

# Effects of granulated baits on meso and macrofauna in soybean soil system

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## Abstract

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Studies were done to see the effects of five toxic baits based on metaldehyde and/or carbaryl on the taxonomic composition of soil meso and macrofauna, and determined the density of beneficial organisms in soybean under no tillage. Six treatments were tested and soil monoliths were taken before application and at 45 and 75 days after application (DAA). Before application, 1601 individuals were recorded, belonging 1086 to mesofauna and 515 to macrofauna. In soil mesofauna such as Annelida: Enchytraeidae (44%), Nematoda (33%) and Arthropoda (23%) were found. In the macrofauna Arthropoda 93% and Annelida: Oligochaeta, Megadrilli 7% were observed. No differences were observed among the treatments ( $p > 0.05$ ) in the total mesofauna density, at 45 and 75 DAA. The density of enchytraeids, mites, collembolans and symphylids showed no differences at 45 and 75 DAA ( $p > 0.05$ ). The total macrofauna density showed differences ( $p < 0.05$ ) at 45 and 75 DAA. The highest density was obtained with 4 kg ha<sup>-1</sup> MataBibos Acay at 45 and 75 DAA. Earthworm and diplopod densities at 45 and 75 DAA showed no differences ( $p > 0.05$ ). The diversity of meso and macrofauna was not affected at 45 and 75 DAA. Bait application did not affect either the abundance or the diversity of soil beneficial invertebrates.

## Key words

Toxic baits, Metaldehyde, Carbaryl, No tillage, Soil mesofauna and macrofauna

## Introduction

Cultivation of agricultural soil has until recently predominantly been achieved by intensive agricultural practices. The tendency at present is to carry out conservation tillage practices, such as no tillage (NT), reduced tillage and minimum tillage, because these systems contribute to protect soil from wind and water erosion (Holland, 2004). Thus, no-tillage (direct-drill or zero tillage) is the extreme form of reduced tillage where essentially no soil manipulation is performed prior to planting and prior crop residues are left on the soil surface (Stinner and House, 1990; Studdert and Echeverría, 2000a). Moreover, plowing acts as a physical disturbance affecting the horizontal and vertical distribution of soil biota. In a general

sense, plowing should be viewed as a perturbation to soil invertebrate communities and has been associated with a reduction in the abundance and the diversity of several invertebrate and plant species (Stinner and House, 1990).

As noted Lavelle (2000), soil invertebrates play an essential role in suppressing specific plant pathogens, improving soil physical properties, decomposing organic matter and supplying nutrients to plants. Also, they are partly responsible for degradation of detritus and cycling of organic matter, whose nutrients are required by other organisms (Giesy *et al.*, 2000). In addition, soil fauna and biodiversity prevent pest outbreaks in agroecosystems by balancing the predator-herbivore ratio (Tillman *et al.*, 2004).

Soil invertebrates have relatively limited abilities to digest soil organic detritus, and rely, to a large extent, on micro-organisms to derive the assimilates they need from soil organic resources. Additionally, the attraction of micro-organisms by larger organisms appears to be essential to the maintenance and enhancement of the activity of microbial communities (Lavelle, 1997). This determines that one way to understand soil biota functions is to classify the biota on the basis of their body size *i.e.* micro-, meso and macrofauna (Swift *et al.*, 1979). Moreover, their responses to extreme climatic conditions, ploughing, indiscriminate agrochemical use and monoculture, suggest that larger organisms are more sensitive than smaller ones (Holland, 2004).

In cropping systems under no-tillage, soil organisms benefit from the reduction in soil disturbance; crop residues concentrate on the surface creating more stable microclimate conditions regarding soil humidity and temperature, which provides a more suitable habitat for the increase of beneficial organisms and harmful pests (Wilson-Rummenie *et al.*, 1999). For example, slugs [*Deroceras reticulatum* (Müller), *D. laeve* (Müller), and *Milax gagates* (Draparnaud) (Mollusca, Pulmonata: Stylommatophora)] and pillbugs [*Armadillidium vulgare* (Latreille) (Crustacea: Isopoda)] frequently cause damage to crops such as oilseed rape, soybean and sunflower (Clemente *et al.*, 2008; Larsen *et al.*, 2007; Garavano *et al.*, 2009). Slug and pillbug control is performed mainly by toxic granulated baits formulated as pellets with food attract and metaldehyde as molluscicide (2,4,6,8-tetramethyl-1,3,5,7-tetrazocane) or carbaryl (1-naphthol N-methyl carbamate) as crustacide (Salvio *et al.*, 2008). Molluscicide and crustacide application could also be toxic for the organisms beneficial to soil meso and macrofauna. However, very little is known about the effect of metaldehyde and carbaryl on non-target organisms (Iglesias *et al.*, 2003; Bieri *et al.*, 1989). Field evaluations on the impact of molluscicides and crustacides over beneficial organisms are thus required.

The aims of this investigation were to examine the effects of five baits based on metaldehyde and/or carbaryl on the taxonomic composition (diversity) of soil meso and macrofauna, and to determine the density of beneficial organisms.

### Materials and Methods

**Study site:** The experiment was carried out in a field located in Balcarce, Southeast of Buenos Aires Province, Argentina [37° 45' S, 58° 18' N, altitude 130 m (a.s.l.)]. The climate is mesothermal, subhumidity-humidity with a mean temperature varying between 22°C for the hottest month (January) and 8°C for the coldest one (July). Mean annual rainfall is 928 mm (mean annual rainfall 1970-2000) with a rainy season from September to February. The field had a fine, mixed, thermic Typic Argiudoll (USDA classification) and the surface horizon had a pH of 6 (1:2.5 in water), loamy texture, 33.1 cmol kg<sup>-1</sup> cation-exchange capacity, 5.0 mg kg<sup>-1</sup> Bray and Kurtz P and 37.7 g kg<sup>-1</sup> soil organic C contents (Studdert and Echeverría, 2000b). The study was carried out on a soybean

(*Glycine max* L.) crop cultivated under no-tillage (NT) to a 5 cm depth with a rotary tiller. The area presented a slug density of 8 ind m<sup>-2</sup> of *Milax gagates* (Draparnaud), and an isopod *Armadillidium vulgare* (Latreille) density of 70 ind m<sup>-2</sup>.

**Experimental design and cropping system:** On November 2007, commercial soybean (*Glycine max* L. var. Nidera 4200) was sowed at a density of 450.000 p ha<sup>-1</sup> at 42 cm row spacing on a cropping system under no-tillage. Chemical fertilizers were applied at a sowing rate of 80 kg ha<sup>-1</sup> diamonic phosphate and 150 kg ha<sup>-1</sup> urinate. Glyphosate, at a dose of 1-1.5 l ha<sup>-1</sup> was also applied as herbicide.

The commercial products used (formulated as pelleted baits) were metaldehyde 4%+ carbaryl 8% Dual Acay, molluscicides 4% metaldehyde Acay and crustacides 8% carbaryl Mata Bibos Acay (Acay Agro S.R.L.) and 5% metaldehyde Clartex and 8% carbaryl Clartex BB (Rizobacter Argentina S.A.).

Experimental design was arranged in randomized blocks with three replications. The treatments were: 6 kg ha<sup>-1</sup> Dual Acay; 4 kg ha<sup>-1</sup> molluscicide Acay; 4 kg ha<sup>-1</sup> molluscicide Clartex; 4 kg ha<sup>-1</sup> Mata Bibos Acay; 4 kg ha<sup>-1</sup> Clartex BB and control. The size of each experimental plot was 400 m<sup>2</sup>, and the baits were applied by hand on 19 November, 2007.

**Sampling technique:** From the study area, 10 soil monoliths (25.2 cm side; 30 cm depth) were taken randomly before baits application. The sampling was done at 45 and 75 days after bait application (DAA) and in each plot, one soil monolith was taken on each sampling date (total samples = 18). In order to separate specimens, the soil was sequentially sifted through two screens of 10 and 2.5 mm opening diameter. Stones and large pieces of debris were removed by hand from the first screen; smaller organisms were removed from the second screen. Then, the soil was separated in visually homogeneous layers 1-4 mm thick, which were analyzed by hand-sorting. They were recorded and examined by means of a stereomicroscope at a 160 magnification and of a microscope at a 400 magnification to verify species identification of the meso and macrofauna.

**Statistical analysis:** Macro and mesofauna density data were analyzed using generalized lineal models assuming a binomial negative distribution. Poisson distribution was used for nematodes. Both analyses were made using the SAS GLM procedure (SAS v. 8 software 2001). When significant effects were detected among treatments, contrasts were carried out with control ( $\alpha = 0.05$ ) (McCullagh and Nelder, 1989).

Meso and macrofauna family diversity was obtained applying the Shannon-Weaver index ( $H'$ ), and evenness Pielou index (E) (Brévault *et al.*, 2007) at both 45 and 75 days after application (DAA) of baits.

### Results and Discussion

**Mesofauna and macrofauna before bait application:** A total of 1601 individuals were recorded, belonging 1086 to mesofauna

and 515 to macrofauna. Mainly 3 phyla were found in soil mesofauna (Table 1). Annelida organisms were the most abundant, accounting for 44%, followed by nematoda and arthropoda with 33 and 23%, respectively. In Insecta, collembola was the most abundant class, accounting for 13% of the arthropod density and there were few Acarina 7% and Symphyla 3% (Table 1). Regarding macrofauna, 2 phyla were observed, Arthropoda 93% and Annelida 7%. Within Arthropoda, Diplopoda (54%) and Insecta (31%) were the most numerous arthropod groups.

Diplopoda essentially consisted of Polydesmida, and Insecta; mainly of Diptera, Coleoptera larvae and adults of Hymenoptera (Table 1).

In general, the Arthropoda was the most abundant organism group of soil meso and macrofauna in soybean crop under no-tillage before bait application (Table 1). In agreement with Rodriguez *et al.* (2006), the arthropod density in conservation tillage practices, such as no tillage (NT), is higher.

**Table - 1:** Density (ind m<sup>-2</sup>) of mesofauna and macrofauna before bait application (BBA), 45 and 75 days after application (DAA)

	Phylum	Class	Order	Family	Density (ind m <sup>-2</sup> )				
					BBA	45 DAA	75 DAA		
<b>Mesofauna</b>	Annelida	Oligochaeta		Enchytraeidae	1203	227	73		
	Nematoda				907	334	303		
	Arthropoda	Insecta	Collembola	Onychiuridae	189	93	157		
				Isotomidae	157	71	106		
				Cryptostigmata= Oribatida	28	13	8		
		Arachnida	Acarina	Prostigmata= Actinedida	106	38	28		
Mesostigmata= Gamasida				61	8	3			
	Symphyla		Scutigereilidae	93	81	51			
<b>Macrofauna</b>	Arthropoda	Chilopoda	Lithobiomorpha	Lithobiidae	5	0	0		
			Scolopendromorpha	Scolopendridae	23	43	18		
			Diplopoda	Juliformia	Julidae	298	268	245	
				Polydesmida	Polydesmidae	407	147	20	
		Insecta	Diptera	Diverse Diptera larvae		139	149	51	
				Coleoptera	Scarabaeidae	3	0	0	
				Curculionidae		28	5	0	
				Elateridae		0	5	0	
				Chrysomelidae		5	3	5	
				Staphylinidae		48	8	10	
				Histeridae		20	0	0	
				Carabidae		0	8	5	
				Cucujidae		10	10	18	
				Dasytidae		3	10	0	
					Hymenoptera	Formicidae		111	83
			Homoptera	Psyllidae		28	45	76	
				Aphididae		10	8	0	
			Crustacea	Isopoda	Armadillidae		53	20	13
			Arachnida	Araneae	Diverse Araneae		23	20	23
Annelida	Oligochaeta	Megadrilli	Diverse Megadrilli		88	73	136		

**Table - 2:** Abundance, taxonomic richness, diversity and evenness index of soil mesofauna in the different treatments at both 45 and 75 days after application (DAA)

Treatments	Number of individuals m <sup>-2</sup>		Number of families		Shannon-Weaver index		Evenness Pielou index	
	45 DAA	75 DAA	45 DAA	75 DAA	45 DAA	75 DAA	45 DAA	75 DAA
Control	36	34	6	6	2.28	2.06	0.88	0.80
6 kg ha <sup>-1</sup> Dual Acay	29	26	5	6	1.88	2.22	0.81	0.86
4 kg ha <sup>-1</sup> Molluscicide Acay	26	20	7	4	2.48	1.93	0.88	0.97
4 kg ha <sup>-1</sup> Molluscicide Clartex	26	28	6	5	2.28	2.06	0.88	0.89
4 kg ha <sup>-1</sup> MataBibos Acay	29	16	5	4	1.65	1.59	0.71	0.80
4 kg ha <sup>-1</sup> Clartex BB	33	21	5	6	1.96	2.26	0.84	0.87

**Table - 3:** Abundance, taxonomic richness, diversity and evenness index of soil macrofauna in the different treatments at both 45 and 75 days after application (DAA)

Treatments	Number of individuals m <sup>-2</sup>		Number of families		Shannon-Weaver index		Evenness Pielou index	
	45 DAA	75 DAA	45 DAA	75 DAA	45 DAA	75 DAA	45 DAA	75 DAA
	Control	58	89	9	8	2.91	2.61	0.92
6 kg ha <sup>-1</sup> Dual Acay	97	72	11	10	3.15	3.00	0.91	0.90
4 kg ha <sup>-1</sup> Molluscicide Acay	50	49	7	6	2.29	2.24	0.82	0.87
4 kg ha <sup>-1</sup> Molluscicide Clartex	42	45	7	9	2.60	2.99	0.93	0.94
4 kg ha <sup>-1</sup> MataBibos Acay	180	99	11	9	2.67	2.77	0.77	0.87
4 kg ha <sup>-1</sup> Clartex BB	62	61	7	7	2.35	2.61	0.84	0.93

Similar to the findings of Rodriguez *et al.* (2006), collembolans were the most abundant arthropods within soil mesofauna. Under NT, these organisms probably find a favourable environment because of the reduction of soil disturbance and of the increase of crop residues on the soil surface. These crop residues form a soil protection surface layer increasing the development of bacteria and fungi, main feeding resources of these organisms (López-Fando and Bello Pérez, 1997; Doles *et al.*, 2001). Our findings were in consistent with those of Baquero *et al.* (2003) and López *et al.* (2005) who also reported enchytraeids as the most frequent in soil mesofauna in the southeast of Buenos Aires Province (Argentina). Moreover, Manetti *et al.* (2008) and Gizzi *et al.* (2009) reported that there are collembolans in addition to enchytraeids, and that, in macrofauna, soil insects are the most abundant. However, in our study, as well as in what has been reported by Wyss and Glasstetter (1992), a low density of earthworms was obtained under no tillage, whereas the enchytraeid density was higher: 1203 ind m<sup>-2</sup>, as has previously been mentioned (Table 1).

In view of the above, the lower density of earthworms obtained (Table 1) favours the dominance of enchytraeids to a large extent due to the abundant feeding resources and highly stable environment, without perturbations. As a result, the low density of earthworms was compensated by a higher density and activity of enchytraeids, which may account for an increased soil porosity and biogenic structures (cast and fecal pellets) (Parmelee *et al.*, 1990).

**Mesofauna and macrofauna after bait application:** The population of mesofauna showed a higher density, 864 ind m<sup>-2</sup> at 45 DAA, than at 75 DAA, 728 ind m<sup>-2</sup>, expressed as the sum of the individuals from all treatments. In this case, samples were collected in summer when the abundance of mites and collembolans was low (Table 1). It was observed that, during this period, the rainfalls were scarce and the soil was dry.

Total density of mesofauna from 45 to 75 DAA did not differ among treatments ( $P = 0.6822$  and  $P = 0.1396$ ) (Fig. 1).

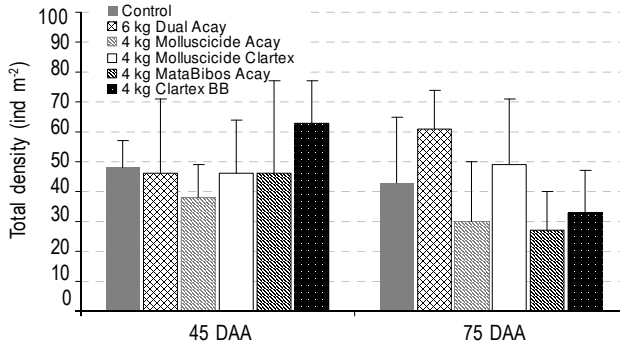
Within mesofauna, the enchytraeid, mite, collembolan and symphylid densities were not significantly different ( $p > 0.05$ ) between 45 and 75 DAA, averaging density from 1 to 18 ind m<sup>-2</sup>.

The population density of nematodes was significantly different at both 45 and 75 DAA; ( $P = 0.0123$  and  $p < 0.001$ , respectively). Nematode density with 4 kg ha<sup>-1</sup> of molluscicide Clartex and Clartex BB differed significantly from the control. In the other treatments, the baits did not affect the density of nematodes as it increased throughout and differences in relation to the control were not observed (Fig. 2). There was a higher density in the population of nematodes at 75 DAA with 6 kg ha<sup>-1</sup> Dual Acay and 4 kg ha<sup>-1</sup> molluscicide Clartex  $p < 0.05$ , 35 and 22 ind m<sup>-2</sup>, respectively (Fig. 2). The action of baits did not affect the organisms in the other treatments, as a similar or relatively higher density in relation to the control was observed.

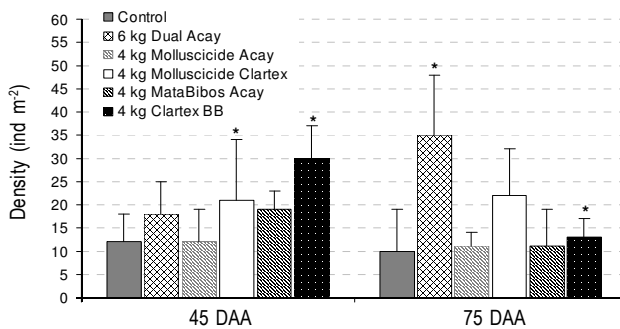
The community diversity index of soil mesofauna at 45 DAA with 6 kg ha<sup>-1</sup> Dual Acay, 4 kg ha<sup>-1</sup> MataBibos Acay and 4 kg ha<sup>-1</sup> Clartex BB decreased in relation to the control and there was a difference in the population diversity of 0.4, 0.63 and of 0.32, respectively (Table 2). As regards molluscicides it was observed that the diversity increased or was similar to the control. At 75 days after application, diversity index with 4 kg ha<sup>-1</sup> MataBibos Acay decreased to 0.47, in relation to the control.

The highest diversity was obtained at 45 DAA with 4 kg ha<sup>-1</sup> molluscicide Acay and with 4 kg ha<sup>-1</sup> Clartex BB at 75 DAA (Table 2).

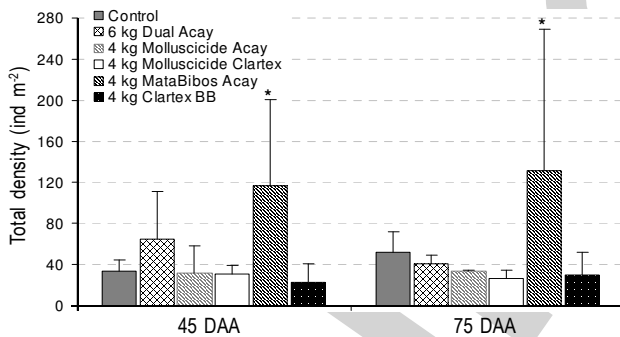
In cropping systems, higher rates of pesticides (particularly insecticides) and fertilizer application results in higher mortality, and in some cases, the disappearance of soil organisms (Bedano and Ruf, 2007). However, our results demonstrated that the soil mesofauna density was not affected by pesticides formulated as bait pellets (Fig. 1). In accordance with Iglesias *et al.* (2003), the density of microarthropods as mites and collembolans after application of metaldehyde (molluscicide) was not reduced. The molluscicide application at doses of 50 g metaldehyde ha<sup>-1</sup> did not produce any negative effect on those organisms (Iglesias *et al.*, 2003). On the other hand, spreading carbaryl at 1.1 and 11.3 kg active ingredient (A.I.) ha<sup>-1</sup> produced a variation on density depending on each different population of organisms (Spain, 1974). When the symphylid density was compared at different doses, it was observed that the population density was affected at the highest dose of carbaryl. On the contrary,



**Fig. 1:** Total density (ind m<sup>-2</sup>) of mesofauna at both 45 and 75 days after application (DAA) in different treatments. Values are mean of 3 replicates ± SD



**Fig. 2:** Density (ind m<sup>-2</sup>) of nematodes between treatments at 45 and 75 days after application (DAA) in different treatments. \* significant differences with the control are indicated by  $p < 0.05$ . Values are mean of 3 replicates ± SD



**Fig. 3:** Total density (ind m<sup>-2</sup>) of macrofauna at both 45 and 75 days after application (DAA) in different treatments. \* significant differences with control are indicated by  $p < 0.05$ . Values are mean of 3 replicates ± SD

a reduction in the collembolan density was found at both carbaryl doses, whereas the effect over mites was insignificant (Cryptostigmata, Mesostigmata and Prostigmata). Barret (1968) considered that 2.24 kg carbaryl ha<sup>-1</sup> reduced the decomposition of crop residues, main feeding resource of detritivores microarthropods, and indirectly affected the number of these organisms. The indirect action of carbaryl took place when the crop residues came into contact with the carbaryl once it had degraded. However, in this work, carbaryl baits did not affect the density or the taxonomic

composition (diversity) of the microarthropod community, probably due to the different formulation type and application mode (Figs. 1 and 2; Table 2).

The total density of macrofauna at 45 and 75 days after application was 904 and 942 ind m<sup>-2</sup> respectively, expressed as the sum of the individuals from all the treatments. Phylum Arthropoda animals were the most abundant group (Table 1).

The total density of macrofauna at 45 and at 75 DAA showed significant differences among treatments,  $P = 0.0648$  and  $P = 0.0168$ , respectively (Fig. 3). With 4 kg ha<sup>-1</sup> MataBibos Acay the highest density was obtained, 117 and 131 ind m<sup>-2</sup> ( $p < 0.05$ ) at 45 and 75 DAA, respectively, in relation to the control (Fig. 3).

Two groups of soil organism macrofauna were considered according to their trophic function and density: Oligochaeta: Megadrilli (earthworms), and Diplopoda, mainly Julidae. Earthworms and diplopods showed no significant differences ( $p > 0.05$ ) in the populations at both sampling times, 45 and 75 days after applications. The average density of earthworms was between 1-8 and 3-14 ind m<sup>-2</sup> and for the diplopods 13-67 and 5-29 ind m<sup>-2</sup> at 45 and 75 DAA, respectively.

The diversity index decreased with metaldehyde or carbaryl alone at 45 days after application, while at 75 days after application with molluscicide Acay 4 kg ha<sup>-1</sup>, the index decreased to 0.37 in relation to the control (Table 3). A higher diversity was obtained at both 45 and 75 DAA, with 6 kg ha<sup>-1</sup> Dual Acay, 3.15 and 3.00, respectively.

The molluscicides based on metaldehyde and applied under field conditions did not reduce the density of *Lumbricus* spp. (Iglesias *et al.*, 2003). As has been observed by Bieri *et al.* (1989), when earthworms are exposed to a metaldehyde dose similar to the one used in the field, the negative effect is insignificant. However, these organisms can present signs such as discoloration and lesions on the tegument, due to a prolonged contact with the pellets. Furthermore, the abundance of spiders, diplopods, ants, staphylinid and carabid beetles larvae was not affected by the baits. In this study, Acay and Clartex molluscicides showed a similar behavior over the density of organisms previously cited.

There was no available information about the effects that crustacides formulated as baits with carbaryl as active ingredient on beneficial organisms. Nevertheless, when carbaryl is used as insecticide, Stenersen *et al.* (1992) and Ville *et al.* (1997) reported that earthworms as *Eisenia fetida andrei* presented signs as tremor, size reduction, neurotoxicity and immunomodulator effects after exposure to the chemical. However, in this study, as carbaryl was formulated as bait, the earthworm density was not affected.

Bait application did not affect either the abundance or the diversity of edaphic beneficial organisms as assure their conservation

to maintain the biological balance in the soybean crop system under no tillage.

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