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Biological control with *Trichogramma pretiosum* increases organic maize productivity by 19.4%

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Abstract

Spodoptera frugiperda is a major pest causing maize yield loss in Brazil. There is therefore a need for control methods, notably for organic farming because classical pesticides are not allowed. A potential solution for organic maize is to apply the biological control agent *Trichogramma pretiosum* to reduce *S. frugiperda* populations. Here, we tested the application of one, two, or three releases of *T. pretiosum*. We measured plant damage ratings, egg masses parasitized, and grain yield. Results show that 79.2% of egg masses were parasited. Maize yields for parasited plots increased of 701 kg/ha versus control plots. This result equals a 19.4% gain of productivity and US $96.5 gain per hectare. Therefore, biological control with egg parasitoids is a promising alternative to control *S. frugiperda* in organic maize.

Keywords – Biological control, Egg parasitoid, IPM, Monitoring, Organic farming, Pheromone trap

1 Introduction

Maize is of primary importance to Brazilian agribusiness, and it is grown widely in the national territory; the crop has a variety of socioeconomic interests across the country. Maize is a fundamental component in a chain of organic food production, which includes milk, beef, and eggs. Despite the increase in geographic area occupied by maize in Brazil and the market demand, research in bringing organic production to sustainable and profitable levels in the country remains inadequate. Therefore, the development and subsequent adoption of alternative pest control strategies for organic agroecosystems poses a substantial challenge, particularly when combined with goals for increasing maize yield.

The fall armyworm, *Spodoptera frugiperda*, is a key pest causing yield reductions in maize production systems (Cruz et al. 2010). Biological control via the release of natural enemies such as egg parasitoids has been proposed as a viable method to control damaging Lepidopteran pests of maize in Brazil (Parra and Zucchi 2004). *S. frugiperda* deposit eggs on the plants. Their larvae emerge and feed on young leaves 3 days after oviposition. Injury to young plants can result in stand reduction with subsequent yield loss of up to 54.5% in the conventional maize agroecosystem (Figueiredo et al. 2006).

The use of natural enemies, particularly parasitoids, has shown promising results in reducing damage from insect pests (Mills et al. 2000; Mills 2010). Favorable results in biological control use have facilitated research opportunities to develop new commercial insectaries, and various natural enemies are commercially available for direct application (i.e., inundative release) in pest management (Cruz et al. 2013; Van Lenteren 2000).

Andow (1997) pointed out that the use of *Trichogramma* to control European corn borer in the USA was limited by the low cost of chemical pesticides, even with substantial improvements in efficiency and a reduction in cost of the parasitoid. This fact is not the case in Brazil. The relatively low value of both the commercial product and labor for release of *Trichogramma* makes the technology viable, even when compared to the use of chemical insecticide. In addition to economic competitiveness, environmental benefits obtained from the use of biological control favor the use of the egg parasitoid *Trichogramma* (Fig. 1), to control *S. frugiperda* (Parra 2010).

The release in a timely manner and appropriate density is imperative for successful pest management. Furthermore, interactions between the natural enemy community and fall armyworm population dynamics in maize fields must be considered in developing an integrated pest management (IPM) program for this crop (Wyckhuys and O'Neil 2006). This approach has been shown to reduce pest population densities, resulting in less plant injury and yield loss. It is believed that conservative measures (Wäckers et al. 2007) taken for biological control in organic systems may promote the survival and the performance of the natural enemy in the area (Wyckhuys and O'Neil 2006). Additionally, the absence of chemical pesticides in organic systems will help increase the population of beneficial insects, as many of these chemicals compromise the efficacy of augmentative releases of biological control agents, as well as their natural occurrence (Ables et al. 1979; Knutson 1998). However, in most production systems, the number of eggs parasitized by native populations of *Trichogramma* and other biological control agents are not sufficient to prevent the pest from reaching damaging levels (King et al. 1985).

Despite progress in biological control worldwide, this alternative is not widely used compared to chemicals (Popp et al. 2013). There are several reasons for this; many problems must be overcome before the product reaches the end user (Parra 2010). Its efficiency depends on several factors, including the kind, quality and suitability of the biological agent, released number, method and the time of release, and the complex interactions between the parasitoid, the target pest, culture, and environmental conditions (Ables et al. 1979; Knutson 1998).

Figure 1. Female of *Trichogramma pretiosum* parasitizing egg mass of *Spodoptera frugiperda* at maize leaf

Insects captured in pheromone traps have been used as a tool in IPM decision-making and to initiate chemical control and natural enemy releases (Ameline and Frérot 2001). A trap containing the *S. frugiperda* sex pheromone is considered the best method to determine if a maize crop requires insecticide treatment (Cruz et al. 2012) and can also be used to ascertain the most suitable *Trichogramma pretiosum* release time. The trap detects moth arrival in the target area, and consequently, female parasitoid release can proceed at the appropriate time. The objective was to evaluate the efficiency of releases of *T. pretiosum* in reducing the population of *S. frugiperda* and increase crop yield in organic corn.

2 Materials and methods

2.1 Study area

The study was conducted at the Centro Nacional de Pesquisa de Milho e Sorgo/CNPMS-Empresa Brasileira de Pesquisa Agropecuária/EMBRAPA, in Sete Lagoas, State of Minas Gerais, Brazil (19° 28′ 00′′ S and 44° 15′ 00′′ W) at an altitude of 820 m.

2.2 Experimental design and treatments

A randomized block experimental design with four *T. pretiosum* release treatments and five replicates (twenty plots) was employed in the study. The parasitoid treatments were applied (0, 1, 2, and 3 releases) when *S. frugiperda* pheromone trap catches reached the action threshold (see section 2.4 below). Each plot (cultivar "BR 106") had 30 rows of maize 20 m long and 0.70 m equidistant (420 m^2). Maize was planted in a no-tillage system. Each experimental unit was separated by buffer plots of equal size to reduce the probability of parasitoids going to neighboring plots.

2.3 Parasitoid

The parasitoids were obtained from a climate-controlled laboratory room maintained at 27±3 °C, 80±10 % RH, and 12:12 h (L:D) photoperiod according to the methodology of Cruz et al. (2013). The colony of *T. pretiosum* was initiated by obtaining adults originated from eggs of *S. frugiperda* and subsequently maintained in the laboratory on eggs of the factitious host Anagasta kuehniella (Cruz et al. 2013). The parasitoid was identified by taxonomist Roberto Antonio Zucchi, and individuals are held in the entomology museum at the Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP) and the Empresa Brasileira de Pesquisa Agropecuária).

The experimental goal was to release five *T. pretiosum* females per square meter maize plot, equivalent to 4200 females and males per plot or 100,000 ha^{-1} based on the assumption of a 1:1 sex ratio. Prior to each release, five random samples of 100 adult parasitoids each (n = 500) were taken from the laboratory colony to determine the sex ratio and estimate the number of females released in the field. The results indicated that an overall mean of 4329 *T. pretiosum* adults/plot (55,706 females ha^{-1}) were released on each date.

2.4 Monitoring of S. frugiperda adults and T. pretiosum release

The initial moth appearance and frequency throughout the experiment was determined using a delta-type trap containing a plastic sachet with BIO SPODOPTERA® (Chem Tica International, SA), a synthetic sex pheromone of *S. frugiperda*. Only one trap was set in the middle of the experimental area beginning at maize shoot emergence according to the manufacturer's instructions. The trap initially was suspended from a stake 0.91 m above ground level. As the maize grew, the trap was raised to remain above the plant canopy, based on the manufacturer's recommendations. Male *S. frugiperda* captured in the traps were counted daily for 25 days. The pheromone lure and the sticky trap base were replaced twice each seven-day period. The trap was used to monitor male moth arrival in the study area and to estimate the pest's temporal proximity to oviposition.

The timing of the first parasitoid release was based on reaching an action limit of three or more moths (cumulatively) per pheromone trap (Cruz et al. 2012). The second and third releases of *T. pretiosum* were performed at a minimum of seven-day intervals, based on continued male adult captures in the trap (i.e., ≥3 males cumulative per trap) after each parasitoid release. The seven-day interval between releases was based on the longevity of the *T. pretiosum* and a mean interval of 10 days between parasitoid generations.

Twenty-four hours after emergence, parasitoids were transported in glass containers in the morning to the target area. The release was conducted in the center of each plot, by opening the containers for adult dispersal. Samplings to detect egg masses of *S. frugiperda* parasitized in 100 plants per plot were initiated 5 days after the first release of *T. pretiosum*. Each plant identified with *S. frugiperda* egg masses was given a numbered and dated label. Plants with egg masses were identified bearing a label stuck on a wooden stake, nailed in the ground (next to the plant), with red pvc tape for easy viewing in the field. Four evaluations were performed to identify egg masses in the area. The first one (Jan. 17), 5 days after the first release of *T. pretiosum*; second (Jan. 19), the first 2 days after. The third (Jan. 24), 5 days after the second, and fourth (Jan. 26), 2 days after the third. The eggs masses found were evaluated within 5 days after the release of *T. pretiosum*, verifying the presence of dark egg (blackened) characteristic of parasitism. In the last release of parasitoid, the presence of egg masses in plants and the occurrence of parasitism were not evaluated. Egg masses were not removed from plants in the experimental area to maintain natural infestation of *S. frugiperda* and to measure the cumulative effects of *T. pretiosum* releases on insect damage and grain yield.

2.5 Assessment of insect damage and grain yield

Efficiency of field releases of *T. pretiosum* are evaluated by measuring egg parasitism, larval densities, crop damage, and economic return relative to similar fields treated with insecticides or not treated (Knutson 1998).

The crop damage was evaluated 25 days after the first parasitoid release. One hundred plants in each experimental plot were randomly assessed for larval damage to six leaves in the central part of the plant using the visual scale reported by Figueiredo et al. (2006): 0, no visible damage; 1, pinhole-type damage; 2, shot hole-type damage; 3, leaf portions eaten away, with some damage in the whorl; 4, plant with the whorl destroyed; and 5, dead plant. The grain yield was obtained by sampling an area containing four rows of 5 m through harvesting, threshing, and manual weighing of grain of corncobs of all plants for all treatments. The grain weight was corrected to 14.5% moisture (Brazilian standard).

2.6 Naturally occurring biological control agents of S. frugiperda

The presence of natural biological control agents was verified by sampling carried out in a contiguous area of maize with the same agronomic conditions of the experimental area. Four subareas of 420 square meters were demarcated in this contiguous area. In each subarea, for four consecutive weeks, 20 plants were sampled and all insects present were counted. The plants were cut at ground level, bagged, and brought to the laboratory. The larvae of *S. frugiperda* were individually placed in 50-ml plastic cups containing artificial diet until the emergence of egg-larval, larva, and larval-pupal parasitoids. The predatory insects collected on plants and the parasitoids after emergence in the laboratory were identified and incorporated into the entomological museum of Centro Nacional de Pesquisa de Milho e Sorgo.

2.7 Statistical analyses

The data were subjected to analysis of variance using the program SISVAR, and the means were compared using the Scott-Knott range test at 5% probability for statistical significance. Regression analyses were performed using Origin 8.1 statistical software.

3 Results and discussion

3.1 Monitoring of S. frugiperda in the trap, egg masses on plants, and parasitoid releases

The presence of *S. frugiperda* male moths in the target area was detected 3 days after plant emergence, initiating the first release of *T. pretiosum* in the experimental plots (Fig. 2a). Moth capture decreased during the remaining 15 days of the trapping period with only 16 of the total 60 moths captured in this period. Furthermore, the presence of egg masses in the experimental area coincided with the increase in the number of males caught in the traps. The following evaluations were performed on January 19, 24, and 26. Incidentally, in the plots without release of the parasitoid, the total number of egg masses (6) was smaller than the total number of egg masses found in plots with parasitoid (13–14) (Table 1). No parasitized egg mass was observed in the plots without release, indicating the absence or low natural population of the *T. pretiosum* in the experimental area. Of the 41 egg masses observed in the plots with *T. pretiosum* releases, 23 were parasitized, i.e., more than 50% had at least one parasitized egg (Table 1). Of the 13 egg masses observed in the treatment with two releases, 10 were parasitized. In plots with three releases, 14 egg masses were observed, 8 of which contained parasitized eggs. The average egg masses parasitized with one or two releases of *T. pretiosum* was 72.58%. Lower larval density in the target area can be further reduced by the presence of other natural enemies such as parasitoids and predators that working together will result less damage to the crop and consequently lower loss in income.

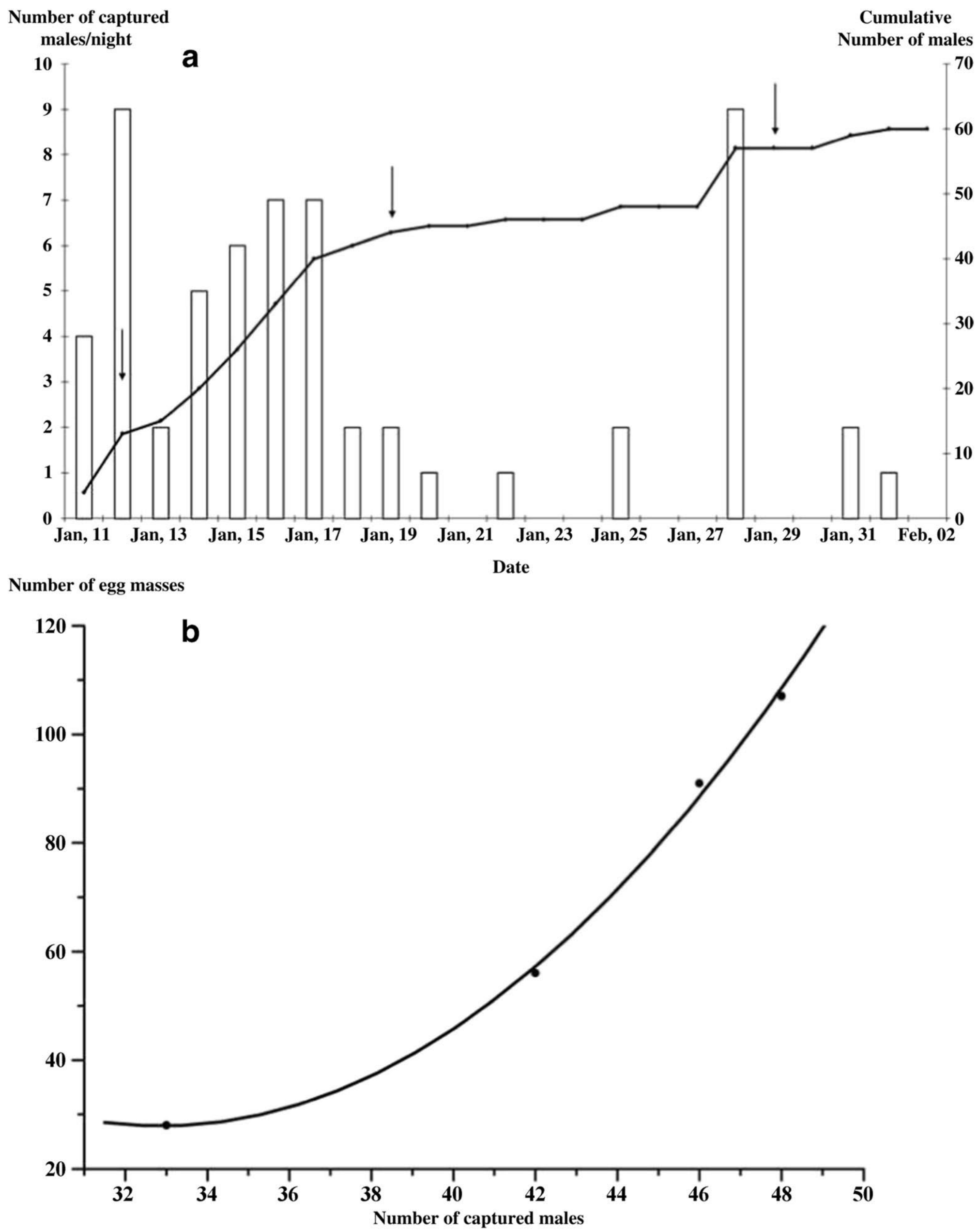

Figure 2. a) Number (daily and cumulative) of *S. frugiperda* moths collected in a pheromone trap. Arrows indicate *T. pretiosum* release dates; **b)** *S. frugiperda* moths captured in pheromone trap and analysis of egg masses in plants observed for 4 days. Regression equation is $y= 408.18 -23.14X + 0.35X^2$; $R^2=0.99$; $P<0.05$

Table 1. Eggs mass of *S. frugiperda* on corn with different numbers of *T. pretiosum* releases

Evaluation (day)	Releases			
	Without	One (Jan.12)	Two (Jan. 12 and 19)	Three (Jan. 12, 19, and 29)
	Parasitism (egg mass parasitized/total egg masses)			
Jan. 17	0/2	1/8	2/4	2/6
Jan. 19	0/1	1/1	3/3	1/1
Jan. 24	0/1	2/3	3/3	3/4
Jan. 26	0/2	1/2	2/3	2/3
Total	0/6	5/14	10/13	8/14
	Percentage of egg masses parasitized			
Jan. 17	0	12.5	50	33.3
Jan. 19	0	100	100	100
Jan. 24	0	66.7	100	75
Jan. 26	0	50	66.7	66.7
Average	0	69.8	79.2	68.75

Egg mass numbers on maize plants increased significantly over the next 16 days with increasing cumulative *S. frugiperda* captured in the pheromone trap following a quadratic relationship (R2=0.99, P<0.05) (Fig. 2b). In plots with release, parasitism was on average 69.8, 79.2, and 68.75% in treatments with one, two, and three releases, respectively (Table 1). It should be noted that the monitoring of egg masses, and the presence of at least one parasitized egg, was only performed until after the second release. The sampling was not performed to determine the occurrence and parasitism in egg masses after the third release. This precludes the conclusion here that three releases would provide more masses of parasitized eggs. Based on the results, the occurrence of a peak of the moth pest in the field required the third release. Therefore, an upward trend in the number of egg masses in the target area can be assumed. It can be expected that with three egg parasitoid releases, larval density on plants tend to be lower than in other larval density plots (0, 1, or 2 releases). Due to the proximity in time between an increase in the number of moth caught in the trap and the third release time of the parasitoid, the plots with three releases could have a greater number of parasitized egg masses.

Three days following trap installation, four males were captured, indicating time for the first parasitoid release. Therefore, the following day (Jan. 12), parasitoids were released into the target area. At this time, the cumulative number of captured males was 13, indicating a high and increasing incidence of moth in the target area (Fig. 2a). Seven days following the first parasitoid release, the second release was performed (19 Jan. 2006). The number of moths captured in the trap between 13 Jan. 2006 (1 day following first release) and 18 Jan. 2006 was 29, far above the cutoff of three moths per trap. This result showed a large adult moth population in the target area. The second and third release of *T. pretiosum* was performed, respectively, 7 days after the first release and 10 days after the second release (29 Jan. 2006). The third and last release was conducted after a second major peak occurrence of moth, similar to that observed at the first release of the parasitoid (Fig. 2a).

It is also worth noting that, after the second release of the parasitoid, the flow of moths in the area was relatively low for about 8 days (Fig. 2a). However, on the ninth day after this second release and 1 day after the third one, the second peak of moth occurred. The relatively high number of captured moths indicated re-infestation and real need for a new release of the parasitoid. The third release of the parasitoid *T. pretiosum* was essential to control the new re-infestation of *S. frugiperda*. This re-infestation was detected only by the efficiency of the pheromone trap. For the experimental premise, the plots without release or with one or two releases of *T. pretiosum* would be subject to damage by the pest, whose intensity would depend on the action of agents of natural occurrence in the experimental area.

It is vital to determine the most appropriate time to release the egg parasitoid *T. pretiosum*. The synchronization of host and *T. pretiosum* is the most essential criterion for efficacious pest control. Pheromone trap might be a tool optimize to biological control. The efficiency of this natural enemy is closely linked to their ability to seek and parasitize the eggs of the moth, to prevent the population growth of larvae in the target plant. Only releases carried out at the right time can bring the expected benefits. Errors in the release will result in failure of control, and this leads to loss of credibility of this pest control tactic that tends to reduce the pest population density in the initial stage of development of the host plant.

3.2 Pest damage

The damage caused by larvae of *S. frugiperda* was low, even in the control, where *T. pretiosum* was not released. Considering the onset of oviposition soon after the arrival of *S. frugiperda*, the average incubation period of the eggs was 3 days, and the flow of moths relatively constant for at least 1 week was expected relatively higher leaf damage than observed, especially in plots without release (Table 2).

The lowest average leaf damage was observed in the plots with three releases. The average leaf damage score was intermediate in the plots with one and two releases of *T. pretiosum* with no significant difference between these two treatments (Table 2). The interaction of natural enemies already present in the area, along with the augmentative releases of *T. pretiosum*, adds an effective control in organic maize cultivation, since the absence of chemical insecticides in the target area benefits both the native and introduced biological control. The low occurrence of leaf damage makes it clear that the release of *T. pretiosum* caused a reduction in the population of larvae. This reduction of larvae in the early stages of plant development (Table 1) probably resulted in a gain in final yields in plots where parasitoids were released. In organic farming, there are added environmental gains from the conservative and augmentative biological control that benefit the crop, as well as providing additional income, which is not always obtained in areas where it employs non-selective chemical insecticides. Even in conventional agriculture, where the use of this chemical are permitted by law, the selectivity of insecticides should be considered in areas treated with releases of natural enemies, as well as conservative biological control.

3.3 Naturally occurring biological control agents of S. frugiperda

The predatory insects observed in samples taken in the subareas of maize adjacent to the experimental area were Doru luteipes (21.57%), Orius sp. (1.22%), and coccinellids (0.5%) that feed on eggs and small larvae of *S. frugiperda*.

The incidence of parasitoids on larvae of *S. frugiperda* was 53.1, 31.3, 29.2, and 33.9% in the first, second, third, and fourth sampling, respectively. In the first sampling, the main parasitoids found were Hymenoptera, Eiphossoma laphygmae (23.96%), Chelonus insularis (20.8%), Campoletis flavicincta (3.1%), Exasticolus fuscicornis (2.08%), and Diptera (3.13%). The same species were found in the subsequent sampling. C. insularis (12.5%), E. laphygmae (8.33%), E. fuscicornis (1.04%), and Diptera (9.38%) were the species found in the second sampled period. In the third sampling, the incidence of Diptera (14.58%) outperformed the E. laphygmae (7.29%), C. insularis (5.25%), and C. flavicincta (2.1%). In the fourth and final sampling, Diptera also predominated (14.79%), followed by E. fuscicornis (1.04%) and C. insularis (1 %). By physical proximity between the two areas of maize, it is possible to consider that the presence of these biological control agents was important in suppressing the pest in addition to the *T. pretiosum* released. The egg-larval parasitoid C. insularis competes with *T. pretiosum*, as both parasitize eggs (Cabello et al. 2011) and its performance in the target area was relevant (20.8% parasitism) in the first sampling. In addition to the egg parasitism, some species of predators feed on both healthy and parasitized eggs, and their occurrence in the production system could result in intraguild competition. Of course, the beneficial effects of parasitoids were observed in egg masses where their presence was noted.

The role of natural control, particularly when maize is produced under different production systems, must be considered for any biological control program. The importance of species complexes of natural enemies that suppress *S. frugiperda* populations was reported by Molina-Ochoa et al. (2001), Hoballah et al. (2004) (parasitoids), and Gross and Pair (1986) (predators). Figueiredo et al. (2006), using the technique of exclusion of natural enemies with cages, confirmed the importance of these organisms in suppressing *S. frugiperda*. When protected for 16 days from the egg stage, the insect infringed severe damage to the plant compared to the damage caused to plants when the insect pest was not protected against their natural control agents.

The experimental area used in this study has been under organic cultivation for more than 15 years without any agrochemicals. This would support increased biodiversity, including natural enemies of insect pests. In fact, both *S. frugiperda* and natural enemies are present in different developmental stages in the area, as observed in other experiments conducted by Figueiredo et al. (2006). This fact can be corroborated with the presence of several biological

Table 2. Injury caused by *S. frugiperda* larvae on corn leaves and grain yield after release of *T. pretiosum*. Means followed by different letters in the same column are significantly different between treatments (Scott-Knott; $p<0.05$)

Number of releases of egg parasitoid *T. pretiosum*	Assessment of injury caused by the larvae of *S. frugiperda** (score 0–5)	Grain yield* (Kg ha^{-1})
0	1.61±0.013 A	3618.85±36.49 A
1	1.53±0.010 B	3887.50±17.58 B
2	1.51±0.008 B	3915.00±32.11 B
3	1.35±0.006 C	4319.97±31.44 C

*Values are mean±standard error

control agents observed in larvae that were collected in the nearby area. Caterpillars parasitized by C. insularis and C. flavicincta (Cruz 2002) substantially reduce their food intake before they were killed. This assumption may be correct, especially considering the results obtained in terms of yield in plots where they held up to three releases of *T. pretiosum*.

The synergistic effects that natural enemies exert over the pest population in an agroecosystem should not be underestimated, mainly when releasing a biological control agent such as *T. pretiosum*. Therefore, at the time of assessment of leaf damage, the larvae originating from eggs of *S. frugiperda* were still in their early instars, and thus, their damage was not yet significant in the evaluation. This assumption can be correct, especially considering the results obtained in terms of grain yield in plots where there were three releases of *T. pretiosum*.

3.4 Yield

Grain yield varied according to the number of releases of *T. pretiosum*. In plots with three releases, grain yield was significantly greater than yield obtained from other treatments (Table 1), averaging >4300 kg ha^{-1}. On the other end of the scale, the smallest yield was obtained in plots where *T. pretiosum* were not released, with an average of 3650 kg ha^{-1}. In plots with one or two releases, the grain yield was intermediate, averaging between 3800 and 3950 kg ha^{-1}, with no significant difference between these averages. The maize yield was significantly and positively correlated with *T. pretiosum* releases (R2=0.84, P<0.05) (Fig. 3). The grain yields obtained in the plots with three releases of parasitoid was 701.25 kg ha^{-1} higher than the yield obtained in plots without release. Grain yield in plots with one or two releases was respectively 268.65 and 296.15 kg ha^{-1} higher than yield observed in witness plots.

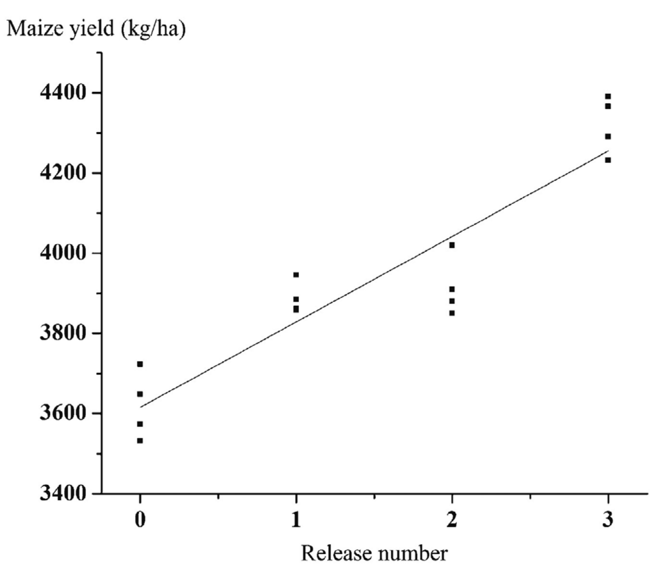

Figure 3. Maize yield after release of *T. pretiosum* to control *S. frugiperda* (Kg ha^{-1}). Regression equation is y=3615.7 + 213.0X; R^2=0.84; P<0.05

According to Smith (1996), the effectiveness of *Trichogramma* releases has been often inconsistent due to the model adopted for assessment and suggests that the crop yield is one of the parameters to be used to validate the effect of this biological control agent. The action limit, based on the capture of moths in traps, was calculated from data on the biology and ecology of *S. frugiperda*, considering periods of life including incubation, larval development, and ability to cause economic damage. The action limit set at three moths caught in pheromone traps corresponds to a level of infestation of 10% of damaged plants (Cruz et al. 2012). Obviously, when the target to be reached is the egg stage by the use of *T. pretiosum*, the release must be immediate as in the study. One cannot deny the accuracy of the method for monitoring the pest through the pheromone trap, considering the specificity of the product and the difficulty of monitoring the pest using other methods. In a conventional system, where chemical control is regularly adopted, usually the only source of the pest population reduction is the insecticide, whose efficiency depends on several factors including type, dose, method of application, etc., where usually the natural enemies are also eliminated. In organic farming, the environmental conditions without chemical insecticide favor the increased population of natural enemies to the point of reducing the population of phytophagous insects to an acceptable level. It should be noted that Spinosad was lethal for all *Trichogramma* species evaluated (Ksentini et al. 2010) and that the application of this and other insecticides should be avoided in agroecosystems where *Trichogramma* are dominant or will be released (Liu and Zhang 2012).

It is possible that natural control would not be able to reduce the density of *S. frugiperda*. Thus, the release of the *T. pretiosum* would be needed to elicit an immediate impact, reducing the *S. frugiperda* population. This fact was evident in the plots with three releases of *T. pretiosum*, because the release was made after a peak population of moths. In fact, 84% of grain yield was explained by the release of the parasitoid. The presence of *T. pretiosum* parasitism in egg masses shows that the natural enemy was able to seek and parasitize their eggs in the experimental area (Table 1). The reduction of pest damage on plant leaves in the initial stage of crop development (although low in all treatments) was significant in the plots with release of parasitoids. The major effect of parasitoid can be seen in the plots with three releases. Although an assessment of damage to leaves was not performed after the third release, the most indicative of the beneficial effect of parasitoids can be seen by the significant increase in grain yield in these plots in relation to grain yields obtained in the other plots, including those where there were no parasitoids released or in fewer releases.

Kuhar et al. (2002) considered *Trichogramma* ostriniae the primary factor in the mortality and subsequent suppression of *Ostrinia nubilalis*, proving that in areas where the parasitoid was released, the control level of the pest in sweet maize was 92%. Hoffmann et al. (2002) also reported similar results after inoculative releases of T. ostriniae to control *O. nubilalis*. Figueiredo et al. (2002) reported positive interactions after releasing the Telenomus remus in maize. An increase of 21% in the control of *S. frugiperda* resulted from the natural occurrence of *Trichogramma* spp., and together, the two parasitoids responded for 97.5% mortality of pest eggs.

In this study, the cost of the wasp and its commercial release for the standard measure adopted in the country, which is the hectare, is US $15.76 for single release parasitoids. In addition to commercial plants, there are in Brazil *Trichogramma* production laboratories under the farmers' association control for use in corn, cotton, soybeans, and tomatoes in each production region. In this scheme, besides reducing control costs, it facilitates the distribution for the various properties. Easy access to parasitoids at the right time and their efficiency has shown that the trend of its use in Brazil is expanding, especially now with the recent occurrence of Helicoverpa armigera in the country in corn and other crops of commercial interest.

Ultimately, both the action of natural biological control agents in the target area and the release can provide a positive impact on pest suppression. This management approach is recommended for control of *S. frugiperda* in organic agriculture. This system experiences less interference than conventional farming, and *T. pretiosum* is commercially available in Brazil. Multiple *T. pretiosum* releases are more cost-effective and exhibit increased efficacy against *S. frugiperda* relative to a single release, and the initial pest distribution and parasitoid functional response are key factors in the biological control effectiveness and decreased environmental impact. Based on comparable efficacy between chemical and biological methods applied to control *S. frugiperda* in agricultural maize fields, Gitz (2008) demonstrated notable advantages to *T. pretiosum* release, including an approximately 78% lower cost of the biological control approach.

The commercial availability of natural enemies of phytophagous insects is a limiting factor to the use of biological control in agricultural areas. However, in many countries, there has been a significant increase in the installation of factories dedicated to the production and sale of different species of beneficial insects for agricultural use. Among the most common species, there is emphasis on the egg parasitoids.

According to Parra (2010), in general, the labor is cheaper in Brazil than in many other countries. One release of T. galloi to control Diatraea saccharalis in sugarcane costs around $14, including labor for release into the field. This cost is lower than in Europe and USA. Over 300,000 ha of sugarcane have been treated with this parasitoid to control D. saccharalis. The species *T. pretiosum* and *Trichogramma* atopovirilia were released in tomatoes and maize in over 50,000 ha in 2007–2008 (Parra 2010). Therefore, biological control is a solid strategy for use in organic farming and integrated pest management programs.

The international scientific literature includes *Trichogramma* species as an excellent alternative to control pests, particularly Lepidoptera (Hassan 1993; Van Lenteren 2000). However, extensive knowledge about different species efficacy to control pests and commercially available natural enemies has been the major restriction in adoption, particularly for organic maize agro ecosystems. The adoption of the pheromone trap along the corn plots is a key to adjust the release of *Trichogramma* in the presence of *S. frugiperda* eggs and avoid the failure of parasitoid releases. Improving the quality of biological agents as well as the correct adoption of biological control with other pest control tactics tend to rise by pressure from consumers concerned with environmental quality and food safety. Despite conflicting results, the adoption of augmentative releases with *Trichogramma* has presented mostly satisfactory results, and with larger studies and techniques may provide better results than before.

Organic farming practices must expand to meet consumer demands for organically grown foods as well as the deployment of new commercial insectaries due to the effectiveness of biological control agents.

4 Conclusion

Monitoring of *S. frugiperda* by capturing males in the trap with synthetic sex pheromone can be used to set the time of parasitoid release, facilitating the management in maize, especially in the area of organic farming, providing gains in grain yield. The release of the parasitoid is due to the presence of independent egg masses of plant development stage.

Three releases of *T. pretiosum* on corn under organic cultivation produced higher grain yields and profitability of the production system.

In this study, the *T. pretiosum* release strategy provided substantial economic benefits to the producer, as up to three releases contributed US $96.476 ha^{-1} gains.

Economic returns and environmental benefits indicate that biological control with egg parasitoids is a viable alternative in organic maize.

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