Control of *Anticarsia gemmatalis* (Hübner: 1818) (Lepidoptera: Erebidae) and *Chrysodeixis includens* (Walker: 1858) (Lepidoptera: Noctuidae) Through Insecticides Applied to Soybean Seeds

E. C. S. Vieira¹, C. J. Ávila¹, L. M. Vivan³, I. F. Silva¹, M. C. S. Vieira¹ & P. G. Silva¹

¹ Programa de Pós-graduação em Entomologia e Conservação da Biodiversidade, Universidade Federal da Grande Dourados, Dourados, MS, Brazil
² Empresa Brasileira de Pesquisa Agropecuária (Embrapa Agropecuária Oeste), Dourados, MS, Brazil
³ Fundação MT, Rondonópolis, MT, Brazil

Correspondence: E. C. S. Vieira, Programa de Pós-graduação em Entomologia e Conservação da Biodiversidade, Universidade Federal da Grande Dourados, Dourados, MS, Brazil. Tel: 55-67-99939-3545. E-mail: elizete.cavalcanteufgd@gmail.com

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**Abstract**

Brazil is the second largest producer of soybeans in the world, with the state of Mato Grosso having the highest production in the country. The soybean crop faces major phytosanitary difficulties throughout its cultivation, as is the case of pest incidence, especially the defoliating caterpillars that can cause intense defoliation of soybean and affect its productivity. Thus, the objective of this work was to evaluate the effect of different insecticides when applied in soybean seed treatment, on leaf consumption and survival of *Anticarsia gemmatalis* (Lepidoptera: Erebidae) and *Chrysodeixis includens* (Lepidoptera: Noctuidae) caterpillars. For this purpose, two trials were installed in the field, one in the Juscimeira Municipality/MT and the other in the Municipality of Itiquira/MT. The soybean seeds were treated with the insecticides (in g a.i./ha): chlorantraniliprole (62.5), imidacloprid + thiodicarb (75.0 + 25.0), fipronil (50.0), thiamethoxam (70.0) and cyantraniliprole + thiamethoxam (60.0 + 70.0) and a control treatment (without insecticide). Soybean leaflets were collected at different times after plant emergence and were offered to *A. gemmatalis* and *C. includens* caterpillars in the laboratory to evaluate leaf consumption and mortality. Cyantraniliprole + thiamethoxam (60.0 + 70.0) and chlorantraniliprole (62.5) treatments were found to reduce leaf consumption of *A. gemmatalis* and *C. includens* caterpillars. The highest mortality values of *A. gemmatalis* and *C. includens* were observed in the treatment cyantraniliprole + thiamethoxam (60.0 + 70.0), followed by chlorantraniliprole (62.5) when compared to the control treatment. The results showed that the treatment of soybean seeds with insecticides can protect the crop against defoliation and provide control of defoliating caterpillars, these effects being more pronounced in sandy soils than in clay soils.

**Keywords:** defoliating caterpillars, *Glycine max* (L.), mortality, leaf consumption, seed treatment, soil

1. **Introduction**

With a world production of grains exceeding 360 million tons, the soybean crop (*Glycine max* L. Merr.) is very important economically (USDA, 2019). Grains of this leguminous are the main source of vegetable protein, an essential component in animal feed production, as well as widespread use in human food (Cattelan & Dall’Agnol, 2018). Brazil is the second largest producer of soybeans in the world, growing about 35 million hectares in the 2018/2019 season, with the state of Mato Grosso having the largest production in the country (Lima et al., 2019). However, throughout the production cycle of the crop, there are phytosanitary factors that compromise its productivity. Among them, the constant presence of insect pests stands out, which can occur from sowing until the maturation stage of the grains, being characterized as one of the main limiting factors for the exploitation of the crop, and consequently, of its production (Ávila & Schlick-Souza, 2015; Husch et al., 2018).
Among the pest insects that occur in soybean, defoliating caterpillars stand out, which especially during the vegetative stage of the plants can cause defoliation and cause economic losses, as well as difficulty of control, since some species are more and more tolerant to the synthetic insecticides commonly used in this crop (Chang & Hartman 2017). In addition, caterpillars of the species *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae) due to the habit of remaining sheltered under the canopy of soybean plants staying less exposed to spraying with insecticides, thus compromising their control efficiency (Bernardi et al., 2012; Zulin et al., 2018). In addition, both *C. includens* and *Anticarsia gemmatalis* (Hübner, 1818) caterpillars (Lepidoptera: Erebidae) when feeding of soybean plants reduce the photosynthetically active leaf area, which may compromise the filling of the pods and, consequently, the productivity of the crop (Moscardi et al., 2012; Bortolotto et al., 2015; Specht et al., 2015; Santos et al., 2017a, 2017b).

There is a possibility of control of defoliating caterpillars in soybean cultivation through seed treatment and, consequently, reduce the damage caused by these insects especially in the initial stages of development of the plants, thus guaranteeing a better initial development of the crop with direct reflexes in its productivity (Balardin et al., 2011; Cox & Cherney, 2011; Camargo et al., 2019).

Thus, seed treatment can reduce the number of insecticide sprays in soybean, especially in the vegetative phase of the crop, reducing the impact to the agroecosystem, and not compromising the development of the natural enemies’ complex present in the crop (Castro et al., 2008). In addition, some insecticides may confer physiological effects on soybean, providing a more vigorous initial development of the plants, thus ensuring a better stand establishment and crop yield (Tavares et al., 2014; Tonin et al., 2014; Milosavljević et al., 2019).

Therefore, the objective of this work was to evaluate the effect of different treatments of soybean seeds with insecticide on the management of *Anticarsia gemmatalis* and *C. includens*.

2. Method

2.1 Seeds Treatments

Five insecticides registered for soybean cultivation in Brazil were evaluated (MAPA, 2019), consisting of the following treatments (in g a.i./100 kg of seeds): T1: chlorantraniliprole (62.5); T2: thiodicarb + imidacloprid (75 + 25); T3: fipronil (50); T4: thiamethoxam (70); T5: cyantraniliprole + thiamethoxam (60 + 70) and T6: control treatment (without application of insecticide in seeds). All the soybean seeds used in the trials were also treated with the fungicides carbendazim + thiram (15 + 35 g a.i./100 kg of seeds) to avoid possible problems with pathogens, especially those present in the soil.

2.2 Field Trials

Soybean plants, in which the seeds were treated or not with insecticides, were used as food sources of the *A. gemmatalis* and *C. includens* caterpillars, coming from two distinct areas of the State of Mato Grosso, MT, Brazil, during the 2015/2016 season, as follows:

2.2.1 Mirandópolis Farm

Located in the municipality of Juscimeira, MT (16°21’49.82" S and 55°04’30.40" W); has an altitude of 545 meters and soil characterized as Dystrophic Red Latosol of clayey texture (Table 1). The soybean cultivar “TMG 1180 RR” was used in the planting of this area, and the sowing was done with a spacing of 0.45 meter between the soybean lines. The fertilization of the area was 350 kilos of the formulated fertilizer 02-23-00 (NPK), applied in the base at the time of sowing, added with 150 kilos of KCl, applied over the soil.

2.2.2 São Miguel Farm

Located in the municipality of Itiquira, MT (17°10’02.3" S and 54°37’15.6" W), with an altitude of 522 m and soil with sandy texture (Table 1). Seeding was carried out using soybean cultivar “TMG 1179 RR”, with a spacing of 0.45 meter in the soybean line. The fertilization consisted of 81 kilos/ha of P₂O₅ + 94 kilos/ha of K₂O + 17 kilos/ha of sulfur (S) applied in the sowing line, plus 90 kilos/ha of KCl, applied over the soil.
Tabela 1. Components of the texture (clay, silt and sand) and organic matter (OM) of the soils of São Miguel Farm (Itiquira, MT) and Mirandópolis Farm (Juscimeira, MT) used for soybean cultivation

| Locality              | Clay  | Silt  | Sand  | OM*   |
|-----------------------|-------|-------|-------|-------|
| Fazenda São Miguel    | 168.0 | 53.0  | 779.0 | 15.0  |
| Fazenda Mirandópolis  | 567.0 | 150.0 | 283.0 | 33.0  |

Note. *Organic matter.

2.3 Laboratory Bioassays

In the laboratory, leaf consumption and mortality of *A. gemmatalis* and *C. includens* caterpillars were evaluated when fed with soybean leaves treated with or without insecticides in the seeds from the two growing areas. For that, soybean leaves were collected at 7, 14, 21, 28 and 35 days after emergence of the plants in the experimental area. After collection, the leaves were initially hygienized in the laboratory and later, circular discs with 10 mm diameter were prepared and offered to newly hatched caterpillars of *A. gemmatalis* and *C. includens*, reared in the laboratory of the MT Foundation. Each caterpillar was confined in a test plate, containing the soybean leaf disc and kept at a temperature of 25±2 °C, RH. 60±10% and 12-hour of photophase.

The leaf disc consumption and the mortality of caterpillars were determined every 7 days after the test installation. In order to evaluate the leaf consumption of *A. gemmatalis* and *C. includens* caterpillars, the area consumed in the leaf disc was estimated from 0 to 100% relative to the disk originally offered intact to the caterpillars. The trials were conducted in the completely randomized design with the six treatments and four replicates, being each replicate represented by 4 caterpillars (four test plates) of each caterpillar species.

The foliar consumption and mortality values of the caterpillars were submitted to analysis of variance (ANOVA) and, when a significant treatment effect was found, the means were compared by the Tukey test at 5% of probability.

3. Results

The percentage of leaf area consumed (LAC) and mortality (M) of caterpillars of *C. includens* at 7 DAE (days after emergence) in the municipality of Itiquira were significant only for the treatment cyantraniliprole + thiamethoxam (60 + 70 g a.i.) in relation to the control, which presented, respectively, lower leaf consumption and higher mortality of caterpillars (Table 2). In the municipality of Juscimeira, there was no significant influence on leaf consumption and mortality of *C. includens* caterpillars for all treatments applied in soybean seeds at all times of evaluation (Table 3). At 14 DAE, in the municipality of Itiquira, the highest percentage of *C. includens* defoliation was observed in the treatment with fipronil (50 g a.i.), which was not different from the control treatment, but was superior in comparison to chlorantraniliprole treatments (62.5 g a.i.) and cyantraniliprole + thiamethoxam (60 + 70 g a.i.), which also presented higher mortality of caterpillars (Table 2).

At 21 DAE, cyanuthoheylamido + thiamethoxam (60 + 70 g a.i.) continued to present lower soybean defoliation intensity and higher larval mortality compared to control as well as thiodicarb + imidacloprid treatment (72.5±31.7). In the other evaluations (28 and 35 DAE) there was no significant difference between treatments evaluated for the intensity of defoliation and mortality of *C. includens* caterpillars (Table 2).

In the test conducted with *A. gemmatalis* caterpillars in the municipality of Itiquira, there was no significant effect of the treatments for leaf caterpillar consumption at 14 DAE. However, the mortality of caterpillars was 100% in the treatments chlorantraniliprole (62.5 g a.i.), fipronil (50 g a.i.) and cyantraniliprole + thiamethoxam (60 + 70 g a.i.), differing from the other treatments that did not present any mortality of caterpillars at this time (Table 4).

At 28 DAE, the control treatment showed the highest foliar consumption and the lowest mortality of caterpillars, while the other treatments applied in soybean seeds had low defoliation intensity and 100% mortality of caterpillars, without differing among themselves (Table 4). In the last evaluation of foliar consumption and mortality of caterpillars carried out with soybean plants from Itiquira (35 DAE), the treatment fipronil (50 g i.a.) presented the highest leaf consumption and the lowest mortality of caterpillars, being these equivalent to the
control, while the cyantraniliprole + thiamethoxam (60 + 70 g i.a.) mixture had the lowest leaf consumption (Table 3).

Table 2. Percentage of leaf area consumed (LAC) and mortality (M) of *Chrysodeixis includens* caterpillars, evaluated in the laboratory at 7, 14, 21, 28 and 35 days after plant emergence (DAE) in different treatments applied to soybean seeds in the municipality of Itiquira/MT

| Treatments (g a.i./ha) | 7 DAE  | 14 DAE  | 21 DAE  | 28 DAE  | 35 DAE  |
|------------------------|--------|--------|--------|--------|--------|
| Control (without insecticide) | 100.0±0.0a | 0.0±0.0b | 75.7±7.1abc | 62.8±7.1ab | 93.8±6.5a |
| Chlorantraniliprole (62.5) | 52.0±21.6ab | 62.5±32.3ab | 51.5±13.7c | 43.8±12.5c | 94.1±11.9a |
| Tiodicarbe + imidacloprido (75+25) | 72.5±31.7ab | 80.8±18.0ab | 93.8±12.5a | 6.3±12.5c | 100.0±0.0a |
| Fipronil (50) | 69.7±21.3ab | 91.9±4.6a | 61.6±16.5bc | 37.5±25.0ab | 89.4±12.3a |
| Thiamethoxam (70) | 81.6±22.0a | 57.4±17.9abc | 71.9±22.7abc | 25.0±20.4abc | 85.9±9.8a |
| Cyantraniliprole + thiamethoxam (60+70) | 25.9±16.1b | 54.7±17.9bc | 50.0±4.3c | 50.0±0.0a | 86.6±12.9a |
| CV (%) | 31.1 | 71.3 | 17.5 | 45.6 | 18.2 |

Note. Averages±Standard Error (SE) followed by the same letter in the column did not differ statistically from each other by the Tukey test (p < 0.05).

Table 3. Percentage of leaf area consumed (LAC) and mortality (M) of *Chrysodeixis includens* caterpillars, evaluated in the laboratory at 7, 14, 21, 28 and 35 days after plant emergence (DAE) in different treatments applied to soybean seeds in the municipality of Juscimeira/MT

| Treatments (g a.i./ha) | 7 DAE  | 14 DAE  | 21 DAE  | 28 DAE  | 35 DAE  |
|------------------------|--------|--------|--------|--------|--------|
| Control (without insecticide) | 84.6±18.5a | 97.0±5.9a | 84.4±6.3a | 85.3±11.3a | 15.9±1.9a |
| Chlorantraniliprole (62.5) | 67.5±23.6a | 80.6±13.0a | 94.7±10.6a | 100.0±0.0a | 89.4±12.3a |
| Tiodicarbe + imidacloprido (75+25) | 82.2±11.9a | 81.3±12.5a | 93.8±12.5a | 94.7±10.6a | 17.5±2.3a |
| Fipronil (50) | 88.1±13.8a | 87.5±14.4a | 100.0±0.0a | 14.1±1.6a | 6.3±12.5a |
| Thiamethoxam (70) | 83.1±11.3a | 94.1±11.9a | 94.1±11.9a | 16.3±1.5a | 0.0±0.0a |
| Cyantraniliprole + thiamethoxam (60+70) | 77.5±15.5a | 93.8±12.5a | 93.8±12.5a | 16.6±3.0a | 0.0±0.0a |
| CV (%) | 22.5 | 92.1 | 12.8 | 160.1 | 17.3 |

Note. Averages±Standard Error (SE) followed by the same letter in the column did not differ statistically from each other by the Tukey test (p < 0.05).

Table 4. Percentage of leaf area consumed (LAC) and mortality (M) of *Anticarsia gemmatalis* caterpillars, evaluated in the laboratory at 14, 21, 28 and 35 days after plant emergence (DAE) in the different treatments applied in the soybean seeds of the municipality of Itiquira/MT

| Treatments (g a.i./ha) | 14 DAE  | 21 DAE  | 28 DAE  | 35 DAE  |
|------------------------|--------|--------|--------|--------|
| Control (without insecticide) | 7.8±4.3a | 37.5±19.6a | 65.0±13.0a | 37.2±21.6ab |
| Chlorantraniliprole (62.5) | 5.0±0.0a | 3.4±0.6b | 4.4±0.7b | 7.5±2.3bc |
| Tiodicarbe + imidacloprido (75+25) | 9.7±5.6a | 34.7±13.4a | 5.0±0.0b | 13.1±12.1bc |
| Fipronil (50) | 6.6±1.2a | 17.8±11.0ab | 5.0±0.0b | 47.2±20.8a |
| Thiamethoxam (70) | 9.4±5.4a | 25.9±5.0ab | 5.0±0.0b | 10.6±3.6bc |
| Cyantraniliprole + thiamethoxam (60+70) | 5.0±0.0a | 5.0±0.0b | 5.0±0.0b | 5.9±3.1c |
| CV (%) | 55.6 | 51.8 | 35.4 | 66.4 |

Note. Averages±Standard Error (SE) followed by the same letter in the column did not differ statistically from each other by the Tukey test (p < 0.05).
In the test conducted with soybean plants from the Municipality of Juscimeira with *A. gemmatalis*, it was verified that the caterpillars that fed 7 DAE, presented reduced leaf consumption in the treatments chlorantraniliprole (62.50 g a.i.), thiamethoxam (70 g a.i.) and with the mixture of cyantraniliprole + thiamethoxam (62.50 g a.i.), when compared to the control. However, all chemical treatments showed relatively high mortality of *A. gemmatalis* caterpillars, surpassing the mortality observed in the control (Table 5).

At 14 DAE, all chemical treatments showed a relatively lower defoliation of soybeans compared to the control (Table 5), with emphasis on the treatments chlorantraniliprole (62.5 g a.i.) and cyantraniliprole + thiamethoxam (60 + 70 g a.i.), however the highest caterpillar mortalities were also observed with chlorantraniliprole (62.5 g a.i.) and the mixture of cyantraniliprole + thiamethoxam (60 + 70 g a.i.). At 21 DAE, there was no treatment effect on the defoliation caused by *A. gemmatalis* caterpillars, however, the higher mortality was presented with the treatment cyantraniliprole + thiamethoxam (62.50 g a.i.), which surpassed the mortality verified in the control and the thiamethoxam treatment (70 g a.i.) (Table 5). In the evaluations performed at 28 and 35 DAE, no significant treatment effect was observed for both leaf consumption and caterpillars mortality of *A. gemmatalis* (Table 5).

4. Discussion

It is known that almost all the soybean seeds currently marketed in Brazil present some type of chemical treatment aimed at controlling especially pests or diseases (Pereira et al., 2011). The use of insecticides and fungicides, applied to the seeds, can significantly improve the soybean stand as well as the grain yield of the crop (Rossman et al., 2018; Milosavljević et al., 2019). In the case of insecticides, the use of active ingredients isolated or mixed in the seed treatment for pest control is an ancient practice (Munkvold et al., 2006).

Some of the active ingredients used in the seeds, such as the neonicotinoids (thiamethoxam, imidacloprid, clothianidin), provide an improvement in the agronomic characteristics of some crops, resulting in increased productivity (Jeschke et al., 2011; Szczepaniec et al., 2013; Afifi et al., 2014; Alford & Krupke, 2017). Neonicotinoids are persistent insecticides and provide long-term protection for seeds and seedlings of different species, and can control pests that are difficult to manage right after planting (Cherry et al., 2017; Milosavljević et al., 2019). Neonicotinoids are toxins that act on the acetylcholine receptors (nAChR) of insects, and as a result cause collapse due to a rupture of the nerve signals, leading the insect to death. These insecticides when applied to seeds provide precise targeting through systemic action in the plant, which favors the agroecosystem, as they can reduce the number of sprays in the aerial part of the plants, as well as the risk of deleterious effects in non-target organisms, such as pollinators and the complex of natural enemies (Bonmatin et al., 2015; Douglas & Tooker, 2015; Furlan & Kreutzweiser, 2015).

Regarding the results obtained at the two sites where the experiments were conducted (Juscimeira and Itiquira) one can observe a highlight for the insecticides of the diamid group. This group acts as an activator of the rianodine receptors, and during the mode of action in lepidopteran larvae causes deregulated release of internal calcium in the cell is verified, this causes rapid cessation of feeding, lethargy, partial paralysis and consequently the death of the insect (Cordova et al., 2006; Lahm et al., 2007). These possible effects corroborate with the results obtained for the insecticides cyantraniliprole and chlorantraniliprole, for which during almost all the evaluations in which caterpillars were fed with leaves of soybean whose seeds had been treated with these products presented a smaller consumption of the leaf area and a greater mortality of caterpillars. Regarding leaf consumption and, consequently, damage caused by defoliating caterpillars in soybean cultivation, there is a constant concern, since only one caterpillar during its larval phase, can have leaf consumption of 85 to 150 cm² of which could compromise the development of the crop (Bueno et al., 2011).

The effect of the insecticides cyantraniliprole and chlorantraniliprole when applied in treatment of soybean seeds against *A. gemmatalis* caterpillars has been reported by Rodrigues et al. (2014). Thrash et al. (2013) found that *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) caterpillars when fed with soybean leaflets, where the seeds received treatment with chlorantraniliprole and cyantraniliprole also had a high larval mortality. When submitting caterpillars of *Mythimna unipuncta* (Haworth) (Lepidoptera: Noctuidae) fed with corn leaves in which the seeds were treated with chlorantraniliprole, alone or in combination, the larval mortality of this species occurred earlier, compared to the treatment where there was no application of this insecticide (Carscallen et al., 2019). Suzana et al. (2017) also verified that the insecticides cyantraniliprole and chlorantraniliprole, used in the proportion of 60 and 62.5 g a.i./100 kg of seeds, demonstrated potential for the control of *H. armigera* caterpillars even 13 days after the emergence of the plants of soybean.
The inability of the insecticide fipronil to prevent leaf consumption of insecticides, such as carbamates and pyrethroids, for example, but with the benefit of having a more favorable toxic action for insects when compared to fast acting caterpillars, shortly after their introduction in the US market, showed a high risk for the development resistance to this mode of action (Owen et al., 2013).

The inability of the insecticide fipronil to prevent leaf consumption of *A. gemmatalis* and *C. includens* in this study indicates a limitation of the use of this active principle in the dose tested when applied to soybean seeds. However, its toxic effect through the inhibition of neuronal receptors in lepidopterans has been observed (Colliot et al., 1992; Carneiro et al., 2014).

When analyzing the effect of soil textures in the two environments in which the tests were conducted, there is generally a lower percentage of leaf area consumed and higher mortality of both species of caterpillars for soybean cultivated in Itiquira compared to soybean cultivated in Juscimeira, especially for the treatments chlorantraniliprole (62.5 g a.i.) and cyantraniliprole + thiamethoxam (60 + 70 g a.i.). The soil of Itiquira is characterized by a sandy texture (Table 1), which may have favored a greater availability of the insecticides in the soil solution and, consequently, a better absorption by the soybean plants and thus providing a higher mortality and lower leaf consumption of caterpillars. Felsot and Lew (1989), when studying soil types, solubility and adsorption, which allows a greater absorption of the molecules of the products by the plants, as probably occurred in this work for the soil of Itiquira. The termite species *Campotermes formosanus* (Isoptera: Rhoentermitidae) can rapidly acquire the lethal dose of chlorantraniliprole in treated sandy soil, but this effect cannot occur when the insects are in soils with high organic matter content (Hannig et al., 2009). Also, in tests of caterpillar mortality, it has been verified that the insecticide cyantraniliprole demonstrates that its oral toxicity is superior to its toxicity by contact, thus evidencing that the ingestion of this active ingredient is important for the control of these insects, which emphasizes and their importance in seed treatment (Bird, 2016; Dong et al., 2016; Suzana et al., 2017).

It is worth noting that chlorantraniliprole has the same toxic action for insects when compared to fast acting insecticides, such as carbamates and pyrethroids, for example, but with the benefit of having a more favorable toxicological and ecotoxicological profile (Hannig et al., 2009). Also, in tests of caterpillar mortality, it has been verified that the insecticide cyantraniliprole demonstrates that its oral toxicity is superior to its toxicity by contact, thus evidencing that the ingestion of this active ingredient is important for the control of these insects, which emphasizes and their importance in seed treatment (Bird, 2016; Dong et al., 2016; Suzana et al., 2017). However, this group of diamidas deserves attention, since studies carried out with different populations of caterpillars, shortly after their introduction in the US market, showed a high risk for the development resistance of *C. includens* to this mode of action (Owen et al., 2013).

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Another factor that could have affected the absorption of insecticides in these two environments in which this study was conducted was probably related to the availability of water in the soil, as a function of the degree of precipitation that occurred in the two localities where the study was conducted. It was verified that in the São Miguel Farm of the Municipality of Itiquira the intensity of precipitation was much greater than the one observed in the Mirandópolis Farm of the Municipality Juscimeira (Figure 1). This greater availability of water in the soil of Itiquira may have favored the absorption by the plants of the products applied in the seeds and, consequently, have had a more significant effect on foliar consumption and mortality of the caterpillars when compared to the soil of Juscimeira. The species *Acheta pennsylvanica* (Burmeister) (Orthoptera: Gryllidae) had its effective control from insecticides applied to soils considered wet in comparison to dry soils (Harris, 1966).

![Figure 1. Monthly precipitation (mm) observed from November 2015 to February 2016 in the areas of the São Miguel Farm (Itiquira/MT) and Mirandópolis Farm (Juscimeira/MT)](image)

In general, the control of the *A. gemmatalis* and *C. includens* caterpillars in this study were higher in the early stages of the crop, when the soybean seeds were treated with different insecticides. Thus, the use of this control technique may be an alternative to reduce the initial attack of caterpillars in soybean crop, especially when associated with other management tactics (Ávila & Santos, 2018). In addition, seed treatment usually uses a low dose of active ingredients per hectare and is therefore a selective control tactic for most of the natural enemies and beneficial insects associated with this crop (Santos et al., 2006; Cunha et al., 2015).

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