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Control of *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) with entomopathogenic fungus and insecticides using two sampling methods on sugarcane fields

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The sampling of *M. fimbriolata* aims to estimate the population level and optimum timing for spittlebug control in the sugarcane fields. Thus, this study was conducted to determine the cost and efficiency of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) and insecticides utilized in *M. fimbriolata* control using two sampling techniques. The experiments were performed between November 2012 and April 2013, in a sugarcane field. In each experiment, apart from the control, treatments included Thiamethoxan (250 g ha^{-1}), Imidacloprid (700 g ha^{-1}), PL 43 (*M. anisopliae* - 2.0×10^{12} con mL^{-1}), ESALQ E9 (*M. anisopliae* -2.1×10^{12} con mL^{-1}) and IBCB 425 (*M. anisopliae* - 1.4×10^{12} con mL^{-1}). In the first experiment, the *M. fimbriolata* nymphs and adults were monitored, while on the other experiment, the nymphs sampled were the small, medium, large, and the adults of *M. fimbriolata* with objective was to identify the timing of the application of treatments. In general, by monitoring the small, medium and large nymphs and the adult spittlebugs, the timing of the application was optimized, which increased the efficiency of *M. fimbriolata* control.

Key words: Biological control, imidacloprid, *Mahanarva fimbriolata*, *Metarhizium anisopliae*, thiamethoxan.

INTRODUCTION

Mahanarva fimbriolata (Stål, 1854) (Hemiptera: Cercopidae) is one of the major pests in sugarcane fields of Brazil (Dinardo-Miranda et al., 2008; Tiago et al., 2012; Garcia et al., 2011). Nymphs and adults of *M. fimbriolata* can cause injuries in sugarcane plants compromising the productivity and quality of this crop (Dinardo-Miranda et al., 2004a, b; Madaleno et al., 2008; Carvalho et al., 2011; Korndörfer et al., 2011).

In order to reduce the *M. fimbriolata* populations (Dinardo-Miranda et al., 2004a, b; Loureiro et al., 2005;

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Cuarán et al., 2012) insecticides and entomopathogenic fungi like *Metarhizium anisopliae* (Metschnikoff, 1879; Sorokin, 1883) (Hypocreales: Clavicipitaceae) are used to protect the sugarcane plantations. Control of the spittlebug begins with monitoring the pest, immediately after the first spring rains (Almeida et al., 2007). Using the conventional sampling method, the *M. fimbriolata* nymphs and adults are monitored at 3 points per hectare on 2 linear feet of row sugarcane planting and an ideal frequency of 15 days (Mendonça, 2005; Dinardo-Miranda et al., 2007).

However, the sampling of the nymphs and adults can impede the identification of the end of the life cycle as well as the timing of control of *M. fimbriolata* (Kassab et al., 2012). Highly infested areas could render the sample data unreliable, while regular rainfall could trigger the resurgence of the spittlebug populations, negating the effects of the insecticide and/or bioinsecticide applications during the control of *M. fimbriolata*. Therefore, Kassab et al. (2012) suggested modifying the *M. fimbriolata* sampling by monitoring the small nymphs (up to 5 mm), medium (6-10 mm) and large ones (over 10 mm) as well as the adult spittlebugs. This proposal enables the observation of the end of the cycle of *M. fimbriolata* generations and the best timing at which to control this pest, the period when the large nymphs are more numerous than the small and medium nymphs. It is at this time, which the *M. fimbriolata* adults can also be sampled in the pest population, which may indicate that eggs of the spittlebug had favorable to emergence of this insect.

Regardless, the new sampling method proposed for *M. fimbriolata* (Kassab et al., 2012) is yet to be compared with the conventional method (Mendonça, 2005; Dinardo-Miranda et al., 2007) and this indicates the importance of understanding the efficiency of the entomopathogenic fungi and insecticides in the control of *M. fimbriolata* by employing both these methods.

The aim of this study was evaluating the cost and efficiency of *M. anisopliae* and the insecticides used in controlling *M. fimbriolata* employing two sampling methods.

MATERIALS AND METHODS

A sugarcane field owned by Energética Santa Helena Ltda., a company in Nova Andradina, Mato Grosso do Sul State, Brazil was the site where the experiments were conducted between November 2012 and April 2013. The experimental area (S 22º13´58´´, W 53º20´34´´ and 380 m asl) was planted with the SP81-3250 variety of sugarcane, with no defects in the sprouting plants.

In this study, we followed the randomized blocks design (RBD) with six treatments and four replications of each, that is, with and without the conventional sampling of *M. fimbriolata*. The plots included 10 lines of sugarcane spaced 1.4 m apart and 10 m long, an area of 140 m^2.

The treatments in each experiment were represented by the control, Thiamethoxan (250 g ha^{-1}), Imidacloprid (700 g ha^{-1}), PL 43 (*M. anisopliae* - 2.0×10^{12} con mL^{-1}), ESALQ E9 (*M. anisopliae* - 2.1×10^{12} con mL^{-1}) and IBCB 425 (*M. anisopliae* - 1.4×10^{12} con mL^{-1}). The isolates of entomopathogenic fungi were used according to the manufacturers' recommendation. The isolates PL 43, ESALQ E9 and IBCB 425 are present in the commercial products Biometha WP Plus®, Metarril WP® and Metiê WP®, respectively. The manufacturers of products are "Biotech Controle Biológico Ltda" (Biometha WP Plus®), K oppert Biological Systems (Metarril WP®) and "Ballagro Agro Tecnologia Ltda" (Metiê WP®).

Using the conventional sampling method, the experiment with *M. fimbriolata* was performed with the weekly monitoring of the spittlebug nymphs and adults (Mendonça, 2005; Dinardo-Miranda et al., 2007). The first application of control for *M. fimbriolata* was done on 23 November, 2012 and the second on 15 Janand, 2013, when the degree of control of *M. fimbriolata* was achieved (Mendonça, 2005). To conduct the experiment with the proposed new sampling method, weekly sampling was done of the small, medium and large nymphs and adult individuals of *M. fimbriolata* to identify the end of the cycle of the spittlebug generations (Kassab et al., 2012) which occurred on 15 January 2013.

Coastal sprayers calibrated for a flow rate of 150 L ha^{-1} (Mendonça, 2005) were used and the insecticide was directed at the stump bases so that 30% of the spray volume reached the stems and 70% reached the sugarcane plant roots (Loureiro et al., 2005). The surfactant Tween® (0.01% polysorbate 80) was used to treat the fungal suspensions.

The *M. fimbriolata* nymphs were sampled every two weeks, up to 60 days after treatment (DAT) in the two linear meters of the furrow-planted sugarcane, in each plot, in the experiments using the two sampling methods. The *M. fimbriolata* nymphs found on the basal sugarcane internodes were counted after removing the residual straw.

Climatic conditions including average temperature, relative humidity and rainfall were represented with data from INMET (Instituto Nacional de Meteorologia) (Figure 1) to determine the relationship between the abiotic factors and *M. fimbriolata* infestation. The total value of recoverable sugar (TRS) according to Landell et al. (1999) was obtained from the sugarcane stalks randomly selected on 15 April 2013.

The tonne per ha value (TRS × TRS quote), estimated a yield per ha [68 tonnes (productivity of Mato Grosso do Sul State (Unica, 2013) × value of a tonne per ha], maintenance costs of the sugarcane (MCS) without the product and the cost involved in the application of *M. fimbriolata* control (Udop, 2013), cost control (including product and application expenses) and earnings per ha (estimated production per ha − MCS) were calculated in dollars (US$) for both the experiments. The expenses for the services outsourced and purchase of products to improve the sugarcane plantation were obtained from consulting firms and the UDOP agricultural database of 2013 (Table 1).

The population data for the *M. fimbriolata* nymphs recorded between 23 November 2012 and 15 January 2013 were subjected to the analysis of variance and the means were compared using the Scott-Knott test at 5% probability. Further, using Abbott's formula (Abbott, 1925) treatment efficiency was calculated and the means were compared by the Scott-Knott test at 5% probability. The average of the *M. fimbriolata* nymphs and the efficiency control (Abbott, 1925), post the 15 January 2013 treatment in the experiments following the two sampling methods were compared by the analysis of variance (ANOVA - F TEST) at 5% probability.

RESULTS

Experiment with conventional sampling: between 23 November 2012 and 15 January 2013

The number of nymphs and efficiency of *M. fimbriolata*

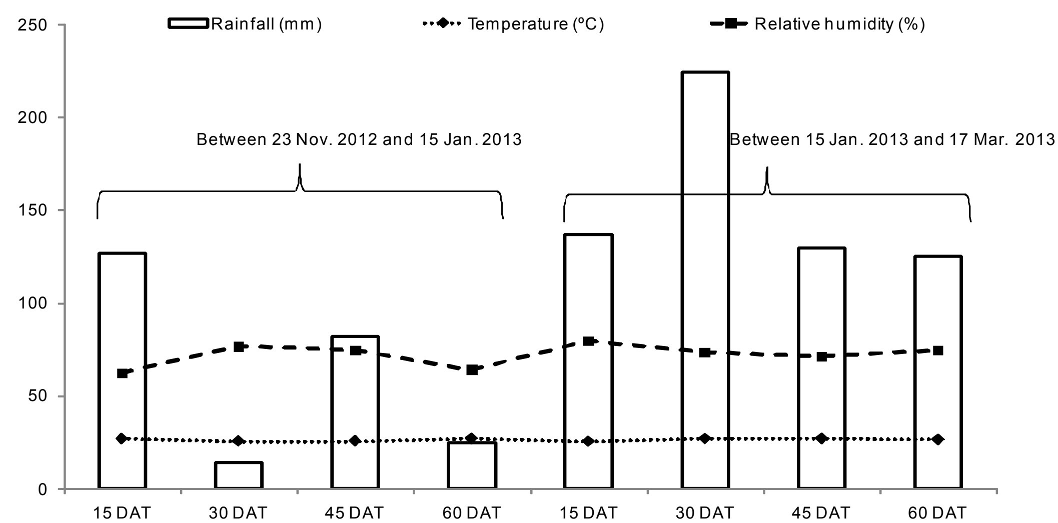

Figure 1. Rainfall (mm), average temperature (°C) and relative humidity (%) in the experimental area.

Table 1. Maintenance costs of sugarcane (MCS) per ha.

MCS	Value (US$)
Agricultural inputs for cultivation	548.50
Mechanized operations (applications)	59.78
Manpower	16.01
Administrative expenses	21.30
Total	645.59

control per treatment did not differ in the ratings of 15 DAT, 30 DAT and 45 DAT (Table 2 and Figures 2A, B and C). At 60 DAT, the treatments with the fungus *M. anisopliae* showed no difference and the nymphal infestations of *M. fimbriolata* were 6.50±1.75, 8.25±1.86 and 9.00±2.04 with the isolates IBCB 425, PL 43 and ESALQ E9, respectively (Table 2). Furthermore, at 60 DAT, the control efficiency of the *M. fimbriolata* recorded nymphs showed similarity between the treatments with isolates IBCB 425, PL 43 and ESALQ E9 with values of 33.75±13.34, 38.75±11.55 and 44.00±14.47. At 60 DAT, the insecticide Imidacloprid and thiamethoxan attained the highest efficiencies and lowest infestations of the *M. fimbriolata* nymphs.

Conventional sampling × new sampling method: Between 15 January 2013 and 17 March 2013

There was no difference in the number of nymphs of *M. fimbriolata* between the treatments during the experiments, both with and without the conventional sampling, at 15 and 30 DAT (Table 3). The control efficiency of the *M. fimbriolata* nymphs did not differ between treatments with and without conventional sampling in the assessments at 15 and 30 DAT (Figures 3A and B).

At 45 and 60 DAT, all the treatments utilizing the new sampling method showed significantly less infestation (Table 3). The treatment efficiency of the new sampling method was higher when compared with the conventional method (Figures 3C and D). Imidacloprid insecticide and the isolate IBCB 425 of *M. anisopliae* were which achieved the highest efficiencies of *M. fimbriolata* control at 45 and 60 DAT (Figures 3C and D). The increase in the number of *M. fimbriolata* nymphs parasitized by *M. anisopliae* (Figure 4), at 45 and 60 DAT, was due to the higher rainfall (mm), temperature (°C) and relative humidity (%) (Figure 1).

The total recoverable sugar (TRS) values, which included the expenses incurred per tonne and the earnings received per ha were higher in all the treatments employing the new sampling method (Table 4). The values regarding the acquisition and application of the products in the treatments with the conventional method were higher than in the other method, because two applications had been done for the control of *M. fimbriolata,* and therefore, lesser profits were made with the conventional method (Table 4).

DISCUSSION

Experiment with conventional sampling: Between 23 November 2012 and 15 January 2013

The lower variation recorded regarding the number of *M. fimbriolata* nymphs following the conventional sampling method, for 15 DAT, 30 DAT and 45 DAT can be related to the monitoring method used for this insect. In this method, the application of the insecticides and entomopathogenic fungi can occur in the initial stage of the first cycle of the spittlebug generations, that is, September/October (Almeida et al., 2007). During this

Table 2. Infestation by the *Mahanarva fimbriolata* (Hemiptera: Cercopidae) nymphs in the treatments with Thiamethoxan, Imidacloprid and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) following the conventional sampling method from 23 November 2013 to 15 January 2013.

Treatments	15 DAT	30 DAT	45 DAT	60 DAT
Control (untreated)	4.50 ± 0.64^{a}	1.50 ± 0.85^{a}	5.25 ± 1.37^{a}	14.00 ± 2.80^{a}
Thiamethoxan (250 g ha^{-1})	1.50 ± 1.19^{b}	1.25 ± 0.47^{a}	1.50 ± 0.28^{b}	2.50 ± 1.55^{b}
Imidacloprid (700 g ha^{-1})	1.75 ± 0.75^{b}	1.00 ± 0.49^{a}	1.50 ± 0.28^{b}	0.75 ± 1.55^{b}
PL 43 ($2,0\times10^{12}$ con mL^{-1} of M.a)	0.75 ± 0.47^{b}	2.00 ± 0.67^{a}	3.00 ± 0.81^{b}	8.25 ± 1.86^{a}
ESALQ E9 ($2,1\times10^{12}$ con mL^{-1} of M.a)	1.75 ± 1.03^{b}	0.25 ± 1.18^{a}	3.00 ± 0.78^{b}	9.00 ± 2.04^{a}
IBCB 425 ($1,4\times10^{12}$ con mL^{-1} of M.a)	1.50 ± 0.85^{b}	2.00 ± 1.02^{a}	1.75 ± 0.75^{b}	6.50 ± 1.75^{a}
CV	67.32	83.58	71.48	56.20

Means followed by the same letter per column were compared using the Scott-Knott test at 5% probability; CV, coefficient of variation; DAT, number of days after treatment; M.a., *M. anisopliae*.

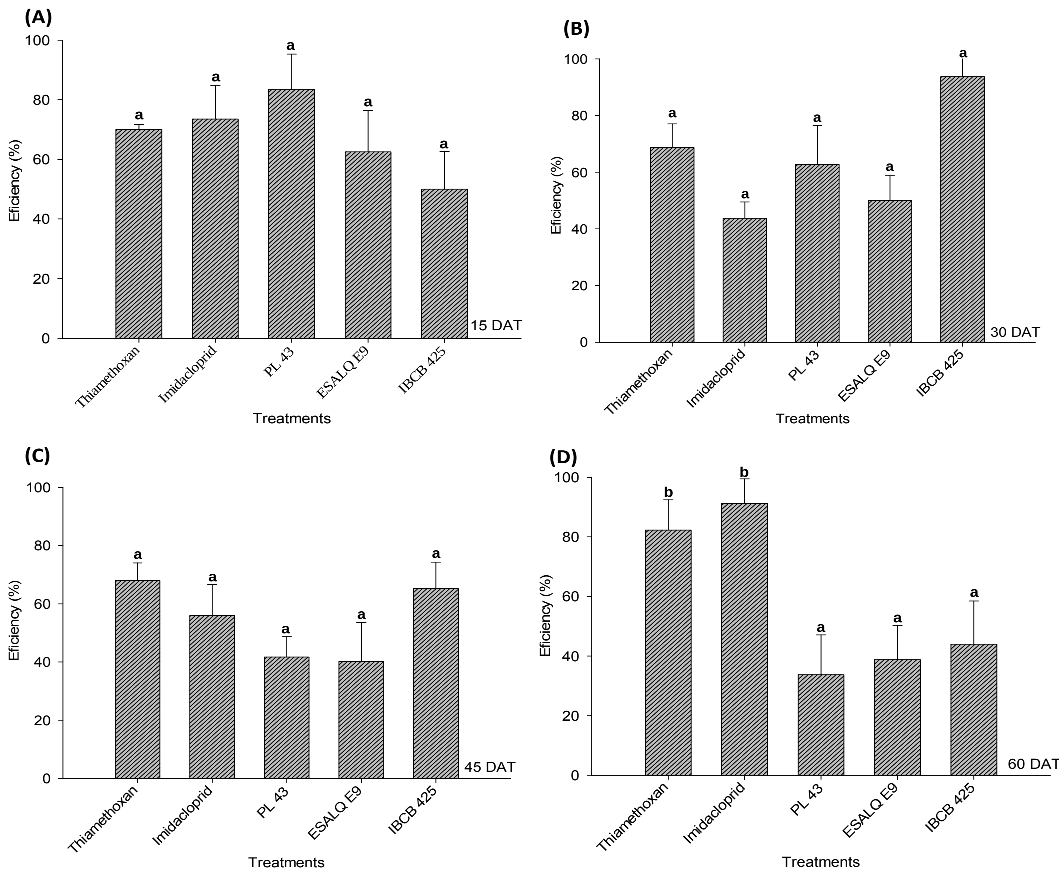

Figure 2. Efficiencies in control of *M. fimbriolata* (Hemiptera: Cercopidae) nymphs in treatments with Thiamethoxan, Imidacloprid and *M. anisopliae* (Hypocreales: Clavicipitaceae) using the conventional method of sampling from 23 November 2013 to 15 January 2013. Means followed by the same letter, in each bar, were compared using the Scott-Knott test at 5% probability

period, most of the *M. fimbriolata* diapausing eggs which are not hatched and due to the greater volume and regularity of rainfall between November and December can increase the possibility of reapplication of these products to control this pest (Kassab et al., 2012).

At 60 DAT, a lesser degree of infestations of the *M. fimbriolata* nymphs was obtained with Imidacloprid and thiamethoxan possibly as a result of the action of these insecticides, which may have contributed to a more efficient control of the spittlebug. The chemical molecule has a greater residual effect than that of the entomopathogenic fungi, which implies that this product

Table 3. Comparative infestation of the *Mahanarva fimbriolata* nymphs (Hemiptera: Cercopidae) between experiments with the conventional method and the new sampling proposal from 15 Jan. 2013 to 17 Mar. 2013.

Treatments	15 DAT		30 DAT	
	AC	NPA	AC	NPA
Thiamethoxan (250 g ha^{-1})	0.50±0.25^{a}	1.00±0.57^{a}	0.25±0.25^{a}	0.25±0.25^{a}
Imidacloprid (700 g ha^{-1})	0.50±0.50^{a}	0.50±0.28^{a}	0.00±0.00^{a}	0.75±0.47^{a}
PL 43 (2,0×10^{12} con mL^{-1} of M.a)	1.50±3.71^{a}	2.50±1.04^{a}	3.00±0.43^{a}	2.25±0.94^{a}
ESALQ E9 (2,1×10^{12} con mL^{-1} of M.a)	1.25±0.47^{a}	2.25±0.71^{a}	1.50±1.19^{a}	1.50±0.64^{a}
IBCB 425 (1,4×10^{12} con mL^{-1} of M.a)	2.25±0.75^{a}	2.05±0.85^{a}	1.75±0.81^{a}	2.00±0.85^{a}

Treatments	45 DAT		60 DAT	
	AC	NPA	AC	NPA
Thiamethoxan (250 g ha^{-1})	19.50±3.43^{a}	4.50±1.87^{b}	22.50±2.22^{a}	7.25±1.93^{b}
Imidacloprid (700 g ha^{-1})	15.00±0.50^{a}	3.75±2.49^{b}	23.75±2.27^{a}	9.25±0.85^{b}
PL 43 (2,0×10^{12} con mL^{-1} of M.a)	14.25±2.09^{a}	4.25±2.78^{b}	22.00±2.31^{a}	11.50±1.47^{b}
ESALQ E9 (2,1×10^{12} con mL^{-1} of M.a)	13.25±1.75^{a}	5.50±1.32^{b}	28.50±3.12^{a}	10.25±1.08^{b}
IBCB 425 (1,4×10^{12} con mL^{-1} of M.a)	10.75±2.21^{a}	3.25±2.13^{b}	18.50±1.93^{a}	9.50±0.75^{b}

Means followed by the same letter, in each line, were compared using F test at 5%; DAT, Number of days after treatment; AC, sampling conventional; NPA, new sampling proposal; M.a., *M. anisopliae*.

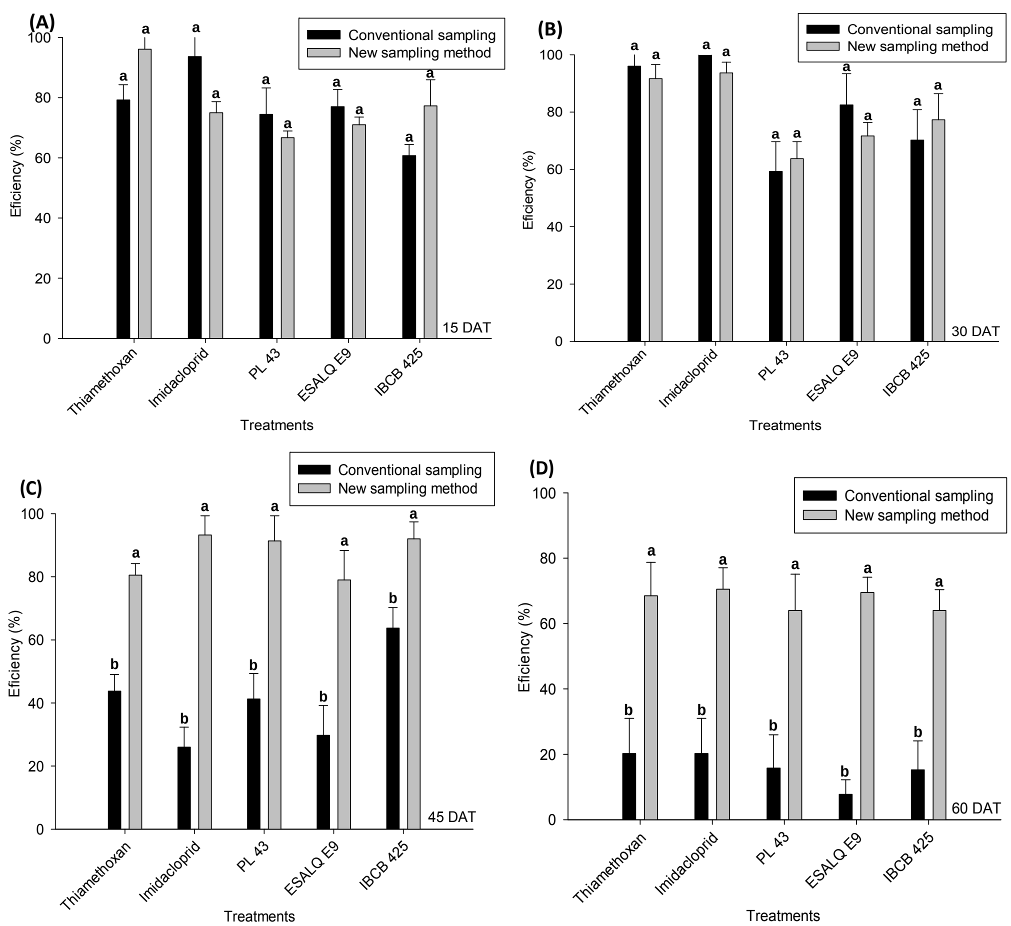

Figure 3. Comparison of the efficiency of control of *Mahanarva fimbriolata* (Hemiptera: Cercopidae) nymphs utilizing the conventional method and new proposal of sampling from 15 Jan. 2013 to 17 Mar. 2013. Means followed by the same letter, in each bar, were compared by the F test at 5%.

Table 4. Total recoverable sugar (TRS), TRS cost (Cot. TRS), price per tonne (Tonne. Price.), production estimate per ha (Prod. ha), maintenance cost of sugarcane plantations (MCS), cost control of *Mahanarva fimbriolata* (Cost. Contr.), profit per ha (L. ha) in the treatments with insecticides and *M. anisopliae* (Hypocreales: Clavicipitaceae) utilizing the conventional and proposed new sampling methods with the values expressed in dollars (US$).

Conventional sampling							
Treatments	**TRS**	**Cot. TRS**	**Tonne. Price.**	**Prod. ha**	**MCS**	**Cost. Contr.**	**L. ha**
Thiamethoxan (250 g ha^{-1})	88	0.19	16.72	1136.96	645.59	107.65	383.72
Imidacloprid (700 g ha^{-1})	90.72	0.19	17.23	1171.64	645.59	58.51	467.54
PL 43 (2,0×10^{12} con mL^{-1} of M.a)	85.02	0.19	16.15	1098.20	645.59	68.10	384.51
ESALQ E9 (2,1×10^{12} con mL^{-1} of M.a)	89.8	0.19	17.06	1160.08	645.59	62.12	452.37
IBCB 425 (1,4×10^{12} con mL^{-1} of M.a)	91.32	0.19	17.35	1179.80	645.59	59.57	474.64
New sampling method							
Treatments	**TRS**	**Cot. TRS**	**Tonne. Price.**	**Prod. ha**	**MCS**	**Cost. Contr.**	**L. ha**
Thiamethoxan (250 g ha^{-1})	89.23	0.19	16.95	1152.60	645.59	53.82	453.19
Imidacloprid (700 g ha^{-1})	94.57	0.19	17.96	1221.28	645.59	29.15	546.54
PL 43 (2,0×10^{12} con mL^{-1} of M.a)	90.31	0.19	17.15	1166.20	645.59	34.05	486.56
ESALQ E9 (2,1×10^{12} con mL^{-1} of M.a)	96.16	0.19	18.27	1242.36	645.59	31.06	565.71
IBCB 425 (1,4×10^{12} con mL^{-1} of M.a)	97.09	0.19	18.44	1253.92	645.59	29.80	578.53

Plant age = 8 months; Cot. TRS (Quotation) - "União dos Produtores de Bioenergia" (UDOP); Price Tonne. = TRS × Cot. TRS; Prod. Ha = value of tonne. × 68 (average production of Mato Grosso do Sul State); MCS = maintenance cost of sugarcane fields without product and application for control of *M. fimbriolata*; Cost. Contr. = Product and ground application; L. ha = Prod. ha - (MCS + Cost Control); M.a.-*M. anisopliae*.

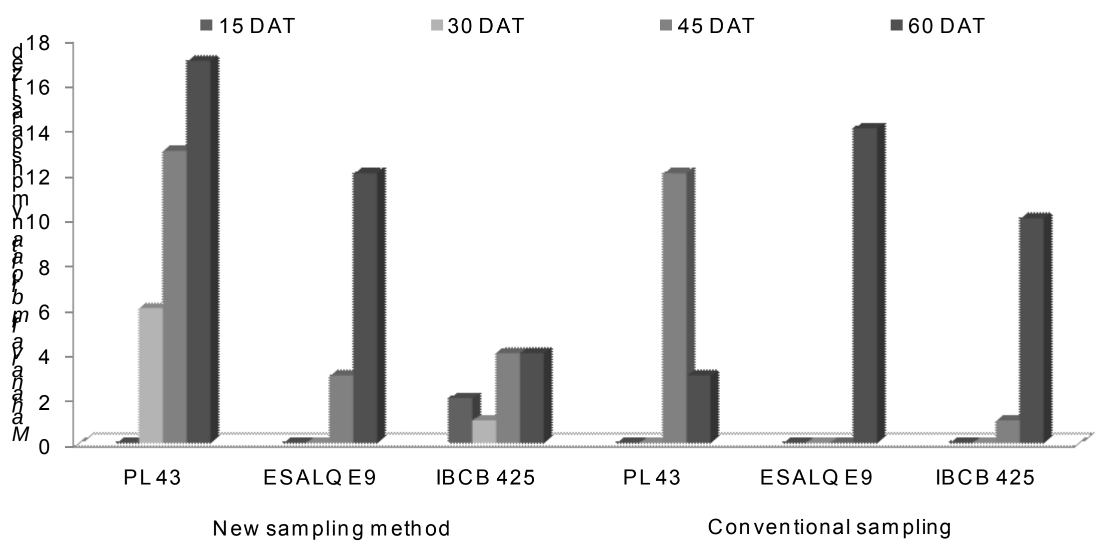

Figure 4. *M. fimbriolata* (Hemiptera: Cercopidae) nymphs parasitized by *M. anisopliae* (Hypocreales: Clavicipitaceae) in the experiments implementing the new proposed and conventional sampling methods from 15 January 2013 to 17 March 2013.

can persist longer on the crop and more effectively control this pest (Dinardo-Miranda et al., 2004b; Carvalho et al., 2011).

The entomopathogenic fungi, however, lose their viability after the application which could influence the effectiveness of the treatments on the isolates of *M. anisopliae* (Lopes et al., 2011; Guerrero-Guerra et al., 2013).

There were fewer *M. fimbriolata* nymphs parasitized by *M. anisopliae* in the experiment following the conventional sampling method, which can be explained by the timing of the treatment application. The conventional sampling method not considers the biotic potential of the area (Kassab et al., 2012) and the insecticide and/or bioinsecticide application in *M. fimbriolata* control occurs in the low density nymphs

(Mendonça, 2005). This simply means that if, in the first application, during spittlebug control the temperature and humidity favor the development of the *M. fimbriolata* nymphs (Freire et al., 1968), new applications of biological and chemical insecticides will be necessary. This is similar to what was observed in this experiment with the first application in *M. fimbriolata* control on 22 Nov. 2012, followed by the second application on 15 January, 2013.

Conventional sampling × new sampling method: between 15 January 2013 and 17 March 2013

The number of *M. fimbriolata* nymphs did not differ between the treatments followed in the two sampling methods, at 15 and 30 DAT. This indicates that the entomopathogenic fungi and insecticides may have a protracted effect in the field (Dinardo-Miranda et al., 2004a; Loureiro et al., 2005; Tiago et al., 2012). However, although generalizations cannot be made, the cumulative effect is controlled by the weather, the active constituent of the insecticide, the chemical mode of action, and the *M. anisopliae* isolated to control *M. fimbriolata* (Dinardo-Miranda et al., 2004a; Loureiro et al., 2005). On other hand, the genetic constitution of the pest population (Quinelato et al., 2012), adaptations and mechanisms of insect resistance (Dubovskiy et al., 2013) may influence the efficiency of the control techniques.

At 45 and 60 DAT, the treatments utilizing the new sampling technique were significantly less infested and revealed greater efficiency in the control of *M. fimbriolata*. The insecticide Imidacloprid and the isolate of *M. anisopliae* (IBCB 425) showed the highest control efficiency. This result was anticipated, as the late application of the insecticides to control the *M. fimbriolata* reduced the spittlebug infestations and increased the sugarcane productivity (Dinardo-Miranda et al., 2004a; Madaleno et al., 2008). Furthermore, the application occurred towards the end of the cycle of *M. fimbriolata* generations and, it is now accepted, that the normal and diapausing eggs of this pest, experienced conditions suitable for the emergence of nymphs which, in turn, may have enabled greater control efficiency (Kassab et al., 2012).

The number of *M. fimbriolata* of nymphs parasitized by *M. anisopliae* rose with the increase in rainfall (mm), temperature (°C) and relative humidity, which can be explained by the action of the climate-dependent entomopathogenic fungi (Almeida et al., 2007). The entomopathogenic fungi can also increase their density in the crop by infecting healthy individuals (Bruck, 2005, Bruck and Donahu 2007). Furthermore, the insects destroyed by *M. anisopliae* remain in the field, which can reduce the possibility of the resurgence of these pests (Guerrero-Guerra et al., 2013).

The value of the total recoverable sugar (TRS), the expenses per tonne and the earnings per ha were higher in all the treatments which employed the new sampling method. Moreover, the isolates PL 43 and IBCB 425 obtained a higher TRS value, which may be a result of the action of the *Metarhizium* ssp. to translocate the nitrogen of the parasitized insect to the plants (Behie et al., 2012). Besides, plant age can also influence the sugarcane productivity (Dinardo-Miranda et al., 2008). Thus, the plants at a more advanced developmental stage may show a higher TRS yield. The TRS value was estimated using 8-month-old plants, although it may be higher for the older individuals.

Careful monitoring of the small, medium and large nymphs and the *M. fimbriolata* adults can optimize the time of application and raise the efficiency of spittlebug control. Besides, from this study, monitoring the *M. fimbriolata* populations is best done after the first spring rains, following the diapause period of the spittlebug eggs. Areas with a history of *M. fimbriolata* infestations should be given priority in the monitoring programs of this pest and the timing of the insecticide application must be synchronized with the end of the spittlebug lifecycle, thus, lowering the likelihood of reapplication of insecticides in its control.

Conclusion

The use of *M. anisopliae* and insecticides along with monitoring the small, medium and large nymphs and adult *M. fimbriolata* is the most suitable method to control the spittlebug populations by providing greater efficiency and lower cost per hectare.

Conflict of Interest

The authors have not declared any conflict of interest.

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