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ORIGINAL CONTRIBUTION

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Transportability of non-target arthropod field data for the use in environmental risk assessment of genetically modified maize in Northern Mexico

J. L. Corrales Madrid[1] | J. L. Martínez Carrillo[2] | M. B. Osuna Martínez[2] | H. A. Durán Pompa[3] | J. Alonso Escobedo[4] | F. Javier Quiñones[5] | J. A. Garzón Tiznado[1] | L. Castro Espinoza[2] | F. Zavala García[3] | A. Espinoza Banda[4] | J. González García[5] | C. Jiang[6] | C. R. Brown[6] | J. M. de la F. Martínez[7] | O. Heredia Díaz[6] | J. E. Whitsel[6] | P. Asiimwe[6] | B. M. Baltazar[6] | A. Ahmad[6]

[1]Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, Mexico

[2]Instituto Tecnológico de Sonora, Dirección de Recursos Naturales, Obregón, Sonora, Mexico

[3]Facultad de Agronomía, Universidad Autónoma de Nuevo León, Escobedo, Nuevo León, Mexico

[4]Unidad Laguna, Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, Mexico

[5]Facultad de Ciencias Agrícolas y Forestales, Universidad Autónoma de Chihuahua, Delicias, Chihuahua, Mexico

[6]Monsanto Company, St. Louis, MO, USA

[7]Monsanto Comercial, Park Plaza torre II, Mexico D.F, Mexico

Correspondence
Aqeel Ahmad, Monsanto Company, St. Louis, MO, USA.
Email: aqeel.ahmad@monsanto.com

Abstract

In country, non-target arthropod (NTA) field evaluations are required to comply with the regulatory process for cultivation of genetically modified (GM) maize in Mexico. Two sets of field trials, Experimental Phase and Pilot Phase, were conducted to identify any potential harm of insect-protected and glyphosate-tolerant maize (MON-89Ø34-3 × MON-88Ø17-3 and MON-89Ø34-3 × MON-ØØ6Ø3-6) and glyphosate-tolerant maize (MON-ØØ6Ø3-6) to local NTAs compared to conventional maize. NTA abundance data were collected at 32 sites, providing high geographic and environmental diversity within maize production areas from four ecological regions (ecoregions) in northern Mexico. The most abundant herbivorous taxa collected included field crickets, corn flea beetles, rootworm beetles, cornsilk flies, aphids, leafhoppers, plant bugs and thrips while the most abundant beneficial taxa captured were soil mites, spiders, predatory ground beetles, rove beetles, springtails (Collembola), predatory earwigs, ladybird beetles, syrphid flies, tachinid flies, minute pirate bugs, parasitic wasps and lacewings. Across the taxa analysed, no statistically significant differences in abundance were detected between GM maize and the conventional maize control for 69 of the 74 comparisons (93.2%) indicating that the single or stacked insect-protected and herbicide-tolerant GM traits generally exert no marked adverse effects on the arthropod populations compared with conventional maize. The distribution of taxa observed in this study provides evidence that irrespective of variations in overall biodiversity of a given ecoregion, important herbivore, predatory and parasitic arthropod taxa within the commercial maize agroecosystem are highly similar indicating that relevant data generated in one ecoregion can be transportable for the risk assessment of the same or similar GM crop in another ecoregion.

KEYWORDS
Bacillus thuringiensis, data transportability, environmental risk assessment, genetically modified crop, non-target arthropods

1 | INTRODUCTION

Biotechnology-derived (genetically modified, GM) crops are the most rapidly adopted crop technology in the last 21 years with acreage increasing more than 100-fold since it was first commercialized (James, 2016). In recent years, crop varieties with two or more GM traits have become important in global agriculture and reached about 75.4 million hectares equivalent to 41% of the 185.1 million hectares planted with GM crops worldwide in 2016 (James, 2016). Maize (*Zea mays* L.) is the most important staple food crop in Mexico with approximately 8 million hectares (ha) planted annually, of which 83.0% is rainfed, and 26.6% of the total area is grown with proprietary hybrid seed (Blanco et al., 2014; Turrent, Wise, & Garvey, 2012). Despite this, production constraints including drought, high weed, disease and insect pressure (Blanco et al., 2014) coupled with growing demand from an increasing population have resulted in a need to complement local maize production with imports. Mexico imports about 10 million metric tons of maize primarily from the United States each year (Turrent et al., 2012). The deficit in Mexico's maize production has led to the need to adopt modern agricultural technologies, including biotechnology, as a means of overcoming some of the above-mentioned production challenges and ultimately increasing yields (Vargas-Parada, 2014).

Monsanto Company has developed the combined trait maize products, MON-89Ø34-3 × MON-88Ø17-3 and MON-89Ø34-3 × MON-ØØ6Ø3-6 by traditional breeding of GM parental inbred lines derived from maize transformation events: MON-89Ø34-3 (YieldGard® VT Pro), MON-88Ø17-3 (YieldGard® VT Rootworm/Roundup Ready® 2) and MON-ØØ6Ø3-6 (Roundup Ready® 2). Both combined trait maize products have provided substantial benefits to growers in North and South America by limiting yield losses from targeted lepidopteran and coleopteran insects as well as from weed pressure, while concomitantly reducing the risk to humans and the environment through reductions in insecticide use and mycotoxins in maize grain (Brookes & Barfoot, 2011).

The core regulatory data for assessing potential non-target arthropod effects of insect-protected GM crops are produced by technology developers (industry and academic scientists) according to the tiered approach of ecological risk assessment (ERA) where, in the earliest tier, a battery of key non-target arthropods (NTAs) belonging to different taxonomic orders and functional groups with both agricultural and worldwide relevance are tested at doses well above those typically expressed in the plant. If the results of the first-tier studies require refinement then subsequent tiers are used to clarify previous results under progressively more realistic situations, ultimately under field conditions if needed (Duan, Lundgren, Naranjo, & Marvier, 2010; Romeis et al., 2008; U.S. Environmental Protection Agency, 2007; Wolt et al., 2010). In the case of insecticidal proteins (Cry1A.105, Cry2Ab2, and Cry3Bb1) expressed in MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MON-ØØ6Ø3-6, the tiered testing has not progressed beyond the early tiers due to the restricted activity spectrum of these proteins (Lundgren & Wiedenmann, 2002; Whitehouse, Wison, & Fitt, 2005). In addition, field studies to date have revealed that insect-protected and herbicide-tolerant traits either single event or in stacked product do not adversely affect biodiversity, populations of natural enemies and other ecologically important NTAs (Ahmad et al., 2016; Al-Deeb & Wilde, 2003; Devos, De Schrijver, De Clercq, Kiss, & Romeis, 2012; Li & Romeis, 2009, 2011; Lundgren & Wiedenmann, 2002; Naranjo, 2005a,b, 2009; Schier, 2006; Svobodova, Shu, Habustova, Romeis, & Meissle, 2017; Wolfenbarger, Naranjo, Lundgren, Bitzer, & Watrud, 2008). However, local NTA field evaluations are commonly required for cultivation approvals of GM crops in some countries often without consideration for data already available. This data may include tiered approach data, or field data from well-designed studies conducted for the ERA of the same GM crop, related traits or GM crop/trait combinations where the ecological assessment endpoints (e.g., NTA) are similar. Results from field studies obtained from multiple geographies for GM soya bean (Horak et al., 2015) and GM maize (Ahmad et al., 2016; Heredia Díaz et al., 2017; Nakai, Hoshikawa, Shimono, & Ohsawa, 2015) demonstrate the utility of generating relevant data that are transportable across geographic regions for the ERA of GM crops. Leveraging existing, relevant ERA data of GM crops across countries will facilitate the efficient use of regulatory data, minimize redundancy and support conclusions with high certainty for assessing potential environmental risk from the commercial release of a GM crop.

Mexico is a "mega-diverse" country and is one of 17 nations that contain nearly 70% of global diversity of plants and animal species (Sarukhán et al., 2009). Mexican territory has been divided into ecological regions (ecoregions) as geographic units with flora, fauna and characteristic ecosystems (CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad), 2009; INEGI-CONABIO-INE (Instituto Nacional de Estadística, Geografía e Informática–Comisión Nacional para el Conocimiento y Uso de la Biodiversidad–Instituto Nacional de Ecología), 2008; Wiken, Jiménez Nava, & Griffith, 2011). The boundaries of an ecoregion are not fixed, but rather encompass an area where important ecological and evolutionary processes generally interact. In contrast, field studies to characterize GM crops are typically implemented in areas devoted to agricultural production. These agricultural areas have relatively homogeneous characteristics (e.g., climate, soils, water availability, infrastructure) and are contained within the larger, usually more heterogeneous, ecoregions. Prior to cultivation of a GM crop in Mexico, local field trials are required to assess the potential adverse effects of the GM crops on its receiving environment, relative to a non-GM control. The focus of these trials is to examine whether the GM crop has potential to

become a plant pest (i.e., weediness characteristics) or to have other adverse environmental impacts (e.g., effects on non-target organisms). Requirements include a stepwise field evaluation of GM crops at multiple sites in each ecoregion, starting with small plots at the experimental phase followed by larger plots at the pilot phase prior to commercial plantings. Local field evaluations on non-target arthropods (NTAs) reported here are used by risk assessors and regulators to determine whether cultivation of a GM crop is acceptable in Mexico.

In this study, we summarize studies performed to evaluate the effect of maize breeding stacks (MON-89Ø34-3 × MON-88Ø17-3 and MON-89Ø34-3 × MON-ØØ6Ø3-6) and single event (MON-ØØ6Ø3-6) on the abundance of NTAs relative to its conventional control in maize production areas located within four ecoregions in Northern Mexico. We also sought to determine the similarity of taxa across ecoregions to evaluate whether the concept of data transportability, where results on NTA data can be leveraged across ecoregions to support ERA, is applicable.

2 | MATERIALS AND METHODS

2.1 | Site description

Thirty-two studies, 18 Experimental Phase (smaller trials) and 14 Pilot Phase (larger trials), were conducted in maize growing regions of the Mexican states of Sinaloa, Sonora, Chihuahua, Coahuila and Durango (Comarca Lagunera) and Tamaulipas, during the 2009-2013 crop seasons (Table 1). The selected areas represented ecoregions level IV as defined by the National Commission for Biodiversity (CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad), 2009; INEGI-CONABIO-INE (Instituto Nacional de Estadística, Geografía e Informática–Comisión Nacional para el Conocimiento y Uso de la Biodiversidad–Instituto Nacional de Ecología), 2008). The four ecoregions where trials were planted included the following: 9.5.1.2 Tamaulipas coastal plain with xeric shrubland or apparent barren land; 10.2.2.8 Floodplain of Yaqui, Mayo and Fuerte rivers with xerophytic scrubland and mesquite; 10.2.4.1 Central plains of Chihuahuan Desert with xerophytic microphyllous halophytic shrubland; 14.3.1.2 Sinaloa coastal plain with low thorn forest (Figure 1; INEGI-CONABIO-INE (Instituto Nacional de Estadística, Geografía e Informática–Comisión Nacional para el Conocimiento y Uso de la Biodiversidad–Instituto Nacional de Ecología), 2008; INEGI 2012).

2.2 | Test and control material

The test materials were GM maize hybrids MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MONØØ6Ø3-6 and MON-ØØ6Ø3-6, and the control materials were corresponding conventional (non-GM) isohybrids. Studies comparing GM hybrids and controls in the same hybrid background minimize sources of variability and allow appropriate comparisons to best assess the potential environmental risks of introduced GM traits. Within each study, the GM maize hybrid and the conventional maize control hybrid were in the same genetic background. At all but one site (Chihuahua), the hybrids were in a genetic background broadly adapted to the environmental conditions of northern Mexican states; at Chihuahua, an early-maturing hybrid background was used. GM hybrid MON-89Ø34-3 × MON-88Ø17-3 expresses three *Bt* proteins (Cry1A.105, Cry2Ab2 and Cry3Bb1) that confer resistance against aboveground lepidopteran insect pests and belowground local *Diabrotica* spp. (Chrysomelidae). It also expresses the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein, which confers tolerance to glyphosate herbicide. GM hybrid MON-89Ø34- 3 × MON-ØØ6Ø3-6 expresses two *Bt* proteins (Cry1A.105 and Cry2Ab2) that confer resistance against aboveground lepidopteran insect pests and expresses the EPSPS protein. GM hybrid MONØØ6Ø3-6 expresses only the EPSPS protein.

2.3 | Production practices

Fields were managed according to the recommendations contained in the technical guide developed by the National Research Institute for Forestry, Agriculture and Livestock (INIFAP) (Mendoza, Macías, & Cortez, 2003). All experiments were conducted under irrigation condition and were located in major corn growing areas in northern Mexico. Planting dates were typical of the local area with some exceptions due to weather, the timing of planting approvals or other considerations. Row spacing varied from 0.65 to 0.92 m, with a seeding rate of 5 to 10 seeds per metre and seed planting depth of 2 to 9 cm, which encompass planting practices in commercial maize production in Mexico. The main soil textures varied across locations and included clay, silty clay, clay loam, sandy loam, sandy clay loam and sandy silt (Table 1). Details of the agro-ecological characteristics are included in Table S1.

Crop management practices included seedbed soil preparation, fertilization, irrigation, and insect and weed control as per regional best practices. Agronomic practices (e.g., fertilizer, irrigation, pesticides) were conducted uniformly across all entries within a study in the Experimental Phase trials to eliminate an additional source of variation on the arthropod abundance. However, in the Pilot Phase trials, insect and weed control practices were conducted according to each material's phenotype, that is, the insect-protected and glyphosate-tolerant hybrids MON-89Ø34-3 × MON-88Ø17-3 and MON-89Ø34-3 × MON-ØØ6Ø3-6 GM did not require conventional insecticide applications for target lepidopteran insect pests, but MON-ØØ6Ø3-6 (glyphosate-tolerant only) and the conventional hybrid required two to four applications of conventional insecticides to control lepidopteran pests across most sites (Data S1). Weed control was also different between the GM hybrids (all glyphosate-tolerant) and the conventional control hybrid. Across all sites, one or two over-the-top applications of Faena Fuerte® with Transorb®[1] (540 g a.i. L^{-1}), a glyphosate-containing herbicide, were made on the three GM hybrids at rates of 2 to 4 L/ha. Weed control for the conventional control was mechanical (cultivator or manual) and/or by applications of selective herbicides.

[1]Registered trademark of Monsanto Technology LLC. Equivalent to Roundup Ultra®.

TABLE 1 Collection method and number of collections from field trials evaluating non-target arthropod abundance on MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MON-ØØ6Ø3-6, MON-ØØ6Ø3-6, and the conventional control conducted during 2009-2013 in northern Mexico

Ecoregion[a]	State	Study type[b]	Year	Site[c]	Planting date	Soil texture	Plot size[d](m^2)/dimensions/Number of replicates	Collection method and (Number of collections)
10.2.2.8	Sonora	Experimental	2009	LO	30 Oct., 09	Clay	100.8/11.2 × 9/4	Pitfall (8), Sticky (8),Visual (3)
				MF	2 Nov., 09	Silty clay	100.8/11.2 × 9/4	Pitfall (6), Sticky (6),Visual (3)
				MG	31 Oct., 09	Clay	100.8/11.2 × 9/4	Pitfall (7), Sticky (7),Visual (3)
		Experimental	2011	BASO	19 Mar., 11	Clay loam	200.0/8 × 25/3	Sticky (6)
				SOCO	5 Mar., 11	Clay	288.0/9.6 × 30/3	Sticky (7)
		Pilot[e]	2012	SON_02	23 Oct., 12	Clay	2160.0/18 × 120/4	Pitfall (4), Sticky (5), Visual (3)
				SON_10	-	-	-/-/4	Pitfall (2), Sticky (2)
				SON_12	12 Oct., 12	Clay loam	2772.0/18.48 × 150/4	Pitfall (3), Sticky (4), Visual (1)
14.3.1.2	Sinaloa	Experimental	2009	LF	8-9 Nov., 09	Clay	105.0/10.5 × 10/4	Pitfall (7), Sticky (7), Visual (3)
				SM	9-10 Nov.,09	Clay	105.0/10.5 × 10/4	Pitfall (7), Sticky (7), Visual (3)
		Experimental	2011	SILM	16 Feb., 11	Clay	128.0/6.4 × 20/3	Sticky (7)
				SIPE	1 Mar., 11	Clay	128.0/6.4 × 20/3	Sticky (7)
		Experimental	2012	SIVJ	25 Mar., 12	Clay	72.0/9 × 8/3	Pitfall (5), Sticky (5), Visual (3)
		Pilot[e]	2012	SIAG	11 Feb., 12	Clay	1020.0/6 × 170/3	Pitfall (6), Sticky (6)
				SICL	27 Jan.,12	Clay loam	990.0/18 × 55/3	Pitfall (7), Sticky (7)
				SIGU	4 Feb., 12	Clay	540.0/18 × 30/3	Pitfall (6), Sticky (6)
		Pilot[e]	2012-2013	SIN_72	12 Dec., 12	Clay	1641.6/27.36 × 60/3	Pitfall (7), Sticky (7)
				SIN_77	14 Jan., 13	Clay	1800.0/12 × 150/2	Pitfall (7), Sticky (7)
9.5.1.2	Tamaulipas	Experimental	2010	TAHU	2 Feb., 10	Clay	114.8/11.48 × 10/4	Pitfall (5), Sticky (5), Visual (3)
				TAVA	14 Feb., 10	Clay	114.8/11.48 × 10/4	Pitfall (5), Sticky (5), Visual (3)
		Experimental	2012	TAVH1	18 Mar., 12	Silty clay	384.0/9.6 × 40/3	Pitfall (5), Sticky (5), Visual (3)
				TAVH2	19 Mar., 12	Sandy silt	384.0/9.6 × 40/3	Pitfall (5), Sticky (5), Visual (3)
		Pilot[e]	2013	TAMPS_15	5 Feb., 13	Sandy clay loam	3888.0/25.92 × 150/4	Pitfall (6), Sticky (6), Visual (3)
				TAMPS_21	4 Feb., 13	Sandy loam	2592.0/25.92 × 100/4	Pitfall (6), Sticky (6), Visual (3)

(Continues)

TABLE 1 (Continued)

Ecoregion[a]	State	Study type[b]	Year	Site[c]	Planting date	Soil texture	Plot size[d](m^2)/dimensions/Number of replicates	Collection method and (Number of collections)
10.2.4.1	Chihuahua and La laguna (Coahuila and Durango)	Experimental	2011	CHIH1	7 Jul., 11	Sandy clay loam	110.4/11.04 × 10/4	Pitfall (4), Sticky (5), Visual (3)
				CHIH2	9 Jul., 11	Sandy loam	97.2/9.72 × 10/4	Pitfall (4), Sticky (5), Visual (3)
				LALA1	21 Jul., 11	Sandy clay loam	90.0/9 × 10/4	Pitfall (4), Sticky (7)
				LALA2	23 Jul., 11	Sandy loam	90.0/9 × 10/4	Pitfall (4), Sticky (7)
		Pilot[e]	2012	CHIH_3	7 Aug., 12	Clay loam	1152.0/14.4 × 80/3	Pitfall (5), Sticky (5), Visual (2)
				CHIH_18	2 Aug., 12	Sandy clay loam	1456.0/14.56 × 100/3	Pitfall (4), Sticky (4), Visual (2)
				LAG_09	10 Aug., 12	Silty clay	1657.5/19.5 × 85/3	Pitfall (4), Sticky (5)
				LAG_12	11 Aug., 12	Sandy clay loam	398.7/9 × 44.3/3	Pitfall (4), Sticky (5)

[a]Ecoregion as described by the National Commission for Biodiversity (CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad), 2009; INEGI-CONABIO-INE (Instituto Nacional de Estadística, Geografía e Informática–Comisión Nacional para el Conocimiento y Uso de la Biodiversidad–Instituto Nacional de Ecología), 2008). 14.3.1.2=Coastal plain of Sinaloa; 10.2.2.8=Floodplain of the rivers Yaqui, Mayo and Fuerte; 9.5.1.2=Coastal plain Tamaulipeca; 10.2.4.1=Central plains of Chihuahuan Desert.

[b]The studies were defined as experimental or pilot. These are the steps required by Mexican Regulators to obtain de-regulation of a GM trait. Total experimental area sizes varied across study types and years and ranged from 0.23 ha for experimental to 5 ha in size for pilot trials.

[c]Site was designated by combining the first letters of the state where the trials were conducted or the first two letters of the name of the owner of the land and the number of the trial in each particular site.

[d]*n* = 3 or 4 replications for each material at each site.

[e]Only MON-ØØ6Ø3-6 and control hybrids were treated with insecticide to control Lepidopteran pests. All arthropod observations or collections (sticky trap and pitfall deployment) were separated from insecticide application by a minimum of 10 days.

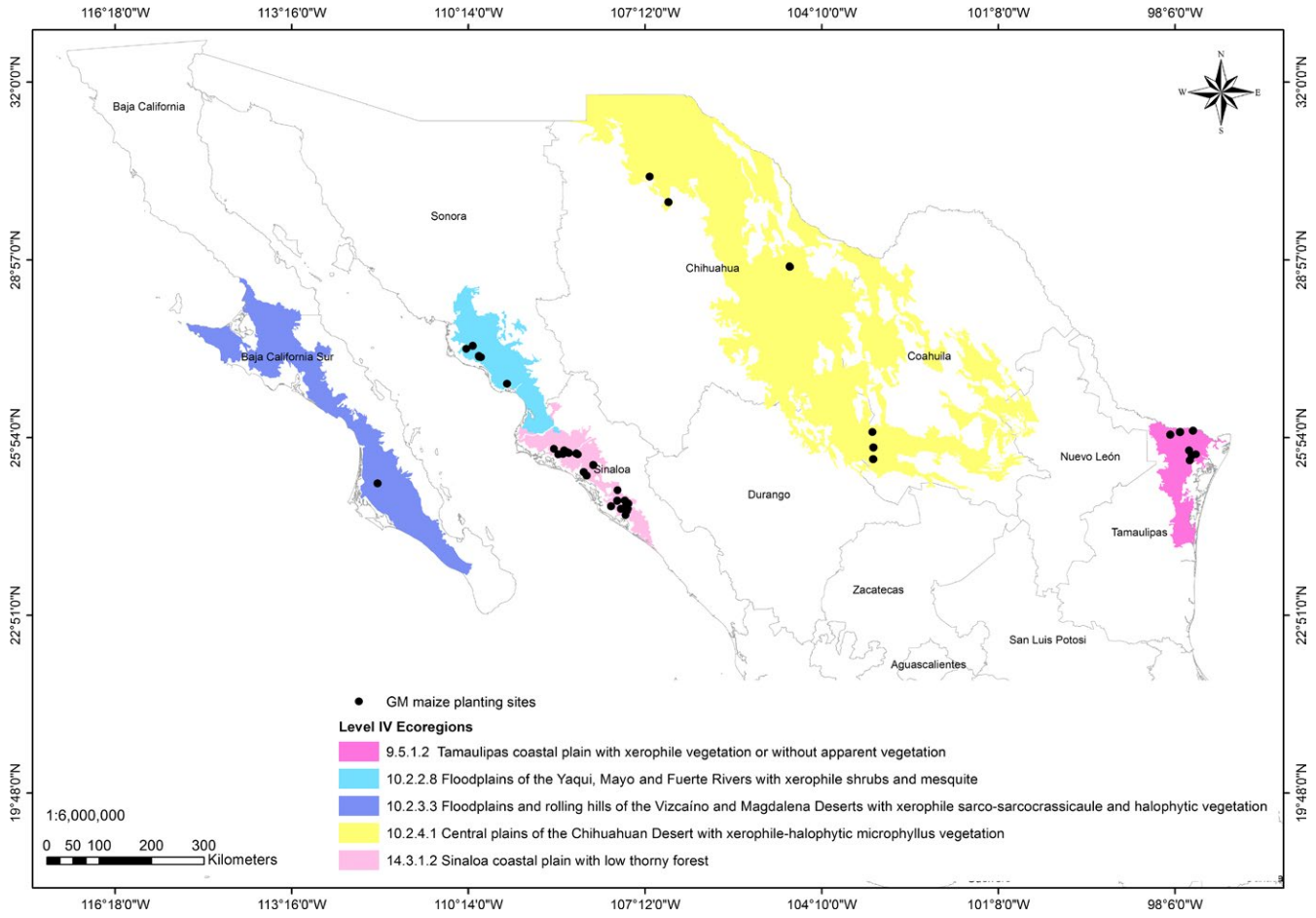

FIGURE 1 Ecoregions where maize field trials were conducted [Colour figure can be viewed at wileyonlinelibrary.com]

2.4 | Experimental design and data collection

Genetically modified maize hybrids MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MON-ØØ6Ø3-6, and MON-ØØ6Ø3-6 and a corresponding conventional isohybrid control were planted in each of 32 studies (18 Experimental Phase, 14 Pilot Phase) in a randomized complete block design (RCBD) with three to four replications and up to four locations per ecoregion per year (Table 1). Individual plot sizes ranged from 100.0 m^2 to 384.0 m^2 (Experimental Phase) and 398.7 m^2 to 4128 m^2 (Pilot Phase) (Table 1). In all cases, NTA data were collected from the central area of each plot. NTA abundance was assessed on all plots from collections performed at different times at each site using yellow sticky traps (Pherocon AM, no-bait sticky traps; Great Lakes Integrated Pest Management, Vestaburg, MI), pitfall traps and/or visual counts (Table 1). NTA abundance was assessed from collections performed from two up to eight times using yellow sticky traps and pitfall traps and one up to three times based on visual counts during the growing season at each site. The yellow sticky traps (2-4 per plot) were deployed every other week starting at approximately V7-V8 growth stages through reproductive growth stage or R3-R5 in each plot. The sticky traps were placed in row at the approximate mid-point between the ground level and the top of the plant canopy. Once the main ear was visible, the sticky traps were deployed at the approximate maize ear level for the remainder of the arthropod collections. Each sticky trap was collected and taken to the laboratory for identification and enumeration of NTAs. Pitfall traps (2-3 per plot) consisted of two uncovered plastic cups, filled with soapy water and placed in the ground between two adjacent rows at approximately V4 growth stages through R3-R5 within each plot. Twenty-four to forty-eight hours later, the pitfall traps were collected and taken to the laboratory for identification and enumeration. Visual counts for arthropod abundance were made by examining the stalk, leaf blade, leaf collar, ear tip, silk and tassel of each plant (ten random plants/plot). Visual observations were conducted during the growing season at approximately V18-VT, R1 and R2 growth stages of development. NTA abundance was assessed from collections performed up to eight times using sticky traps and pitfall traps and three times based on visual counts during the growing season at each site. The majority taxa were identified to the genus level; however, some were not identified beyond the family or order level as each of these was treated as a functional group for analysis. This focused method of taxa selection is intended to present clear results from representative taxa of recognized importance and/or taxa that are directly or indirectly exposed to the proteins expressed in GM maize.

TABLE 2 Abundance of arthropods[a] (Mean/plot) associated with MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MON-ØØ6Ø3-6, MON-ØØ6Ø3-6, expressing Cry1A.105, Cry2Ab2, Cry3Bb1 and EPSPS, and the conventional control in field trials across ecoregions

Order: Family: Genus	Ecoregions[c]	Number of Sites	Mean (*SE*)[b] abundance per plot					
			MON 89Ø34-3 × MON-88Ø17-3	Control	MON-89Ø34-3 × MON-ØØ6Ø3-6	Control	MON-ØØ6Ø3-6	Control
Ground dwelling arthropods (pitfall traps)								
Acari	CH, SIN, TAM	6	3.9 (0.5)	4.6 (0.4)	4.6 (0.8)	5.5 (0.5)	4.2 (0.3)	4.7 (0.4)
Araneae	CH, SIN, TAM, SON	15	2.2 (0.2)	2.6 (0.4)	1.8 (0.2)	2.3 (0.4)	2.3 (0.2)	2.7 (0.4)
Coleoptera								
Carabidae	CH, SIN, SON	10	2.0 (0.2)*	2.8 (0.2)	2.3 (0.2)	2.6 (0.2)	2.8 (0.2)	2.7 (0.2)
Staphylinidae	CH, SIN, TAM	11	12.1 (1.4)	10.9 (1.4)	10.1 (0.9)	11.3 (1.6)	10.5 (1.0)	11.0 (1.4)
Collembola	CH, SIN, TAM, SON	28	113.8 (7.4)	127.7 (6.6)	135.4 (13.5)	133.8 (10.1)	109.7 (8.9)	142.5 (10.3)
Dermaptera								
Forficulidae	SIN, TAM, SON	5	13.0 (1.4)	15.8 (1.3)	15.7 (0.7)	16.3 (1.5)	14.8 (2.6)	17.3 (1.5)
Orthoptera								
Gryllidae	CH, SIN	7	4.6 (0.4)	4.4 (0.2)	3.5 (0.3)	3.3 (0.2)	4.1 (0.3)	4.4 (0.2)
Canopy dwelling arthropods (sticky traps)								
Coleoptera								
Chrysomelidae								
Chaetocnema spp.	CH, SIN, TAM, SON	19	46.9 (1.9)*	51.3 (2.9)	60.8 (2.7)	60.5 (4.1)	56.8 (2.8)	55.0 (3.5)
Diabrotica spp.	CH, SIN, TAM, SON	12			4.1 (0.3)	4.5 (0.3)	3.9 (0.2)	4.0 (0.2)
Coccinellidae	CH, SIN, TAM, SON	22	6.7 (0.2)	7.0 (0.3)	2.9 (0.1)	2.9 (0.2)	7.2 (0.2)	7.5 (0.3)
Diptera								
Otitidae								
Euxesta spp.	CH, SIN, TAM, SON	25	47.7 (1.8)*	56.6 (1.8)	53.1 (2.0)	61.7 (2.2)	62.6 (2.3)	61.3 (1.9)
Syrphidae	CH, TAM, SON	6	22.4 (1.4)	30.9 (2.2)	44.9 (2.6)	46.5 (2.7)	37.2 (3.2)	34.0 (2.2)
Tachinidae	CH, SIN	10	5.4 (0.3)	5.9 (0.4)	6.4 (0.6)	6.5 (0.5)	6.8 (0.5)	5.7 (0.4)
Hemiptera								
Anthocoridae								
Orius spp.	CH, SIN, TAM, SON	23	6.5 (0.3)	6.4 (0.3)	4.7 (0.2)	4.8 (0.3)	6.9 (0.3)	6.6 (0.3)
Aphididae	CH, SIN, TAM, SON	12	10.1 (0.9)	9.3 (0.8)	9.2 (1.0)	8.7 (0.9)	10.9 (0.6)	10.2 (0.8)
Cicadellidae								
Dalbulus spp.	CH, SIN, TAM, SON	24	113.8 (6.5)	124.6 (4.7)	118.4 (5.0)	106.3 (7.3)	112.9 (4.9)	101.5 (5.8)
Miridae	CH, SIN, SON	7	2.8 (0.2)	3.0 (0.2)	1.6 (0.1)	2.1 (0.2)	2.9 (0.2)	2.8 (0.2)

(Continues)

TABLE 2 (Continued)

Order: Family: Genus	Ecoregions[c]	Number of Sites	Mean (*SE*)[b] abundance per plot					
			MON 89Ø34-3 × MON-88Ø17-3	Control	MON-89Ø34-3 × MON-ØØ6Ø3-6	Control	MON-ØØ6Ø3-6	Control
Hymenoptera								
Parasitic wasp	CH, SIN, SON	23	13.2 (0.9)*	18.2 (1.2)	9.2 (1.0)*	12.5 (1.3)	13.1 (0.9)	14.6 (0.8)
Neuroptera								
Chrysopidae								
Chrysopa spp.	CH, SIN, SON	15	4.9 (0.2)	5.0 (0.2)	3.3 (0.2)	3.3 (0.2)	5.0 (0.1)	5.1 (0.2)
Thysanoptera								
Thripidae	CH, SIN, TAM, SON	18	278.4 (12.5)	277.9 (9.9)	143.1 (7.0)	133.2 (7.1)	339.5 (15.2)	350.8 (11.3)
Canopy dwelling arthropods (visual counts)								
Coleoptera								
Chrysomelidae								
Chaetocnema spp.	CH, TAM	6	12.4 (1.1)	14.2 (1.2)	14.3 (0.7)	16.9 (3.6)	16.4 (1.4)	18.5 (4.2)
Coccinellidae	SIN, TAM, SON	6	2.0 (0.2)	1.9 (0.2)	2.2 (0.2)	1.9 (0.2)	1.4 (0.1)	1.5 (0.1)
Hemiptera								
Anthocoridae								
Orius spp.	CH, SIN, SON	8	20.3 (0.8)	19.4 (1.6)	18.3 (1.4)	20.3 (1.3)	17.8 (1.0)	21.0 (1.6)
Cicadellidae								
Dalbulus spp.	CH, SON	8	19.0 (1.9)	18.4 (1.3)	20.7 (1.6)	18.1 (1.2)	22.2 (1.4)	20.7 (1.5)
Neuroptera								
Chrysopidae								
Chrysopa spp.	CH, SIN, TAM, SON	9	2.2 (0.1)	2.0 (0.2)	2.0 (0.2)	1.8 (0.2)	2.3 (0.2)	1.8 (0.1)

*Indicates significant difference between GM maize hybrid and its conventional isogenic control ($p < .05$).

[a]Arthropods observed that were most abundant and occurred in at least two of the four ecoregions and in at least five sites across regions.

[b]*SE* is standard error.

[c]Ecoregions are as follows: CH=Chihuahua, Coahuila and Durango, ecoregion 10.2.4.1; SIN=Sinaloa, ecoregion 14.3.1.2; SON=Sonora, ecoregion 10.2.2.8; TAM=Tamaulipas, ecoregion 9.5.1.2.

2.5 | Statistical analysis

2.5.1 | Non-target arthropod abundance

The primary focus of the study was on the effects of GM maize hybrids MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MON-ØØ6Ø3-6 and MON-ØØ6Ø3-6 and a corresponding conventional control on the mean count of each arthropod taxon during the entire season in each region (Data S2). For an appropriate comparison between the GM and the control maize hybrids, the following two-part inclusion criteria were applied before fitting the statistical model to the data and making the comparisons. First, a site inclusion criterion was applied for each site where a mean count of ≥ 1 per plot across all collection times, all material, and all replicates was required for each site to be included in the analysis. Secondly, a taxa inclusion criterion was applied justifying an across-site analysis, that is, presence at ≥5 sites from at least two regions (Comas, Lumbierres, Pons, & Albajes, 2014). Data combinations with counts below these criteria were excluded from significance testing but summarized in Table S2.

The differential insecticide regime used between GM and control plots in Pilot studies may have impacted arthropod abundance differently. An interaction term with insecticide regime was added to the model to determine whether there were any significant effects of insecticides on abundance within a site. Only two of 93 comparisons demonstrated significant interaction. Therefore, data were combined across sites for a combined-site analysis.

The following model was used in a combined-site analysis:

$$y_{ijklm}=\mu+R_i+S_{j(i)}+B_{k(ij)}+M_l+(RM)_{il}+C_{m(ij)}+(SM)_{jl(i)}+(MC)_{lm(ij)}+e_{ijklm} \quad (1)$$

where y_{ijklm}=square root of the observed arthropod count; μ=overall mean; R_i=fixed region effect; $S_{j(i)}$=random site effect within region; $B_{k(ij)}$ =random replicate effect within each site; M_l=fixed GM treatment effect; $(RM)_{il}$=fixed interaction effect of region and GM treatment; $C_{m(ij)}$=random collection time effect within each site; $(SM)_{jl(i)}$=random interaction effect of GM treatment and site; $(MC)_{lm(ij)}$=random interaction effect of GM treatment and collection time; and e_{ijklm}=random residual effect. A square root transformation was applied to the count data prior to analysis to achieve approximate normality and variance homogeneity. The transformed data were analysed with a mixed linear model. SAS procedures (PROC MIXED) were used for computation of the model parameters and statistics for each taxon sampled by each of the three collection methods (Demidenko, 2004; Littell, Henry, & Ammerman, 1998; SAS Institute, 2002 – 2012). The GM treatment effect (insect protection, herbicide tolerance or a stacked combination) was tested across multiple sites. Due to differences in the number of the GM and control hybrids across sites, the analysis was conducted for each paired comparison separately, using only the GM hybrid and the corresponding control data from the available sites. In all analyses, a Type I (α) significance level of 5% was used to test the two-sided null hypothesis.

2.5.2 | Statistical power

A 50% detectable difference in the abundance of a taxonomic group was used to assess the statistical power (Blumel et al., 2000; Perry, Rothery, Clark, Heard, & Hawes, 2003). Methods similar to Duan et al. (2006) were used with additional random effect terms in model (1). Let x_1 and x_2 represent the observed insect count, and μ_{x1} and μ_{x2} represent the expected mean counts for the control and the test lines, respectively. Then detectable difference (d_x) relative to the control implies $d_x=\mu_{x1}-\mu_{x2}=0.5\mu_{x1}$ when $\mu_{x1} > \mu_{x2}$ or $d_x = -0.5\mu_{x1}$ when $\mu_{x1} < \mu_{x2}$. If y is the square root of x, the corresponding difference in y, that is d_y, can be obtained from the following equations:

$$\begin{cases} d_{ya}=\mu_{y1}-0.5\sqrt{4\mu_{y1}^2-2\left(\mu_{y1}^2+\sigma_y^2\right)} & \text{for } d_y>0 \\ d_{yb}=\mu_{y1}-0.5\sqrt{4\mu_{y1}^2-2\left(\mu_{y1}^2+\sigma_y^2\right)} & \text{for } d_y<0 \end{cases}$$

where μ_{y1} and σ_y^2 are the control mean and the total variance of all random effects in model (1) in square root scale. The power calculation used $d_y = \min(d_{ya}, -d_{yb})$, where min represents the minimum of the two quantities in parenthesis.

Next, a two-sample t test with a significance level of α was used for a detectable difference d_y. The calculation substituted the parameters in the power calculation with the corresponding estimates from the combined-site analysis using model (1). A customized SAS program was used for the estimation of different statistical parameters and the subsequent calculations of the power.

3 | RESULTS

The interaction of region with maize hybrids was only observed for 4.49% of the total comparisons ($p < .05$). This is within the nominal error rate of 5% and indicates that arthropod response to GM and non-GM hybrids was similar across regions. The "regional" differences were influenced by differences in categorization of arthropod taxa across researchers, year-to-year fluctuations of arthropod populations, as well as fluctuations in arthropod abundance that would be expected across regions. Overall, a high degree of similarity of taxa across regions was observed especially for the most abundant taxa representing the ecological functions of herbivores, predators and parasitoids in maize fields (Table 2 and Table S3).

Across all ecoregions, twenty invertebrate taxa (comprising 11 taxonomic orders and 17 families) were relevant and sufficiently abundant to evaluate the effects of GM maize on NTAs (Table 2). The ground-dwelling NTAs collected in pitfall traps primarily belonged to seven different taxa: soil mites (Acari), spiders (Araneae), predatory ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae), springtails (Collembola), predatory earwigs (Dermaptera: Forficulidae) and field crickets (Orthoptera: Gryllidae). The foliage-dwelling NTAs collected in sticky traps and

visual counts primarily belonged to 13 different taxa: ladybird beetles (Coleoptera: Coccinellidae); corn flea beetles, *Chaetocnema* spp. (Coleoptera: Chrysomelidae); rootworm beetles, *Diabrotica* spp. (Coleoptera: Chrysomelidae); cornsilk flies, *Euxesta* spp. (Diptera: Otitidae); syrphid flies (Diptera: Syrphidae); tachinid flies (Diptera: Tachinidae), minute pirate bugs, *Orius* spp. (Hemiptera: Anthocoridae); aphids (Hemiptera: Aphididae); leafhoppers, *Dalbulus* spp. (Hemiptera: Cicadellidae); plant bugs (Hemiptera: Miridae); parasitic wasps (Hymenoptera); lacewings, *Chrysoperla* spp. (Neuroptera: Chrysopidae); thrips (Thysanoptera: Thripidae). Additionally, these taxa were widely distributed across the ecoregions, with majority of the important herbivorous, predatory and parasitic taxa occurring in at least three of the four ecoregions (Table 2 and Table S3). The statistical power analysis conducted on these widely distributed taxa demonstrated that the majority of the taxa (19 of 20) had higher than 80% power to detect a 50% difference in arthropod abundance (Table S4). Therefore, given the scale and intensity of the sampling, any significant impacts of GM maize on populations of widely distributed taxa across ecoregions should have been detectable within this study.

Across all GM maize hybrids, no significant differences in NTA abundance were detected for 69 (93.2%) of the 74 statistical comparisons (Table 2). Of the 20 taxa individually analysed, a total of five significant differences were detected with only four taxa, consisting of two pest arthropods (*Chaetocnema* spp. and *Euxesta* spp.) and two beneficial arthropods (Carabidae and parasitic wasps). Fewer *Chaetocnema* spp. ($F_{1,123} = 13.12$, $p = .0004$) and *Euxesta* spp. ($F_{1,17.7} = 19.07$, $p = .0004$) were detected for MON-89Ø34-3 × MON-88Ø17-3 compared to the control.

Fewer Carabidae were observed for MON-89Ø34-3 × MON-88Ø17-3 compared with the control ($F_{1,27.2} = 6.18$, $p = .0193$). Fewer parasitic wasps were also detected for MON-89Ø34-3 × MON-88Ø17-3 and MON-89Ø34-3 × MON-ØØ6Ø3-6 compared with their respective conventional controls ($F_{1,29.7} = 6.68$, $p = .0149$ and $F_{1,19.9} = 7.46$, $p = .0129$, respectively).

4 | DISCUSSION

Each GM crop undergoes a scientifically sound ERA prior to commercialization to assess for potential ecological impact of the introduced trait(s) with the purpose of demonstrating the GM crop is "as-safe-as" non-GM comparators. To date, across commercialized GM crops and their respective inserted genes (e.g., *Bt* genes, *cp4 epsps* gene), no evidence of unacceptable risks to the environment has been documented which is aligned with extensive commercial experience with these GM crops worldwide (Pilacinski et al., 2011; Weber et al., 2012). Despite the history of safe use, rapid adoption of GM crops in several geographies, and the fact that risk assessors and regulators have access to environmental assessment data generated on the crop and trait in other geographies, extensive local field evaluations are still required prior to making informed decisions on the cultivation approval of GM crops in Mexico.

Assessment of MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MON-ØØ6Ø3-6 and MON-ØØ6Ø3-6 was conducted based on regulatory guidance laid out in Biosafety Law for Genetically Modified Organisms in Mexico (DOF (Diario Oficial de la Federación), 2005; DOF (Diario Oficial de la Federación), 2008). We conducted a comprehensive field evaluation in diverse maize growing regions representative of four ecological regions in Mexico and assessed non-target arthropods that were ecologically relevant, sufficiently abundant to detect differences and with potential for direct and/or indirect exposure to the GM traits (Prasifka et al., 2008; Rauschen, Schaarmschmidt, & Gathmann, 2010; Rauschen, Schultheis et al., 2010; Romeis, Van Driesche, Barratt, & Bigler, 2009; Romeis et al., 2014). The purpose of these evaluations is to confirm the results of the early-tier laboratory testing and address any uncertainties in the risk assessment by collecting meaningful data on NTAs that are closely associated with the plant (Romeis, Meissle, & Bigler, 2006; Romeis et al., 2008). The results of the NTA assessments in multisite and multiecoregion field trials demonstrate the absence of adverse effects when NTA communities are exposed to maize MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MON-ØØ6Ø3-6 and MON-ØØ6Ø3-6. The reductions in abundance observed for two pest species, *Chaetocnema* spp. and *Euxesta* spp., in MON-89Ø34-3 × MON-88Ø17-3 do not imply increased susceptibility (adverse environmental impact) of this GM crop to these pests. Similar reductions in *Chaetocnema* spp. have been observed in Cry3Bb1 maize, MON-ØØ863-5 and for *Euxesta* spp. in MON-89Ø34-3 maize containing Cry1A.105 and Cry2Ab2 and were probably an indirect response, with *Chaetocnema* spp. and *Euxesta* spp. being attracted to the conventional control plots with a measurable feeding damage caused by target pests (Bhatti et al., 2005b; Goyal et al., 2012). Among the beneficial arthropods, the observed reduction in abundance of Carabidae in MON-89Ø34-3 × MON-88Ø17-3 may have resulted from decrease in prey availability due to efficient control of target pests (Leslie, Biddinger, Mullin, & Fleischer, 2009; Riddick & Barbosa, 1998) since Carabidae are known to have a density-dependent relationship with prey populations (Ellsbury et al., 1998). Additionally, early-tier laboratory and field studies indicated no adverse effect of the coleopteran-active *Bt* protein, Cry3Bb1 expressed in MON-89Ø34-3 × MON-88Ø17-3 on various species of Carabid beetles (Duan et al., 2006; Priesnitz, Benker, & Schaarschmidt, 2013). Given the host-specific nature of parasitoids, the lower abundance in the GM hybrids was most likely due to the reduction in their lepidopteran prey (Liu et al., 2015). Similar prey-mediated effects on parasitoids have been reported by other studies where these results were actually because of nutritionally poorer prey rather than any direct toxic effect of the *Bt* proteins (Chen et al., 2008; Walker, Cameron, MacDonald, Madhusudhan, & Wallace, 2007; Wolfenbarger et al., 2008). These few statistical differences in NTA abundance, such as might occur from a subtle and unforeseen interaction, are unlikely to have adverse implications for environmental safety. Thus, the results support the conclusion of no adverse effects on NTA communities from deployment

of MON-89Ø34-3 × MON-88Ø17-3, MON-89Ø34-3 × MON-ØØ6Ø3-6 and MON-ØØ6Ø3-6 for cultivation.

Our results agree with prior published literature that demonstrate the absence of adverse effects on NTA independently for Cry1A.105 + Cry2Ab2 (Hendriksma, Härtel, & Steffan-Dewenter, 2011; Rosca & Cagan, 2013; Schuppener, Mühlhause, Müller, & Rauschen, 2012; Whitehouse et al., 2005), Cry3Bb1 (Ahmad, Wilde, Whitworth, & Zolnerowich, 2006; Ahmad, Wilde, & Zhu, 2005; Al-Deeb & Wilde, 2003; Bhatti et al., 2005a,b; Comas et al., 2014; Devos et al., 2012; ILSI-CERA 2014; Lundgren & Wiedenmann, 2002) and CP4 EPSPS (Comas et al., 2014; ILSI-CERA 2010; Reyes, 2005; Rosca, 2004; Schier, 2006). Additionally, these studies confirm findings of no adverse effects on NTA when dual modes of insecticide action, or insecticide and herbicide-tolerant traits are combined through conventional breeding (Comas et al., 2014; Devos et al., 2012; Marvier, McCreedy, Regetz, & Kareiva, 2007; Svobodova et al., 2017). Taken together, our results confirm findings from both lower-tier laboratory testing and confirmatory field studies demonstrating no adverse effect on arthropod communities representing the ecological functions of herbivores, predators and parasitoids in maize agro-ecosystems of Mexico.

It is important that regulators have access to and utilize relevant data produced in one geographical region to support a risk assessment on the crop and trait for another geographical region (Garcia-Alonso et al., 2014; Horak et al., 2015; Roberts, Devos, Raybould, Bigelow, & Gray, 2014). Several recent reports have provided empirical evidence for when data can be transported from one geographical region to another for the ERA of a GM soya bean (Horak et al., 2015) and GM maize (Ahmad et al., 2016; Heredia Díaz et al., 2017; Nakai et al., 2015). These studies demonstrate that the environmental safety conclusions from comparative assessments between GM and conventional counterparts are consistent across geographies, including those differing in climate and production practices. Using similar measurement endpoints is a key to enable transportability, making risk assessments conducted based on this kind of data robust enough to use in different geographies.

A key principle of ERA is risk-based testing (CropLife International (CLI), 2016; Wolt et al., 2010), in which testing is limited to those scenarios under which there is plausible scientific rationale for an adverse environmental effect. The consistent findings of transportability of field trial conclusions across diverse geographies confirm that in the absence of a plausible hypothesis for an interaction between trait and environment that would increase adverse environmental impact, data are transportable regardless of differences in climate or production practices. The need to consider the similarity of climatic conditions or agronomic practices to enable transportability, as the conceptual framework by Garcia-Alonso et al. (2014) proposes would only be relevant in cases of specific risk hypotheses in the environment to which the conclusions will be transported.

In this study, a comparison of the arthropod taxa across ecoregions revealed that the most relevant and abundant taxa were similar across ecoregions and represented key functional groups including herbivores, predators, parasitoids and decomposers. These arthropod taxa fit the concept of representative taxa for field tests and meet the recommendations of Knecht et al. (2010), Albajes, Lumbierres, Pons, and Comas (2013) and Comas, Lumbierres, Pons, and Albajes (2013, 2015) on abundance consistency and capacity to detect potential effects of insect-protected maize on non-target arthropods. This similarity of non-target arthropod taxa indicates that the data are readily transportable for use in risk assessment between these ecoregions, therefore eliminating duplication of ERA efforts in each ecoregion. Additionally, the most abundant taxa observed in local field studies in Mexico are similar to those observed in NTA studies for the environmental risk assessment of GM maize in the United States, Argentina and Brazil (Ahmad et al., 2016) indicating that these taxa are representative of maize growing ecosystems across ecoregions and countries. The results from this and other similar studies in other countries indicate that the key non-target arthropod taxa are similar across geographies irrespective of climatic, soil and environmental variations (Ahmad et al., 2016). Therefore, risk assessments of GM maize already conducted in any of these geographies are sufficient for use in Mexico and other geographies with similar fauna. The few differences in taxa that may occur across ecoregions or geographies are not barriers to data transportability but require appropriate consideration in the context of problem formulation, specificity and safety of the proteins from the tiered risk assessment in the ERA.

In summary, the results of this study indicate that the abundance of non-target arthropods was not adversely affected by the single or stacked insect-protected and herbicide-tolerant GM maize hybrids relative to conventional controls. Additionally, the similarity of key non-target taxa across ecoregions indicates that repetitive field studies across ecoregions and agricultural ecosystems are not testing novel scenarios. Therefore, the current number of field sites across different ecoregions required to evaluate potential environmental impacts of GM maize hybrids may not provide additional relevant information in an environmental risk assessment in Mexico. Several of the key non-target taxa here have also been found in other world areas where similar environmental risk assessments have been conducted, providing further justification for transportability of field non-target arthropod data on maize with these same traits from one geography (country) to another for the environmental risk assessment.

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AUTHOR CONTRIBUTION

Conceptualization: AA, CRB, OHD, JMM, BMM. Formal analysis: CJ. Investigation: JLCM, JLMC, MBOM, HADP, JAE, FJQ, JAGT, LCE,

FZG, AAEB, JGG. Writing – original draft: AA, JEW, PA, BMM, CJ, CRB, OHD, JMM.

ORCID

A. Ahmad http://orcid.org/0000-0001-8854-419X

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SUPPORTING INFORMATION

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