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Population fluctuation of *Spodoptera frugiperda* eggs and natural parasitism by *Trichogramma* in maize

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ABSTRACT. This study aimed to evaluate the fluctuation of *S. frugiperda* eggs and natural parasitism by *Trichogramma* and identify and quantify parasitoid species in maize fields in the state of Rio Grande do Sul, Brazil. Surveys were carried out in two growing seasons: in 2006/2007 we assessed one growing season (late sowing) in Santa Maria, and in 2007/2008 we assessed two growing seasons (early and late sowing) in Santa Bárbara do Sul. Daily air temperature and rainfall were recorded at both locations. In each evaluation, entire plants were examined for the presence or absence of *S. frugiperda* egg masses, which were analyzed for larvae hatching or parasitoid emergence. The number of *S. frugiperda* eggs is higher between 8 and 30 days after plant emergence, decreasing in the later stages of maize growth; the occurrence of parasitoids begins about two days after pest egg occurrence; *S. frugiperda* egg parasitism is low; and eggs are parasitized by *Trichogramma pretiosum* and *Trichogramma atopovirilia*, with marked predominance of the first and possible occurrence of both parasitoid species in the same egg mass.

Keywords: biological control, fall armyworm, Noctuidae, Trichogrammatidae, *Zea mays* L.

Flutuação populacional de ovos de *Spodoptera frugiperda* e parasitismo natural por *Trichogramma* em milho

RESUMO. O presente trabalho objetivou estudar a flutuação de ovos de *S. frugiperda* e do parasitismo natural por *Trichogramma*, além de identificar e quantificar as espécies de parasitoides encontradas em cultivos de milho no Rio Grande do Sul. Para isso, foram realizados levantamentos em dois anos agrícolas: 2006/2007 numa época de semeadura (do tarde) em Santa Maria, e 2007/2008 em duas épocas de semeadura (do cedo e do tarde) em Santa Bárbara do Sul. Diariamente, a temperatura do ar e a precipitação pluviométrica foram registradas em ambos os locais. Em cada avaliação, toda a parte aérea das plantas foi examinada quanto à presença ou ausência de posturas de *S. frugiperda*, as quais foram analisadas quanto à eclosão de lagartas ou à emergência de parasitoides. O número de ovos de *S. frugiperda* foi maior entre 8 e 30 dias após a emergência das plantas, diminuindo nas fases mais adiantadas da cultura; a ocorrência de parasitoides iniciou cerca de dois dias após a ocorrência de ovos da praga; o parasitismo de ovos de *S. frugiperda* é baixo; e os ovos são parasitados por *Trichogramma pretiosum* e *Trichogramma atopovirilia*, com acentuado predomínio do primeiro e com a possibilidade de ocorrência de ambas as espécies de parasitoides em uma mesma postura.

Palavras-chave: controle biológico, lagarta-do-cartucho, Noctuidae, Trichogrammatidae, *Zea mays* L.

Introduction

Spodoptera frugiperda (J. E. Smith, 1797) (Lepidoptera, Noctuidae), also known as fall armyworm, is the main insect pest of maize in Brazil. It occurs in all producing regions during early and late sowings and causes plant damage from plant emergency to reproductive phases (BESERRA et al., 2002). *S. frugiperda* lays its egg masses at night in layers (three or more) (LEIDERMAN; SAUER, 1953), preferring the lower regions of the plant and the abaxial leaf surface during the early stages of maize growth (V4-V6 stages) (BESERRA et al., 2002). In later stages (V8-V10), the eggs are found in the middle and upper plant regions and on adaxial leaf surfaces.

Because they are poliphagous and resistant to the control methods used (DIEZ-RODRÍGUEZ; OMOTO, 2001), problems with *S. frugiperda* have intensified, necessitating the use of alternative tools for integrated pest management (IPM), such as natural or applied biological controls. In the presence of natural enemies, fall armyworms cause less damage and may make insecticide spraying unnecessary (FIGUEIREDO et al., 2006b).

Although the literature describes a great number of *S. frugiperda* parasitoids (ANDREWS, 1988; FIGUEIREDO et al., 2006a), few have been effectively researched in Brazil, especially for maize crops (SÁ et al., 1993; FIGUEIREDO et al., 1999).

Among these, microhymenopteran egg parasitoid *Trichogramma* (Hymenoptera, Trichogrammatidae) species have been widely used in biological control programs in several regions of Brazil; inundative release might provide great results because this parasitoid reduces insect pest populations in the field before they enter the larval phase (NAVA; NACHTIGAL, 2010; ZUCCHI; PARRA, 2004). In Rio Grande do Sul (RS) State, such extension programs are developed by local technical assistance and rural extension services (EMATER), especially with small producers. However, the biological material used comes from other Brazilian regions, which reduces the chances of success because it disregards species and races that occur naturally under subtropical conditions that are adapted to maize fields in southern Brazil.

So far, records of natural parasitism by *Trichogramma* spp. in *S. frugiperda* eggs in Brazil include: *Trichogramma atopovirilia* Oatman & Platner, 1983, *Trichogramma pretiosum* Riley, 1879 and *Trichogramma rojasi* Nagaraja & Nagarkatti, 1973 (CAMERA et al., 2010; ZUCCHI et al., 2010). Thus, we aimed to identify and quantify parasitoid species in *S. frugiperda* eggs, as well as evaluate parasitoid fluctuation and its relationship with the host (*S. frugiperda*) egg fluctuation in maize crops in Rio Grande do Sul State.

Material and methods

The study was undertaken in Rio Grande do Sul State during two growing seasons: 2006/2007 in Santa Maria city during one season (late sowing), and in 2007/2008 in Santa Bárbara do Sul city during two seasons (early and late sowing) (Table 1). The climate in both areas according to the Köppen classification, is 'Cfa', which is characterized by rain throughout the year and a mean temperature greater than 22°C in the hottest month (MORENO, 1961).

Both regions cultivated the hybrid maize Pioneer 3069 in an area of 30 × 30 m (900 m^2). All the local technical recommendations for this culture were adopted except those regarding insect pest control. The experimental design included randomized blocks with samples in plots. Each crop season was considered a repetition. All areas were divided into 16 plots, and six plants in each plot were assessed for a total of 96 plants per area. In each evaluation, all aerial parts of the plant were examined to determine whether *S. frugiperda* egg masses were present. Initially, the assessments were made on a daily basis, but this frequency was reduced over time as the number egg masses declined. The *S. frugiperda* egg masses and part of the attached leaf were collected, stored in individual plastic bags, numbered, and transferred to the laboratory.

Table 1. Details of locals (cities) and maize crop areas location in Rio Grande do Sul State.

Locals	Santa Maria	Santa Bárbara do Sul	
Coordinates	29°39"23,7'S; 53°39"14,5'W	28°32'34.21"S; 53°16'1.30"W	
Altitude	126 m	428m	
Growing years	2006/2007	2007/2008	2007/2008
Sowing	late	early	late
Sowing (date)	January 13th 2007	December 15th 2007	January 08th 2008
Emergence (date)	January 18th 2007	December 20th 2007	January 14th 2008
Surroundings	North: native vegetation South: a small maize plantation East: a small area with the same maize genotype used in the experiment West: eucalyptus plantation	North: fig plantation South: soybean plantation East: a small area with the same maize genotype used in the experiment West: soybean plantation	North, South and East: soybean plantation West: a small area with the same maize genotype used in the experiment

In the laboratory, the egg masses were labeled and individually packed in jelly capsules (0.8 cm diameter × 2.0 cm length). Afterwards, we determined the total number of eggs in each egg mass by counting hatched larvae, emerged parasitoids, no hatched eggs and the number of eggs with a parasitoid exit hole. The emerged parasitoids were identified according to Querino and Zucchi (2011).

For Santa Maria, data about air temperature were collected from the 8th District of Meteorology (DISME) station located at the Federal University of Santa Maria. It is nearly 10 kilometers from the experimental area, and the precipitation data were collected *in loco* through a pluviometer (rain gauge). In Santa Bárbara do Sul, the air temperature and precipitation data were collected with a digital thermometer and a pluviometer, respectively.

The data analysis was carried out with an 'SOC - NTIA' statistics package (EMBRAPA, 1997). The data obtained were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Bartlett's test), and t-tests were performed to compare means. Differences were considered significant at $p < 0.05$.

The fluctuation of *S. frugiperda* egg masses (parasitized and total) was graphed along with temperature and precipitation data for the different counties. For each date, we compared the egg numbers and the meteorological data using Pearson's correlation tests.

Results and discussion

Number of *S. frugiperda* egg masses, eggs and parasitized eggs

The mean number of *S. frugiperda* egg masses per plant ranged from 0.58 in Santa Maria to 1.93 in Santa Bárbara do Sul, Rio Grande do Sul State, early sowing (ES). The number of eggs per plant ranged from 61.80 in Santa Maria to 270.00 in Santa Bárbara do Sul ES. In spite of the large variation in egg number, the mean number of parasitized eggs did not show a wide range and were between 1.46 and 1.97 per plant (Table 2).

Table 2. Mean number of *Spodoptera frugiperda* egg masses, eggs and parasitized eggs by trichogrammatids per maize plant. Santa Maria, Rio Grande do Sul State, 2006/2007 (late sowing); Santa Bárbara do Sul, Rio Grande do Sul State, 2007/2008 (early and late sowings).

	Santa Maria (late sowing)	Santa Bárbara do Sul (early sowing)	Santa Bárbara do Sul (late sowing)
Egg masses	0.58 b	1.93 a	1.76 a
Eggs	61.80 b	270.00 a	194.15 a
Parasitized eggs	1.46 a	1.94 a	1.97 a

*Means followed by the same letters in lines are not significant (5%) by t-tests of two means with equivalent variances.

The large number of eggs in Santa Bárbara do Sul ES and late sowings (LS) (Table 2) might be related to the fact that these two areas are located next to large fields of soybean crops. Maize is the favorite host for oviposition of *S. frugiperda* moths (PITRE et al., 1983), which are also found in soybean areas (CASMUZ et al., 2010).

The percentage of *S. frugiperda* parasitized eggs reflects the occurrence of natural parasitism in both places, corresponding to 2.36, 0.72 and 1.01% of eggs found in Santa Maria, Santa Bárbara do Sul ES and Santa Bárbara do Sul LS, respectively. These results were similar to parasitism rates of approximately 2.21% found by Beserra et al. (2002) in Brazil in the São Paulo State and by Toonders and Sánchez (1987), who observed values from 0 to 10% in Mexico.

Most of the collected egg masses had layers of eggs that were covered by scales. These variables might have influenced the low number of parasitized eggs, in accordance with Beserra et al. (2002) and Beserra and Parra (2004), who highlighted that *S. frugiperda* egg masses have three or more egg layers covered by scales. Such behavior is a strategy that females employ to avoid egg parasitism that prevents their progeny's development. Another aspect that might have led to low parasitism rates was noted by Noldus (1989), ho verified that *S. frugiperda* eggs are rarely attacked by *T. pretiosum* because this parasitoid does not respond to its semiochemicals.

Population fluctuation of *S. frugiperda* eggs and parasitism by *Trichogramma* spp.

Spodoptera frugiperda appeared in all areas, with egg masses from the fourth day after emergence (DAE) of maize plants (Figures 1, 2 and 3). This appearance was earlier than observed by Sá and Parra (1994), who recorded egg masses from the 11th DAE.

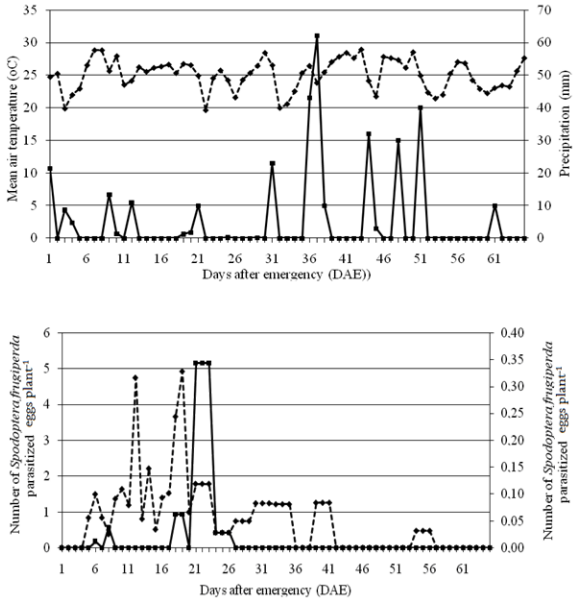

Figure 1. Mean air temperature (---) and precipitation (—) during the maize crop and population fluctuation of *Spodoptera frugiperda* eggs: total (---) and parasitized by *Trichogramma* spp. (—). Santa Maria, 2006/2007.

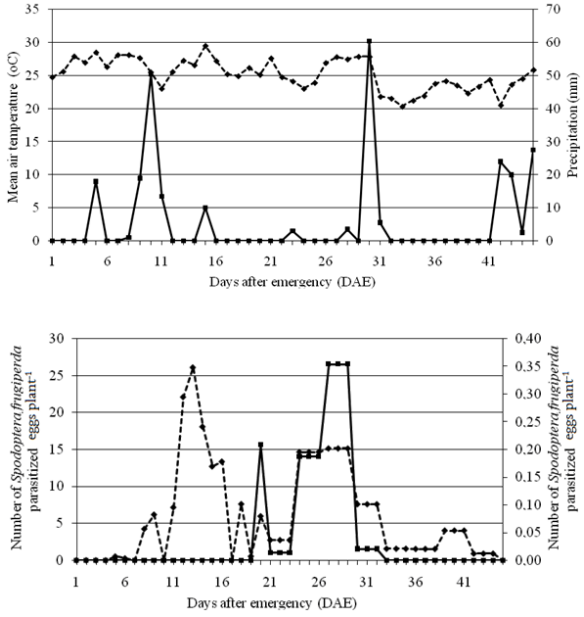

Figure 2. Mean air temperature (---) and precipitation (—) during the maize crop and population fluctuation of *Spodoptera frugiperda* eggs: total (---) and parasitized by *Trichogramma* spp. (—). Santa Bárbara do Sul, early sowing, 2007/2008.

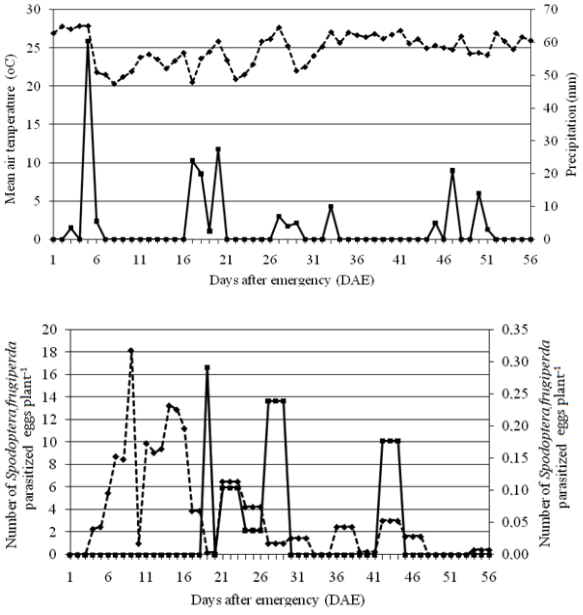

Figure 3. Mean air temperature (---) and precipitation (—) during maize crop and population fluctuation of *Spodoptera frugiperda* eggs: total (---) and parasitized by *Trichogramma* spp. (—). Santa Bárbara do Sul, late sowing, 2007/2008.

The peak of *S. frugiperda* egg population (26.15 eggs plant^{-1}) occurred in Santa Bárbara do Sul ES at 13 DAE (Figure 2). In each location, the moth oviposition apparently followed a pattern in which the number of eggs was higher during the initial maize plant stages compared to the later stages, when a decrease in the egg population was observed. The population peaks occurred from 8 to 30 DAE (Figures 1, 2 and 3).

These values differed from those reported by Pitre et al. (1983), who found that *S. frugiperda* moths preferred maize plants between 54 and 64 days compared to younger ones (22 to 42 days).

This difference is likely due to the different meteorological conditions in the regions (e.g., temperature) because the period observed in the current paper corresponded to stage V4, which is the preferred stage for *S. frugiperda* oviposition (BESERRA et al., 2002).

In contrast, parasitism occurred from 6 to 26 DAE in Santa Maria (Figure 1), from 20 to 33 DAE in Santa Bárbara do Sul ES (Figure 2) and from 19 to 45 DAE in Santa Bárbara do Sul LS (Figure 3). Sá and Parra (1994) observed parasitism from 12 to 53 DAE, a period that is similar to what we observed in the present study considering all three areas. Natural parasitism was low during crop development and was not observed in some periods, such as the initial vegetative phase, which has density-dependent synchronization with the host, and after 45 DAE. The maximum number of parasitized eggs was 0.35 per plant, and this occurred from 21 to 23 and 26 to 30 DAE in Santa Maria and in Santa Bárbara do Sul ES, respectively (Figures 1 and 2). The results suggest that in Rio Grande do Sul State, applied biological control programs using egg parasitoids such as *Trichogramma* should release natural enemies in maize crop areas beginning in the period within the first and the fourth week after crop emergence. This is when the population peaks occur and natural parasitism is low.

Apparently, the largest *S. frugiperda* egg populations were found when the pluviometric precipitation was lower (Figures 1, 2 and 3). However, the correlation analysis between the total number of eggs and the precipitation indicated that there was no linear relationship between these factors (Table 3). Conversely, the mean air temperature during the crop cycles did not seem to influence the *S. frugiperda* population. However, the data collected from Santa Bárbara do Sul during LS suggest a negative correlation of temperature and the total number of eggs of *S. frugiperda* (Table 3).

Table 3. Values of r (Pearson's correlation) for the total number of *Spodoptera frugiperda* eggs and trichogrammatid-parasitized eggs versus mean air temperature ('mT') and precipitation ('precip.') in the study locations. Santa Maria, Rio Grande do Sul State, 2006/2007 (late sowing); Santa Bárbara do Sul, Rio Grande do Sul State, 2007/2008 (early and late sowings).

Locals	Total number of eggs		Number of parasitized eggs	
	x mT	x precip.	x mT	x precip.
Santa Maria	0.15	-0.02	-0.18	-0.10
Sant Bárbara do Sul (early sowing)	0.27	-0.08	0.22	-0.11
Santa Bárbara do Sul (late sowing)	-0.56	-0.18	-0.01	-0.10

S. frugiperda egg parasitoids

Among 500 *S. frugiperda* parasitized eggs, including those that showed evidence of emergence and those that only had vestigial exit holes, we were able to specifically identify 141 parasitoids.

All emerged parasitoids belong to Trichogrammatidae family; 117 were *Trichogramma pretiosum* Riley (1879), and 24 were *Trichogramma atopovirilia* Oatman & Platner (1983), corresponding to 82.73 and 17.27%, respectively. A total of 45 egg masses which there was emergency of parasitoids (13 in Santa Maria, 20 in Santa Bárbara do Sul ES and 12 in Santa Bárbara do Sul LS) and six egg masses (13.33%) were parasitized simultaneously by both species.

The predominance of *T. pretiosum* (93.79% of parasitized eggs) was also reported by Beserra et al. (2002), in Piracicaba, São Paulo State, followed by

T. atopovirilia (2.07%). Davies and Zalucki (2008) found that it accounted for 97% of collected parasitized sample in north Australia. In Venezuela, there is a reference of an occurrence of *T. atopovirilia* parasiting eggs of *S. frugiperda* (MORALES et al., 2007; RÍOS; TERÁN, 2003), but they did not quantify the level.

Despite being found in a smaller proportion, *T. atopovirilia* is more aggressive to *S. frugiperda* and presents a higher specificity than *T. pretiosum* (BEZERRA; PARRA, 2004) because it has a greater capability of parasitism in egg masses with different physical barriers (egg layers and scale densities over egg masses). Thus, programs of applied biological control in Rio Grande do Sul State must consider the usage of this parasitoid species for *S. frugiperda* control in maize crops once its adaptation is ensured based on its natural occurrence.

Conclusion

Based on these results, it is possible to conclude the following:

- the number *S. frugiperda* eggs is highest from 8 to 30 days after crop emergence and decreases in the advanced stages of maize development;

- the occurrence of parasitoids begins around two days after the insect pest eggs are laid;

- the natural parasitism of *S. frugiperda* eggs is low (between 0.72 and 2.36%);

- *S. frugiperda* eggs are parasitized by *Trichogramma pretiosum* and by *Trichogramma atopovirilia*; the first species is highly predominant over the second, and both can occur in the same egg mass;

- the occurrence of *T. atopovirilia* in Rio Grande do Sul State areas suggest that this parasitoid species must be considered in applied biological control programs of *S. frugiperda*.

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