# Gauging the effect of transgenic maize and cotton on non-target soil arthropods in Colombia

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## INTRODUCTION

The Colombian Ministry of Agriculture and Development, through the Instituto Colombiano Agropecuario (ICA), designed a scheme to determine the viability of incorporating GMOs into the agricultural production process. In 1998, ICA published resolution 03492 to regulate and establish the procedures for the introduction, production, liberation and commercialization of GMOs. Through Agreement 013/98 and 0002/02, ICA created the National Technical Council for Agricultural Biosecurity (NTC) to function in the regulation of GMOs.

Since the establishment of those regulations, applications have been submitted for *Brachiaria*, carnations, cassava, coffee, cotton, maize, rice, *Stylosanthes* and sugar cane. Of these, only four have been approved to date: (i) carnations for cut-flower production, (ii) cotton for commercial production, (iii) rice for small scale field trials, and (iv) maize for biosecurity tests (Diaz 2003).

At present, the biosafety information available to researchers and regulators in Colombia stems from studies conducted in other countries that largely represent temperate regions. That ex-situ experience has to be effectively transferred to the tropical and developing country arenas if we are to successfully gauge the magnitude of GMO effects on the abundance, diversity and ecological function of non-target arthropods.

## OBJECTIVE

Evaluate and compare the impact of GMO and non-GMO plant protection technologies on non-target soil arthropods in Colombian maize and cotton.

## MATERIALS AND METHODS

Due to delays in the approval of Bt-transgenic maize, an initial study was conducted on the soil insecticide chlorpyrifos in conventional maize. The research was conducted at the International Center for Tropical Agriculture (CIAT), located at 3º31' N, 76º21' W, 956 m elevation, mean annual rainfall 1000 mm, mean temperature 24ºC, and Holdridge life zone classification Dry Tropical Forest. There were eight experimental plots (43 x 43 m each) evaluated over two consecutive cycles of maize (second semester 2002 and first semester 2003). The two treatments were maize (commercial hybrid "Master" from Syngenta) with and without insecticide incorporated at planting to control soil-active lepidopteran pests, in particular the impact of *Spodoptera frugiperda (*Noctuidae) as a cutworm.

In a second phase of activities, in collaboration with ICA's division of Agricultural Regulation and Protection, field studies were initiated in cotton to establish the effects of Bollgard® technology (Bt-transgenic cotton insecticidal to lepidopteran pests). The first of three consecutive cycles (first semester 2003), in rotation with soybean, was conducted at the ICA research station in Palmira, located at 03°31'N, 76°19'W, 975 m elevation, annual precipitation 1295 mm, mean temperature 24ºC, relative humidity 76%, and Dry Tropical Forest. There were 24 experimental plots (15 x 15 m each) with four replicates of six treatments based on plant material (Bollgard® technology represented by the var NuCont 33B with the Cry1A(c) gene and conventional technology represented by var DP 5415) and insecticide regime (conventionally applied insecticides, insecticides to control non-lepidopteran pests, and Bt-based insecticides). Because economic thresholds were never reached in the first cycle, no insecticides were applied and the data were analyzed as two plant variety treatments.


**Fig. 1. Pitfall traps showing (A) fixed component, (B) removable component and (C) lid.**

Information was gathered from two types of samples: pitfall traps to sample surface-active arthropods (maize and cotton) and soil cores extracted with berlese funnels to sample soil-active arthropods (only cotton). Pitfall traps were located between plants within the rows; eight were put out in each experimental plot (Fig. 1) and these were opened to sampling for a 24-hour period each week. Soil samples were taken with a cup cutter (10 cm diam, 10 cm depth) every 2 wk from within the row between plants (Fig. 2). Four samples were taken from each experimental plot. Samples were placed in berlese funnels for 24 hours, then arthropods were sorted from the debris and stored in 70% ethyl alcohol until analysis (CIAT 2003; Mojocoa 2003; Rodriguez & Peck 2004). The statistical model used for the analysis of the data was a completely randomized block design.


**Fig. 2. Field collection of samples for berlese extraction of arthropods (A) cup cutter, (B) soil sample, (C) berlese funnels.**

With this design an ANOVA will be used to determine differences in abundance among treatments and determine the effect of their interactions. In addition, for the most abundant groups we will conduct an analysis of the area under the population curve (accumulated insect-days) to determine differences among treatments during the trial. We will also compare the diversity and abundance among treatments using various indices of taxonomic diversity, dominance and equity.

## RESULTS

**Surface-active arthropods (pitfalls):** In the two-cycle maize study a total of 11,850 arthropods were captured and sorted from pitfall traps, representing five taxonomic classes and 18 orders; 58.7% of individuals were captured in the insecticide plots (Table 1). Poduromorpha and Acarina were the most abundant orders with 37.8 and 19.7% of specimens, respectively. Treatments had a significant effect on two orders, Acarina (more abundant with insecticide) and Thysanoptera (more abundant without insecticide). There were significantly more pitfall captures in the first cycle compared to the second.

In cotton, 438,934 specimens were captured in the first cycle, representing eight classes and 20 orders (Table 1); 54.3% of individuals were captured in NuCotn 33B (Bt-transgenic) plots. Sixty-five different species have been identified and only three of these were not present in both NuCotn 33B and DP 5415 (Table 2). The most abundant class was Collembola with 52.3% of total captures (Table 1). Poduromorpha, Hymenoptera and Acarina were the most abundant orders with 50.4, 29.7 and 17.2% of specimens, respectively. Treatments had a significant effect on two orders, Collembola and Isopoda, each more abundant in NuCotn 33B.

**Table 1. Number of individuals and composition of invertebrate classes caught in pitfall traps in maize (2002-2003) and cotton (2003).**

| Taxonomic class | Maize | | | Cotton | | |
|---|---|---|---|---|---|---|
| | With insecticide | Without insecticide | Total | NuCotn 33B | DP5415 | Total |
| Aracnida | 1,978 | 1,236 | 3,214 | 37,321 | 38,677 | 75,998 |
| Chilopoda | 12 | 18 | 30 | 6 | 2 | 8 |
| Collembola | 3,417 | 2,027 | 5,444 | 138,061 | 91,364 | 229,425 |
| Diplopoda | 102 | 94 | 196 | 17 | 29 | 46 |
| Insecta | 1,452 | 1,514 | 2,966 | 62,666 | 70,443 | 133,109 |
| Malocostraca | 0 | 0 | 0 | 112 | 48 | 160 |
| Nematoda | 0 | 0 | 0 | 25 | 108 | 133 |
| Oligochaeta | 0 | 0 | 0 | 0 | 1 | 1 |
| Symphyla | 0 | 0 | 0 | 27 | 27 | 54 |
| **Total** | **6,961** | **4,889** | **11,850** | **238,235** | **200,699** | **438,934** |

**Table 2. Abundance of invertebrate orders (mean number of individuals captured per evaluation date) in maize (2002-2003) and cotton (2003).**

| Order | Maize | | Cotton | |
|---|---|---|---|---|
| | With Insecticide | Without Insecticide | NuCotn 33B | DP5415 |
| Acarina | 11.38±15.23 a | 7.44±8.59 b | 10.65±14.79 a | 8.08±9.35 a |
| Araneae | 4.57±24.61 a | 2.53±8.66 a | 4.98±25.59 a | 2.03±1.48 b |
| Blattaria | 0.02±0.15 a | 0.01±0.09a | 0 | 0.03±0.18 |
| Chilopoda[1] | 0.10±0.39 a | 0.15±0.55 a | 0.13±0.54 a | 0.11±0.41 a |
| Coleoptera | 3.60±6.46 a | 2.49±4.21 a | 3.38±6.02 a | 2.67±4.82 a |
| Dermaptera | 0.10±0.45 a | 0.04±0.24 a | 0.09±0.44 a | 0.05±0.25 a |
| Diplopoda[1] | 0.82±1.79 a | 0.76±1.64 a | 0.25±0.69 b | 1.37±2.23 a |
| Diptera | 0.91±2.07 a | 0.85±1.85 a | 0.43±0.97 b | 1.36±2.55 a |
| Entomobyomorpha | 3.18±3.62 a | 3.26±4.83 a | 1.22±1.81 b | 5.35±5.03 a |
| Hemiptera | 0.98±2.43 a | 0.87±1.79 a | 1.43±2.79 a | 0.38±0.75 b |
| Homoptera | 0.31±0.63 a | 0.43±0.88 a | 0.36±0.87 a | 0.38±0.64 a |
| Hymenoptera | 4.32±8.37 a | 5.91±15.08 a | 5.71±15.89 a | 4.48±6.21 a |
| Isopoda | 0.52±1.06 a | 0.63±1.42 a | 0.56±1.42 a | 0.58±1.06 a |
| Lepidoptera | 0.02±0.13 a | 0.02±0.13 a | 0.01±0.09 a | 0.03±0.16 a |
| Nematoda | 0.14±0.41 a | 0.19±0.50 a | 0.13±0.42 a | 0.20±0.50 a |
| Neuroptera | 23.66±112.35 a | 12.48±84.12 a | 34.77±136.23 a | 0.26±1.05 b |
| Oligochaeta | 0.72±1.86 a | 0.60±1.41 a | 1.02±2.13 a | 0.28±0.71 b |
| Orthoptera | 0.05±0.22 b | 0.19±0.62 a | 0.20±0.60 a | 0.04±0.20 b |
| Poduromorpha | 0.75±1.46 a | 0.60±0.97 a | 0.81±1.54 a | 0.53±0.79 a |
| Symphyla[1] | 11.38±15.23 a | 7.44±8.59 b | 10.65±14.79 a | 8.08±9.35 a |
| Symphypleona | 4.57±24.61 a | 2.53±8.66 a | 4.98±25.59 a | 2.03±1.48 b |
| Thysanoptera | 0.02±0.15 a | 0.01±0.09a | 0 | 0.03±0.18 |
| Unidentified | 0.10±0.39 a | 0.15±0.55 a | 0.13±0.54 a | 0.11±0.41 a |

For each row and crop, means followed by different letters are statistically different at P<0.05 (Tukey-Kramer test for multiple comparasions);[1]Taxonomic class.

**Soil-active arthropods (berlese):** To date, 44% of the samples collected in the first cycle of cotton have been evaluated, numbering 80,541 specimens representing 11 classes and 21 orders (Table 3). The most abundant classes were Arachnida and Insecta with 65.0 and 20.4% of total specimens, respectively. The most abundant order was Acarina, with 65% of total captures and 1.2 times more abundant in DP 5415.

**Diversity indices:** In maize, the species richness index (S) was not significantly between treatments or between semesters. The Shannon diversity index and Simpson dominance index were significantly different between semesters but not between treatments. In terms of species similarity, the Jaccard index showed that 97 and 91% of orders were in common between treatments and semesters, respectively.

In cotton, the species richness, Shannon and Simpson indices were not significantly different between the treatments NuCotn 33B and DP 5415. Values were 14.1, 1.0 and 0.4, respectively. The Jaccard index showed that 80% of orders were in common between the two treatments.

**Table 3. Number of individuals and composition of invertebrate classes extracted from soil cores in cotton (2003).**

| Taxonomic class | NuCotn 33B | DP 5415 | Total |
|---|---|---|---|
| Arachnida | 24,127 | 28,218 | 52,345 |
| Chilopoda | 36 | 44 | 80 |
| Collembola | 4,709 | 4,638 | 9,347 |
| Diplopoda | 14 | 42 | 56 |
| Diplura | 48 | 70 | 118 |
| Insecta | 7,679 | 8,717 | 16,396 |
| Malacostraca | 46 | 63 | 109 |
| Nematoda | 12 | 20 | 32 |
| Oligochaeta | 155 | 215 | 370 |
| Protura | 3 | 4 | 7 |
| Symphyla | 729 | 952 | 1,681 |
| **Total** | **37,558** | **42,983** | **80,541** |

## CONCLUSIONS

- These studies have identified a high abundance and diversity of soil-active and surface-active fauna associated with the cotton crop under the conditions of the Cauca Valley, Colombia.
- Pitfall traps are an appropriate method for measuring the abundance of surface-active arthropods and comparing their activity and diversity across treatments.
- Extracting soil cores with berlese funnels is an adequate method for measuring the abundance of soil-active arthropods and comparing their activity and diversity across treatments.
- The various indices of taxonomic diversity, richness, dominance and equity are useful tools for comparing ecological communities and will allow us to make long-term comparison of the effects of different plant protection technologies under the conditions of the Cauca Valley, Colombia.
- The abundance differences observed between treatments in the first cycle of cotton should be studied in more detail to define how GMOs affect those differences. The protocols established in the first cycle will therefore be implemented in two additional cycles to better describe abundance effects over time, and to gather information to compare differences in species composition of key groups such as the springtails.
- Although abundance and diversity differences may exist in response to GMO technology, it is important to determine whether the magnitude of those differences is ecologically relevant, i.e. have an effect on ecological function or overall soil health.

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