecticides as a model system in Iowa. Next, Naranjo presents the results of a long-term study in Arizona to assess the impact of transgenic *Bt* cotton on the abundance and functional activity of arthropod natural enemies and to contrast the effects of *Bt* cotton to conventional systems using selective and broad-spectrum insecticides. He further examines the influence of plot size, sampling method, and statistical power to provide guidance for the conduct of future nontarget evaluations in transgenic crops. The next three studies were conducted in commercial cotton fields in New South Wales, Australia, and Georgia, Alabama, and South Carolina in the United States. They primarily compared nontarget effects in *Bt* and conventional non-*Bt* fields subject to typical grower practices. Whitehouse et al. evaluated a wide taxonomic range of foliar-dwelling nontarget arthropods including natural enemies, herbivores, and detritivores and contrasted abundance and diversity among treatments, whereas Torres and Ruberson contrasted abundance

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Table 1. Summary of studies contributed to the special section on field studies assessing arthropod nontarget effects in *Bt* transgenic crops

Authors	Crop	Toxin	Years/ sites	Plot size (ha)	Replications	Contrasts	No. of nontarget taxa	Sampling methods	Analyses
Prasifka et al.	Field corn	NA	2/1	0.01–0.53	4–10	Plot size and isolation effects; Insecticide and control treatments	25 (spiders, insect predators, parasitoids, herbivores, detritivores)	Pitfall traps, whole plant, sticky traps	RM-ANOVA[a], PRC[b]
Naranjo	Cotton	Cry1Ac	6/1	0.12–2.0 main plots	4	Non-*Bt* parent cultivar; Insecticide-treated positive controls	23 (spiders, insect predators, parasitoids, herbivores)	Whole plant, sweep net, leaf sample	RM-ANOVA[a], multi-year ANOVA, PRC[b], power analysis
Whitehouse et al.	Cotton	Cry1Ac, Cry1Ac+Cry2Ab	3/1	1.0–80.0 Grower fields	2–3	Non-*Bt* cultivar; Insecticide-treated non-*Bt* (commercial practice)	132 (spiders, insect predators, parasitoids, herbivores, detritivores)	Suction sampler	RM-ANOVA[a], PRC[b], splines, diversity analysis
Torres and Ruberson	Cotton	Cry1Ac	3/1	5.0–11.0 Grower fields	3	Insecticide-treated non-*Bt* cultivar (commercial practice)	28 (spiders, insect predators)	Whole plant, drop cloth, pitfall traps	RM-ANOVA[a], PRC[b], power analysis
Head et al.	Cotton	Cry1Ac	3/4	5.0–30.0 Grower fields	3–4	Insecticide-treated non-*Bt* cultivar (commercial practice)	9 (spiders, insect predators, herbivores)	Whole plant, drop cloth, beat-bucket	RM-ANOVA[a]
Dively	Field corn	Cry1Ab+VIP3A	3/1	0.4	3	Non-*Bt* isogenic hybrid; Insecticide-treated positive controls	203 (spiders, insect predators, parasitoids, herbivores, detritivores)	Whole plant, sticky traps, leaf sample, pitfall traps, emergence traps	RM-ANOVA[a], PRC[b], diversity analysis
Daly and Buntin	Field corn	Cry1Ab	2/2	0.053	4	Near isogenic non-*Bt* hybrids	23 (spiders, insect predators, herbivores)	Whole plant, pitfall traps, ear samples	RM-ANOVA[a], multi-year ANOVA
Pilcher et al.	Field corn	Cry1Ab	3/3	0.12–0.17 main plots	4	Non-*Bt* hybrids	5 (insect predators and parasitoids)	Sticky traps	RM-ANOVA[a], multi-year ANOVA
Lopez et al.	Field corn	Cry1Ab	2/2	0.39	3	Non-*Bt* isogenic hybrid; Insecticide-treated positive controls	37 surveyed, 3 analyzed (all Carabidae)	Pitfall traps	RM-ANOVA[a], power analysis
Bhatti et al.	Field corn	Cry3Bb1	3/1	0.134 main plots	4	Non-*Bt* isogenic hybrid; Insecticide-treated positive controls	71 surveyed, 25 analyzed (spiders, insect predators, herbivores, detritivores)	Pitfall traps, pan traps, sticky traps	RM-ANOVA[a], multi-year ANOVA
Bitzer et al.	Field corn	Cry3Bb1	2/2	0.033–0.10 main plots	4	Non-*Bt* isogenic hybrid; Insecticide-treated positive controls	216 (all Collembola)	Pitfall traps, soil cores	RM-ANOVA[a], diversity analysis

[a] Repeated-measures analysis of variance.
[b] Multivariate principal response curves.

of a subset of epigeal and foliar-dwelling arthropod natural enemies, primarily predators. Head et al. concentrated on comparing abundance of a smaller set of foliar-dwelling nontarget predators and herbivores at sites in multiple states. In lepidopteran-active corn, Dively conducted extensive taxonomic evaluations of nontarget arthropod natural enemies, herbivores, and detritivores in Maryland and contrasted effects of *Bt* toxins to use of conventional insecticides on abundance and diversity. This was the only study to examine plants producing a *Bt* toxin, VIP3A, which is not yet commercially available. The next three studies examined nontarget effects of a smaller number of taxa in Cry1Ab-producing field corn. Daly and Buntin examined a subset of epigeal and foliar-dwelling nontarget arthropod natural enemies and herbivores at two sites in Georgia, whereas Pilcher et al. evaluated five taxa of foliar-dwelling insect natural enemies at three sites in Iowa. These two studies compared nontarget abundance in *Bt* and non-*Bt* cultivars that did not receive any insecticide applications. Lopez et al. surveyed multiple species within a single nontarget taxonomic group (Carabidae) and contrasted the effects of *Bt* corn and insecticide-treated corn on the density of three of the more abundant species. The final three studies evaluated nontarget effects in transgenic corn producing beetle-active toxins. Bhatti et al. surveyed nontarget epigeal and foliar-dwelling arthropod natural enemies, herbivores, and detritivores in Illinois. Their study contrasted the effects of *Bt* corn and insecticide-treated corn on the density of a subset of the more abundant taxa. Finally, Bitzer et al. conducted extensive surveys of nontarget Collembola at sites in Iowa and Illinois and contrasted effects of *Bt* corn and insecticides on the richness and diversity of the collembolan community and abundance of a subset of the more dominant species.

These 13 papers represent only a small portion of past and on-going research efforts dedicated to the assessment of nontarget effects of transgenic crops worldwide, but they characterize the diversity of taxonomic breadth, experimental design, and analytical methodology being brought to bear on this important environmental issue. The unique aspect of many of these studies is their long-term nature that begins to address concerns about possible chronic effects of transgenic crops arising from subtle sublethal effects on nontarget organisms that may only become apparent after repeated exposure of multiple generations to plant-produced toxins. Collectively, these studies show the highly selective activity of the most widely used δ-endotoxins from *B. thuringiensis*. Minor changes in abundance of a few nontarget taxa were shown to occur with the cultivation of *Bt* corn and cotton, but almost all these effects were explained by expected changes in target pest populations. Furthermore, many studies documented that the alternative use of insecticides with broad-spectrum activity was many times more damaging to the nontarget arthropod community. Methodological issues of plot size, replication, and statistical power were examined explicitly in several studies, and implicitly when the results of all the studies are considered collectively. The findings are consistent over a range of plots sizes from fractions of a hectare to multi-hectare commercial fields. Thus, although some species may rapidly recolonize relatively small plots, there may still be sufficient independence to measure real effects with sufficient replication within sites and over time. As the adoption and use of transgenic crops continues to grow in the future, so will research to examine unintended effects. Collectively, these studies lends strong support to the conclusion that extant cultivars of transgenic *Bt* cotton and corn pose a relatively low risk to nontarget arthropods, and they further serve to improve and refine future research questions addressing nontarget impacts.

Acknowledgments

We thank T. Sappington (Subject Editor) for sharing editorial duties, the many anonymous referees who contributed their time, and A. Cameron (Editor-in-Chief) for being receptive to the idea of a special section on this topic, for spearheading the development of "Transgenic Plants and Insects" as a new subject area in the journal, and for providing expert guidance and encouragement throughout the editorial process.

References Cited

James, C. 2004. Preview: global status of commercialized biotech/GM crops: 2004. ISAAA Briefs No. 32. ISAAA, Ithaca, NY. URL: http://www.isaaa.org.

U.S. Department of Agriculture (USDA). 2004. Acreage. National Agricultural Statistic Service, Agricultural Statistics Board, U.S. Department of Agriculture, Washington, DC. URL: http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bba/acrg0604.pdf.

Received for publication 28 June 2005; accepted 14 July 2005.