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CROP SCIENCE

Natural enemies recovered from *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) larvae infesting the cartridge, ear and stem of corn plants under conventional and organic farming systems in Brazil

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Abstract: The corn cropping system can influence the natural enemy identity and the number of *Spodoptera frugiperda* J.E. Smith, 1797 (Lepidoptera: Noctuidae) individuals infesting the cartridge, ear and stem of this plant. The objectives were to identify the *S. frugiperda* natural enemies and differences in the number of individuals infesting the cartridge, ear and stem of corn plants under conventional and organic cropping systems, in Brazil after an initial collection of adult males through semiochemical traps. We also evaluated key morphometrical parameters of the larva and factors contributing with the viability of *S. frugiperda*. A total of 16 and 136 adult males was trapped, and 1,124 and 1,112 larvae was recovered from conventional and organic systems with 4.7 and 6.7% of them parasitized by dipteran and hymenopteran, respectively. Most of the parasitoids recovered had occurrence in both cropping systems, except Cremastinae and *Ophion flavidus* Brullé, 1846 (Hymenoptera: Ichneumonidae), which were recorded only in organic and *Campoletis* sp. (Hymenoptera: Ichneumonidae) only in conventional corns. The number of parasitoids recovered was similar in corn plant samples between both cropping systems. A total of 152 and 31 larvae was recovered from corn ears under conventional and organic systems, respectively. *Doru luteipes* (Scudder, 1876) (Dermaptera: Forficulidae) was recovered from all samples under conventional system. The larva length was overall similar between cropping systems. Parasitism by dipteran and hymenopteran, infection by microorganisms, larva mortality by undetermined causes, and differences in viability of *S. frugiperda* stages were factors contributing with the supression of this pest. The new associations and parasitoids reported represent possibilities of expanding the biological control strategies to manage *S. frugiperda* in corn crops.

Key words: cropping system, fall armyworm, natural enemy, biological control, corn field.

INTRODUCTION

Corn, *Zea mays* L. (Fabales: Fabaceae) is one of the most important crops in the world because of its use as a food to human and animals (Yongfeng & Jane 2016, Ely et al. 2016, Bordini et al. 2019). The corn cultivation under conventional system, which is usually implemented at a large scale, is mainly used for the production of grains or silage. The grains can be used as seeds to cultivate the next cropping season, while the silage to prepare animal feed (Mike 2015, Spetter et al. 2018). Organically grown corn grains are consumed fresh by humans in the form of baby corn, boiled corn and pickles, as well as being a source of foods such as ice cream, juices, popsicles, and sweet corn cake (Revilla et al. 2015, Oliveira et al. 2016). Low- and medium-income corn farmers, with reduced investment capital for insecticide and other chemical use in their production, generally use family labor for its cultivation (Capellesso et al. 2016, Mendoza et al. 2017). The use of biological control of insect pests becomes important in areas of corn cultivated under organic system to prevent the consumption of chemical-contaminated cereals and ensure food safety (Tavares et al. 2016, Peterson et al. 2018, Abdallah et al. 2018).

The fall armyworm, *Spodoptera frugiperda* J.E. Smith, 1797 (Lepidoptera: Noctuidae) causes serious damage to corn and other plants in many countries on most continents (Mallapur et al. 2018, Sisodiya et al. 2018, Baudron et al. 2019). This pest attacks various corn plant parts including cartridge, ear and stem, and it causes specific and similar damage from other lepidopteran pests in this crop (Vettorazzi et al. 2018, Deole & Paul 2018). The number of *S. frugiperda* larvae and the damage they cause may vary between the attacked plant part and climatic conditions (Sharanabasappa et al. 2018, Midega et al. 2018). In addition, different natural enemy species may occur on the pest according to the attacked plant part and cultivation system (Shylesha et al. 2018, López et al. 2018). Despite being a widely studied pest, *S. frugiperda* may present unknown natural enemies that could contribute to its biological control (Salas-Marina et al. 2018, Hernández-Trejo et al. 2018). A number of *S. frugiperda* natural enemy species is known and some of them are reared in biofactories at a large scale for releases onto infested areas (Tavares 2010, Vieira et al. 2017). The use of natural enemies can be effectively combined with the application of biological, botanical and synthetic insecticides, as well as other control methods (Perez-Zurubi et al. 2016, Sisay et al. 2018), with the monitoring of *S. frugiperda* males performed using the sex pheromone of the female of same species (Garcia et al. 2018).

Corn plants grown under the organic system could create better conditions for actions of infection (i.e. by entomopathogens), parasitism (i.e. by parasitoids) and predation (i.e. by predators) on all stages of *S. frugiperda* (Camargo et al. 2015, Figueiredo et al. 2015, Tavares et al. 2016). This is due to the ban in the use of synthetic products in areas under agroecological system, which would provide greater abundance, diversity, reproductive capacity, and survival of natural enemies (Kebede & Shimalis 2018). On the other hand, *S. frugiperda*'s attack on corn plants is expected to be less under the conventional system due to the use of advanced management techniques including the application of selective pesticides (Aguirre et al. 2015, Frizzas et al. 2017). The objectives of this study were to identify the *S. frugiperda* larva natural enemies and differences in the number of individuals infesting the cartridge, ear and stem of corn plants cultivated under conventional and organic cropping system areas in Sete Lagoas, Minas Gerais state, Brazil after an initial collection of adult males of this species through semiochemical traps. We also evaluated the key morphometrical parameters of the larva and factors contributing with the viability of *S. frugiperda*.

MATERIALS AND METHODS

Experimental site

Corn crops were established in the 2010-2011 wet (i.e. growing) season, in an area of Cerrado (Savannah-type) biome in Sete Lagoas (19° 28' S × 44° 15' W, 776.73 m above sea level). This experimental site belongs to *Embrapa Milho e*

Sorgo of the *Ministério da Agricultura, Pecuária e Abastecimento* (MAPA) of Brazil. Evaluations on cartridge, ear and stem of corn plant samples were carried out in the *Laboratório de Criação de Insetos* of *Embrapa Milho e Sorgo*.

Sowing

Corn seeds were sown in a morning of a land area under conventional cropping system and in another under organic cropping system in the 2010-2011 season, with both areas being managed under their respective systems for about 40 years.

Conventional cropping system had a total area of one hectare planted with the corn cultivar BR 106 (Supplementary Material - Table SI) and organic system another area of 1.0 hectare planted with the same corn cultivar. The corn cultivar was commercially deployed in 1985 by *Embrapa Milho e Sorgo* and has been improved genetically every crop cycle by selection for yield traits. Sowing was carried out at 5 cm depth, with a population of 40 thousand corn plants per hectare in both cropping systems. The experimental areas in both cropping systems were located at a distance of about 3 Km from one another. Five plots of 0.2 ha each were set per experimental area and they were divided into 24 equal-sized sub-plots, each consisting of 10 20-m long rows with a gap of 70 cm between them. No synthetic chemicals were used from 40 days after sowing in the conventional area.

Soil and climate

The soil in both cropping systems is of dystrophic, red-dark latosol type, with a clayey texture (Galvão et al. 2016). The climate is classified as humid subtropical (Cwa) according to the Köppen–Geiger classification system (Kottek et al. 2006), with a rainy season from October to March and a drought season from April to September. Total rainfall and average annual air temperature are 1,272 mm and 20.9 °C, respectively. July is the coldest month with an average temperature of 17.5 °C, while February is the hottest, with an average temperature of 22.9 °C (Galvão et al. 2017).

Management

Corn was planted in the conventional system in an area of no-tillage under the straw of the corn from the previous crop and without removing weeds. The first and second weeding were carried out with the application of herbicide using knapsack sprayers. Sowing, planting fertilization, liming, and cover fertilization were carried out using a no-till seeder/ fertilizer applicator machine coupled with a tractor. Fertilization and liming were performed according to a soil chemical analysis carried out in the *Laboratório de Fertilidade do Solo* of the *Embrapa Milho e Sorgo* in Sete Lagoas and following the nutritional requirements of this crop (Michalovicz et al. 2014). The crop was irrigated using a sprinkler system with water from a nearby canal. The irrigation frequency and volume were determined with the irrigation software *IrrigaFácil* developed by *Embrapa Milho e Sorgo*.

In the organic system, corn was established in an area with soil covered by dry straw of sunn hemp, *Crotalaria juncea* L. (Fabales: Fabaceae). Plants of this legume were cut using a sickle before its flowering and were left covering the ground uniformly (Tavares et al. 2011a, b, Costa et al. 2012). Land preparation and sowing were performed using a manual no-till seeder machine, and the first and second weeding were carried out using a hoe. No additional fertilization, besides nutrients provided by *C. juncea* (Fosu et al. 2004, Yuliana et al. 2015, Subaedah et al. 2016), was performed.

Pionus Wagler (Psittacidae: Psittacidae) and other harmful birds were controlled in both cropping systems using scarecrows and fireworks without harming them (Tavares et al. 2016). The impact of small mammals and rodents was also controlled in both crops by planting additional corn plants in strategically pre-determined areas near the refuges of these animals.

Monitoring of *S. Frugiperda* adult males

Immediately after planting corn, a DELTA-type trap (Ferocon 1C®), containing the sachet-type synthetic *S. frugiperda* sex pheromone (BIO SPODOPTERA®), was installed in the center of each experimental area (Cruz et al. 2012). The traps and pheromones were obtained from ChemTica International, S.A. (Heredia, Santa Rosa, Costa Rica). The traps were installed one meter above the ground level. They were dynamically raised before plants reached the trap height, always keeping them slightely above the plant tip, as recommended by the manufacturer. The synthetic *S. frugiperda* sex pheromone was replaced by a new one every 15 days. The sticky surface of the traps was replaced by a new one when it was covered in insects or debris.

Collection of *S. Frugiperda* larvae

As soon as the first *S. frugiperda* moth was detected in the trap, systematic collections of corn plants were started. Three collections were performed per week, with the first one carried out after the appearance of the first adult male in the trap and the last at the end of the corn plant cycle. Ten plants were harvested per sub-plot with random selection, totaling 240 plants per collection. Twenty collections were performed per plot over the study period. Each plant collected was placed individually in a 2-Kg polypropylene bag and taken to the *Laboratório de Criação de Insetos* in Sete Lagoas where they were kept at 25 ± 2 °C, 70 ± 10% RH and under a 12:12 (L:D) h photoperiod.

Ten samples, each comprised by a plant with ear, were taken weekly from both cropping systems, starting 15 days after the initial appearance of the ear. The ears were cut from the plants manually in the laboratory. Only the most developed ear was selected from plants with more than one ear.

All 10 stems sampled per collection were evaluated. The detection of *S. frugiperda* larvae in the stem was performed after the longitudinal opening of the stems using a knife and on the ears after the removal of the shank, silk and grains. Larvae were taken from these plant parts using a brush and tweezers. The larvae collected were placed individually in a 50 mL plastic cup, each with 7 g of a cube-shaped solidified artificial diet developed for *S. frugiperda* (Tavares et al. 2013a, b), sealed with transparent acrylic covers, where they were kept until its death, or moth or adult parasitoid emerged.

Evaluations on *S. Frugiperda* bioecology

The following parameters were evaluated: date of the first adult male trapped and the total, average, maximum, and minimum numbers of these insects/collection using traps; mean number of larvae/sample/cropping system; mean length (cm) of larvae/sample/cropping system at the time of collection; percentage of adults that emerged from the larvae collected/sample/cropping system; percentage of the larvae collected, killed in the laboratory by microorganisms up to the end of the larval stage/sample/cropping system; percentage of the larvae collected, killed in the laboratory by undetermined causes up to the end of the larval stage/sample/cropping system; percentage of inviable pupae up to the end of the pupal stage/sample/cropping system in the laboratory; percentage of the larvae collected, parasitized by dipteran and hymenopteran up to the end of the larval stage/sample/cropping system;

parasitoids distribution/cropping system; and total and average numbers of predators recovered from corn plants collected.

Mounting, identification and deposit of natural enemies

The natural enemies recovered were preserved in 20-mL glass tubes filled with 70% ethanol. Subsequently, the insects were dried at 25 ºC and mounted using entomological pins.

The larva parasitoids were identified after analysis on keys and taxonomic descriptions of the external body morphology: *Archytas* Jaennicke, 1867, *Hyphantrophaga* Townsend, 1892 and *Winthemia* Robineau-Desvoidy, 1830 (Diptera: Tachinidae: Tachininae and Exoristinae, respectively) by Nihei (2016), Inclán et al. (2016) and Zetina et al. (2018); *Campoletis* Förster, 1869, *Eiphosoma* Cresson, 1865, *Microcharops* Roman, 1910 and *Ophion* Fabricius, 1798 (Hymenoptera: Ichneumonidae: Campopleginae, Cremastinae and Ophioninae, respectively) by Onody et al. (2009), González-Moreno & Bordera (2012), Melo et al. (2012), Fernandes et al. (2014), and Camargo et al. (2015); Cremastinae Förster, 1869 (Hymenoptera: Ichneumonidae) by Khalaim et al. (2018); *Cotesia* Cameron, 1891, *Dolichozele* Viereck, 1911, *Exasticolus* van Achterberg, 1979 and *Glyptapanteles* Ashmead, 1904 (Hymenoptera: Braconidae: Microgastrinae, Macrocentrinae and Homolobinae, respectively) by López-Martínez et al. (2011), Gadallah et al. (2015a), Cerântola et al. (2016), and Salgado-Neto et al. (2018); *Apsylophrys* (Hymenoptera: Encyrtidae: Encyrtinae) by Zuparko (2015) and Fallahzadeh & Japoshvili (2017); and *Euplectrus* Westwood, 1832 (Hymenoptera: Eulophidae: Eulophinae) by Yefremova (2015) and Gadallah et al. (2015b). Predators were also identified after analysis on keys and taxonomic descriptions of the external body morphology: *Doru* Burr, 1907 (Dermaptera: Forficulidae) by Kamimura & Ferreira (2017) and *Orius* Wolff, 1811 (Hemiptera: Anthocoridae) by Ostovan et al. (2017).

After the identification of the natural enemies, part of the specimens was deposited at the *Coleção Entomológica* of the *Departamento de Ecologia e Biologia Evolutiva* of the *Universidade Federal de São Carlos* in São Carlos, São Paulo state, Brazil, and the other part at the *Museu de Insetos* of the *Embrapa Milho e Sorgo*.

Images

Insect images were taken using a Leica DFC295 digital camera attached to a Leica M205_C stereomicroscope (Wetzlar, Germany) with the Leica Application Suite Arquive application.

Statistical analysis

The data of the total, average, maximum, and minimum numbers of adult males trapped/month/cropping system were presented. The following data were also presented per sample: (a) the number of larvae collected; (b) the body length of larvae collected; (c) the percentage of larvae that reached adulthood; (d) the percentage of larvae collected, killed by microorganisms; (e) the percentage of larvae collected, killed by undetermined causes; (f) the percentage of unviable pupae; (g) the percentage of larvae collected, parasitized by dipteran and hymenopteran; (h) parasitoids distribution; and (i) the number of predators recovered. Data were separated into groups to evaluate differences between conventional (1) and organic (2) systems. Averages were compared between cartridge, ear and stem samples per group of data 1 and 2. Data were submitted to the analysis of variance (one way ANOVA) after assumptions were checked (data experimental errors were normally distributed, equal variances between treatments and independence of samples) through Burr-Foster Q (Burr & Foster

1972) and Shapiro-Wilk W (Shapiro & Wilk 1965) tests. Transformation, when applied, was used following criteria suggested by Ostle & Mensing (1975). Means were compared using the Scott-Knott hierarchical clustering algorithm at 5% probability (Scott & Knott 1974). Analyses were carried out using the software SISVAR (Ferreira 2011). Data were presented as mean ± SD.

The Shannon Entropy H (nat) (Shannon 1948) was used to compare the diversity index of parasitoid species recovered from cartridge, ear and stem samples between conventional and organic cropping systems. The analysis was run using the software Business Performance Management Singapore (BPMSG) (Goepel 2020).

RESULTS AND DISCUSSION

S. Frugiperda adult males collected in sex pheromone traps

The total of *S. frugiperda* adult males, collected over the collection period, was 16 in conventional and 136 in organic corn, with an average of 0.1 and 1.1 individuals per collection, respectively. The maximum number of males captured in a collection was three and 27, and the minimum was zero and zero, respectively (Figures 1a-1b). This was expected because the restriction in the use of synthetic pesticides in organic areas leads to a higher population of this pest. The *S. frugiperda* male collection through traps represents a monitoring tool as well as capable to reduce the chances of mating (Malo et al. 2018). The number of adult males

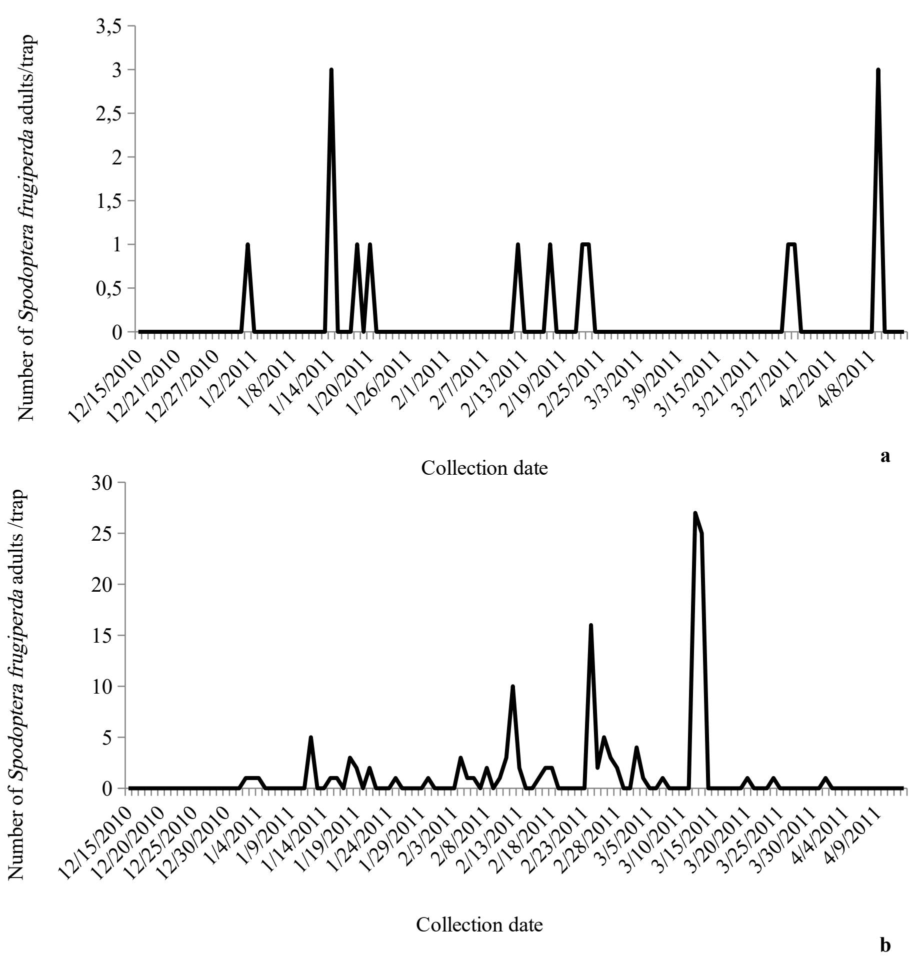

Figure 1. Monthly number of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) adult males collected in sex pheromone traps in corn plants, *Zea mays* (Poaceae) under conventional (a) and organic (b) systems in Sete Lagoas, Minas Gerais state, Brazil.

collected in the traps was low in conventional and high in organic corns in the presente study, with an economic injury level of *S. frugiperda* in corn crops achieved when three males are captured per trap in a night (Cruz et al. 2012). Natural enemies are the main controllers of *S. frugiperda* in organic cropping systems; however, the natural biological control has to be combined with other measures for a successfull management of this pest (Figueiredo et al. 2015).

Number, larva length, mortality factors, and viability of *S. frugiperda* on corn plants under conventional and organic systems

A total of 1,124 and 1,112 larvae was recovered from conventional and organic systems, with an average of 56.2 ± 3.8 and 55.6 ± 6.6 individuals per collection, respectively. The average larvae length was 1.3 cm in both cropping systems. A total of 4.7 and 6.7% of the larvae collected was parasitized by dipteran and hymenopteran, 5.9 and 5.4% killed by microorganisms, 2.1 and 1.3% killed by undetermined causes, 1.8 and 1.2% origined inviable pupae, and 85.5 and 85.4% reached adulthood in conventional and organic corns, respectively (Figures 2a-2b and Table SII). The greatest parasitism rate by hymenopteran can be explained by the fact that this order has a high number of species and presence of groups of these parasitoids able to parasitize the *S. frugiperda* larvae with different sizes/instars (Agboyi et al. 2020). The high efficacy of parasitism on the final instars of lepidopteran and fecundity explain the great parasitism rate of *S. frugiperda* larvae by dipteran (Sisay et al. 2018). The low number of *S. frugiperda* males in

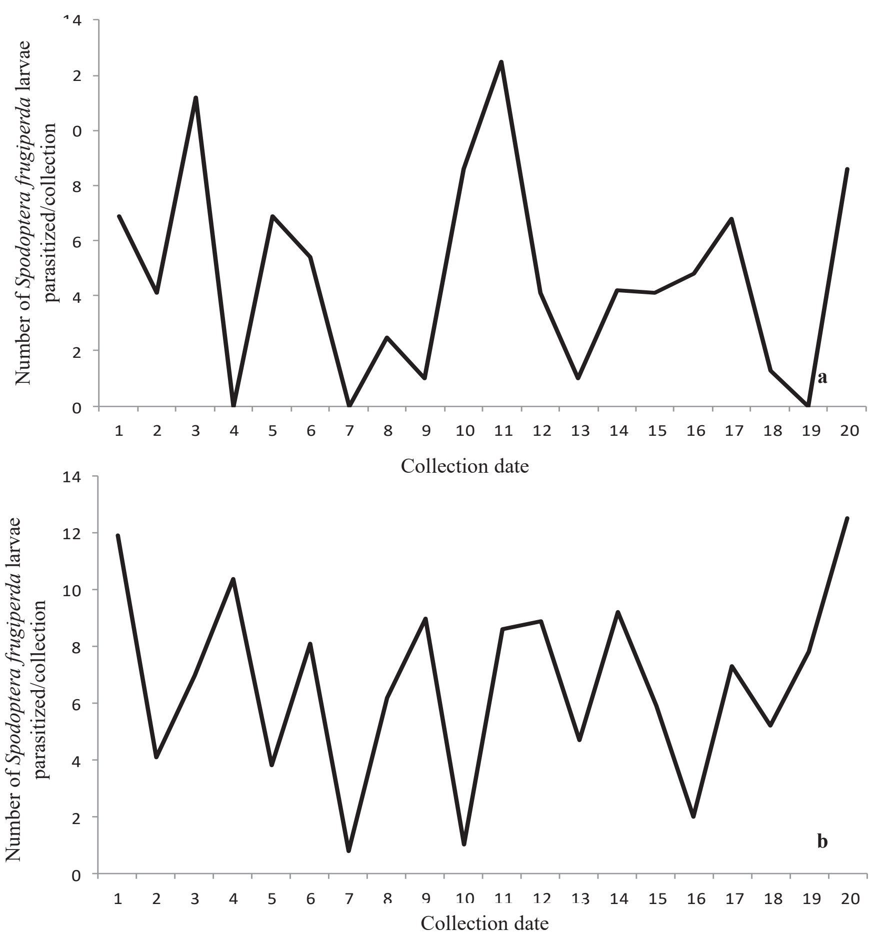

Figure 2. Percentage of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae killed by parasitoids per collection in corn plants, *Zea mays* (Poaceae) under conventional (a) and organic (b) systems in Sete Lagoas, Minas Gerais state, Brazil.

the conventional corn and the similar of larvae collected and mortality factors of larvae in the laboratory between cropping systems suggest a high mortality rate of the pupal stage of this pest in the convential corn.

The percentage of larvae killed by microorganisms was similar between conventional and organic corns, but no larva collected in the third collection in both cropping systems of this study was killed by these microbes. The existing microorganisms in the field are able to act as entomopathogens and they can be also applied to the crops. The most common entomopathogens used in control strategy of *S. frugiperda* with high efficacy in terms of infection rate include *Bacillus thuringiensis* (Berliner, 1915) (Bacillales: Bacillaceae) (da Silva et al. 2016), baculoviruses (Baculoviridae) (Sousa et al. 2018), *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912) (Hypocreales: Cordycipitaceae), and *Metarizium anisopliae* (Metchnikoff) Sorokin (1883) (Hypocreales: Clavicipitaceae) (Gutiérrez-Cárdenas et al. 2019).

Parasitoids recovered from *S. Frugiperda* larvae infesting corn plants under conventional system and their distribution

The parasitoids recovered from larvae on corn plants, under conventional system, were *Archytas* sp.1 (Figure 3a), *Archytas* sp.2 (Figure

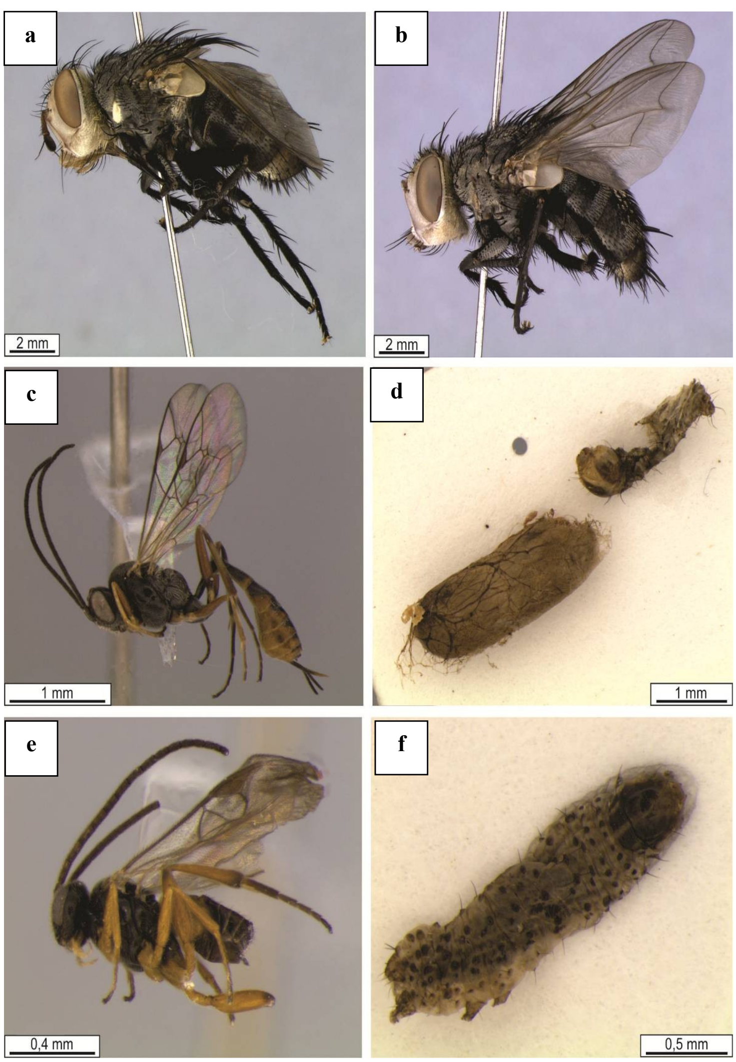

Figure 3. Parasitoids recovered from *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae infesting corn plants, *Zea mays* (Poaceae) under conventional system: *Archytas* sp.1 (Diptera: Tachinidae: Tachininae) (a), *Archytas* sp.2 (b), adult (c) and cocoon (d) of *Campoletis* sp. (Hymenoptera: Ichneumonidae: Campopleginae), and adult (e) of *Cotesia* sp. (Hymenoptera: Braconidae: Microgastrinae) and *S. frugiperda* larva dead after emergence of this parasitoid (f).

3b), *Campoletis* sp. (Figures 3c-3d), *Cotesia* sp. (Figures 3e-3f), *Dolichozele koebelei* Viereck, 1911 (Figures 4a-4c), *Eiphosoma laphygmae* Costa Lima, 1953 (Figures 4d-4e), *Eiphosoma* sp.1 (Figure 4f), *Eiphosoma* sp.2 (Figure 4g), *Euplectrus* sp. (Figures 5a-5b), *Exasticolus* sp. (Figures 5c-5d), *Hyphantrophaga* sp. (Figure 5e), and *Winthemia* sp. (Figure 5f). *Archytas* is reported as a parasitoid of *S. frugiperda* larvae in the Americas and Caribbean Basin with records in Puerto Rico (Pantoja et al. 1985), United States of America (USA) (Gross & Pair 1991), Argentina (Murúa et al. 2006), Brazil (Bortolotto et al. 2014), Mexico (Gurrola-Pérez et al. 2018), Paraguay (Cabral-Antúnez et al. 2018), and other nations. *Campoletis* is also recorded as a *S. frugiperda* larval parasitoid with reports in Brazil (Zanuncio et al. 2013), Mexico (Contreras-Cornejo et al. 2018), India (Sharanabasappa et al. 2019), Senegal (Tendeng et al. 2019), and other countries. Besides other territories, *Cotesia* is recorded as a *S. frugiperda* larval parasitoid in Nigaragua (Gladstone 1991), USA (Desneux et al. 2010) and Ethiopia, Kenya and Tanzania (Sisay et al. 2019). *Spodoptera frugiperda* larvae is recorded as being parasitized by *D. koebelei* (da Silva et al. 2014) and *E. laphygmae* (Figueiredo et al. 2006) in Brazil. Other *Eiphosoma* species are recorded as *S. frugiperda* larval parasitoid in countries such as Brazil (Melo et al. 2012) and

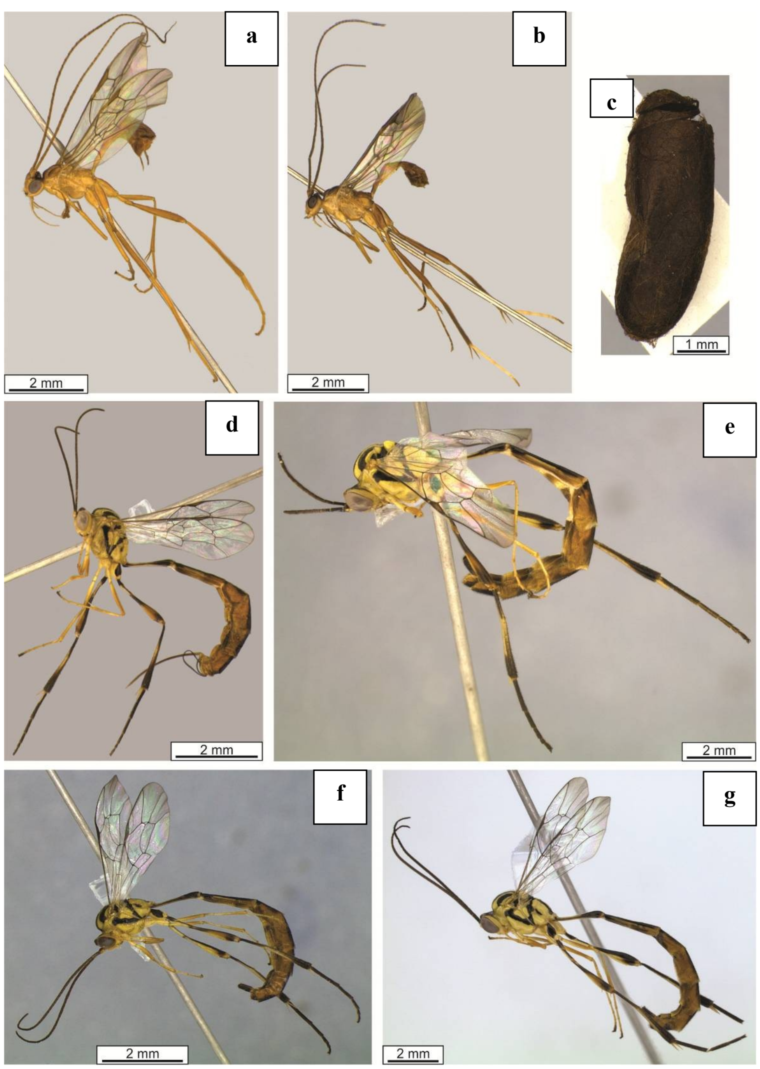

Figure 4. Parasitoids recovered from *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae infesting corn plants, *Zea mays* (Poaceae) under conventional system: female (a), male (b) and cocoon (c) of *Dolichozele koebelei* (Hymenoptera: Braconidae: Macrocentrinae), female (d) and male (e) of *Eiphosoma laphygmae* (Hymenoptera: Ichneumonidae: Cremastinae), *Eiphosoma* sp.1 (f), and *Eiphosoma* sp.2 (g).

Mexico (Salas-Marina et al. 2018). The genus *Euplectrus* is recorded as a larval parasitoid of *S. frugiperda* in Brazil (Sturza et al. 2013), USA (Hay-Roe et al. 2013), Mexico (Ordóñez-García et al. 2015b), and other lands. The genus *Exasticolus* is recorded as a *S. frugiperda* larval parasitoid in nations including Brazil (Figueiredo et al. 2006) and Paraguay (Cabral-Antúnez et al. 2018). Cuba and USA (Molina-Ochoa et al. 2003) are countries recorded as having *S. frugiperda* larvae parasitized by *Hyphantrophaga*, and Peru (Palomino 1965), USA (Rohlfs & Mack 1985), Mexico (Ruíz-Nájera et al. 2007), Brazil (Bortolotto et al. 2014), and Paraguay (Cabral-Antúnez et al. 2018) by *Winthemia*.

The parasitoids recovered from larvae on corn plants, under conventional system, were distributed into 1.0 ± 0.4, 0.1 ± 0.0, 0.1 ± 0.0, 0.1 ± 0.0, 0.2 ± 0.0, 0.2 ± 0.0, 0.1 ± 0.0, 0.05 ± 0.0, 0.05 ± 0.0, 0.05 ± 0.0, 0.3 ± 0.0, and 0.2 ± 0.0 individuals per collection, respectively (Table SIII).

Parasitoids recovered from *S. Frugiperda* larvae infesting corn plants under organic system and their distribution

The parasitoids recovered from larvae on corn plants, under organic system, were *Archytas* sp.1, *Archytas* sp.2, *Cotesia* sp., Cremastinae (Figures 6a-6b), *D. koebelei*, *E. laphygmae, Eiphosoma* sp.1, *Eiphosoma* sp.2, *Euplectrus* sp., *Exasticolus*

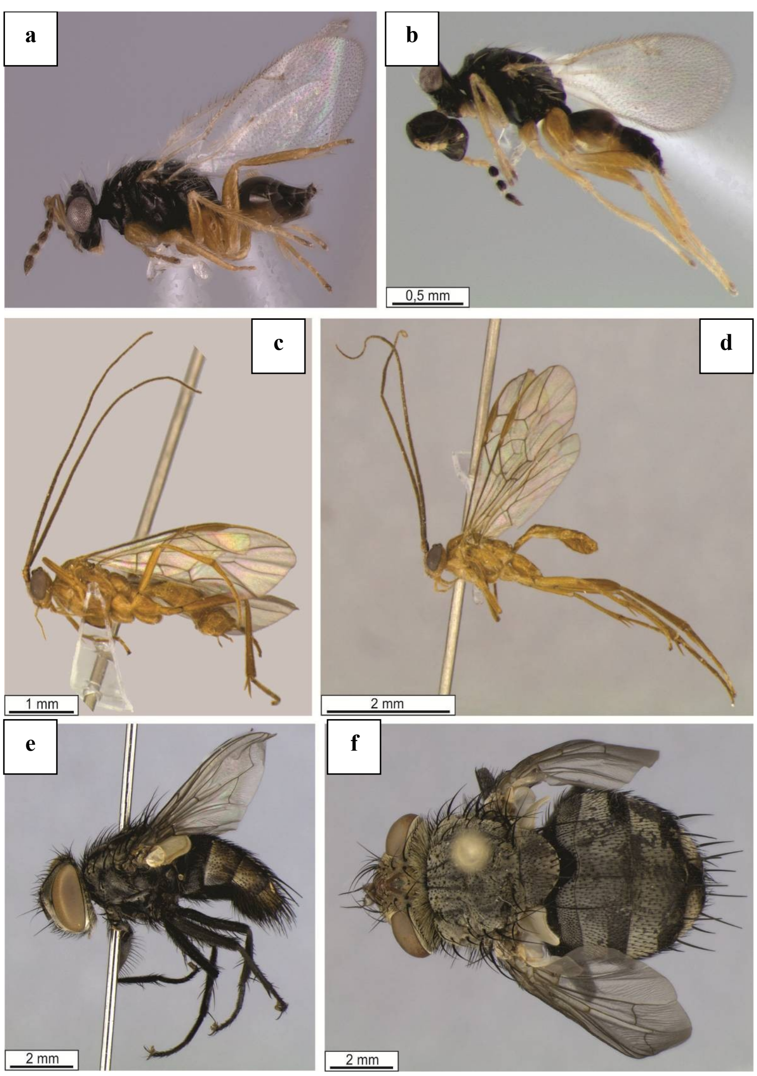

Figure 5. **Parasitoids recovered from *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae infesting corn plants, *Zea mays* (Poaceae) under conventional system: female (a) and male (b) of *Euplectrus* sp. (Hymenoptera: Eulophidae: Eulophinae), female (c) and male (d) of *Exasticolus* sp. (Hymenoptera: Braconidae: Homolobinae), *Hyphantrophaga* sp. (Diptera: Tachinidae: Exoristinae) (e), and *Winthemia* sp. (Diptera: Tachinidae: Exoristinae) (f).**

sp., *Hyphantrophaga* sp., *Ophion flavidus* Brullé, 1846 (Figures 6c-6e), and *Winthemia* sp. Several Cremastinae species are recorded as larval parasitoids of *S. frugiperda* in countries including Brazil (Melo et al. 2012) and USA (Meagher Jr. et al. 2016). *Ophion flavidus* is recorded as a *S. frugiperda* larval parasitoid in USA (Rohlfs & Mack 1985, Gross & Pair 1991, Hay-Roe et al. 2016), Nicaragua (Gladstone 1991), Brazil (Fernandes et al. 2014), and Mexico (Ordóñez-García et al. 2015a).

The parasitoids recovered from larvae on corn plants, under organic system, were distributed into 0.6 ± 0.1, 0.4 ± 0.0, 0.2 ± 0.0, 0.05 ± 0.0, 0.4 ± 0.1, 1.0 ± 0.3, 0.3 ± 0.0, 0.05 ± 0.0, 0.1 ± 0.0, 0.2 ± 0.0, 0.1 ± 0.0, 0.05 ± 0.0, and 0.1 ± 0.0 individuals per collection, respectively (Table SIII).

Most of the parasitoids recovered had occurrence in both cropping systems of this study, except Cremastinae and *O. flavidus*, which were recorded only in organic corn. *Campoletis* sp. was recovered only in conventional corn. The number of parasitoids recovered was similar in corn plant samples between both cropping systems, except *E. laphygmae*, which occurred in a higher number in organic corn (Table SIII). Although Cremastinae and *O. flavidus* occurred only in organic and *Campoletis* sp. only in convential corn, a low number of individuals of these species was recovered. *Ophion flavidus* has a sazonal distribution with the highest number of individuals recovered from corn crops in Tifton, Georgia, USA by mid-June and ability to parasitize the fourth, fifth and sixth instars with equal success (Gross & Pair 1991). *Campoletis* requires normally a high number of its individuals and of available hosts (i.e. third instar larva) for successful parasitism and its sex ratio is largely affected by host body size (Patel & Habib 1987, Matos Neto et al. 2004). The similar number of parasitoids recovered between organic and conventional corns can be explained by the use of modern cultivation

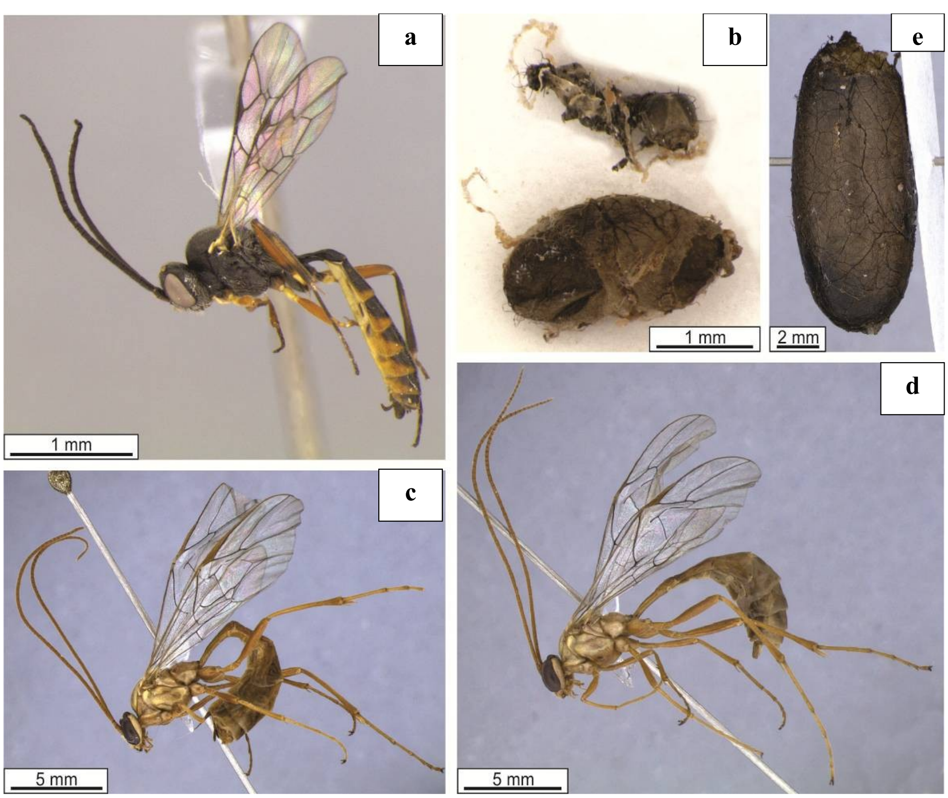

Figure 6. Parasitoids recovered from *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae infesting corn plants, *Zea mays* (Poaceae) under organic system: adult (a) and cocoon (b) of Cremastinae (Hymenoptera: Ichneumonidae) and female (c), male (d) and cocoon (e) of *Ophion flavidus* (Hymenoptera: Ichneumonidae: Ophioninae).

techniques in the latter with low impact on natural enemies such as no-till system, selective pesticides and presence of areas preserved surrounding the corn crop as a refugy for natural enemies. The highest number of *E. laphygmae* in organic corn agrees with report of the greatest abundance, richness and diversity indexes of *Eiphosoma* species in organic cropping systems with median intensity than those with higher intensity of management (Onody et al. 2012).

Number, larva length, mortality factors, and viability of *S. Frugiperda* on corn ear and stem, under conventional system

A total of 152 and four larvae was recovered from corn ears and stems, with an average of 30.4 and 1.3 individuals per collection and an average larva length of 1.6 and 0.6 cm, respectively under conventional system (Tables SIV and SV). This result confirms the greater preference of *S. frugiperda* larvae to infest the cartridge and ear over the stem of corn plants (Silva et al. 2020).

A total of 0.6% of the larvae recovered from corn ears, under conventional system, was parasitized by dipteran and hymenopteran, 9.2% killed by microorganisms, 4.6% killed by undetermined causes, 7.9% origined inviable pupae, and 77.7% reached adulthood (Table SIV).

Collections of larvae from corn stems, under conventional system, resulted in 25.0% of them killed by microorganisms and 75.0% reached adulthood (Table SV). Although being a minor pest on corn ears, the numbers of *S. frugiperda* observed on ears are lower than that of the corn earworm, *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) (Rodríguez-del-Bosque et al. 2010). *Spodoptera frugiperda* larvae are able to feed on the ear peduncle, preventing grain formation; and cause direct damage to the grains by feeding on the ear top (Cruz et al. 2012, da Silva et al. 2014, Figueiredo et al. 2015).

Number, larva length, mortality factors, and viability of *S. Frugiperda* on corn ear, under organic system

A total of 31 larvae was collected from corn ears, under organic system, with an average of larvae per collection of 6.4 and an average body length of 1.7 cm. A total of 6.4% of the larvae was parasitized by dipteran and hymenopteran, 3.2% killed by microorganisms, 3.2% origined inviable pupae, and 87.2% reached adulthood (Table SIV).

Parasitism of *S. Frugiperda* larvae on corn plants under conventional and organic systems

Spodoptera frugiperda larvae were parasitized by dipteran and hymenopteran, confirming them as the most prevalent parasitoids of this pest (Hay-Roe et al. 2016, Meagher Jr. et al. 2016, Sisay et al. 2018). The percentage of larvae with emergence of parasitoids, in conventional corn, ranged from 0.0 to 12.5%. The lowest percentage of parasitism (0.0%) was obtained in the fourth, seventh and 19th collections, while the highest (12.5%) in the 11th collection (Figure 2a). The percentage of larvae with emergence of parasitoids, in organic corn, ranged from 0.8 to 12.5%. The lowest percentage of parasitism (0.8%) was obtained in the seventh collection, while the highest (12.5%) in the 20th collection (Figure 2b).

The percentage of larvae parasitized was similar between conventional and organic corns, although no parasitized larva was found in the fourth, seventh and 19th collections in conventional corn (Table SII). The percentage of *S. frugiperda* larvae parasitized was similar between conventional and organic corns with parasitism rate by 11.3% in Mexico (Molina-Ochoa et al. 2001). The similarities in percentage of *S. frugiperda* larvae parasitized between the two studied cropping systems can be explained by the similar number of parasitoid individuals in these systems.

Parasitoids recovered from *S. Frugiperda* larvae infesting corn ear under conventional and organic systems and their distribution

The parasitoids recovered from larvae on corn ears, under conventional system, were *Glyptapanteles* sp. (Figures 7a-7b) and *Hyphantrophaga* sp. *Glyptapanteles* is reported as a larval parasitoid of *S. frugiperda* in countries such as Mexico (Molina-Ochoa et al. 2003) and India (Shylesha et al. 2018). The current study reports, for the first time, the parasitism of *S. frugiperda* by *Glyptapanteles* in Brazil.

The parasitoids recovered from larvae on corn ears, under conventional corn, were distributed into an average of 0.2 individuals per parasitoid species (Table SVI).

The parasitoids recovered from larvae on corn ears, under organic system, were *Hyphantrophaga* sp. and *Microcharops* sp. (Figures 8a-8b). They were distributed into 0.2 individuals per parasitoid species per collection (Table SVI).

Percentage of parasitoids recovered from *S. Frugiperda* larvae infesting corn ear, under conventional and organic systems

The percentage of parasitoids recovered from larvae on corn ears was 50% of *Glyptapanteles* sp. and 50% of *Hyphantrophaga* sp. under conventional system, while it was 50% of *Hyphantrophaga* sp. and 50% of *Microcharops* sp. under organic system (Table I)

Percentage of parasitoids recovered from *S. Frugiperda* larvae infesting corn cartridge, under conventional and organic systems

The most numerous parasitoid of larvae on corn plants, under conventional system, was *Archytas* sp.1 (35.5%), followed by *Hyphantrophaga* sp. (11.8%), *D. koebelei* and *Winthemia* sp. (9.8%), *E. laphygmae* (7.8%), and *Archytas* sp.2 and *Eiphosoma* sp.1 (5.9% each). Less numerous parasitoids were *Campoletis* sp. and *Cotesia* sp. (3.9% each), *Eiphosoma* sp.2, *Euplectrus* sp. and *Exasticolus* sp. (1.9% each) (Table II).

The most numerous parasitoid of larvae on corn plants, under organic system, was *E. laphygmae* (26.7%), followed by *Archytas* sp.1 (16.0%), *Archytas* sp.2, *D. koebelei* and *Eiphosoma* sp.1 (10.7% each), and *Cotesia* sp. and *Exasticolus* sp. (5.3% each). Less numerous parasitoids were *Euplectrus* sp. and *Winthemia* sp. (4.0% each), *Hyphantrophaga* sp. (2.7%), and Cremastinae,

Figure 7. Parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae infesting corn ears, *Zea mays* (Poaceae) under conventional system: adult (a) and cocoons (b) of *Glyptapanteles* sp. (Hymenoptera: Braconidae: Microgastrinae).

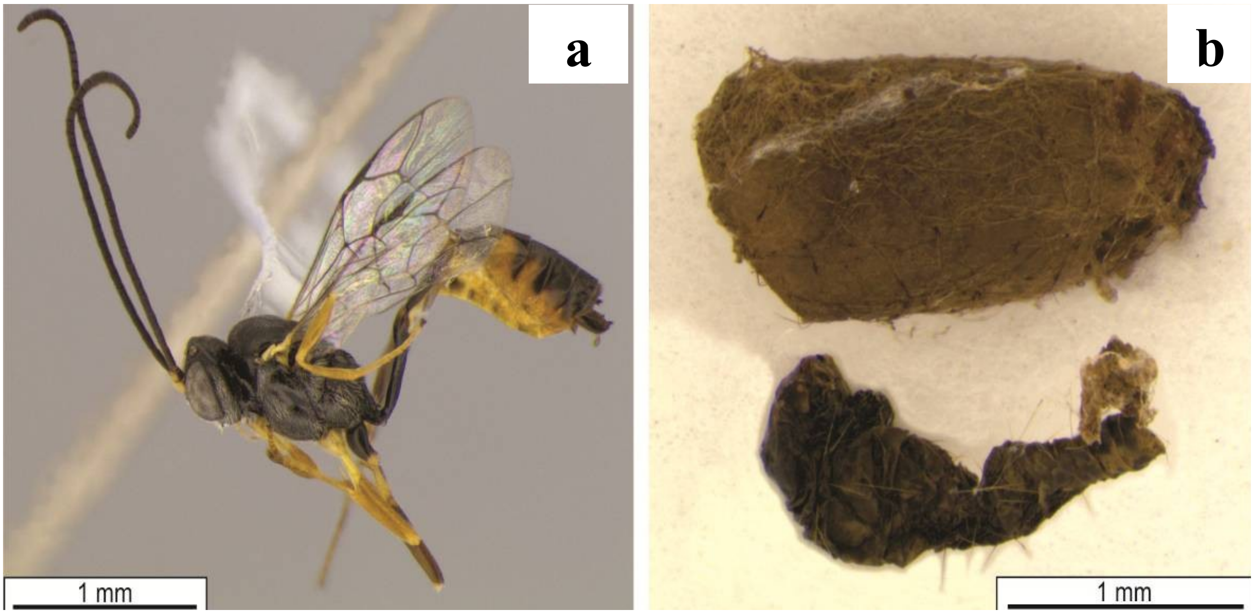

Figure 8. **Parasitoid of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae infesting corn ears, *Zea mays* (Poaceae) under organic system: adult (a) and coccon (b) of *Microcharops* sp. (Hymenoptera: Ichneumonidae: Campopleginae).**

Table I. **Percentage of parasitoids emerged and mean length of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae, recovered from corn ears, *Zea mays* (Poaceae) under conventional and organic systems in Sete Lagoas, Minas Gerais state, Brazil.**

Parasitoids	Corn production systems			
	Conventional (%)	Mean length of the larva parasitized (cm)	Organic (%)	Mean length of the larva parasitized (cm)
Glyptapanteles sp.	50.0	1.5	-	-
Hyphantrophaga sp.	50.0	1.8	50.0	2.0
Microcharops sp.	-	-	50.0	1.5
Total	100.0		100.0	

Eiphosoma sp.2 and *O. flavidus* (1.3% each) (Table II).

Archytas sp.1 was prevalent in conventional corn, while *E. laphygmae* was prevalent in organic corn. Dipteran parasitoids were prevalent in conventional corn, while hymenopteran parasitoids were prevalent in organic corn.

Predators of *S. Frugiperda* on corn plants, under conventional and organic systems

The earwig, *Doru luteipes* (Scudder, 1876) (Dermaptera: Forficulidae: Forficulinae) adults and/or nymphs (Figures 9a-9b) were recovered from all samples, under conventional system, with a total of 99 specimens distributed into 4.2 individuals per collection. A low number of the minute pirate bug, *Orius* sp. (Hemiptera: Anthocoridae: Anthocorinae) was found with individuals recovered from few samples, in conventional corn (Figure 9c). *Doru luteipes*

Table II. **Percentage of parasitoids emerged and mean length of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae, recovered from corn plants, *Zea mays* (Poaceae) under conventional and organic systems in Sete Lagoas, Minas Gerais state, Brazil.**

Parasitoids	Corn production systems			
	Conventional (%)	Mean length of the larva parasitized (cm)	Organic (%)	Mean length of the larva parasitized (cm)
Archytas sp.1	35.5	1.9	16.0	1.7
Archytas sp.2	5.9	2.0	10.7	1.8
Campoletis sp.	3.9	0.7	-	-
Cotesia sp.	3.9	1.0	5.3	0.9
Cremastinae	-	-	1.3	0.5
Dolichozele koebelei	9.8	0.8	10.7	1.5
Eiphosoma laphygmae	7.8	1.2	26.7	1.1
Eiphosoma sp.1	5.9	1.7	10.7	1.1
Eiphosoma sp.2	1.9	1.9	1.3	1.0
Euplectrus sp.	1.9	1.9	4.0	1.3
Exasticolus sp.	1.9	1.9	5.3	1.2
Hyphantrophaga sp.	11.8	1.9	2.7	2.0
Ophion flavidus	-	-	1.3	2.0
Winthemia sp.	9.8	2.0	4.0	1.7
Total	100.0		100.0	

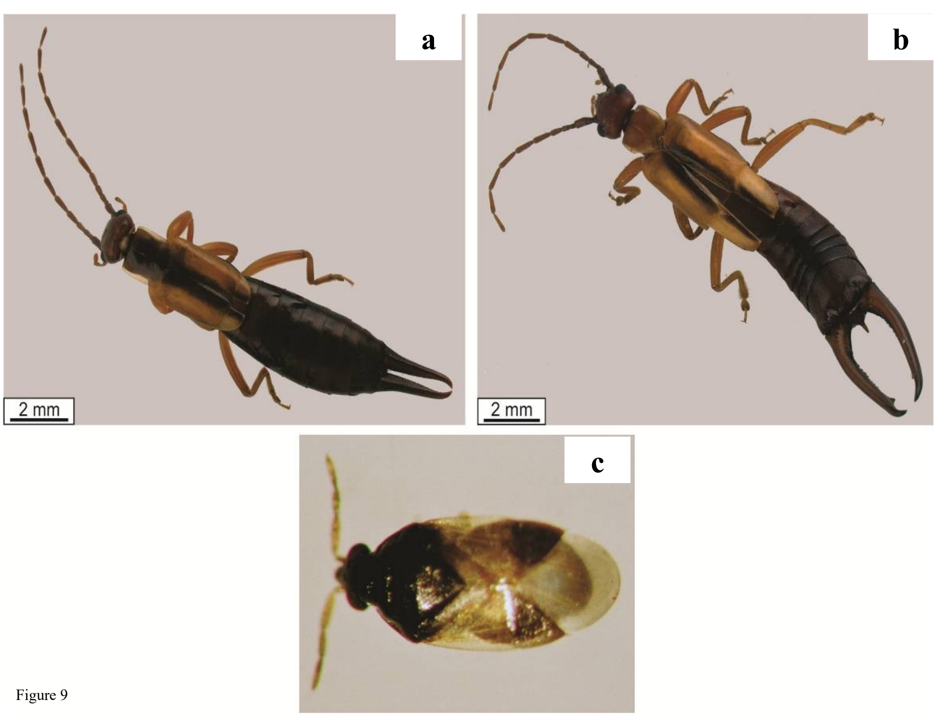

Figure 9. **Predators of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) recovered from corn plants, *Zea mays* (Poaceae): female (a) and male (b) of earwig, *Doru luteipes* (Dermaptera: Forficulidade: Forficulinae) and adult of the minute pirate bug, *Orius* sp. (Hemiptera: Anthocoridae: Anthocorinae) (c).**

is recorded as an important predator of *S. frugiperda* eggs and small larvae in Brazil (Reis et al. 1988, Figueiredo et al. 2006).

Adults and/or nymphs of *D. luteipes*, with a total of 137 specimens distributed into 5.7 individuals per collection, under organic corn, were recovered from all collections. A low number of *Orius* sp. was found with individuals recovered from few samples, in organic corn. *Orius* is an important predator of *S. frugiperda* eggs on corn (Varella et al. 2015).

The diversity of parasitoid species was higher in the cartridge of organic corn, followed by cartridge of conventional corn and ear in both conventional and organic corns (Table SVII).

The number of *S. frugiperda* larvae collected over the collection period was low, but the new associations and parasitoid species reported represent possibility of using these natural enemies to manage this pest in corn crops.

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SUPPLEMENTARY MATERIAL

Tables SI-SVII.

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