

## CROP PROTECTION

The Effect of the Glyphosate, 2,4-D, Atrazine e Nicosulfuron Herbicides upon the Edaphic Collembola (Arthropoda: Ellipura) in a No Tillage System<sup>1</sup>VILMA S. LINS<sup>2</sup>, HONÓRIO R. SANTOS<sup>3</sup> AND MANOEL C. GONÇALVES<sup>3</sup>

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## Impacto dos Herbicidas Glifosate, 2,4-D, Atrazina e Nicosulfuron sobre as Populações de Collembola (Arthropoda: Ellipura) Edáficos em Sistema de Plantio Direto

RESUMO - No plantio direto o uso de herbicidas é uma prática comum e intensiva, que influencia direta ou indiretamente a população de artrópodes da mesofauna edáfica. O grau de abundância e diversidade de Collembola comumente é indicado para comprovar a extensão de distúrbios de várias práticas agrícolas, pois esse grupo serve como bioindicador das condições do solo. Esta pesquisa teve como objetivo comparar a influência de alguns herbicidas na flutuação populacional de Collembola, em solo sob o sistema de plantio direto. O trabalho foi realizado em uma área de plantio direto de sequeiro, do Núcleo Experimental de Ciências Agrárias da Universidade Federal de Mato Grosso do Sul (UFMS), Campus de Dourados, em latossolo roxo distroférico com cobertura de milho, durante os meses de outubro de 2002 a janeiro de 2003. Os dados obtidos foram analisados segundo o modelo inteiramente casualizado constituído de tratamentos dispostos no esquema de parcelas subdivididas, onde as parcelas são representadas por uma testemunha mais quatro herbicidas: glifosate, atrazina, 2,4-D e nicosulfuron (totalizando cinco tratamentos) e as subparcelas pelas épocas de coleta (10, 20, 30 e 40 dias após aplicação dos herbicidas). Tanto os herbicidas testados quanto as épocas de coleta influenciaram a população de Collembola. Dependente do período de degradação dos herbicidas no solo, os tratamentos com 2,4-D, Atrazina, foram os que mais influenciaram a abundância de Collembola reduzindo sua população.

PALAVRAS-CHAVE: Fauna edáfica, efeito de agrotóxicos, flutuação populacional, impacto no solo

ABSTRACT - The use of herbicides is a common and intensive practice in no tillage systems. The herbicides can influence, directly or indirectly, the population of edaphic arthropods. Collembola is a group that functions as a bio-indicator of soil conditions. The degree of abundance and diversity of Collembola provides the level of soil disturbance provoked by agricultural practices. This experiment was designed to compare the influence of herbicides on the population fluctuation of Collembola in a no-till soil preparation system. The work was conducted in a non irrigated no-till area at the Núcleo Experimental de Ciências Agrárias of the Universidade Federal de Mato Grosso do Sul (UFMS), Campus de Dourados, in soil planted with corn as a surface covering, during the period of December, 2002 to December, 2003. The data were analyzed according to a completely randomized model, in a split plot design. The plots received four types of herbicides: glyphosate, atrazine, 2,4-D and nicosulfuron. A fifth plot did not receive any herbicide (control), for a total of five treatment types. The sub plots were represented by their collection times (10, 20, 30 and 40 days after the herbicide applications). Both the type of herbicide and the time of data sampling influenced the Collembola population fluctuation. The treatments with atrazine and 2,4-D caused the most reduction of the population of Collembola, depending on the time of application.

KEY WORDS: Edaphic fauna, pesticide effect, population fluctuation, soil impact

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The edaphic mesofauna is composed mainly of Collembola, Acari and Insecta. The most numerous are the acarids Oribatei (Cryptostigmata) and Collembola, which, together, constitute about 72% to 97% of the individuals of the total fauna of Arthropoda of the soil (Singh & Pillai 1975).

According to Bruno *et al.* (1995) and Bzuneck & Santos (1991), the edaphic mesofauna has important roles. Such as a catalyzer in the microbial activity for the organic matter decomposition, soil moistening process, mechanical disaggregation of the decomposing vegetable material, and formation and maintenance of the structure of the soil (Sautter *et al.* 1996). Besides interacting in the biological, biochemical and physical processes, these organisms are also responsible for the replacement of several nutrients in the alimentary food chain (Vieira & Santos 2001).

This group can only be analyzed when comparing similar factors such as soil structure, humidity, texture, amount of organic matter, vegetable covering and, in the case of agricultural environments, the use of pesticides (Bzuneck & Santos 1991). Experimental studies showed that *Cyphoderus* sp. (Cyphoderidae) could be used as a potential bio-indicator of insecticide impact on the soil (Joy & Chakravorty 1991).

Undisturbed ecosystems possess higher bio-diversity of macro- and mesofauna in relation to soil submitted to monocultural practices. Begon *et al.* (1999) points out that species richness varies according to factors such as productivity, spatial heterogeneity, kind of habitat, stage of succession and frequency of disturbance. A larger edaphic species diversity is found in natural ecosystems, (forests, for example), than in systems under intensive cultivation (Vieira & Santos 2001).

Productive demand, in which more areas would be devastated for planting, requires agronomic practices leading to the conservation of the edaphic mesofauna. Today there is an increase in knowledge of the importance of edaphic arthropods with a view to improving soil quality and productivity. In accordance with Vieira & Santos (2001), no tillage systems are more suitable in comparison with those of conventional tillage, due to the greater preservation of the edaphic organisms.

In no tillage systems, the vegetable residues on the surface of the soil are retained, imitating natural ecosystems, in which the temperature and humidity are moderate, making them more favorable to the edaphic fauna and flora (Perdue & Crossley 1989).

In spite of the no tillage systems being indicated as an economically viable method, with the purpose of reducing impact to the soil, fauna, flora and human health, this system was only adopted with the importation of herbicides into Brazil. However, as more no tillage systems are implemented, the use of these products is likely to increase. When these compounds are applied to the soil, the edaphic organisms that are able to use them increase their populations until the decomposition of the products is complete. After that, when there is a reduction in demand of this food source, they return to normal (Almeida & Rodrigues 1988).

Fratello *et al.* (1985), mentions that atrazine, in the doses

of 5 kg/ha and 8 kg/ha, still presented residue in the superficial layer of the soil after five months of application. Both doses induced reduction of the population of Collembola to the depth of 10 cm.

Bitzer *et al.* (2002) affirms that more information is necessary about population dynamics of springtails and the action of pesticides employed in agroecosystems upon these beneficial arthropods. In order to discover the extent of disturbance by these various agricultural practices, the knowledge about the abundance and bio-diversity of arthropods is essential. Therefore, the aim of this research is to compare the influence of some herbicides on the Collembola populational fluctuation in soil under a system of no tillage and no irrigation.

## Material and Methods

This work was developed in the Experimental Nucleus of Agrarian Sciences (NCA) at the Universidade Federal do Mato Grosso do Sul (UFMS), from December, 2002 to December, 2003, in the municipality of Dourados, MS (22° 14,5' S and 54° 49' W and mean altitude of 452m ) (SEPLAN-MS 1990).

**Evaluated area.** The soil of the researched area is classified as Typic Dystropheric Red Latosol, of loamy texture and topography glides (EMBRAPA 1999), whose chemical and physical characteristics were evaluated by the laboratory of soils of NCA-UFMS (Table 1).

The experiment was conducted in soil whose history presents a covered area initially used for pasture, planted with *Brachiaria decumbens*, Stapf, then cultivated with traditional preparation (tillage planting). Beginning with 1994, the area was worked as an irrigated no-till system, and four years later, a non-irrigated no tillage system was used. From that date on, the area was managed using weed control and soil handling for the cultivation of a sequence of crops. From 1998 to 2000, the rotation was corn (summer) and soy (autumn/winter), in 2001 it was soy (summer) and pea (autumn/winter), and for the year of 2002/2003 forage turnip (*Raphanus sativus* L.) was cultivated, (autumn/winter) followed by corn (summer). When this experiment was begun, the surveyed area had been sown with corn, DKB 350 cultivar that was at 21 days after emergence. The previous crop had been forage turnip.

The climate of this region, according to the classification of Köppen, is characterized as Cwa, with summer rains and hot summers, with extremely varied thermal fluctuation during the year and an annual precipitation average of 1,390 mm (Ayoade, 1986). The precipitation, relative humidity and average temperatures were registered during sampling intervals of the experiment, throughout the whole period of the research (Table 2).

The statistic delineation employed was entirely in agreement with the completely randomized model, which consisted of treatments applied in a pattern of subdivided plots, where the plots were the treatments with herbicides (four herbicides and a control) and the sub plots were the

Table 1. Physical and chemical analysis of soil from the experimental area in each place (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> collection) at Núcleo Experimental de Ciências Agrárias da Universidade Federal de Mato Grosso do Sul, municipality of Dourados, MS (December 2002 to January 2003).

Samplings	pH	Al <sup>+3(2)</sup>	H+Al	Ca <sup>+2(2)</sup>	Mg <sup>+2(2)</sup>	K <sup>+(1)</sup>	P <sup>(1)</sup>	Gross sands	Fine sands	Silt	Clay	O.M. <sup>(3)</sup>
	(CaCl <sub>2</sub> )			(mmolc.dm <sup>-3</sup> )			(mg.dm <sup>-3</sup> )	(%)	(%)	(%)	(%)	(g.dm <sup>-3</sup> )
1 <sup>st</sup>	5.6	0	38	60.86	27.1	11.46	33.6	--	--	--	--	39.22
2 <sup>nd</sup>	5.4	0.48	42.4	59.9	26.6	10.46	30	--	--	--	--	40.98
3 <sup>rd</sup>	5.8	0	40	58.66	25.7	11.96	33.4	--	--	--	--	38.48
4 <sup>th</sup>	5.4	0.84	40	62.1	27.9	9.96	30.2	--	--	--	--	41.42
Average	5.55	0.33	40.1	60.38	26.8	10.96	31.8	3.8	12.71	5.75	77.7	40.03

<sup>1</sup>Extractor Mehlich-1; <sup>2</sup>Extractor KCl (Embrapa 1997); <sup>3</sup>Method of Walkley & Black (Tedesco *et al.* 1985)

gathering times (10, 20, 30 and 40 days after the application of the herbicides). Therefore, three plots (36 x 12 m) were selected. Each plot was divided into four equal subplots (9 x 12 m) with a control (2.5 x 2.5 m) without application of a product, inserted between the plots treated with herbicides (Fig. 1).

The treatments applied at random, were: 1 - glyphosate - 900 ml i.a. /ha, 2 - atrazine - 2.250 ml i.a. /ha, 3 - 2,4-D - 744.4 ml i.a. /ha, 4 - nicosulfuron - 44.8 ml i.a. /ha (Fig. 1). In the control, plastic canvases were used (2.8 x 2.8 m) to avoid contaminating this control area with the employed chemical products. The herbicides were applied with a directed jet applicator, a costal pulverizer (Jacto<sup>®</sup>), with a capacity of 20 liters, provided with a fan jet outlet (Jacto 110-05<sup>®</sup>).

The four sets of samplings were made during the period of December, 2002 to January, 2003. The first set was collected 10 days after the application of the herbicides, the second set after 20 days, the third after 30 days and the fourth was collected after a period of 40 days. Each time, the samples were collected in eight randomly selected units, with a minimum distance of 2 m from the edge, totaling 24 unit samples (three plots x eight unit samples) per treatment with 120 unit samples each collection time.

**Extraction and identification of Collembola.** To obtain the edaphic springtails, soil samplings were taken within each treatment subplot. Rings of galvanized iron of 6 cm of height by 7.3 cm of diameter and 0.13 cm of thickness were used. Each ring containing soil was placed in a plastic

bag to avoid the loss of edaphic organisms. The bags were organized in plastic boxes measuring 50 x 40 cm which were taken to the Laboratório de Entomologia, NCA-UFMS, Dourados, MS.

The rings containing the soil samples were placed inside suitably modified Berlese funnels, presenting 28 cm of length, 7.5 cm of diameter and 5 cm of depth, with a capacity for 250 cm<sup>3</sup> of soil. The funnels were installed on a metal exposure table, measuring 2.20 m of length by 1.80 m of height and width 0.40 m, with a surface containing holes of 8 cm diameter. Here they stayed during a period of seven days. In the inferior part of each funnel, an opaque plastic vial of 8 x 5 cm was added, containing conserving liquid (alcohol 70%, water 28% and formolin 2%). The exposure table was provided with five 25W lamps, whose brightness and heat repelled the edaphic organisms of the samples, which migrated to the deepest layers of the funnels and fell into the vials. The superior part of the exposure table containing the material for study was protected with nylon screen and TNT cloth to avoid the entrance of undesirable organisms that would be attracted to the light.

After screening the Collembola, the clarification process was accomplished using plates "Clin" containing lactic acid, in which the specimens were maintained submerged for a period of from 36h to 72h in an oven (model FANEM<sup>®</sup> 315 SE) at 60°C. After that, the Collembola were individually mounted in temporary slides, for identification of the families. Identification keys by Jordana & Arbea (1989) and by Palace-Vargas (1990) were used. The identity of Collembola

Table 2. Mean temperature (°C), relative humidity of the air (%) mean precipitation (mm) in the periods of each one of the four sampling, in the municipality of Dourados, MS (December, 2002 to January, 2003).

Dates	Temperature (°C)		Relative humidity (%)		Accumulated rainfall (mm)
	max	min	max	min	
06/12/02-15/12/02	32.46	21.06	93.7	46.63	2.95
16/12/02-25/12/02	33.57	21.74	93.4	47.07	5.59
26/12/02-04/01/03	33.99	21.43	91.7	41.08	0.72
05/01/03-15/01/03	31.44	21.92	95.53	55.07	7.83

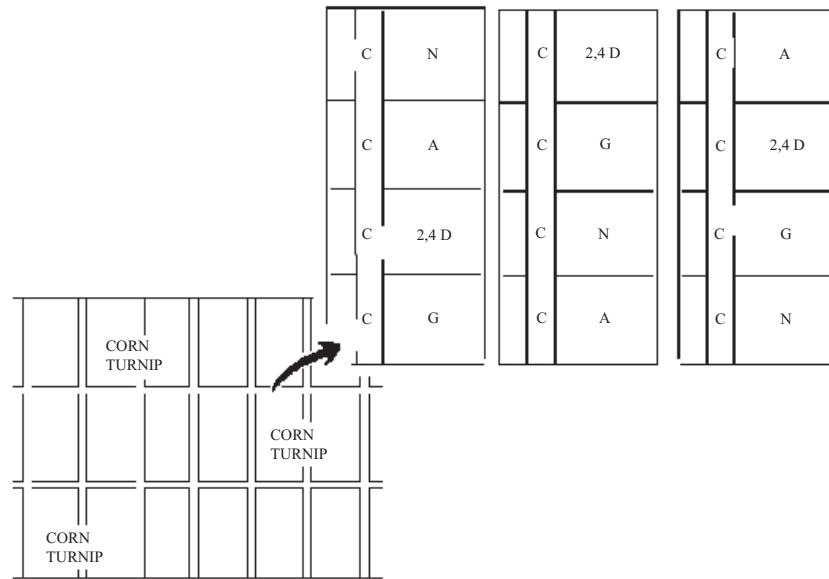


Fig. 1. Schematic of the three plots divided into four subplots, where the five treatments were accomplished (G = glyphosate, A = atrazine, 2,4-D, N = nicosulfuron and C = control). Dourados, MS, 2003.

taxa were confirmed by Dr<sup>a</sup>. Maria Cleide Mendonça of the Departamento de Entomologia, Museu Nacional, Rio de Janeiro (CM/MNRJ), where the voucher specimens were deposited and kept in the collection of Collembola.

The average number of Collembola was calculated by the number of individuals per cm<sup>2</sup>, using the area equation:  $S = 2\pi r$ . After that, the data were extrapolated for m<sup>2</sup>. The obtained data were submitted to the variance analysis (F Test), and to guarantee the normality and the homogeneity, were previously transformed in  $\sqrt{x + 0,5}$  (Gerard & Berthet 1966). The averages were compared by the test of Tukey to 5% of probability (Banzatto & Kronka 1989), also presenting SE (Standard error) compared of the averages.

## Results and Discussion

The total mean number of collected Collembola was 100,921 ind/m<sup>2</sup>, distributed in three orders: Entomobryomorpha, Poduromorpha and Symphypleona. They were represented by the families Entomobryidae, Isotomidae, Cyphoderidae, Neanuridae, Sminthuridae, Bourletiellidae, Onychiuridae, Katiannidae and Sminthurididae. The most abundant organisms belonged to the family Entomobryidae, with about 87.3% of the total mean. The families Isotomidae, Cyphoderidae and Neanuridae added 9.41, 1.47 and 0.86% of the specimens, respectively. The other families represented, altogether, a total of 0.95% (Fig. 2).

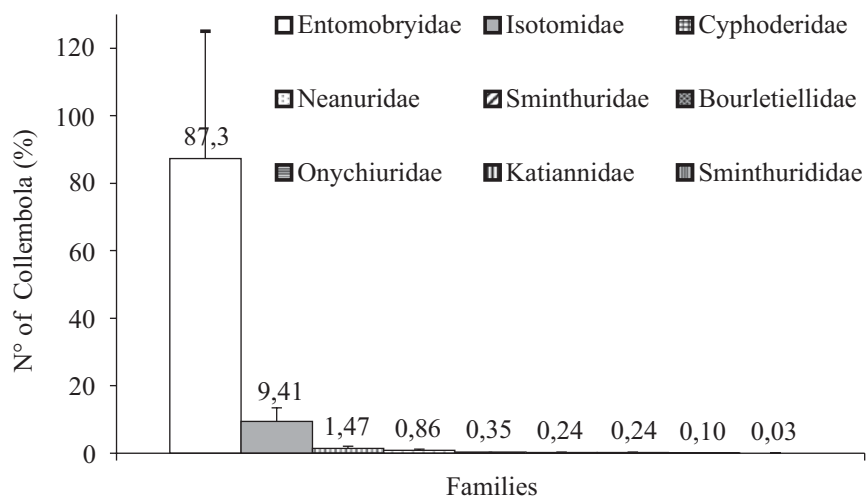


Fig. 2. Total mean number of Collembola families ( $\pm$ SE) collected during all the experiment. Núcleo Experimental de Ciências Agrárias, UFMS, Dourados, MS (December 2002 to January 2003).

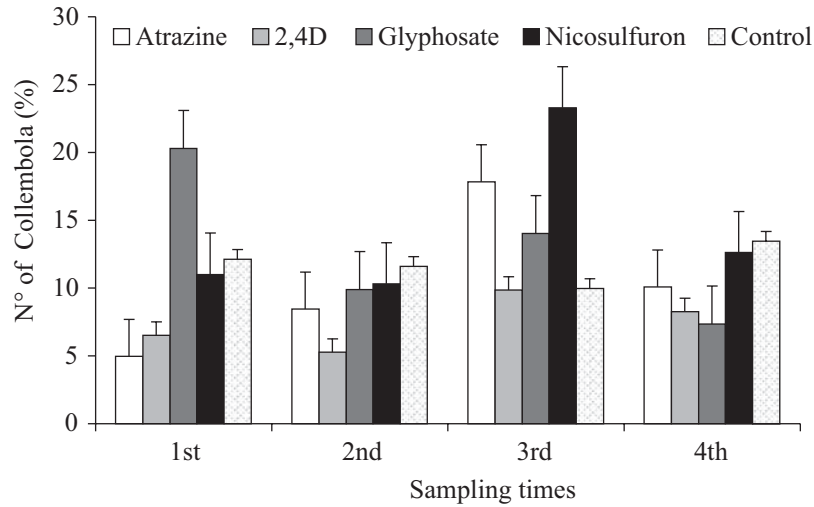


Fig. 3. Mean number of Collembola collected ( $\pm$ SE) in each treatment (Atrazine, 2,4-D, Glyphosate, Nicosulfuron and control) at the different times of sampling (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> sampling). Núcleo Experimental de Ciências Agrárias, UFMS, Dourados, MS (December 2002 to January 2003).

Of the four tested herbicides, the treatment with glyphosate, in the 1st sampling, presented a high number of Collembola. The same result was also observed in the treatments with nicosulfuron and atrazine, in the 3rd sampling (Fig. 3). Atrazine was the herbicide that most reduced the population of springtails in the 1st sampling, and 2,4-D, when compared to the control, reduced the number of Collembola, except in the 3rd sampling. In the 4th sampling, the population of Collembola presented a tendency to stability, probably due to the greater stability of the environment in the corn cultivation, which was 61 days old, and the herbicides presenting 40 days of degradation (Fig. 3).

The variance analysis shows a significant effect ( $P < 0.05$ ) with relationship to the treatment, time, treatment versus time (Table 3).

Table 3. Variance analysis at the level of 5% of significance among the treatments (herbicides and control) and sampling times (10, 20, 30 and 40 days). Núcleo Experimental de Ciências Agrárias, UFMS-Dourados, MS (December 2002 to January 2003).

Variation fount	F.D.	Square mean
Collecting times	3	9.420035 <sup>1</sup>
Treatments	4	12.39184 <sup>1</sup>
Samples	7	1.6219760 <sup>2</sup>
Collecting times x Treatments	12	4.629731 <sup>1</sup>
Collecting times x Samples	21	1.5620380 <sup>2</sup>
Treatments x Samples	28	0.9395474 <sup>2</sup>
Residue	404	2.2272410 <sup>2</sup>
V.C. (%) 48.291		

<sup>1</sup>Level of significance (5%)

<sup>2</sup>No significance at 5% of probability

There was a statistical relationship in the test of comparison of averages for the interaction of the treatments with the sampling times. This indicates that there was a significant effect of the treatments for each sampling time (capital letters) and also, an effect of the times for each treatment (small letters) (Table 4).

The significant influence of the treatments for each sampling time was observed in two of the four sampling times. In the 1st sampling, there was a predominance of the number of collected individuals in the treatment with glyphosate over the other treatments. That increment of springtail population is probably due to the elevation of their reproductive capacity when the amount of food (chemical compounds of herbicides) also increases. Almeida & Rodrigues (1988) pointed out that when the herbicides are applied to the soil, the populations of Acari and Collembola are able to use them, increasing their population density until the decomposition of the product occurs. After the reduction of that resource (residue of herbicides), the population comes back to normal.

In spite of the treatment with glyphosate showing a larger numeric average of Collembola, this herbicide did not differ statistically from the treatments with nicosulfuron and the control in the first sampling time. These last herbicides also did not show differences from the treatments with atrazine and 2,4-D, which presented a low number of springtail specimens.

The number of Collembola, in the 3rd sampling, was high in the treatment with the herbicide nicosulfuron, presenting statistical difference only with the control and 2,4-D, showing results similar to the treatments with atrazine and glyphosate. In the 4th sampling, there was a tendency towards a balance of the populations (Table 4). This can mean a certain resiliency level, i. e., period sufficient for a population to come back to stability after an environmental impact.

In relation to the effect of the sampling times of each treatment, a significant difference in three of the five



Table 4. Mean number of individuals of Collembola sampled in five treatments and their interaction with the sampling times. Núcleo Experimental de Ciências Agrárias, UFMS-Dourados, MS (December 2002 to January 2003).

Treatments	Sampling times (collects)			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Atrazine	2.2311Bb	2.6989Aab	3.6838ABa	3.0964Aab
2,4-D	2.3711Ba	2.2264Aa	3.0247Ba	2.7293Aa
Glyphosate	3.6887Aa	2.9499Aab	3.3487ABab	2.5569Ab
Nicosulfuron	3.1253ABb	3.1804Ab	4.4122Aa	3.4706Aab
Control	3.3627ABa	3.1655Aa	2.9610Ba	3.5249Aa

Equal letters in the same column (capital letters), and equal letters in the same line (small letters), did not differ at 5% of significance by Tukey's test.

treatments was verified. The influence of the 3rd sampling by the treatment with atrazine was observed, having a similarity to the 4th and 2nd samplings. Also, for the treatment with nicosulfuron, the time in which there were certain springtails predominant was that of the 3rd sampling, although this did not differ significantly from the 4th sampling. The treatment with glyphosate manifested a higher average of individuals in the 1st sampling, although there were no significant differences in relation to the times of samplings of the 2nd and 3rd samplings. In the treatments with 2,4-D and control there were no significant variations among gathering times. For the control, the statistical similarities among the times indicates a certain uniformity in the individuals' average in each gathering period (Table 4).

The number of Collembola in the treatments with the herbicides glyphosate, nicosulfuron and control was statistically superior to the other treatments after 10 days of persistence in the soil. After 30 days the number of Collembola was higher in the treatments with nicosulfuron, atrazine and glyphosate. At 40 days after herbicide sprays, a tendency to stability of the populations occurred in the treatments.

The times of samplings that influenced most in the treatment with the herbicide atrazine were those of the 2nd, 3rd and 4th samplings and, for the nicosulfuron, were the 3rd and 4th samplings, culminating in the degradation of the herbicide. On the other hand, the glyphosate presented a higher tendency to an elevation of the number of individuals of Collembola in the times of 10, 20 and 30 days. In the control, the averages number of springtails presented a similarity among the gathering times.

The results obtained in this research (Table 4) are compared to those found by Almeida & Rodrigues (1988) and Bitzer *et al.* (2002). The first authors mention work accomplished with the herbicide 2,4-D, whose species of Collembola were little affected even with successive applications in following years. While Bitzer *et al.* (2002), affirm that the effect of the herbicide glyphosate in a culture of transgenic soy, did not present deleterious action upon Collembola populations.

The results found here demonstrated that, in the first ten days after the application of the products, the herbicides atrazine and 2,4-D were what most affected the Collembola

populations. While the herbicide 2,4 D, after 30 days of persistence in the soil, was that which most reduced the number of those edaphic collembolans. However, at 40 days of degradation of the herbicides, there was a tendency of balance in the populations in all the treatments. Thus, there is a need for larger number of sampling for confirmation of the period necessary for the Collembola populations to establish their balance situation in the agroecosystems.

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