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NATURAL CONTROL OF *Helicoverpa armigera* (LEPIDOPTERA: NOCTUIDAE) PUPAE IN ORGANIC AND CONVENTIONAL MAIZE CROPS

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Abstract – The natural biological control of soil pests is poorly studied. Notably, the control of *Helicoverpa armigera* in the pupae stage is unknown. To increase knowledge about the control of this pest in organic and conventional maize crop, tests were conducted to verify if the duration of pupae availability in days, the type of crop treatment (organic and conventional), the stage of crop development, and the depth of the soil significantly affect predation by natural enemies. The pupae availability time (days) in the soil did not affect their removal by natural enemies. However, in the fallow stage, on the surface and in the reproductive phase, the predation was higher. In organic maize, predation was 15% higher when compared to conventional maize. The rupture of the soil and the possible losses associated with beneficial fauna were the main factors responsible for higher predation during fallow, so conservationist practices usually used in organic treatment are the main reason for higher predation in this type of crop. There is a significant decrease in the control of *H. armigera* pests by natural enemies when maize is grown using conventional practices, what reinforces the importance of the conservation techniques used in maize crops.

Keywords: Natural biological control, Edaphic fauna, Conservation, Old world cotton bollworm, Environmental services.

CONTROLE NATURAL DE PUPAS DE *Helicoverpa armigera* (HÜBNER)(LEPIDOPTERA:NOCTUIDAE) EM MILHO ORGÂNICO E CONVENCIONAL

Resumo - O controle biológico natural de pragas no solo é pouco estudado. Particularmente, o controle da praga *Helicoverpa armigera* na fase de pupa é desconhecido. Para aumentar o conhecimento sobre o controle dessa praga em milho orgânico e convencional, testes foram realizados para verificar se a duração da disponibilidade de pupas em dias, o tipo de tratamento de cultivo (orgânico e convencional), o estágio de desenvolvimento da cultura e a profundidade do solo afetam significativamente a predação por inimigos naturais. O tempo de disponibilidade (dias) das pupas no solo não afetou sua remoção pelos inimigos naturais. No entanto, no estágio de pousio, na superfície e na fase reprodutiva, a predação foi maior. No milho orgânico, a predação foi 15% maior quando comparado com o convencional. A ruptura do solo e as possíveis perdas associadas à fauna benéfica foram o principal fator responsável pelas maiores predações durante o pousio, assim práticas conservacionistas usualmente utilizadas no tratamento orgânico são a principal razão para as predações mais elevadas neste tipo de cultivo. Há uma diminuição significativa no controle de pragas de *H. armigera* por inimigos naturais quando o milho é cultivado usando práticas convencionais. Isto reforça a importância das técnicas de conservação usadas no cultivo de milho.

Palavras-chave: Controle biológico natural, Fauna edáfica, Conservação, Old world cotton bollworm, Serviços ambientais.

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Maize (*Zea mays* L.) is one of the major cereals grown in the world. In Brazil, maize is a crop of both economic and social importance, being produced in the conventional or organic system (Campanha et al., 2012). In the conventional system, there is a strong dependence on external inputs such as insecticides and chemical fertilizers and modified processes of pest and disease self-regulation affecting stability, resilience, and self-sufficiency of agricultural ecosystems (Yu et al., 2018). In the organic system, methods of soil conservation and recovery and natural control of pests and diseases are frequent (Paschoal, 1994). Severe attacks of caterpillars of the species known as *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) have been reported in Brazil (Bueno et al., 2014). Czepak et al. (2013) reported the first occurrence record of *H. armigera* in the country, in the states of Goiás and Bahia, soybean crop, and in Mato Grosso state, cotton crop. The *H. armigera* is a polyphagous species whose larvae have been recorded in more than 60 species of wild and cultivated plants, and about 67 host families including Asteraceae, Fabaceae, Malvaceae, Poaceae and Solanaceae (Pogue, 2004). This wide range of host families signifies, therefore, damage to different economically essential crops such as cotton, legumes in general, sorghum, maize, tomatoes, and ornamental and fruit plants (Moral Garcia, 2006).

H. armigera passes through the developmental stages of egg, larva, pre-pupa, pupa, and adult (Naseri et al., 2011). The pre-pupa phase is comprised between the stage when the caterpillar stops feeding until the pupa phase (Ali & Choudhury, 2009). All pupal development occurs on the ground and, depending on the climatic conditions, it can enter diapause (Karim, 2000). When they are in an optional diapause, pupae can spend up to 140 days on the ground, usually in response to the conditions of short days and high temperatures, varying between 33 and 39ºC, inducing summer diapause (Liu et al., 2006). In the same way, low temperatures also promote winter diapause (Albernaz et al., 2014; Chen et al., 2014). Host plants, in terms of nutritional adequacy, can also affect the hibernation of pupae, and the nutritional adequacy of host plants can affect the potential for hibernation and survival, defining the intensity of pest outbreaks in subsequent years (Liu et al., 2010). Thus, the success of this insect pest, in general, happens as a result of its survival strategies, such as the use of several hosts, high fertility, dispersion capacity and the ability to enter diapause, thus being able to adapt to different conditions of agroecosystems and climate (Cleary et al., 2006). The pupal stage of *H. armigera* develops in soil, where they build tunnels around 10 cm deep and form pupal chambers (Araújo, 1990; Ávila et al., 2013). At this stage, the action of natural enemies as pupal predators or parasitoids (Schiavon et al., 2015) may lead the pest to death. Considering the establishment of *H. armigera* as a pest of maize, the damages caused, and the scarcity of studies on the soil fauna related to the control of agricultural pests, this study intends to investigate its natural control. The objective was to evaluate maize organic or conventional crop effect on the natural control of *H. armigera.*

Materials and Methods

The research was conducted at the experimental fields and the Insect Rearing Laboratory (LACRI) of the Brazilian Agricultural Research Corporation (Embrapa Maize & Sorghum), located in the municipality of Sete Lagoas, state of Minas Gerais, Brazil. The experiment was conducted in two maize crops systems, one under organic treatment (19º28”13’S and 44º10”37’W) and the other under conventional treatment (19º26”46’S and 44º10”11’W). Both areas received the same soil preparation, where they were worked with heavy grid and leveler and were mechanically seeded, using the same BRS 451 maize seed, at a density of six seeds per linear meter of row, being each row spaced 0.70 cm apart. Organic compound was used as fertilizer in the organic production system, while in the conventional area, the formula of chemical fertilizer N:P:K (4-14-8) was used, in the concentration of 500 kg ha^{-1}. Synthetic organic insecticides were not used in any of the areas.

Black plastic pots, 22.5 cm tall and 19 cm in diameter, were used to determine the average pupae burial depth and enable their subsequent burial at the same depths in the field, filled with soil taken from the region near the conventional crop. One *H. armigera* caterpillar of the last instar was placed in each pot, which contained a prepared piece of artificial diet on the soil surface to avoid caterpillar death by starvation. The pots were sealed with the use of tulle with a mesh size of less than 1 mm. The caterpillars naturally inserted themselves into the soil and passed to the pre-pupal stage. Five days later, the pupae were dug up and the depth at which they were found was measured in each pot, after careful removal of soil and measuring of depth.

Because it is a relatively static phase, pupae of the insect were used in the experimental test, thus allowing their recovery in the place where they were left, if not removed from there by some other organism. All pupae used in the field experiment came from a rearing lab colony, where the insects stay in a room at 25±1°C, with relative humidity of 70±10% and a photoperiod of 12 hours. Sampling took place in three moments: before planting, in the vegetative phase V6, and during the reproductive phase, with the maize ear already formed. The implementation of the experiment before the establishment of the crop was because *H. armigera* pupae could be in diapause state buried in the soil. The other two moments are justified because *H. armigera* can cause damage both in the vegetative and in the reproductive stages of maize.

Based on the result of the pre-pupae burying experiment, an average depth of 14 cm was found. Thus, the pupae on the surface and at two other depths are close to the average found in this test with pots. *H. armigera* pupae were located at three depths (0 cm – surface, Fig. 1A; 10cm, Fig. 1B; 20cm, Fig. 1C). The pupae that remained on the surface was representative of when *H. armigera* begins to form the gallery in the soil to bury itself, a stage when the insect can already be preyed on or parasitized by biological control agents. Eighteen holes were opened with an 8 cm diameter drill for each depth, that is, 54 sampling points for each treatment (crop system) received a single pupa, one to two days old, being regularly distributed and spaced 1 m apart (Fig. 2).

Evaluation of pupae condition happened soon after the removal of the soil containing the insect pupa on the fifth and tenth day after insect placement in the soil, in each cultivation period (fallow, vegetative or reproductive phase). Each portion of soil removed corresponded to a sample obtained with the aid of a square template, 25 cm per side and 20 cm high, adjusted to the desired depth (10 or 20 cm), with the use of a mallet for its penetration into the soil. A small trench opened next to the template, at the desired depth, facilitated the soil cut. A trenching shovel cut the soil away from underneath the template, completely removing it (Fig. 3). Removal and registration of pupae occurred for each soil sample after sieving at the laboratory, with three possible responses: 1) pupa absence or presence of its parts, indicating removal by predators; 2) pupa present, but immobile, with signs of perforations that are typical of parasitoid action (with little chance of parasitism due to the absence of gallery made by *H. armigera* when burying itself) and; 3) pupa present and mobile, indicating survival during the period of

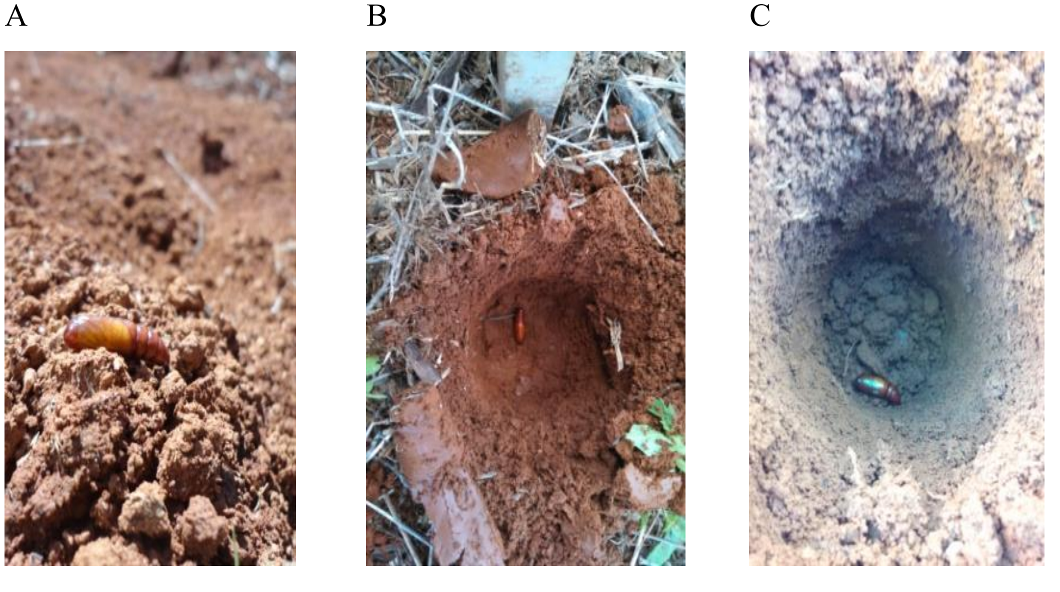

Figure 1. Pupa of *Helicoverpa armigera* on the surface (A), at 10 cm (B) and 20 cm (C) of depth in the soil, in conventional and organic maize cultivation treatments, in an area belonging to Embrapa Maize & Sorghum, in the municipality of Sete Lagoas, Brazil.

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Figure 2. Details of conventional maize crop (A) and organic maize crop (B) in an area belonging to Embrapa Maize & Sorghum, in the municipality of Sete Lagoas, Brazil. Stakes indicate the burial position of each pupa in the areas.

A B

Figure 3. Representation of the template on the ground (A) at the desired depth and template removal with a trench shovel (B). This process was carried out in the maize crop, with conventional treatment and organic treatment, in the area belonging to Embrapa Maize & Sorghum, in the municipality of Sete Lagoas, MG.

natural enemies. Other edaphic macro-organisms identified by date, crop, and sample point stay in a 96% alcohol dilution.

Student's t-test was used to measure *H. armigera* availability period on its removal, where the response variable (continuous, quantitative) was the number of pupae removed and the independent variable (qualitative with

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two levels) was the pupae permanence time (five or ten days) of availability.

All dependent variables presented homogeneity of variance and normal residues. The Analysis of Variance (ANOVA) was used with three factors to evaluate the hypotheses that there is more substantial removal of pupae during the reproductive phase of the maize, in the organic production system, and at lower depths. Confidence Interval of ninety-five percent measured the significant relationships identified in the Analysis of Variance.

Temperature and rainfall data, for the period the pupae remained exposed in the fields (11 consecutive days), were obtained from the automatic weather station available at Embrapa and were compared, using ANOVA, for the three cultivation periods (fallow, vegetative phase, reproductive phase) to assist in understanding the results. A 5% significance level was considered for the tests.

Results and Discussion

The average burial depth of the pupae was 7.8 cm, ranging from 4 to 12.5 cm. Therefore, for the field experiment, depths of 0 (soil surface), 10 and 20 cm, applied for the placement of pupae and their removal analysis, represent a range close to reality.

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There was no significant difference in the removal of *H. armigera* pupae when placed on the soil surface ($t_{var.\ sep.(34)}$= -0.995; p=0.356), or at 10 cm ($t_{(34)}$= -1.001; p= 0.341), or at 20 cm ($t_{(34)}$=-1.195; p=0.259). Thus, the residence period (days) of the pupae in the soil did not alter the rate of removal by predators, probably due to the non-perception of the pest by the beneficial organisms (Fig 4).

With no difference in the removal rate for pupae exposure between 5 and 10 days, the removal data of the two dates were then added, allowing the performance of a three-way ANOVA. This analysis indicated an effect of time ($F_{(2,18)}$=5.707; p=0.012), depth ($F_{(2,18)}$=4.259; p=0.031), cultivation treatment ($F_{(1,18)}$=8.345; p=0.010) and the interaction between depth and time ($F_{(4,18)}$=5.991; p=0.003; Fig 4) for the *H. armigera* pupae removal. However, there was no significant interaction between time and cultivation treatment ($F_{(2,18)}$=0.534; p=0.595), depth and cultivation treatment ($F_{(2,18)}$=1.259; p=0.308) as well as the triple interaction between time, depth and cultivation treatment ($F_{(4,18)}$=0.509; p=0.730).

In the maize crop under organic production system, the pupa removal was 15% higher than in the conventional treatment (Fig 5). Several studies have pointed to the increase of organism richness and diversity in crops with fewer management practices.

The extended availability of *H. armigera* pupae in the soil increases the probability of them being found by their agents of natural biological control and, therefore, increasing their frequency of removal; what is expected according to the

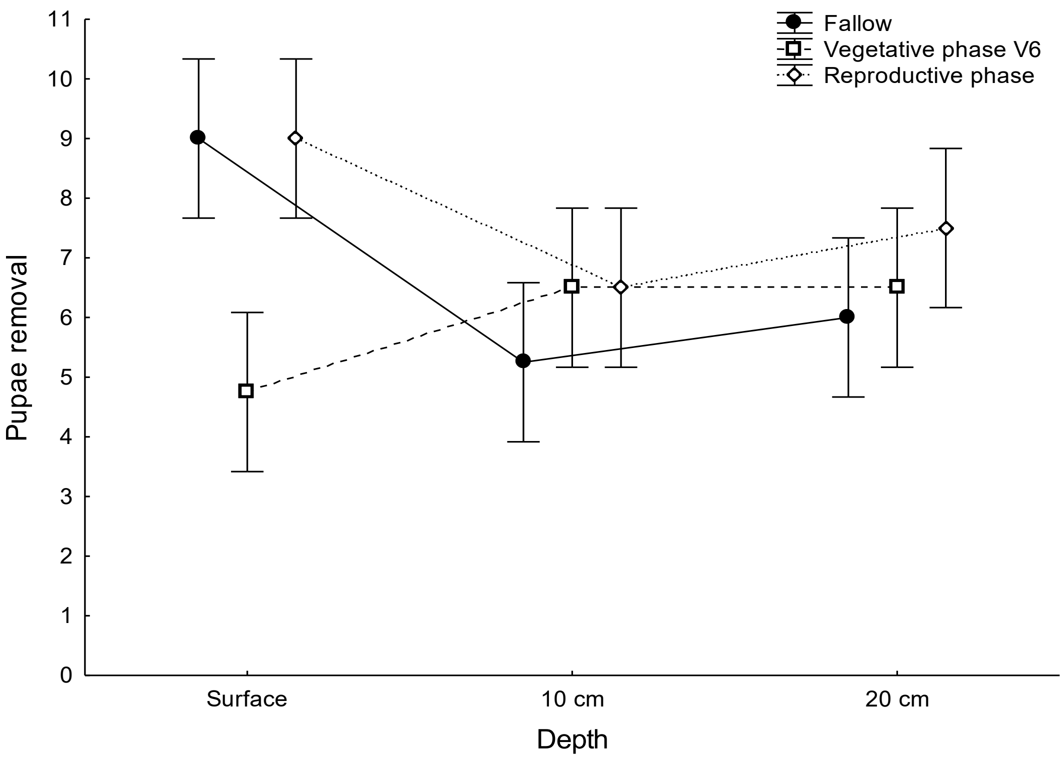

Figure 4. *Helicoverpa armigera* pupae removal rate, at different soil depths (surface, 10 and 20 cm) during fallow, vegetative phase and reproductive phase of maize crops, cultivated under conventional and organic treatments in an area of Embrapa Milho and Sorgo, municipality of Sete Lagoas, MG

ecological theory of predator-prey interactions (Abrams, 2000; Barbosa & Castellanos, 2005). Additionally, a higher rate of removal can be expected in an organic system of maize cultivation, where no chemicals are used to kill or repel beneficial organisms that use the pupae as food sources (Dainese et al., 2017; Krauss et al., 2011; Winqvist et al., 2012). More substantial removal of *H. armigera* pupae closer to the surface may also be expected, since there may be coexistence of organisms that live on the surface or just below it at the site. At greater depths, the characteristics of the habitat are more specific and with smaller number of species, which reduces the probability of *H. armigera* removal. During the vegetative and reproductive stages of the host plant, more significant removal of pupae may occur due to the increased environmental heterogeneity and the availability of temporary insect resources that may be attracted to lead to natural biological control consequently.

No pupae recovered, alive or dead, showed signs of parasitism, confirmed by the emergence of adult insects from live pupae, thus supporting the hypothesis presented in the material and methods section. This was probably due to the

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Figure 5. . *Helicoverpa armigera* pupae removal in maize crops cultivated under conventional and organic treatments in an area belonging to Embrapa Maize & Sorghum, in the municipality of Sete Lagoas, MG.

absence of the gallery that the larva digs to lodge in the ground. This gallery has chemical signals emitted by the pupae, such as kairomones (Parra et al., 2002), which are perceived by the parasitoids and so allowing their encounter.

Farwig et al. (2009) showed that the presence of sacrificed crickets, left for a longer period on the soil surface, increased their perception by vertebrate and invertebrate organisms, which consequently increased their removal. However, the greater availability of *H. armigera* pupae did not increase their removal in conventional or organic maize crop. These pupae were alive and possibly their attractiveness did not increase with time. Still, in this study, the authors do not mention the cricket sacrifice method, which may have influenced the decomposition process, increasing their attractiveness to removers over time. Despite these differences, the expectation for *H. armigera* was that the removal degree would be higher with time, since, with the more extended pupae availability, chances of them being found and removed would increase.

In general, the removal of pupae on the surface was higher in the fallow period and reproductive phase. These removals were also higher than any other at 10 cm or 20 cm of depth, at any stage (fallow, vegetative phase and reproductive phase). These results corroborate the expectations of the study, as they foresaw higher removal on the surface due to the likely higher organism richness and diversity in this location. An exception is the lowest removal value detected at any depth (0, 10 or 20 cm), at any stage (fallow, vegetative phase and reproductive phase) in the fields for the surface pupae removal during the vegetative phase.

Also, a plausible explanation is that, with the tillage using heavy harrow and then disking, the community of beneficial organisms on the soil surface may have had their activity reduced due to the disruption of their populations and consequent interactions. In fact, after the soil disturbance (during soil preparation), there were some birds highly active preying on the exposed organisms. The assessment of removal, during the vegetative stage, took place eight days after disking in the fields, which was probably insufficient time for restructuring the biological community associated with the soil, especially that of the surface. Moreover, when farming practices involve soil preparation, they disrupt the soil natural communities when compared to crops with no-tillage.

In fields with crop rotation, Baretta et al. (2006) evaluated the effect of tillage on edaphic diversity in four treatments: conventional with plowing and disking; minimum tillage with scarification and harrowing; direct seeding; and conventional treatment with no vegetation and no surface crust. The diversity of organisms was higher when applying direct seeding and minimum tillage preparation systems. Similar results were found in studies carried out in the state of Mato Grosso do Sul, Brazil, in agroforestry systems (Heid et al., 2012); in vineyard and maize cultivation in Italy (Gagnarli et al., 2015); and in France with barley, alfalfa, oat, soy, rye and wheat cultivation (Pelosi et al., 2014). Therefore, this present result is in agreement with those found in the literature, where it is evident that soil movement through agricultural practices negatively influences the soil fauna of a particular location and hence compromises potential benefits that this fauna can bring such as, for example, removal of a pest.

Regarding the higher removal during the fallow period, this probably occurred because the availability of resources for remover organisms on the soil surface was low at that time. Thus, the inclusion of pupae in the environment, before maize planting, meant providing resources for those organisms present in the target area, both on the soil surface as well as in the subsurface. The more significant removal of *H. armigera* pupae in the fallow period could be expected, considering that in areas in constant agricultural use, pasture, or even invaded by grasses, the environment is less diverse. In these situations, the environment is also deficient in species as initial time of ecological succession (Whittaker, 1965) when few dominant species have high population abundance, and the community follows a geometric pattern of abundance

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distribution (Magurran, 1988).

In Italy, Simoni et al. (2013) evaluated how the abundance and diversity of arthropods are influenced by maize crops under conventional and organic treatment and found higher abundance and diversity of insects in organic farming areas. In the city of Granada, Spain, Jerez-Valle et al. (2014) found higher insect richness and diversity in organic cultivation without conventional tillage. Therefore, under conventional treatment, the use of agrochemicals and implements appear to affect the beneficial organisms community, thus affecting the consequent benefits from it (Baretta et al., 2014).

In Chapecó, state of Santa Catarina, Brazil, Baretta et al. (2006) studied the influence of soil management in crop rotation, grazing, and Atlantic Forest remnant areas, on the diversity of some insect groups. They found higher abundance and richness of insects in areas with minimum tillage and direct sowing. Cividanes (2002), in an intercropped maize and soybean field in Jaboticabal, state of São Paulo, Brazil, found increased activity of Hymenopteran organisms, especially ants, and greater abundance of Dermapteran in the no-till system. More recently, Ricci et al. (2015), in France, in cereal crop, reported that the intensification of agriculture reduced soil biodiversity in areas with different fertilization and cultivation system and found a higher abundance of earthworms in organic fields without application of pesticides and direct seeding.

Despite all of these studies pointing to an increase in the effectiveness of pest control in conservation and organic farming systems, it is still possible that the removal of *H. armigera* in the experiment was lower than the real capacity of the natural environment to perform it, since they are dealing with a new pest in these growing environments (Czepak et al., 2013). Thus, their perception by predators may also be limited but may increase with time. Following this reasoning, it is assumed that pests that occur in the region over time, such as *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), have a higher frequency of removal by predators, since it is a pest already established in Brazil, when compared to *H. armigera* (Leiderman & Sauer, 1953). This logic is applied to top-down and competition relationships between non-natives and their probable native enemy, expressed as the "enemy release hypothesis" (ERH) that explains the success of invaders in new ecosystems (e.g. Parker & Gilbert, 2007). ERH supports the greater success of non-natives in new environments as *H. armigera* in the study area. However, there are studies reporting fast responses of native species to new preys as, for example, the fruit-eating bugs that evolved different mouthparts, so increasing attack efficiency towards invasive vine (Carroll et al., 2005), or the reduction of heads in Australian snakes, thus preventing them from eating toxic non-native toads and avoiding increasing death probability (Phillips & Shine, 2004). These and other examples led Simberloff & Gibbons (2004) to conclude that native predators commonly cause a decline of non-native populations since they had time to evolve with them. We may be experiencing one of these examples when analyzing H. armigera presence in the study area.

This paper indicates that the type of crop management employed by the farm is essential to the agricultural ecosystem, as it affects beneficial processes that occur within it. The practices adopted in organic farming are more conservative, to the point of positively influencing the natural biological control of a pest that is causing severe damage to various crops, among them, maize. In the literature, there is information on biological control, biodiversity, and on the natural enemies' action in maize shoots. However, the information on how to control this pest in the soil and how environmental factors control it is only now beginning to be known.

The knowledge now available suggests that the temporal availability of *H. armigera* pupa does not affect their removal and that removal on the surface is higher in the fallow and reproductive stages of maize. Additionally, removal of pupae on the surface during the vegetative development stage of maize plant corresponds to the lowest removal value detected at any depth and at any phase; and in maize crops under organic treatment, the removal is higher than in crops under conventional treatment.

It is difficult to obtain precise information about interactions between the pupa stage of insect pests and its natural enemies, mainly when it occurs in the soil, as in the case of *H. armigera*, recently introduced in Brazil and with little information about its natural control agents. Besides, the soil is an environment of meticulous study since any interference can cause behavioral and developmental changes in its associated organisms. Therefore, the present research represents a significant contribution to the incentive of new studies on soil fauna and the advancement of knowledge in this area of science that is still deficient in Neotropical regions.

Conclusions

Considering the knowledge now available, the following stand out: the temporal availability of *H. armigera* pupa does not affect its predation; predation of *H. armigera* pupae on the surface is superior in fallow period and in the reproductive phase of maize; predation of pupae on the surface during the vegetative phase corresponds to the lowest predation value detected at any depth and at any phase, possibly soil preparation effects; in organic maize crops, natural enemies predation on *H. armigera* is superior when compared to conventional crops.

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