SOIL ORGANISMS ASSOCIATED TO THE WEED SUPPRESSANT *Crotalaria juncea* (FABACEAE) AND ITS IMPORTANCE AS A REFUGE FOR NATURAL ENEMIES[1]

Organismos de Solo Associados à Supressora de Plantas Daninhas ***Crotalaria juncea*** *(Fabaceae) e sua Importância como Refúgio para Inimigos Naturais*

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ABSTRACT - Soil organisms play an important role in organic crops of *Crotalaria juncea* (Fabaceae) and are associated with the natural conservation of the environment. The present study was aimed to investigate the population of soil organisms in the organic culture of *C. juncea*, as well as its importance as a refuge for natural enemies. *Dalbulus maidis* (Hemiptera: Cicadellidae), *Diabrotica* sp. (Coleoptera: Chrysomelidae), *Doru luteipes* (Dermaptera: Forficulidae), *Gryllus assimilis* (Orthoptera: Gryllidae), *Lagria villosa* (Coleoptera: Lagriidae), *Melanotus* sp. (Coleoptera: Elateridae), *Meloidogyne incognita* (Tylenchida: Heteroderidae), *Nephila clavipes* (Araneae: Nephilidae), *Orius insidiosus* (Hemiptera: Anthocoridae), *Pheidole* sp. (Hymenoptera: Myrmicidae), *Phyllophaga* sp. (Coleoptera: Scarabeidae), *Procornitermes* sp. (Isoptera: Termitidae), *Solenopsis* sp. (Hymenoptera: Formicidae), and *Utetheisa ornatrix* (Lepidoptera: Arctiidae) were identified in *C. juncea*. The organisms that were found during a 3-month period in 144 trenches in *C. juncea* were pest species (84.47%) and natural enemies (15.53%) as well. Natural enemies had an average of 11.89 individuals per 1.08 m^3 of soil cultivated with *C. juncea*. The abundance of organisms in the pod stage (5.49%) of *C. juncea* was lower than that in the vegetative (83.50%) and flowering (11.01%) stages. *Crotalaria juncea* plants can be used as part of a crop system for Integrated Pest Management.

Keywords: *Crotalaria juncea*, natural enemies, organic agriculture, pests, soil organisms, sun hemp.

RESUMO - Organismos de solo desempenham um importante papel em cultivos orgânicos de ***Crotalaria juncea*** *(Fabaceae) e estão associados com a conservação natural do ambiente. O presente estudo teve como objetivo investigar a população de organismos de solo no cultivo orgânico de* ***C. juncea****, bem como sua importância como um refúgio para inimigos naturais.* ***Dalbulus maidis*** *(Hemiptera: Cicadellidae),* ***Diabrotica*** *sp. (Coleoptera: Chrysomelidae),* ***Doru luteipes*** *(Dermaptera: Forficulidae),* ***Gryllus assimilis*** *(Orthoptera: Gryllidae),* ***Lagria villosa*** *(Coleoptera: Lagriidae),* ***Melanotus*** *sp. (Coleoptera: Elateridae),* ***Meloidogyne incognita*** *(Tylenchida: Heteroderidae),* ***Nephila clavipes*** *(Araneae: Nephilidae),* ***Orius insidiosus*** *(Hemiptera: Anthocoridae),* ***Pheidole*** *sp. (Hymenoptera: Myrmicidae),* ***Phyllophaga*** *sp. (Coleoptera: Scarabeidae),* ***Procornitermes*** *sp. (Isoptera: Termitidae),* ***Solenopsis*** *sp. (Hymenoptera: Formicidae) e* ***Utetheisa ornatrix*** *(Lepidoptera: Arctiidae) foram identificados em* ***C. juncea****. Os organismos que foram encontrados durante um período de três meses em 144 trincheiras em* ***C. juncea*** *foram as espécies de pragas (84,47%) e inimigos naturais (15,53%). Inimigos naturais tiveram uma média de 11,89 indivíduos por 1,08 m^3 de solo cultivado com* ***C. juncea****. A abundância de organismos na fase de vagem (5,49%) de* ***C. juncea*** *foi menor do que nas fases vegetativa (83,50%) e floração (11,01%). Plantas de* ***C. juncea*** *podem ser usadas como parte de um sistema de cultivo para o Manejo Integrado de Pragas.*

Palavras-chave: *Crotalaria juncea*, inimigos naturais, agricultura orgânica, pragas, organismos do solo, crotalária.

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INTRODUCTION

Green manure of legumes is a beneficial cultural practice to production systems (Silva et al., 1998). Mulching of *Crotalaria juncea* (Fabaceae) can improve soil structure, reduce soil erosion, and increase levels of soil organic matter and nutrients (Sharma et al., 2010). Seed germination and the amount of weed dry matter were reduced in soil covered with mulching of *C. juncea* due to increased shading and release of allelochemical substances (Queiroz et al., 2010). These substances repel nematodes, but some of these species were attracted to the roots of *C. juncea* (Wang et al., 2002; Chellemi, 2006). The mortality of pupae of *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae) was higher in soil covered with mulching of *C. juncea* due to the release of allelochemical substances, humidity, and pathogenic microorganisms in the soil (Gill et al., 2010). However, some species of beetle pests can adapt themselves and survive in this crop mulching thanks to their increased resistance to soil edaphic factors (Santos et al., 2008).

Crotalaria juncea plants can be used in cropping systems to attract or repel pests, natural enemies, and other organisms (Santos et al., 2008). Other Fabaceae, including *Acacia caven, Centrosema pubescens*, and *Stizolobium cinereum* are important as refuges for natural enemies such as Araneae, Coccinellidae, Eulophidae, Heteroptera, Hymenoptera, Pteromalidae, and Trichogrammatidae (Rojas-Rousse, 2006; Silva et al., 2007). The architecture of the stems and dense cultures of *C. juncea* favored by increased humidity and lower temperature, providing areas of refuge and shelter for parasitoids and predators, besides beneficial mites and nematodes in soil (McSorley et al., 2009). A greater number of *Chrysoperla externa* (Neuroptera: Chrysopidae) and higher levels of bee pollination were observed during the flowering period of *C. juncea*, because its yellow flowers had large amounts of pollen and gave off an attractive smell (Venzon et al., 2006). Natural enemies associated with *C. juncea* migrated to agricultural crops when the plants were grown in systems of rotation or succession (Gill et al., 2010).

Crotalaria juncea cultures play a significant role in Integrated Pest Management (IPM) programs in weeds and crops (McSorley et al., 2008a). Weeds can be damaged by defoliators associated with *C. juncea*, which affects organic agriculture where the use of synthetic pesticides is forbidden (Gill et al., 2010). Moreover, defoliators of *Phyllophaga cuyabana* (Coleoptera: Melolonthidae) would rather feed on *C. juncea* than on crops (Oliveira & Garcia, 2003; Oliveira et al., 2007). *Crotalaria juncea* plants increased moisture and reduced soil temperatures after the incorporation of this green manure and decaying vegetal material released allelochemical substances, attracting soil pests and repelling air natural enemies (Venette et al., 1997; McSorley et al., 2006). Soil organisms attracted by *C. juncea* cultures are little known. However, due to their relevance they are being studied.

This study was aimed to record edaphic macroinvertebrates associated to the weed suppressant species *C. juncea* and to determine its role as a refuge for natural enemies.

MATERIAL AND METHODS

The research was carried out from December 2006 to April 2007 in experimental fields and in the laboratory of the National Research Center of Corn and Sorghum (CNPMS) of the Brazilian Agricultural Research Corporation in Sete Lagoas, Minas Gerais State, Brazil (19º28'00"S, 44º15'08"W). This region is characterized as semi-humid. Its predominant crop is sun hemp followed by maize. Soil chemical analyses from zero to 20 cm were pH (H_2O) = 5.8, $H+Al^{+3}$= 5.6, Al^{+3} = 0.0, Ca^{+2} = 2.71, Mg^{+2} = 0.51, SB = 3.43 and CTC = 9.03 cmolc dm^{-3}; K= 84 and P= 17 mg dm^{-3}; M.O. = 3.94 dag kg^{-1}, and V = 38 and SatAl = 0.

Insects, nematodes, and spiders in the soil were extracted in a *C. juncea* stand (1.0 ha) isolated that was cultivated in December 2006 with Dystrophic Red Yellow Latosol-type soil. This stand was divided into six plots of 200 m^2 each one (20 m long and 10 m wide) with poles. These plots were equally spaced around the stand. Two trenches (0.15 x 0.15 m^2 to 1.0 m deep) were marked and dug by plot in each sample (three samplings weekly) and the soil

on each one was placed in plastic bags with a maximum capacity of 20 kg and taken to the laboratory where it was dumped on a bench. Soil organisms were extracted with forceps and brush and screened in acrylic tubes with alcohol 70%. Root knot nematodes were not collected in *C. juncea* roots and plants maintained after the samplings. The collection period was from 08:00 to 12:00.

The sampling of soil organisms began when the plants were approximately 10 day old and continued weekly until *C. juncea* presented pods. Weeding was performed on a weekly basis to eliminate weeds. The sun hemp plants were in the vegetative, flowering, or pod stages during sampling. Each one of these sampling was observed in the 12 trenches (two per plot), total of 0.27 m^3 of soil per week or 1.08 m^3 of soil per month. Larvae of *Diabrotica* sp. (Coleoptera: Chrysomelidae), *Melanotus* sp. (Coleoptera: Elateridae) and *Phyllophaga* sp. (Coleoptera: Scarabeidae) and caterpillars of *Utetheisa ornatrix* (Lepidoptera: Arctiidae) were taken to the laboratory and maintained up to the adult stage for identification.

Randomized block design, with six replications was used; each plot consisted of 24 trenches. The foraging activity of ants was expressed as frequency (%) in the trenches dug in the plots, to avoid overestimation of abundance of species with high recruitment capacity close to the nest (Soares et al., 1998). The abundance of other organisms was evaluated. Data were transformed into $\sqrt{X+1}$ and submitted to an analysis of variance (ANOVA), with split plots; plots were represented by trenches and subplots by the phenological stages of *C. juncea*, using the MSTAT-C, version 2.1 software (Supplier: CNPMS) (Russel, 1989).

RESULTS AND DISCUSSION

Fourteen species were identified in *C. juncea*: *Dalbulus maidis* (Hemiptera: Cicadellidae), *Diabrotica* sp., *Doru luteipes* (Dermaptera: Forficulidae), *Gryllus assimilis* (Orthoptera: Gryllidae), *Lagria villosa* (Coleoptera: Lagriidae), *Melanotus* sp., *Meloidogyne incognita* (Tylenchida: Heteroderidae), *Nephila clavipes* (Araneae: Nephilidae), *Orius insidiosus* (Hemiptera: Anthocoridae), *Pheidole* sp. (Hymenoptera: Myrmicidae), *Phyllophaga* sp., *Procornitermes* sp. (Isoptera: Termitidae), *Solenopsis* sp. (Hymenoptera: Formicidae), *U. ornatrix*. These insects were found in the adult (78.37%) or larva (21.63%) stages in this culture, but *Phyllophaga* sp. was found in both the larval and adult stages (Table 1). The higher number of species of the order Coleoptera in the soil of *C. juncea* confirms that these are the most abundant species in soil with green manure *Cajanus cajan* (Fabaceae), *C. juncea*, *S. cinereum*, and *Stylosanthes guianensis* (Fabaceae) (Oliveira & Garcia, 2003; Oliveira et al., 2007; Santos et al., 2008).

Procornitermes sp. (100.72 ± 15.20), *M. incognita* (19.27 ± 2.47), *Diabrotica* sp. (17.58 ± 2.35), *O. insidiosus* (16.25 ± 2.91), and *Phyllophaga* sp. (12.46 ± 1.46) were abundant in the vegetative stage of *C. juncea*; *O. insidiosus* (11.50 ± 2.59), *Procornitermes* sp. (3.29 ± 0.27), *Phyllophaga* sp. (2.27 ± 0.18), *U. ornatrix* (2.11 ± 0.20), and *Diabrotica* sp. (2.01 ± 0.20) in the flowering stage; and *O. insidiosus* (3.31 ± 0.33), *Procornitermes* sp. (3.09 ± 0.27), and *Diabrotica* sp. (2.40 ± 0.20) in the pods stage of this culture. *Doru luteipes*, *N. clavipes*, and *O. insidiosus* were the most common natural enemies found in *C. juncea*, while the most common pests were *Diabrotica* sp., *M. incognita*, *Phyllophaga* sp., and *Procornitermes* sp. The organisms found over a 3-month period in 144 trenches in *C. juncea* were pest species (84.47%) and natural enemies (15.53%). Natural enemies had an average of 11.89 individuals per 1.08 m^3 of soil cultivated with *C. juncea*. The abundance of organisms in the pod stage (5.49%) of *C. juncea* was lower than that in the vegetative (83.50%) and flowering (11.01%) stages (Table 1). Insect pests had lower populations during the flowering and pod stages of *C. juncea* than did those in *Phaseolus lunatus* (Fabaceae), suggesting toxic effects of nectar from flowers of the first plant of these species (Kost & Heil, 2005). Organisms as *Diabrotica* sp. (Jones & Coppedge, 2000, Jackson & Harrison, 2008), *N. clavipes* (Ferro et al., 2006), *O. insidiosus* (Erlandson & Obrycki, 2010), *Phyllophaga* spp. (Lewis et al., 2003), and *U. ornatrix* (Iyengar & Eisner, 2002; Guimarães et al., 2006) were abundant in *C. juncea* and/or other Fabaceae.

Table 1 - Number of individuals (means ± standard error of mean) as function of the stage of *Crotalaria juncea* (Fabaceae) plants[1/]

Taxons	Vegetative	Flowering	Pods
	Herbivores		
Coleoptera Chrysomelidae adults			
Diabrotica sp.	17.58 ± 2.35 a	2.01 ± 0.20 b	2.40 ± 0.20 b
Diabrotica sp./1.08 m^3 soil	5.86 ± 0.78 a	0.67 ± 0.06 b	0.80 ± 0.06 b
Coleoptera Elateridae adults			
Melanotus sp.	9.07 ± 0.94 a	1.26 ± 0.09 b	0.00 ± 0.00 b
Melanotus sp./1.08 m^3 soil	3.02 ± 0.31 a	0.42 ± 0.03 b	0.00 ± 0.00 b
Coleoptera Lagriidae adults			
Lagria vilosa	1.22 ± 0.09 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Lagria vilosa/1.08 m^3 soil	0.40 ± 0.03 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Coleoptera Scarabeidae adults			
Phyllophaga sp.	6.07 ± 0.74 a	2.27 ± 0.18 b	1.41 ± 0.09 b
Phyllophaga sp./1.08 m^3 soil	2.02 ± 0.24 a	0.75 ± 0.06 b	0.47 ± 0.03 b
Hemiptera Cicadellidae adults			
Dalbulus maidis	2.11 ± 0.18 a	1.42 ± 0.09 a	0.00 ± 0.00 a
Dalbulus maidis/1.08 m^3 soil	0.70 ± 0.06 a	0.47 ± 0.03 a	0.00 ± 0.00 a
Isoptera Termitidae adults			
Procornitermes sp.	100.72 ± 15.20 a	3.29 ± 0.27 b	3.09 ± 0.27 b
Procornitermes sp./1.08 m^3 soil	33.57 ± 5.06 a	1.09 ± 0.09 b	1.03 ± 0.09 b
Lepidoptera Arctiidae adults			
Utetheisa ornatrix	1.42 ± 0.09 a	2.11 ± 0.20 a	1.39 ± 0.09 a
Utetheisa ornatrix/1.08 m^3 soil	0.47 ± 0.03 a	0.70 ± 0.06 a	0.46 ± 0.03 a
Orthoptera Gryllidae adults			
Gryllus assimilis	2.52 ± 0.18 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Gryllus assimilis/1.08 m^3 soil	0.84 ± 0.06 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Tylenchida Heteroderidae adults			
Meloidogyne incognita	19.27 ± 2.47 a	0.00 ± 0.00 b	0.00 ± 0.00 b
Nematodes/1.08 m^3 soil	6.42 ± 0.82 a	0.00 ± 0.00 b	0.00 ± 0.00 b
Coleoptera Scarabeidae larvae			
Phyllophaga sp.	12.46 ± 1.46 a	0.00 ± 0.00 b	0.00 ± 0.00 b
Phyllophaga sp./1.08 m^3 soil	4.15 ± 0.48 a	0.00 ± 0.00 b	0.00 ± 0.00 b
Total Herbivores	172.44 ± 23.70 a	12.36 ± 1.03 b	8.29 ± 0.65 b
Herbivores/1.08 m^3 soil[2/]	57.48 ± 7.90 a	4.12 ± 0.34 b	2.76 ± 0.21 b
	Predators		
Araneae Nephilidae adults			
Nephila clavipes	2.12 ± 0.20 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Nephila clavipes/1.08 m^3 soil	0.70 ± 0.06 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Dermaptera Forficulidae adults			
Doru luteipes	1.08 ± 0.09 a	1.42 ± 0.09 a	0.00 ± 0.00 a
Doru luteipes/1.08 m^3 soil	0.36 ± 0.03 a	0.47 ± 0.03 a	0.00 ± 0.00 a
Hemiptera Anthocoridae adults			
Orius insidiosus	16.25 ± 2.91 a	11.50 ± 2.59 a	3.31 ± 0.33 b
Orius insidiosus /1.08 m^3 soil	5.41 ± 0.97 a	3.83 ± 0.86 b	1.10 ± 0.11 b
Total Predators	19.45 ± 3.20 a	12.92 ± 2.68 a	3.31 ± 0.33 b
Predators/1.08 m^3 soil[3/]	6.48 ± 1.06 a	4.30 ± 0.89 a	1.10 ± 0.11 b
	Ants		
Hymenoptera Formicidae			
Pheidole sp. and *Solenopsis* sp.			
Frequency (%)[4/]	16.40 ± 1.75 a	0.00 ± 0.00 b	0.00 ± 0.00 b

[1/] Data transformed in √X+1 before statistical analyses. [1/] Means followed by the same letter per line do not differ by the t test ($p > 0.05$). [2/] Sum of densities of *D. maidis*, *Diabrotica* sp., *G. assimilis, L. vilosa*, *Melanotus* sp., *M. incognita*, *Phyllophaga* sp., *Procornitermes* sp., and *U. ornatrix*. [3/] Sum of densities of *D. luteipes*, *N. clavipes*, and *O. insidiosus*. [4/] Combined occurrence of *Pheidole* sp. and *Solenopsis* sp.

A total of 35.7 adults of *Procornitermes* sp., 7.4 *Phyllophaga* sp., and 7.33 larvae of *Diabrotica* sp. were found per 1.08 m^3 of soil in all evaluations. Overall, there were 50.43 individuals of these species per 1.08 m^3 of soil. Coleoptera species were abundant in the vegetative stage of *C. juncea*, and larvae of Lepidoptera in the flowering stage reached 42.88%. Nematodes had greater abundance in the vegetative stage (100%) of *C. juncea* (Table 1). *Doru luteipes* was found in *C. juncea* and superficially on the soil of crop plants without cartridge, where they feed on different prey (Fenoglio & Trumper, 2006). This predator is common in maize and sorghum where it lay eggs and preys on the main pests, *Diatraea saccharalis* (Lepidoptera: Crambidae) and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Figueiredo et al., 2006; Wyckhuys & O'Neil, 2007; Lima et al., 2010). However, the higher abundance of natural enemies in organic agriculture is also due to lower levels of chemical application in these areas (Birkhofer et al., 2008; Tavares et al., 2009). Natural enemies in *C. juncea* demonstrate the importance of the culture as a refuge for insects and the need for research development on the crops, either in outline or in succession with annual or perennial crops, in order to improve the efficiency of similar agricultural regions.

The lower abundance of *M. incognita* from the flowering and pods stages of *C. juncea* suggests the allelopathic potential of this culture in these periods as recorded for this plant and/or other Fabaceae (Bhan et al., 2010), indicating that the use of these plants as organic fertilizers can reduce infestations of this species (Berry et al., 2009; McSorley et al., 2008a, 2009). The penetration of *Meloidogyne* sp. was above 30% in the roots of *Crotalaria spectabilis* (Fabaceae), but the second-stage juveniles did not reach the third stage because it was not a suitable host, although they did cause necrosis (Sano et al., 1983; Sano & Nakasono, 1986). The incorporation of residues of *Crotalaria longirostrata* (Fabaceae) in the soil reduced the galls by *M. incognita* and *Meloidogyne arenaria* (Tylenchida: Heteroderidae) in tomato roots, due to the release of toxic compounds within plant tissue (Villar & Zavaleta-Mejía, 1990). Plowing the soil with moldboard ploughs during crop rotation with *Gossypium hirsutum* (Malvaceae) and *C. spectabilis* or *C. juncea* can also reduce *M. incognita* populations and *Phyllophaga* sp. by exposing them to natural enemies and the allelopathic potential of these plants (McSorley et al., 2008b).

Solenopsis sp. and *Pheidole* sp. are widely distributed in the crops (Cassill et al., 2005; Wilkie et al., 2010). These ants may exploit food sources, present ability to recruit, and are highly aggressive (Braga et al., 2010). However, oscillations in soil temperature, prey, and soil type also create a favorable environment for ants (Varon et al., 2007). *Pheidole* spp. are ecologically diverse, and it also collects seeds, are omnivorous, predatory and mutualistic in associations with plants (Soares et al., 1998). The higher frequency of ants in a no-till area than in conventional crops suggests that the organic matter in this system increases the populations of Termitidae (Lange et al., 2008).

The ideal time to sow *C. juncea* in the region of Sete Lagoas, Minas Gerais State, Brazil is November and December, in succession with maize. The present study was not performed during these months, but *D. luteipes*, *N. clavipes*, and *O. insidiosus* predators were found to be the primary natural enemies in this culture. The populations of the referred predators needed to control pest populations of predatory insects in *C. juncea* plantations are not known, but individuals in the latter group found primarily in the soil can efficiently control insect pests.

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