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 /m^2; mite populations can approach 200,000/m^2 and species diversity up to 200/m^2 (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 *Euetheola 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 m^2 (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. The date of 50% germination was 5 October 2002 and the date of harvest (20% moisture) was 15 February 2003. Yield was measured according to protocols of CIMMYT's office in Colombia.

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 on 3 October 2002. 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 and 40. 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 **(Figure1A)**. 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)**.

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

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)**.

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. Counting and identifying specimens 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: During the survey period of the first growing cycle (17 Oct 2002 – 29 Jan 2003) 8465 specimens were captured representing 15 orders and 5 classes of arthropods **(Tables 1, 2)**. Of these, 98.9% were identified to order and 71.9% to family. Class Collembola was the most represented, with 56.0% of all individuals evaluated. Class Chilopoda was the least abundant, with 0.2% **(Table 1)**. Of total individuals captured, 59.8% corresponded to the insecticide treatment and 40.2% to the control.

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

Class	Total	%
Arachnida	2000	23.6
Chilopoda	17	0.2
Collembola	4737	56.0
Diplopoda	32	0.4
Insecta	1679	19.8
Sum	8465	100

The orders of greatest abundance were Collembola and Acarina with 56.0 and 16.1% of all individuals captured **(Table 2)** (note: Collembola was considered at the level of both class and order). Only the order Thysanoptera exhibited a significant difference in abundance between treatments **(Table 3)**, while only orders Acarina and Collembola had a significant difference between treatments in area under the curve **(Figures 3, 4)**. The greatest abundance of Collembola was in the insecticide treatment with 30.0% more individuals than the control. Of all Collembola collected, 94.0% of individuals were from the family Podomorpha **(Table 4)**.

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

	With insecticide		Without insecticide		Sum with and without	
Order	Total	%	Total	%	Total	%
Acarina	773	15.3	590	17.4	1363	16.1
Araneae	443	8.7	194	5.7	637	7.5
Chilopoda	6	0.1	11	0.3	17	0.2
Coleoptera	244	4.8	191	5.6	435	5.1
Collembola	3077	60.8	1660	48.8	4737	56.0
Dermaptera	9	0.2	2	0.1	11	0.1
Diplopoda	15	0.3	17	0.5	32	0.4
Diptera	29	0.6	26	0.8	55	0.6
Hemiptera	95	1.9	88	2.6	183	2.2
Homoptera	16	0.3	30	0.9	46	0.5
Hymenoptera	259	5.1	472	13.9	731	8.6
Lepidoptera	27	0.5	45	1.3	72	0.9
Neuroptera	0	0.0	1	0.0	1	0.0
Others	59	1.2	45	1.3	104	1.2
Orthoptera	9	0.2	7	0.2	16	0.2
Thysanoptera	4	0.1	21	0.6	25	0.3
Total	5065	100	3400	100	8465	100

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

Order	With insecticide	Without insecticide
Acarina	12.08±18.0 a	9.22±10.6 a
Araneae	6.92±39.2 a	3.03±12.0 a
Chilopoda	0.09±0.3 a	0.17±0.7 a
Coleoptera	3.81±7.0 a	2.98±4.9 a
Collembola	48.08±153.1 a	25.94±116.0 a
Dermaptera	0.14±0.6 a	0.03±0.2 a
Diplopoda	0.23±0.8 a	0.27±0.6 a
Diptera	0.45±1.0 a	0.41±0.9 a
Hemiptera	1.48±3.2 a	1.38±2.3 a
Homoptera	0.25±0.6 a	0.47±1.1 a
Hymenoptera	4.05±9.9 a	7.38±20.1 a
Lepidoptera	0.42±1.0 a	0.70±1.7 a
Neuroptera	0.00±0.0 a	0.02±0.1 a
Orthoptera	0.14±0.5 a	0.11±0.4 a
Thysanoptera	0.06±0.2 b	0.33±0.8 a

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 Collembola families caught in pitfall traps in second semester maize, 2002, with and without insecticides.

Family	With insecticide	%	Without insecticide	%	Total	%
Entomobryidae	85	2.8	71	4.3	156	3.3
Podomorpha	2922	94.9	1530	92.2	4452	94.0
Sminthuridae	71	2.3	59	3.6	130	2.7
Total	3078	100	1660	100	4738	100

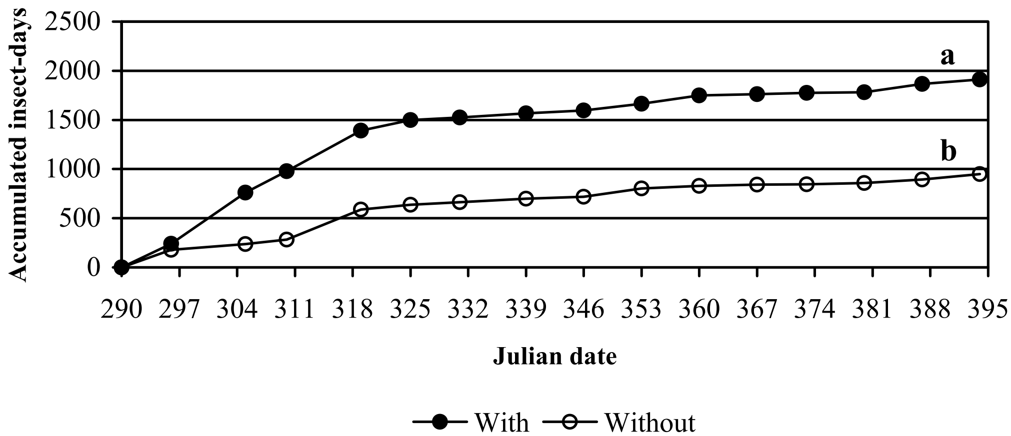

Figure 3. Area under the abundance curve for Acarina in second semester maize, 2002, with and without insecticides.

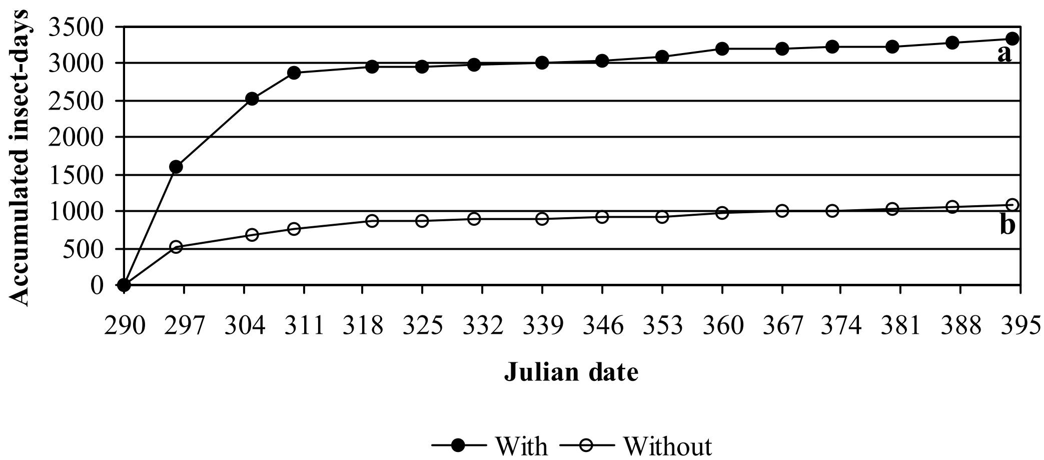

Figure 4. Area under the abundance curve for Collembola in second semester maize, 2002, with and without insecticides.

Of the 321 Coleoptera captured, 56.4% were from the insecticide treatment and 46.3% from the control. Analysis of the area under the curve showed statistically higher accumulated area for the control treatment **(Figure 5)**. The Carabidae and Cicindellidae were the most represented families, comprising 68.8 and 12.5% of all beetles, respectively **(Table 5)**; 69.1% of Carabidae and 13.3% Cicindellidae were captured in the insecticide treatment. The most represented genera of the Carabidae were *Calosoma* (especially *C. granulatum*) with 84.2% of individuals and tribe Galeritini with 9.0%. For the family Cicindellidae all individuals corresponded to the genus *Megacephala* (*Tetracha*).

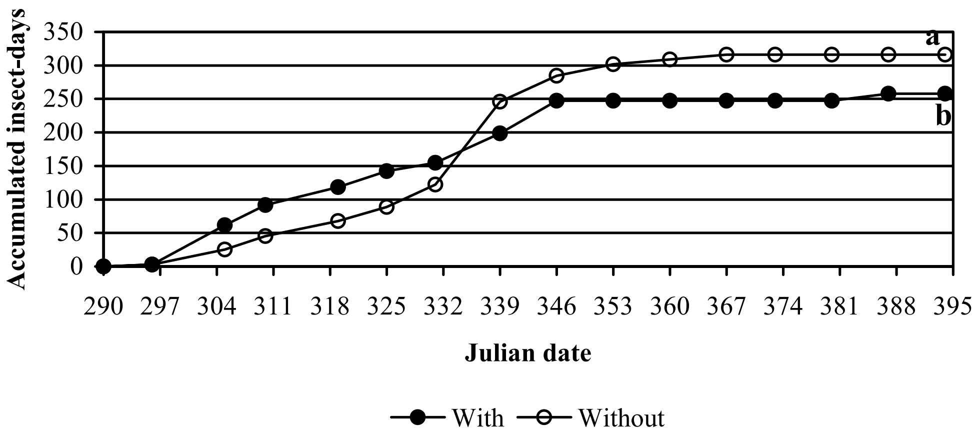

Figure 5. Area under the abundance curve for Coleoptera in second semester maize, 2002, with and without insecticides.

Table 5. Number of individuals and composition of Coleoptera families caught in pitfall traps in second semester maize, 2002, with and without insecticides.

	With insecticide		Without insecticide		Sum with and without	
Family	**Total**	**%**	**Total**	**%**	**Total**	**%**
Carabidae	125	69.1	96	68.6	221	68.8
Cicindellidae	24	13.3	16	11.4	40	12.5
Chrysomelidae	1	0.6	0	0.0	1	0.3
Cucujidae	1	0.6	0	0.0	1	0.3
Geotrupidae	2	1.1	0	0.0	2	0.6
Lycidae	1	0.6	1	0.7	2	0.6
Myxophaga	0	0.0	1	0.7	1	0.3
Nitidulidae	3	1.7	6	4.3	9	2.8
Scarabaeidae	22	12.2	16	11.4	38	11.8
Scolytidae	0	0.0	1	0.7	1	0.3
Staphylinidae	2	1.1	3	2.1	5	1.6
Unidentified	3	1.7	6	4.3	9	2.8
Total	181	100	140	100	321	100

Of the 183 individuals captured from the order Hemiptera, 51.9% were captured in the insecticide treatment **(Table 6)**. The family Pyrrhocoridae was the most represented with 95.6% of total individuals captured, all belonging to the genus *Dysdercus*.

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

Family	With insecticide	%	Without insecticide	%	Total	%
Lygaeidae	1	1.0	1	1.1	2	1.1
Pyrrhocoridae	92	97.0	83	94.3	175	95.6
Reduviidae	1	1.0	2	2.3	3	1.6
Tingidae	1	1.0	2	2.3	3	1.6
Total	95	100	88	100	183	100

The order Hymenoptera represented 8.6% of total individuals captured, with 94.5% representing the family Formicidae where 66.0% were captured in the control treatment. Analysis of the area under the curve showed significant differences in accumulated area in favor of the control **(Figure 6)**.

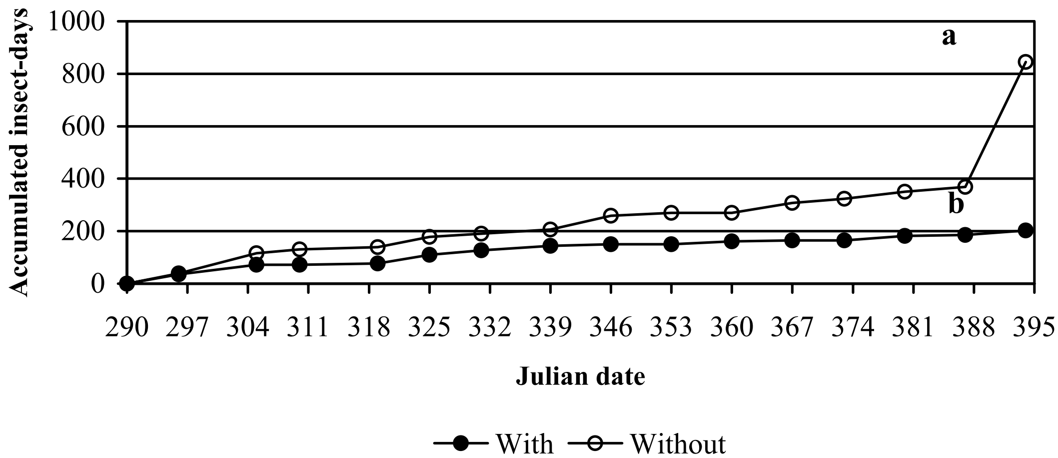

Figure 6. **Area under the abundance curve for Hymenoptera in second semester maize, 2002, with and without insecticides.**

Arthropod Taxonomic Diversity: Taxonomic richness, measured at the level of order, was highest in the control maize without insecticide **(Table 7)**. The Shannon, Margalef and Simpson diversity indices were all lower in the insecticide treatment versus the control. The dominance index was 1.3 times higher in the insecticide treatment; while the equity index was 1.2 times higher in the control treatment.

Table 7. **Indices of arthropod taxonomic (ordinal level) diversity, dominance and equity in second semester maize, 2002, with and without insecticides.**

Index	With insecticide	Without insecticide
Shannon diversity index	1.30	1.58
Margalef diversity index	1.53	1.72
Simpson diversity index	0.59	0.70
Simpson dominance index	0.41	0.30
Equity index	0.50	0.59

Summary of Results

- Over the 16 evaluation dates conducted in the first growing cycle, 19.8% of individuals captured in pitfall traps belonged to the class Insecta.
- For the control and insecticide treatments, 60.8 and 48.8% of the total individuals belonged to the order Collembola, followed in abundance by the orders Acarina, Hymenoptera, Araneae and Coleoptera.
- The most abundant family of the Collembola were the Podomorpha, with 65.6% of podomorpha caught in the insecticide treatment, and 34.4% in the control.

- The most abundant species of the Coleoptera was *Calosoma granulatum*, with 58.1 and 41.9% caught in the insecticide and control treatments, respectively.
- The most abundant family of the Hymenoptera was the Formicidae, with 66.0% of ants caught in the insecticide treatment.
- Abundance differences between treatments was only detected for one (Thysanoptera) of 15 orders the first growing cycle.
- For the area under the curve, Acarina and Collembola were greater in the insecticide treatment, while Coleoptera and Hymenoptera were greater in the control treatment
- Compared to the control treatment, the insecticide treatment had a lower diversity index, higher dominance index, and lower equity index when taxonomic diversity was considered at the level of order

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