

Evaluating the Impact of Biotechnology on Biodiversity: Effect of Transgenic Maize on Non-Target Soil Organisms

Activity 1. Response of Non-Target Soil Arthropods to Chlorpyrifos in Colombian Maize.

Introduction

Quantitatively and qualitatively, arthropods constitute the most important group of soil macro-organisms, whether in terms of number of individuals, biomass, trophic function, or species diversity (Paris 1979, Jaramillo 1997). The majority of these arthropods are detritivores, playing an important role in the transformation and mineralization of organic material (Marasas *et al.* 2001), as well as regulation of microbial populations, decomposition of organic material, and nutrient cycling within the soil (Doles *et al.* 2001).

Mites and springtails constitute nearly half of all soil arthropods (ECA 2001). Springtails can occur in very high abundance, up to 40,000 individuals /m²; mite populations can approach 200,000/m² and species diversity up to 200/m² (Jordan 1996). In some habitats, diplopods and other arthropods such as fly larvae are important, and can represent the principal detritivores at the soil surface when earthworms are absent (Jordan 1996). Overall, arthropods are expected to have a higher diversity and abundance in less perturbed ecosystems such as forests and permanent prairies (Raw 1971).

There are a diversity of beneficial insects that occur in the soil and function in biological control, lowering populations of pest arthropods and being an important component of integrated pest management (Kirsten *et al.* 1998). In agricultural systems, diversity can be viewed as an indicator of agroecosystem balance, where the application of chemical controls to reduce the effect of pest insects in the crop generates a disequilibrium in the populations of beneficial fauna, creating conditions favorable for the increase, resurgence and/or appearance of potential pests (Kirsten *et al.* 1998). In one study that compared the soil surface entomofauna in maize/bean systems (Zanin *et al.* 1995), it was established that insecticide application reduced the population of almost all arthropods in the individual crops, especially when the product was applied to the whole plant versus the soil.

In Colombia, maize was planted on 574,117 ha in 2001, with technified and traditional maize accounting for 26.0 and 74.0% of that area, respectively. National production was 1,239,346 tons, 44.5 and 55.5% corresponding to technified and traditional, respectively. Mean yield was 2.2 tons/ha (Ministerio de Agricultura 2001). The most important pests to maize during the germination and early plant stage are associated with the soil and include the cutworms *Spodoptera frugiperda* (J.E. Smith), *S. eridiana* (Cramer) and *Agrotis ipsilon* (Hufnagel), *Solenopsis* sp. ants, the scarab *Euethela bidentata* (Burmeister) and the chinch bug *Blissus* sp. (Corpoica 2001). In general terms, the attacks are localized and when damage is greater than 10% of the seedlings, some type of control should be initiated (Corpoica 2001).

Spodoptera frugiperda (Lepidoptera: Noctuidae) is considered the most important pest of maize in Colombia and often achieves very high populations (García Roa 1996). Although known as the fall armyworm, *S. frugiperda* acts as a soil-borne cutworm, but also attacks the shoot and fruit (García Roa 1996). Chlorpyrifos (Lorsban) is the most common of the chemical control products used to combat this insect, incorporated into the soil before planting to reduce the impact of *S. frugiperda* as a cutworm (Ospina 1999).

As part of the project “Assessing the Impact of Biotechnology on Biodiversity: Effect of Transgenic Maize on Non-Target Soil Organisms” we conducted a study at CIAT to determine the effect of chlorpyrifos on soil arthropods in Colombian maize over two consecutive growing cycles (2002-2003). We expect that the results of this study will establish the usefulness of pitfall traps as a technique to monitor soil arthropod populations under tropical conditions and will generate data on the fauna associated with maize in the Cauca Valley of Colombia.

Objectives

General Objective: Determine the impact of soil insecticides on non-target soil arthropods in maize.

Specific Objectives

- Evaluate the effect of chlorpyrifos application to non-target soil arthropods in field plots.
- Generate information on the species richness of soil arthropods associated with maize.
- Quantify and compare the biodiversity of soil arthropods in maize with and without the use of soil insecticides.

Establishment and execution of work plan. Research was conducted at the International Center for Tropical Agriculture (CIAT), located at 3°31' N, 76°21' W, 956 m elevation, mean annual rainfall 1000 mm, mean temperature 24° C, and Holdridge life zone classification Dry Tropical Forest.

The experimental area consisted of eight experimental plots each with an area of 1849 m² (43 x 43 m) and evaluated over two consecutive cycles of maize (second semester 2002 and first semester 2003). In the semester previous to the start of the experiment, the plots were planted to *Crotalaria juncea* that was incorporated as a green manure. Planting date was 30 September 2002 and the plant material was the commercial hybrid “Master” from Syngenta. Plants were spaced 0.2 m apart in rows 0.75 m apart for a density of 12,326 plants/plot. At planting the graminicide “Dual” was applied at 1.5 l/ha.

Two treatments with four replicates were evaluated: maize with and without soil insecticides. Once treatments were assigned to field plots, chlorpyrifos (Lorsban 2.5%, 25 g AI/kg, product of Dow AgroSciences) was applied to the corresponding plots. No other pesticides were used and any weed control was done by hand.

Permanent pitfall traps were put out once germination reached 50%. Eight traps were used for each plot, one placed randomly along rows 5, 10, 15, 20, 25, 30, 35. Pitfalls were evaluated every week from germination to harvest except when rainfall interrupted sampling. The pitfall

traps had three components. The fixed part of the trap was a disposable 12 oz plastic cup with mouth diameter 7.5 cm; this was placed in each of the corresponding rows, dug into the soil so the top rim was even with the soil surface (**Figure 1A**). The removable part of the trap was a 4 oz disposable plastic cup with mouth diameter 6.5 cm; this part of the trap was put in for 24 hours and then lidded and brought back to the lab for evaluation (**Figure 1B**). When the traps were not being used for collecting samples they were covered with the lid of a petri dish to prevent arthropods from falling in (**Figure 1C**).

Field samples were brought to the laboratory for their processing on the same day. Larger arthropods were picked out by hand. To recover the microarthropods, the samples were processed in a small funnel lined with a very fine mesh. The field sample was washed into the funnel with water. By capping the end of the funnel, the sample was floated, and the supernatant removed after discarding the larger debris. Then the remaining precipitate was floated again, this time in 35% salt solution and the supernatant removed. Both supernatant samples were then combined and stored in 70% ethyl alcohol until analysis and identification (**Figure 2**).



Figure 1. (A) Fixed component and removable component and (B) lid of the pitfall traps in the field.

The samples were counted and identified under a dissecting scope and with appropriate taxonomic keys. Specimens that could not be identified to family or order were labeled and stored for shipping to Cornell for identification by specialists.



Figure 2. Cleaning and storage of samples in the laboratory.

Analysis of information: The experiment was set up as a completely randomized design. Differences in the abundance of organisms between treatments were tested with an ANOVA.

For the more abundant groups, the area under the abundance curve (accumulated insect-days) was calculated to determine differences between treatments in insect load. To compare arthropod diversity between treatments, we used taxonomic data on the level of order to calculate three indices of diversity (Shannon, Margalef and Simpson), a dominance index (Simpson), and an equitability index.

Results

Arthropod Taxonomic Composition: Over both cycles of evaluation (2002B –2003A), 11,850 specimens were captured representing 16 orders and 5 classes of arthropods (**Tables 1, 2**). Of these, 98.9% were identified to order and 64.8% to family. Class Collembola was the most represented, with 46.0% of all individuals evaluated. Class Chilopoda was the least abundant, with 0.3% (**Table 1**). Of total individuals captured, 58.7% corresponded to the insecticide treatment and 41.3% to the control. The majority of specimens (71.4%) were captured during the first cycle (2002).

Table 1. Number of individuals and composition of arthropod classes caught in pitfall traps in maize, 2002B and 2003A, with and without insecticide.

Class	2002B	%	2003A	%	Total
Arachnida	2,000	23.6	1,214	35.8	3,214
Chilopoda	17	0.2	13	0.4	30
Collembola	4,737	56.0	707	20.9	5,444
Diplopoda	32	0.4	164	4.8	196
Insecta	1,677	19.8	1,289	38.1	2,966
Sum	8,463	100	3,387	100	11,850

Table 2. Number of individuals and composition of arthropod orders caught in pitfall traps in maize, 2002B and 2003A, with and without insecticides.

Order	With insecticide		Without insecticide		Sum with and without	
	Total	%	Total	%	Total	%
Acarina	1,411	20.3	922	18.9	2,333	19.7
Araneae	567	8.1	314	6.4	881	7.4
Blattaria	3	0.0	1	0.0	4	0.0
Chilopoda ¹	12	0.2	18	0.4	30	0.3
Coleoptera	446	6.4	307	6.3	753	6.4
Dermaptera	12	0.2	5	0.1	17	0.1
Diplopoda ¹	102	1.5	94	1.9	196	1.7
Diptera	113	1.6	105	2.1	218	1.8
Entomobryomorpha	394	5.7	404	8.3	798	6.7
Hemiptera	121	1.7	108	2.2	229	1.9
Homoptera	39	0.6	53	1.1	92	0.8
Hymenoptera	536	7.7	733	15.0	1,269	10.7
Lepidoptera	64	0.9	78	1.6	142	1.2
Neuroptera	2	0.0	2	0.0	4	0.0
Others	93	1.3	75	1.5	168	1.4
Orthoptera	17	0.2	23	0.5	40	0.3
Poduromorpha	2,934	42.1	1,548	31.7	4,482	37.8
Symphyleona	89	1.3	75	1.5	164	1.4
Thysanoptera	6	0.1	24	0.5	30	0.3
Total	6,961	100	4,889	100	11,850	100

¹ Taxonomic Class (Including for analysis).

The orders of greatest abundance were Poduromorpha and Acarina with 37.8 and 19.7% of all individuals captured (**Table 2**). Only the orders Acarina and Thysanoptera exhibited a significant difference in abundance between treatments (**Table 3**), while only orders Araneae, Diptera, Diplopoda, Hemiptera and Thysanoptera exhibited a significant difference in abundance between cycles. The orders Acarina and Collembola had a significant difference between treatments in area under the curve during both cycles (**Figures 3, 4**). The greatest abundance of Collembola was in the insecticide treatment with 59.2% more individuals than the control. Of all Collembola collected, 82.3% of individuals were from the family Poduromorpha (**Table 5**).

Of the 753 Coleoptera captured, 59.2% were from the insecticide treatment and 40.8% from the control. Analysis of the area under the curve showed statistically higher accumulated area for the control treatment during first growing cycle (**Figure 5**). The Carabidae and Cicindellidae were the most represented families, comprising 46.6 and 6.6% of all beetles, respectively (**Table 4**); 47.8% of Carabidae and 6.8% Cicindellidae were captured in the insecticide treatment and control, respectively. The most represented genera of the Carabidae were *Calosoma* (especially *C. granulatum*) with 85.8% of individuals and tribe Galeritini with 7.1%. For the family Cicindellidae all individuals corresponded to the genus *Megacephala* (*Tetracha*).

Of the 229 individuals captured from the order Hemiptera, 52.8% were captured in the insecticide treatment (**Table 6**). The family Pyrrhocoridae was the most represented with 79.5% of total individuals captured, all belonging to the genus *Dysdercus*.

Table 3. Abundance of arthropods (mean ± S.E. number of individuals caught per evaluation date) associated with maize, 2002B and 2003A, with and without insecticide.

Order	Between Treatments		Between Semesters	
	With Insecticide	Without Insecticide	2002B	2003A
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
Dermoptera	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
Entomobryomorpha	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
Lepidoptera	0.52±1.06 a	0.63±1.42 a	0.56±1.42 a	0.58±1.06 a
Neuroptera	0.02±0.13 a	0.02±0.13 a	0.01±0.09 a	0.03±0.16 a
Orthoptera	0.14±0.41 a	0.19±0.50 a	0.13±0.42 a	0.20±0.50 a
Poduromorpha	23.66±112.35 a	12.48±84.12 a	34.77±136.23 a	0.26±1.05 b
Symphyleona	0.72±1.86 a	0.60±1.41 a	1.02±2.13 a	0.28±0.71 b
Thysanoptera	0.05±0.22 b	0.19±0.62 a	0.20±0.60 a	0.04±0.20 b
Unidentified	0.75±1.46 a	0.60±0.97 a	0.81±1.54 a	0.53±0.79 a

¹ Taxonomic Class (Including for analysis).

For each row, means followed by different letters are statistically different at P<0.05 (Tukey-Kramer test for multiple comparisons).

Table 4. Number of individuals and composition of Coleoptera families caught in pitfall traps in maize, 2002B and 2003A, with and without insecticides.

Family	With Insecticide		Without Insecticide		Sum With and Without	
	Total	%	Total	%	Total	%
Bruchidae	1	0.2	--	--	1	0.1
Carabidae	213	47.8	138	45.0	351	46.6
Cicindellidae	29	6.5	21	6.8	50	6.6
Chrysomelidae	6	1.3	3	1.0	9	1.2
Cucujidae	1	0.2	0	0.0	1	0.1
Curculionidae	2	0.4	1	0.3	3	0.4
Elateridae	1	0.2	0	0	1	0.1
Geotrupidae	2	0.4	0	0	2	0.3
Immature	138	30.9	86	28.0	224	29.7
Lycidae	1	0.2	1	0.3	2	0.3
Melolonthidae	0	0	1	0.3	1	0.1
Myxophaga	0	0	2	0.7	2	0.3
Nitidulidae	11	2.5	12	3.9	23	3.1
Scarabaeidae	24	5.4	23	7.5	47	6.2
Scolytidae	0	0.0	1	0.3	1	0.1
Staphylinidae	8	1.8	8	2.6	16	2.1
Unidentified	9	2.0	10	3.3	19	2.5
Sum	446	100.0	307	100.0	753	100.0

Table 5. Number of individuals and composition of Collembola orders caught in pitfall traps in maize, 2002B and 2003B, with and without insecticides.

Order	With Insecticide	%	Without Insecticide	%	Total	%
Entomobryomorpha	394	11.5	404	19.9	798	14.7
Poduromorpha	2,934	85.9	1,548	76.4	4,482	82.3
Eysymphyleona	89	2.6	75	3.7	164	3.0
Sum	3,417	100	2,027	100	5,444	100

Table 6. Number of individuals and composition of Hemiptera families caught in pitfall traps in maize, 2002B and 2003A, with and without insecticides.

Family	With Insecticide	%	Without Insecticide	%	Total	%
Gelastocoridae	1	0.8	1	0.9	2	0.9
Immature	97	80.2	85	78.7	182	79.5
Lygaeidae	5	4.1	2	1.9	7	3.1
Pentatomidae	4	3.3	4	3.7	8	3.5
Pyrrhocoridae	4	3.3	1	0.9	5	2.2
Reduviidae	0	0	1	0.9	1	0.4
Tingidae	9	7.4	9	8.3	18	7.9
Unidentified	1	0.8	5	4.6	6	2.6
Sum	121	100.0	108	100.0	229	100.0

The order Hymenoptera represented 10.7% of total individuals captured, with 91.8% representing the family Formicidae where 58.4% were captured in the control treatment. Analysis of the area under the curve showed significant differences in accumulated area in favor of the control during first cycle. During the second cycle the area under the curve showed significant differences in favor of the treatment with insecticide.

Arthropod Taxonomic Diversity: Taxonomic richness, measured at the level of order, was highest in the control maize without insecticide (**Table 7**). The index of species richness was not significantly different between treatments or between semesters. Some of the other measures, however, did exhibit differences between semesters. In terms of species similarity, the Jaccard Index for differences between treatments was 0.93 for 2002 and 1.00 for 2003. Comparing semesters, this index was 0.88 for the insecticide treatment and 0.94 for the control treatment.

Conclusions

- The protocols used to determine differences in abundance of soil active and surface active arthropods were adequate to provide the information needed for statistical analyses.
- Differences in abundance between semesters were greater than the differences between the two treatments.
- Contrary to expected results, the insecticide treatment had a higher overall arthropod abundance than the control.
- Analysis of the area under the population curve showed statistically significant differences between treatments. Only the orders Coleoptera and Hymenoptera exhibited differences between semesters.
- Indices of species diversity and dominance exhibited differences between semesters but not between treatments.
- There was 100% overlap of arthropod orders between treatments for semester 2003A

Table 7. Indices of arthropod taxonomic (ordinal level) diversity, dominance and equity in maize, 2002B and 2003A, with and without insecticides.

Index	Between Treatments		Between Semesters	
	With Insecticide	Without Insecticide	2002B	2003A
Wealth (S)	13.00 a	13.25 a	12.88 a	13.38 a
Shannon diversity index	1.61 a	1.79 a	1.48 b	1.92 a
Margalef diversity index	1.85 a	1.98 a	1.76 a	2.06 a
Simpson diversity index	0.70 a	0.76 a	0.66 b	0.801 a
Simpson dominance index	0.30 a	0.23 a	0.34 a	0.20 b
Equity index	0.63 a	0.69 a	0.58 b	0.74 a

For each row, means followed by different letters are statistically different at $P < 0.05$ (Tukey-Kramer test for multiple comparisons).

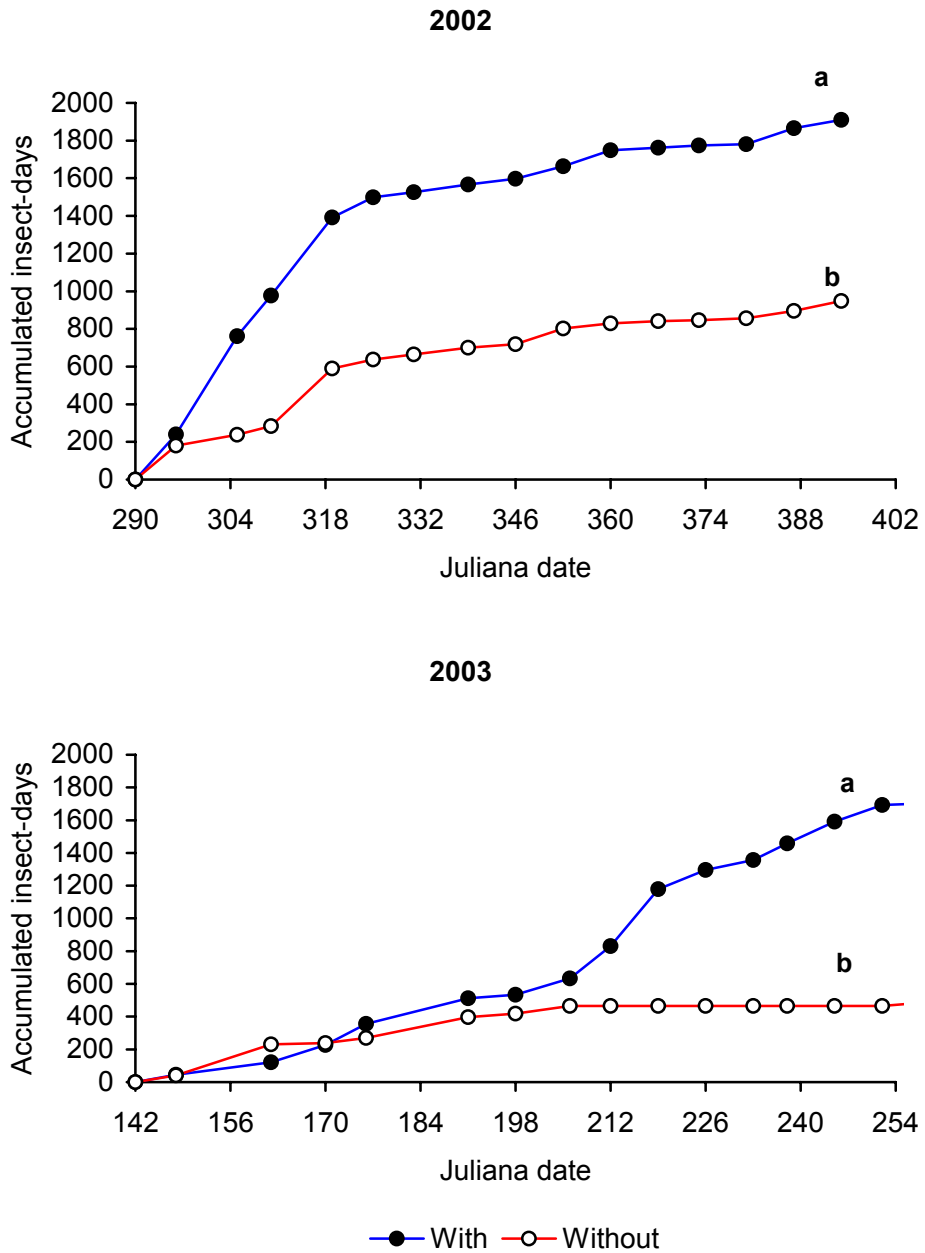


Figure 3. Area under the abundance curve for Acarina in maize, 2002 and 2003, with and without insecticides.

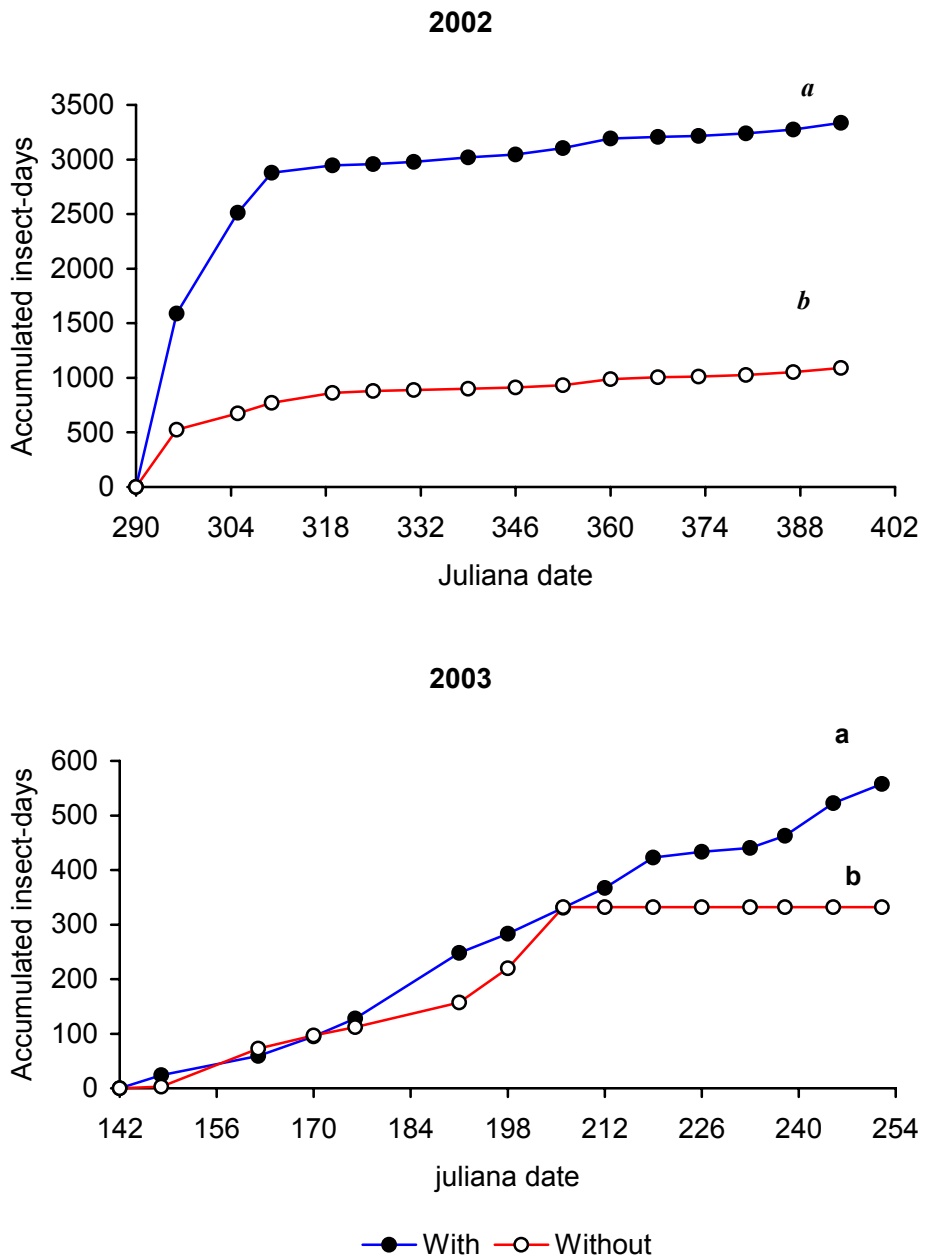


Figure 4. Area under the abundance curve for *Collembola* in maize, 2002B and 2003A, with and without insecticides.

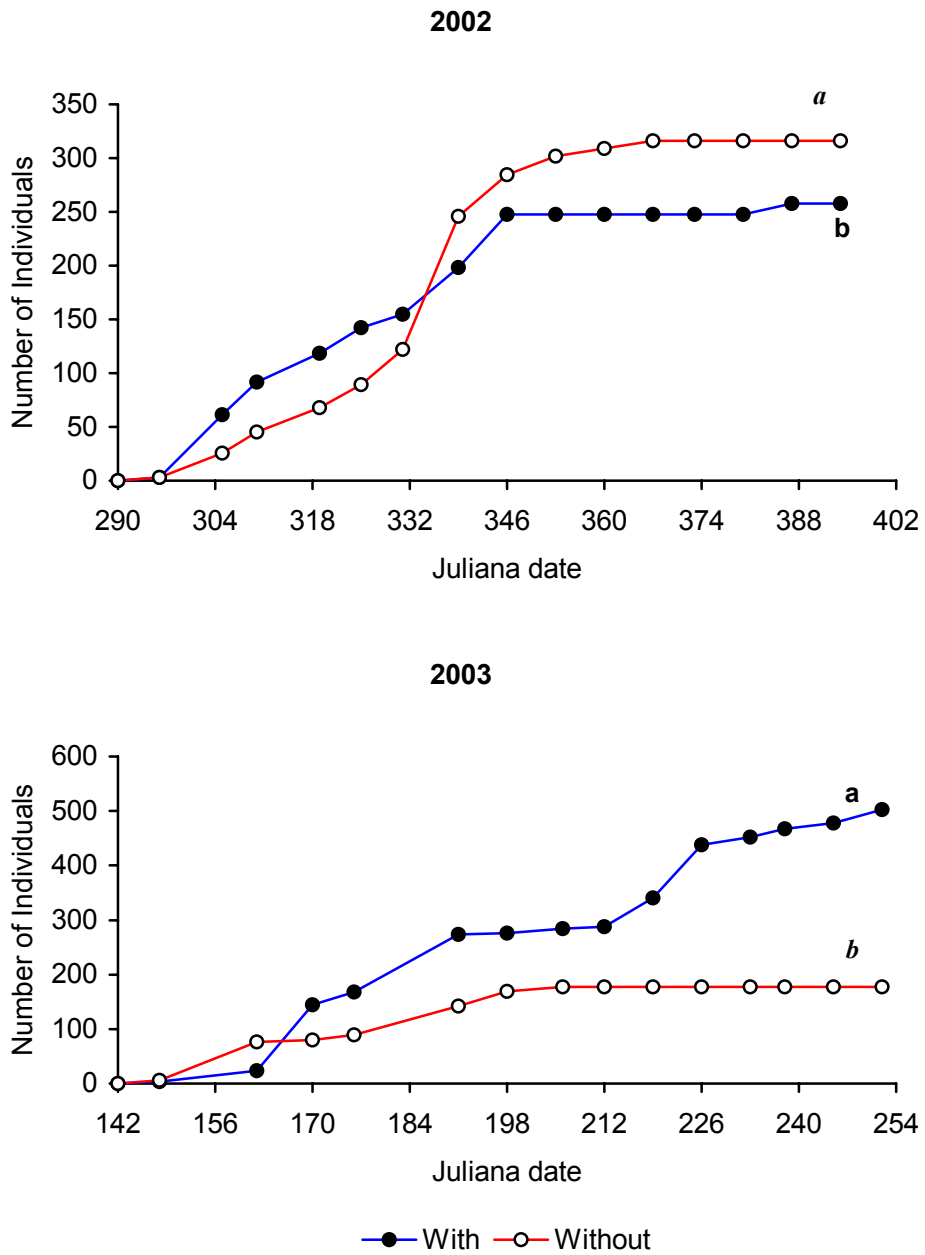


Figure 5. Area under the abundance curve for Coleoptera in maize, 2002B and 2003A, with and without insecticides.

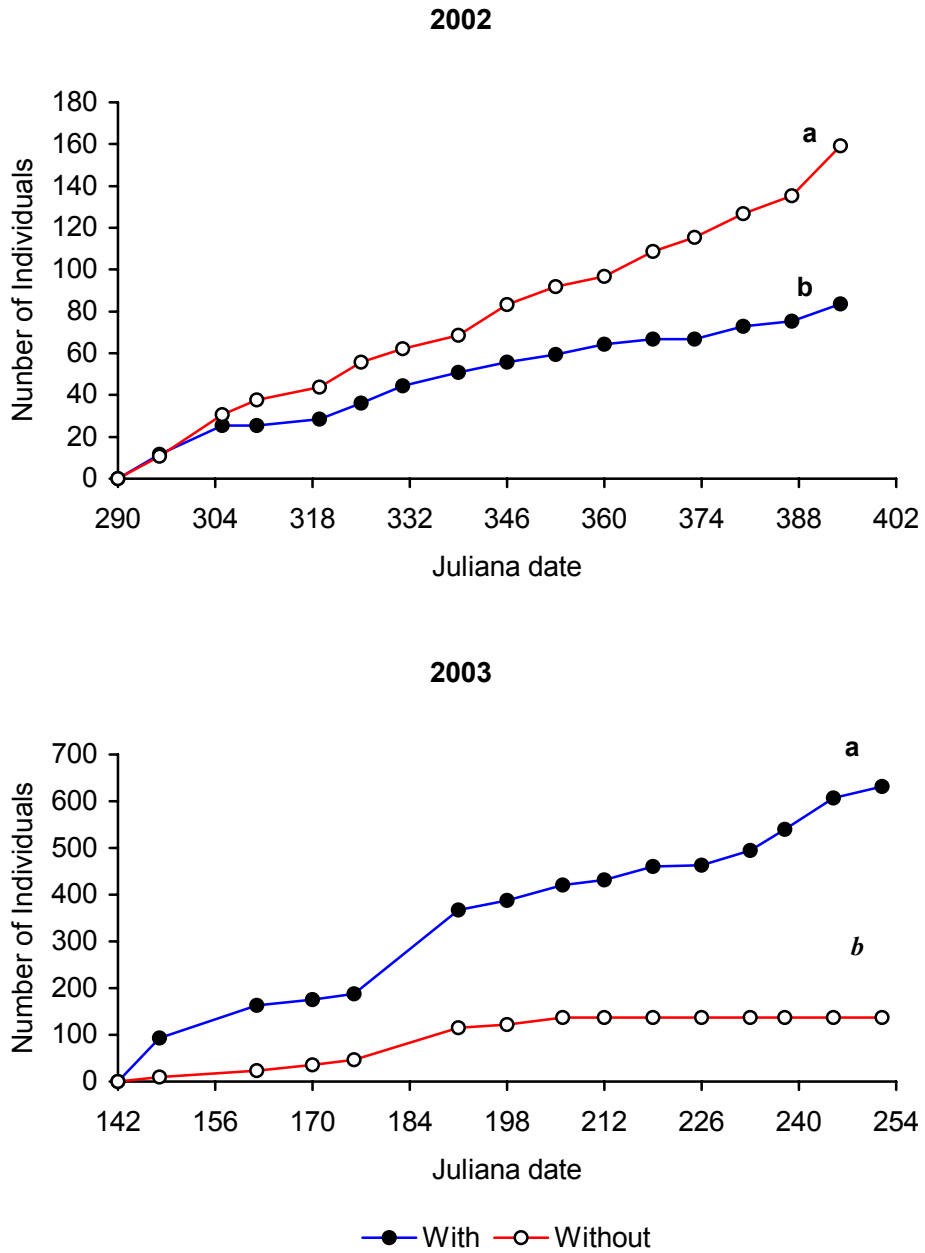


Figure 6. Area under the abundance curve for Hymenoptera in maize, 2002B and 2003A, with and without insecticides.

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Activity 2. Effect of transgenic cotton [Bollgard® Bt Cry1A(c)] on Non-Target Soil Arthropods in the Cauca Valley of Colombia.

Despite the controversy over the use of genetically-modified organisms (GMOs), the number of countries with GM commercial crops has grown from one in 1992 to 13 in 1999 (Shelton *et al.* 2002). From 1996 to 2000, the global area under GMOs increased 42.5 million ha (James 2004). The countries that reported the greatest increases were USA, Canada and Argentina, with 98% of the total area (Shelton *et al.* 2002). During 2003/04 67.7 million ha of GMOs were planted worldwide, of which soybean, maize, cotton and colza occupied 99% of the total area. Soybean plantings made up 61%, followed by maize and cotton with 23 and 11%, respectively (ICAC Recorder 2004).

For 2003/04, the area planted to GM cotton was 6.8 million ha, representing 21% of the total area planted to cotton worldwide. The countries that currently plant GM cotton are Argentina, Australia, China, Colombia, India, Indonesia, Mexico, South Africa and USA. The proportion of cotton that was GMO in 2003/04 was 77% for USA and 58% for China with an increase of 7% with respect to the previous harvest (ICAC Recorder 2004).

The Ministry of Agriculture and Rural Development, through the Colombian Agricultural Institute (ICA), designed a scheme to determine the viability of incorporating GMOs into the agricultural production system. ICA therefore passed Resolution 03492 in 1998 to establish and regulate the process of introduction, production, liberation and commercialization of GMOs. In two other provisions (Agreements 013/98 and 0002/02) ICA created the National Technical Council of Agricultural Biosecurity (CTN) to function in the assessment and support of GMO technology.

Since the establishment of those regulations, applications have been submitted for *Brachiaria*, carnations, cassava, coffee, cotton (resistance to lepidopterans), 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 (Díaz 2003).

For the period 1991-2002, Colombia experienced a reduction of 83% in the area planted to cotton. The 2001/02 harvest only included 39,000 ha in the two cotton-growing regions of Tolima-Valle and Costa-Meta (ICA 2001). One aspect that has greatly influenced the loss of area planted to cotton in Colombia is the high incidence of pests. The greatest losses are caused by the boll weevil (*Anthonomus grandis*, Coleoptera: Curculionidae) that affects 89% of the growing area in the departments of Córdoba, Cesar and Tolima, causing 15% loss of flower heads. *Heliothis virescens* (Lepidoptera: Noctuidae) affects 100% of the cotton planting area of Colombia, causing damage to 15-20% of the flower heads and bolls. Some 10% of the cultivated area is additionally affected by the foliovore “gusano rosado” (*Sacadoses pyralis*, Lepidoptera: Noctuidae) and whiteflies (Homoptera: Aleyrodidae).

Control of these pests is largely based on extensive use of agrochemicals and these represent 23% of the direct costs of the crop to the Colombian producer. In the Atlantic Coast, there was an average of 26 applications of pesticides per crop cycle, with 69.2% of those directed toward the control of lepidopterans. In the Cauca Valley, the number of applications has been reduced

73% to an average of 7 applications per crop cycle, with 57.1% directed towards the control of lepidopterans.

Given this scenario, ICA and the CTN implemented the first studies to determine the effect of the Bollgard® technology (Monsanto) on populations of arthropods and annelids in the cotton zone of Córdoba department in the Caribbean Region. The Bollgard® technology, generated by Monsanto, has the Cry1Ac insert whose target pests include the following lepidopterans: *Alabama argillacea* (Noctuidae, cotton leafworm), *Heliothis virescens* (Noctuidae, tobacco budworm), *Helicoverpa zea* (Noctuidae, corn earworm), *Pectinophora gossypiella* (Gelechiidae, pink bollworm), *Sacadodes pyralis* (Noctuidae, “el gusano rosado colombiano”), *Spodoptera frugiperda* (Noctuidae, fall armyworm), *Trichoplusia* sp. (Noctuidae, looper) and *Bucullatrix* sp. (Lyonetiidae, cotton leaf perforator)

Based on results obtained during the 2001-2002 growing cycle, ICA authorized the first commercial plantings of cotton with resistance to lepidopterans. The department of Córdoba was the first to commercially plant GM cotton with 6,187 ha planted in the second semester of 2003. During the first semester of 2004, 4,495 ha were planted in Tolima-Huila and 696 ha in the Cauca Valley.

Objectives

General Objective: Determine the impact of Bt transgenic cotton on non-target soil arthropods.

Specific Objective

- Evaluate the short-term effect of Bollgard® technology on non-target soil arthropods in field plots.
- Generate information on the species richness of soil arthropods associated with transgenic and non-transgenic cotton in the Cauca Valley.
- Evaluate the long-term effect of Bollgard® technology on non-target soil arthropods in field plots.
- Compare and contrast the non-target effects of traditional chemical and Bt-transgenic plant protection technologies.

Establishment and execution of the work plan: In collaboration with ICA’s division of Agricultural Regulation and Protection, we initiated field studies for the first cycle of cotton at the ICA research station in Palmira, located at 03°31’N, 76°19’W, 975 m elevation, annual precipitation 1295 mm, mean temperature 24°C, relative humidity 76%, and corresponding to the Holdridge life zone of Dry Tropical Forest.

The evaluations were conducted within the methodology implemented by ICA to evaluate the effect of Bollgard® technology on arthropod populations in the cotton crop in the departments of Tolima, Huila and Valle del Cauca.

The experimental units were plots measuring 225 m² (15 m x 15 m) in a completely randomized block design. Each block had 6 plots for a total of 24 plots under evaluation. Plant material was

(1) Bollgard® technology represented by the variety NuCont 33B that contains the Cry1A(c), and (2) the conventional technology represented by variety DP 5415.

Sampling: Information was gathered from two types of samples: pitfall traps and berlese funnels. Pitfall traps were located between plants within the rows; eight were put out in each experimental plot (**Figure 1**). A total of 192 pitfall traps were deployed, and these were opened to sampling for a 24-hour period each week.

Field samples were brought to the laboratory for their processing on the same day. Larger arthropods were picked out by hand. To recover the microarthropods, the samples were processed in a small funnel lined with a very fine mesh. The field sample was washed into the funnel with water. By capping the end of the funnel, the sample was floated, and the supernatant removed after discarding the larger debris. Then the remaining precipitate was floated again, this time in 35% salt solution and the supernatant removed. Both supernatant samples were then combined and stored in 70% ethyl alcohol until analysis and identification (**Figure 2**).

In addition to the pitfall traps, a cup cutter was used to take soil samples every 2 weeks. The cup cutter had a diameter of 10 cm and the sample was taken to a depth of 10 cm in the row between plants (**Figure 2**). Four samples were taken per plot for a total of 96 samples per evaluation. Samples were placed in berlese funnels for 24 hours after which the samples of separated arthropods were stored in 70% ethyl alcohol until analysis. Because only 48 funnels were available, blocks 1 and 2 were done the first period, followed by blocks 3 and 4 which were maintained at 11°C during the interim 24 hours. Arthropod samples were separated, sorted and processed as in the pitfall trap samples.



Figure 1. (A) Fixed component and removable component and (B) lid of the pitfall traps in the field.

Analysis of information: The statistical model used for the analysis of the data was a completely randomized block design. 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.



Figure 2. Field collection of samples for berlese extraction of arthropods using a “Lever Action Hole Cutter.”

Results

Arthropod Taxonomic Composition

Pitfall traps: During the first cycle (2003), 438,934 specimens were captured, belonging to 20 different taxonomic orders in 8 taxonomic classes. Sixty-five different species were identified, with a difference of only three species between those reported in NuCotn 33B and DP 5415 (**Table 2**). The most abundant class was Collembola with 52.3% of total individuals captured (**Table 1**).

Table 1. Number of individuals and composition of arthropod classes caught in pitfall traps and berlese funnels in cotton, during 2003 in the Cauca Valley, Colombia.

Class	Pitfall trap			Berlese funnels		
	NuCotn 33B	DP 5415*	Total	NuCotn 33B	DP 5415*	Total
Aracnida	37,321	38,677	75,998	24,127	28,218	52,345
Chilopoda	6	2	8	36	44	80
Collembola	138,061	91,364	229,425	4,709	4,638	9,347
Diplopoda	17	29	46	14	42	56
Diplura	0	0	0	48	70	118
Insecta	62,666	70,443	133,109	7,679	8,717	16,396
Malacostraca	112	48	160	46	63	109
Nematoda	25	108	133	12	20	32
Oligochaeta	0	1	1	155	215	370
Protura	0	0	0	3	4	7
Symphyla	27	27	54	729	952	1,681
Sum	238,235	200,699	438,934	37,558	42,983	80,541

* 44% of samples evaluated.

Of all individuals captured, 54.3% of those were associated with transgenic cotton and 45.7% with conventional cotton. Of the 20 identified orders, the most abundant were Poduromorpha, Hymenoptera and Acari with 50.4, 29.7 and 17.2%, respectively (**Table 3**). Abundance, in terms of individuals per order, was 1.2 times greater in NuCotn 33B where only Acarina and Hymenoptera were more abundant in DP 5415 (**Table 4**). Only Collembola and Isopoda exhibited differences between the treatments, both being more abundant in NuCotn 33B.

Table 2. Number of individuals and composition of arthropod orders caught in pitfall traps and berlese funnels in cotton, during 2003 in the Cauca Valley, Colombia.

Order	NuCotn 33B		DP 5415	
	Pitfall Traps	Berlese Funnels	Pitfall Traps	Berlese Funnels
Acarina	37,139	24,114	38,497	28,198
araneae	182	13	180	20
Blattaria	27	3	37	9
Chilopoda ¹	6	36	2	44
Coleoptera	257	743	251	768
Dermaptera	3	0	--	1
Diplopoda ¹	17	14	29	42
Diptera	91	224	159	370
Diplura	0	48	0	70
Entomobryomorpha	4,171	829	3,360	928
Hemiptera	61	5	61	7
Homoptera	782	62	878	68
Hymenoptera	61,385	6,311	68,977	7,228
Isopoda	112	46	48	63
Lepidoptera	23	91	31	93
Neuroptera	0	0	2	0
Orthoptera	26	2	38	3
Poduromorpha	133,382	3,880	87,930	3,706
Protura	--	3		4
Symphyla ¹	27	729	27	952
Symphyleona	508	0	74	4
Thysanoptera	9	6	5	2
Unidentified	2	232	4	168
Sum	238,210	37,391	200,590	42,748

¹ Taxonomic Class (Including for analysis).

* 44% of samples evaluated.

Given their overall abundance, the class Collembola was examined in more taxonomic detail. Seven families were identified belonging to three suborders (**Table 5**). Of the springtail orders identified to date, Poduromorpha, Entomobryomorpha and Simphypleona were more abundant in NuCotn 33B.

In the analysis of the area under the population development curve, NuCotn 33B accumulated significantly more area than DP 5415 (**Figure 3**). Among the Hymenoptera, the most abundant family was Formicidae, with 99.9% of total individuals captured. Overall 52.9% of those specimens were captured from DP 5415; DP 5414 also had significantly more accumulated area than NuCotn 33B (**Figure 4**). Although Acarina had 1.36 times more individuals captured in NuCotn 33B, no difference was detected in the area under the curve (**Figure 5**).

Berlese funnels. To date, 44% of the samples collected in the first cycle of cotton (2003) have been evaluated. These number 80,541 specimens representing 11 classes and 21 orders (**Table 1**). 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.

Table 3. Abundance of arthropods (mean \pm S.E. number of individuals caught per evaluation date) associated with cotton, during 2003 in the Cauca Valley, Colombia.

Group	NuCotn 33B	DP5415
Acarina	22.76 \pm 54.60 a	23.59 \pm 50.05 a
Araneae	0.11 \pm 0.33 a	0.11 \pm 0.34 a
Blattaria	0.02 \pm 0.14 a	0.02 \pm 0.16 a
Chilopoda ¹	0.00 \pm 0.06 a	0.00 \pm 0.03 a
Coleoptera	0.16 \pm 0.48 a	0.15 \pm 0.47 a
Dermaptera	0.00 \pm 0.04 a	0.00 \pm 0.00 a
Diplopoda ¹	0.01 \pm 0.10 a	0.02 \pm 0.16 a
Diptera	0.06 \pm 0.33 a	0.10 \pm 1.24 a
Entomobryomorpha	2.56 \pm 10.58 a	2.06 \pm 8.17 a
Hemiptera	0.04 \pm 0.24 a	0.04 \pm 0.22 a
Homoptera	0.48 \pm 1.53 a	0.54 \pm 4.48 a
Hymenoptera	37.61 \pm 79.69 a	42.27 \pm 82.57 a
Isopoda	0.07 \pm 0.50 a	0.03 \pm 0.19 b
Lepidoptera	0.01 \pm 0.18 a	0.02 \pm 0.15 a
Neuroptera	0	0.00 \pm 0.05 a
Orthoptera	0.02 \pm 0.13 a	0.02 \pm 0.17 a
Poduromorpha	81.73 \pm 436.77 a	53.88 \pm 333.46 b
Symphyla	0.02 \pm 0.15 a	0.02 \pm 0.15 a
Symphyleona	0.31 \pm 3.80 a	0.05 \pm 0.30 b
Unidentified	0.00 \pm 0.03 a	0.00 \pm 0.03 a

¹ Taxonomic Class (Including for analysis).

For each row, means followed by different letters are statistically different at $P < 0.05$ (Tukey-Kramer test for multiple comparisons).

Arthropod Taxonomic Diversity: The species richness and Shannon indices were not significantly different between the treatments NuCotn 33B and DP 5415. The Simpson index showed dominance for one species, presenting values of 0.6 and 0.7 for NuCotn 33B and DP 5415, respectively. The Margalef index was 4.8 for NuCotn 33B and 4.6 for DP 5415. In comparing the similarity (Jaccard, Sorenson and Morisita) between NuCotn 33B and DP 5414 in terms of the identified species, values ranged from 0.8 for Jaccard to 0.97 for Morisita.

Table 4. Collembola families collected from pitfall traps during 2003 in the Cauca Valley, Colombia.

Order	Family	Genus
Poduromorpha	Hypogasturidae	<i>Ceratophysella</i>
	Brachystomellidae	<i>Brachystomella</i> *
	Neanuridae	<i>Arlesia</i>
	Cyphoderidae	<i>Cyphoderus</i> **
Entomobryomorpha	Entomobryidae	<i>Seira, Lepidocyrtus</i>
	Isotomidae	<i>Isotoma, Proisotoma, Folsomides</i>
	Paronellidae	<i>Paronella, Salina</i>
Symphyleona	Dicyrtomidae	<i>Calvatomina</i>

* Only on the soil surface (pitfall traps).

** Only in soil (soil cores).

Table 5. Indices of arthropod taxonomic (ordinal level), diversity, dominance and equity in cotton, during 2003 in the Cauca Valley, Colombia.

Index	NuCotn 33B	DP 5415
Species richness (S)	14.1 a	14.2 a
Shannon diversity index	1.0 a	1.0 a
Margalef diversity index	1.3 a	1.4 a
Simpson diversity index	0.6 a	0.6 a
Simpson dominance index	0.4 a	0.4 a
Equity index	0.4 a	0.4 a

For each row, means followed by different letters are statistically different at $P < 0.05$ (Tukey-Kramer test for multiple comparisons)

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.

Recommendation

It is a recommendation of this research group that this information be used in the best possible way to benefit the general community. We highlight that this information is preliminary and should be interpreted as such, keeping in mind that this information.

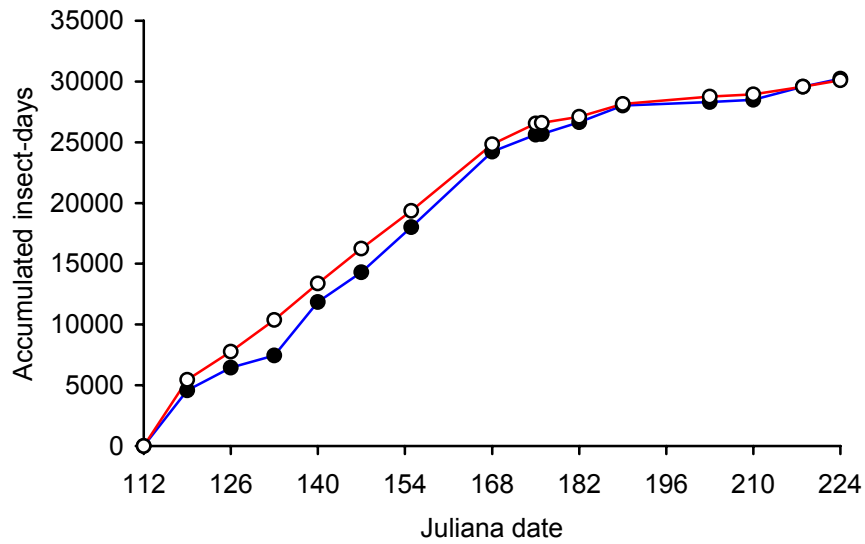


Figure 3. Area under the abundance curve for Acarina in Cotton, 2003 in the Cauca Valley, Colombia.

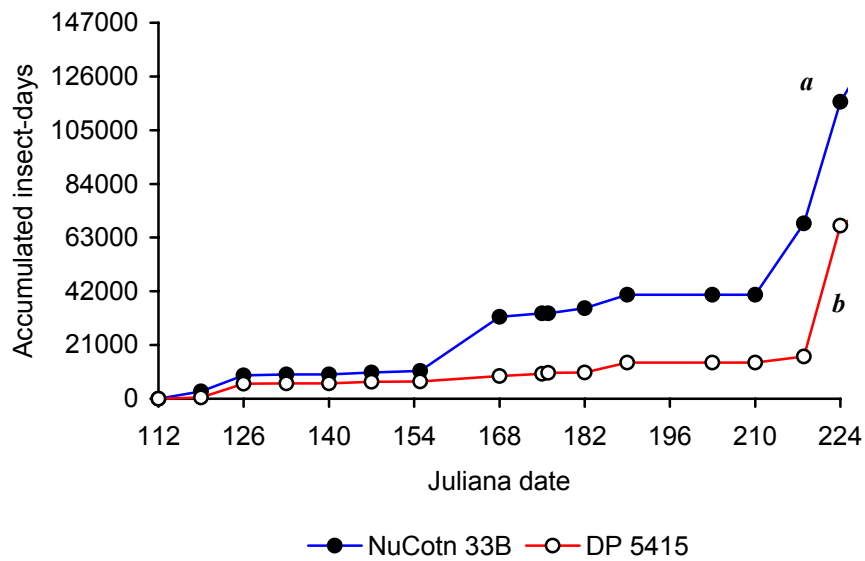


Figure 4. Area under the abundance curve for Collembola in cotton, 2003 in the Cauca Valley, Colombia.

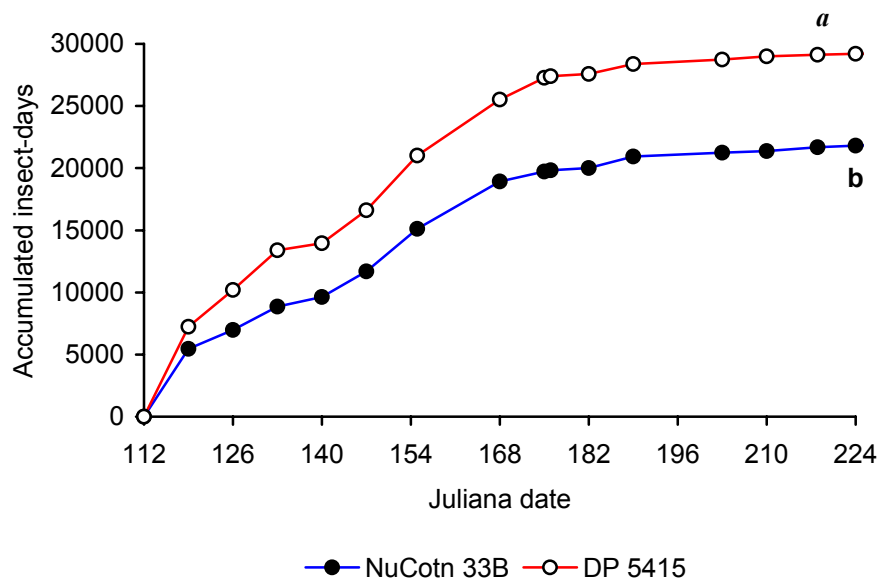


Figure 5. Area under the abundance curve for Hymenoptera in cotton, 2003 and in the Cauca Valley, Colombia.

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