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NOCTUIDAE) EGGS AT DIFFERENT PHENOLOGICAL  
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Source: Florida Entomologist, 85(4) : 588-593

Published By: Florida Entomological Society

URL: [https://doi.org/10.1653/0015-4040\(2002\)085\[0588:DANPOS\]2.0.CO;2](https://doi.org/10.1653/0015-4040(2002)085[0588:DANPOS]2.0.CO;2)

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## DISTRIBUTION AND NATURAL PARASITISM OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) EGGS AT DIFFERENT PHENOLOGICAL STAGES OF CORN

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### ABSTRACT

The oviposition behavior of *Spodoptera frugiperda* (J. E. Smith) and natural parasitism of this pest by *Trichogramma* spp. at different phenological stages of corn were evaluated under field conditions. The distribution of *S. frugiperda* eggs varied according to the phenological stage of the corn. The preferred site for oviposition was the lower region of the plant and the abaxial leaf surface during the early development stages of the crop (4-6 leaves), changing to the middle and upper regions of the plant and the adaxial leaf surface at subsequent stages (8-10 and 12-14 leaves). A larger number of egg masses, and, therefore, of eggs was collected at the 4-6 and 8-10 leaf stages compared to plants in the 12-14 leaf stages. Natural parasitism was low, with a maximum of 2.21% eggs parasitized, especially on the lower and middle parts of the plant. The distribution and degree of parasitism by *Trichogramma* spp. on different regions of the plant were independent of the developmental stage of the crop. *Trichogramma pretiosum* Riley was the most frequent parasitoid, found in 93.79% of the parasitized eggs, followed by *Trichogramma atopovirilia* Oltman & Platner, with 2.07%.

Key Words: fall armyworm, *Trichogramma*, egg parasitoid, oviposition behavior, *Zea mays*

### RESUMEN

Se evaluó el comportamiento de la oviposición de *Spodoptera frugiperda* (J. E. Smith) y en el parasitismo natural de esta plaga por *Trichogramma* spp. a diferentes etapas fenológicas de maíz bajo condiciones del campo. La distribución de los huevos de *S. frugiperda* varió según la etapa fenológica del maíz. El sitio preferida para la oviposición fue la región inferior de la planta y la superficie abaxial (dorsal o del envez) de la hoja durante las etapas tempranas de desarrollo del cultivo (4-6 hojas), cambiando a las regiones medianas y superiores de la planta y la superficie adaxial (ventral o del haz) de la hoja en las etapas subsecuentes (8-10 y 12-24 hojas). Un mayor número de masas de huevos, y, por lo tanto, un mayor número de huevos fueron recolectados en las etapas de 4-6 y de 8-10 hojas en comparación con plantas en la etapa de 12-14 hojas. El parasitismo natural fue bajo, con un máximo de 2.21% de huevos parasitados, especialmente en las partes bajas y medias de la planta. La distribución y el grado de parasitismo por *Trichogramma* spp. en diferentes regiones de la planta fue independiente de la etapa de desarrollo del cultivo. *Trichogramma pretiosum* Riley fue el parasitoida más frecuentemente encontrado con el 93.79% de los huevos parasitados, seguido por *Trichogramma atopovirilia* Oltman & Platner, con el 2.07% de los huevos parasitados.

In Brazil, *Spodoptera frugiperda* (J. E. Smith) is a common species in corn plantations. It is a pest of great economic importance, with production losses reaching 34% (Carnevali & Florcovski 1995). It is found in corn from plant emergence up to the ear stage, within which it is often sheltered, and in many cases has become as important a pest as the corn earworm, *Helicoverpa zea* (Boddie) (Parra et al. 1995).

Morphological and physiological variations occur during the development and maturation of plants, often provoking changes in *S. frugiperda* egg distribution and egg mass characteristics,

such as number of egg layers and scale density. These may be due to nutritional factors that vary as a consequence of physiological changes in the plants. *S. frugiperda* egg mass distribution on the host plant is influenced by the phenological stage of the crop, and can be concentrated within the lower, middle or upper plant regions and on different parts of the leaves and fruiting structures (Ali et al. 1989, Sifontes et al. 1988, Meneses et al. 1991). Sifontes et al. (1988) found that as the rice plant host grew older, *S. frugiperda* egg masses had a higher scale density, as well as a larger number of layers and eggs.

A possible control for *S. frugiperda* is the use of egg parasitoids like *Trichogramma*. These species, however, have difficulties in parasitizing egg masses of this pest because they are covered in scales and the eggs are deposited in layers (Toonders & Sánchez 1987, Cortez & Trujillo 1994). Thus, optimal use of *Trichogramma* spp. to control *S. frugiperda* requires information on egg placement by *S. frugiperda* during development of the corn crop, especially egg distribution in layers and the presence of scales, as well as the degree of natural parasitism by *Trichogramma* spp. The aim of this research was to evaluate the egg distribution and the natural parasitism of *S. frugiperda* eggs at different phenological stages of corn.

#### MATERIALS AND METHODS

The trials were conducted at the Areão Farm, in Piracicaba, state of São Paulo, Brazil, at 22°42'00" South latitude, 47°38'00" West longitude and 546 meters altitude. Data were collected from March—July, 1998 and 1999, October—December, 1998, and from February—April, 2000. Each time a 2-hectare field, with approximately 50,000 corn plants per hectare was studied. The crop received conventional cultural practices recommended for corn, except that no insecticides were applied after the first leaves appeared.

Weekly, 250 plants were sampled, randomly distributed among 10 sampling spots, approximately 30 meters apart. In each spot, 25 plants in an "X" distribution were evaluated, with 5 plants in the center and 5 at each end of the "X". Each group thus included 5 plants, approximately 5 meters from the next group. The plants were evaluated thoroughly and the plant height, the number of leaves per plant and the egg-laying sites were recorded. The eggs were grouped according to the plant region (lower, middle or upper) and leaf surface (abaxial or adaxial) and distribution and natural parasitism were studied at the corn plant phenological stages of 4-6, 8-10 and 12-14 leaves (Cruz & Turpin 1982). The eggs were collected and placed in labeled plastic tubes (3.5 × 1.0 cm), taken to the laboratory, and then kept in glass tubes (8.5 × 2.5 cm). Data were collected on the number of egg layers, the presence or absence of scales and the number of eggs, based on the number of larvae that hatched. Natural parasitism by *Trichogramma* spp. was determined by counting the number of parasitized eggs. The *S. frugiperda* eggs were placed in glass tubes (8.5 × 2.5 cm) in the laboratory for the observation of parasitoid emergence.

The results on the distribution and number of eggs found, the number of eggs and egg layers in each egg mass, and the number of parasitized eggs and their distribution at different phenological stages and on different parts of the corn plant

were subjected to an analysis of variance with the means compared by the PLSD (Protected Least Significant Difference) test ( $\alpha = 0.05$ ). The treatments were arranged in a factorial scheme with two factors: plant age and plant region; the number of parasitized eggs and percentage *S. frugiperda* egg parasitism at different phenological stages were compared by the Tukey test ( $\alpha = 0.05$ ) with a completely randomized design, using SAS software for analysis.

#### RESULTS

The *S. frugiperda* eggs were found at every phenological stage of the corn plant, and over 99% were found on leaves with only two egg masses found on the stem. A significant interaction was found between plant phenological stage and region (lower, middle and upper) ( $F = 2.79$ ;  $P = 0.02$ ) and corn leaf surface (abaxial and adaxial) ( $F = 7.79$ ;  $P = 0.009$ ). That is, the egg distribution among the different parts of the plant varied with the age and development of the corn plant.

At the early developmental stages of the crop (4-6 leaves), the majority of oviposition occurred on the 1st and 2nd leaves (60.4%) and on the abaxial surface of the leaves (83.4%). There were significant differences between the upper, medium and lower regions and the abaxial versus the adaxial leaf surface at the same developmental stage and among the various developmental stages (4-6, 8-10 and 12-14 leaves) (Table 1). As the plant developed to the 8-10 leaf stage, the preferred oviposition site changed and the eggs became more concentrated on the middle and upper plant regions (73.5%) and on the adaxial leaf surface (66.9% of the egg masses). At the 12-14-leaf stage, the greatest concentration of egg masses (61.4%) was observed on the middle region of the plant, significantly greater than on the lower and upper regions. However, there was no significant difference between the abaxial and adaxial leaf surfaces in terms of egg distribution. The middle part of the plant was, to a degree, constant as an oviposition site, with no significant difference between the phenological stages (Table 1).

The mean number of egg masses was significantly ( $F = 4.09$ ;  $P = 0.01$ ) different among the three developmental stages of the corn, with the highest concentration of egg masses at the early developmental stages (4-6 leaves) until approximately 60 days after planting, when the plants had 8-10 leaves, decreasing at the 12-14 leaf stage. However, the mean number of eggs did not differ among the three stages ( $F = 2.76$ ;  $P = 0.01$ ) (Table 2).

A significant interaction was found between plant-age and the number of egg layers in each *S. frugiperda* egg mass ( $F = 2.72$ ;  $P = 0.01$ ). At every phenological stage, most egg masses had more than one layer; the most frequent configuration was

TABLE 1. DISTRIBUTION OF *SPODOPTERA FRUGIPERDA* EGG MASSES ON CORN PLANTS AT DIFFERENT PHENOLOGICAL STAGES DURING THE 1998-2000 GROWING SEASONS IN PIRACICABA, SÃO PAULO STATE.

Phenological stage	Plant age (days)	n	Plant region <sup>1</sup>			Leaf surface <sup>2</sup>		
			Lower <sup>3</sup>	Middle	Upper	Abaxial <sup>3</sup>	Adaxial	
4-6 leaves	14.0-38.0	8	9.7 ± 3.61aA	4.6 ± 0.94bA	2.0 ± 0.92cB	13.8 ± 3.76aA	2.7 ± 1.53bB	
8-10 leaves	22.0-61.0	16	4.3 ± 1.06aB	6.1 ± 1.29aA	6.1 ± 1.31aA	5.4 ± 0.84bB	11.0 ± 2.34aA	
12-14 leaves	36.0-90.0	16	2.0 ± 0.52bB	6.2 ± 1.67aA	1.9 ± 0.61bB	3.9 ± 0.79aB	6.3 ± 1.90aA	

<sup>1,2</sup>Data transformed into  $(x + 1)^{0.1}$  and  $\log x + 1$ , respectively.

<sup>3</sup>Means followed by the same lower-case letter in rows and capital letters in columns do not differ by the PLSD (Protected least Significant Difference) test (alpha = 0.05).

TABLE 2. NUMBER OF EGG MASSES, EGGS AND EGG MASSES WITH VARYING NUMBERS OF LAYERS (1, 2, 3, >4) OF *SPODOPTERA FRUGIPERDA* AT DIFFERENT PHENOLOGICAL STAGES OF CORN, DURING THE 1998-2000 GROWING SEASON IN PIRACICABA, SÃO PAULO STATE.

Phenological stage	n	Average no. of egg masses	Average no. of eggs/collection	Layers/egg mass <sup>1</sup>			
				1	2	3	>4
4-6 leaves	8	16.2 ± 5.19a <sup>2</sup>	2,344.0 ± 882.22a <sup>3</sup>	0.8 ± 0.33bA <sup>4</sup>	6.4 ± 2.58aA	5.0 ± 1.65aA	2.2 ± 1.23bB
8-10 leaves	16	16.5 ± 2.89a	3,647.7 ± 788.25a	0.6 ± 0.15cA	2.7 ± 0.15bB	5.6 ± 0.41aA	5.5 ± 0.37aA
12-14 leaves	15	10.1 ± 2.45b	2,086.2 ± 471.13a	0.6 ± 0.15bA	1.5 ± 0.29abB	2.4 ± 0.64aB	3.2 ± 0.27aAB
Overall average				0.7 ± 0.13b	3.5 ± 0.56a	4.3 ± 0.56a	3.6 ± 0.90a

<sup>1</sup>Data transformed into  $(x + 1)^{0.1}$ .

<sup>2</sup>Means followed by the same letter do not differ by the PLSD (Protected Least Significant difference) and F tests (alpha = 0.05), respectively.

<sup>3</sup>Means followed by the same lower-case letter in rows and capital letters in columns do not differ by the PLSD test (alpha = 0.05).

three layers, with 91.56% covered by scales. At the 4-6-leaf stage the eggs predominantly had 2 or 3 layers and at the following stages - 8-10 and 12-14 leaves, those with 3 or 4 layers prevailed (Table 2).

With respect to parasitism, no significant interaction was observed between plant phenology and plant region ( $F = 1.49$ ;  $P = 0.21$ ), and no significant differences were found among the three developmental stages in the number ( $F = 0.3$ ;  $P = 0.74$ ) and percentage ( $F = 1.37$ ;  $P = 0.25$ ) of *S. frugiperda* egg masses parasitized by *Trichogramma* spp. (Table 3). However, significant differences in parasitism were detected among the three parts of the plant ( $F = 2.91$ ;  $P = 0.05$ ); the greatest number of parasitized eggs occurred on the lower and middle regions of the plant (Table 3). Also the number ( $F = 0.98$ ;  $P = 0.38$ ) and percentage ( $F = 1.84$ ;  $P = 0.17$ ) of parasitized *S. frugiperda* eggs did not differ among the three phenological stages of the corn (Table 4), showing that the distribution and degree of parasitism by *Trichogramma* spp. on different regions of the plant were independent of the developmental stage of the crop.

Overall natural parasitism was very low with a maximum of 2.21% of the eggs parasitized (Table 4), despite the fact that *S. frugiperda* eggs were available at every plant phenological stage. Parasitism remained constant over all densities of eggs observed at the 4-6 and 8-10 leaf stages (Table 2). Few *Trichogramma* species were found parasitizing the *S. frugiperda* eggs. *Trichogramma pretiosum* Riley was most abundant, comprising 93.79%. *Trichogramma atopovirilia* Oatman & Platner occurred in 2.07% of the samples. Multi-parasitism—a frequent phenomenon in *Trichogramma*—was observed, as *T. pretiosum* and *T. atopovirilia* appeared simultaneously in various egg masses. The remaining infested eggs (4.14%) were parasitized by other species of the genus *Trichogramma*. Only one individual of another genus of the family Trichogrammatidae was found. It was not identified because only one fe-

male emerged (the identification is based on males). In addition, this female failed to reproduce in eggs of the factitious host *Anagasta kuehniella* Zeller.

## DISCUSSION

Changes in the oviposition behavior of *S. frugiperda* occurred as the host plant developed. Egg masses were most abundant on the lower region of the plant, and on the abaxial leaf surface, at the early 4-6-leaf stage; and on the middle and upper regions and the adaxial leaf surface at the subsequent stages (8-10 and 12-14 leaves). Pitre et al. (1983) showed that while in grasses such as sorghum and corn, *S. frugiperda* egg masses are more numerous and larger than in other hosts such as soybean and cotton. They also showed that the abaxial leaf surface, and the plant regions with greatest leaf mass, are preferred oviposition sites since they constitute a protected environment. The oviposition strategy of *S. frugiperda* on corn may focus on protection from natural enemies or suitable feeding sites for progeny, since females oviposit in areas near the feeding sites of the larvae. As the plant develops, egg laying shifts to the upper region of the plant, close to the spindle. When this site is not available after bolting, the middle region of the plant, close to the ear, becomes the main oviposition site. Labatte (1993) found that larvae migrate towards the upper region of the corn plant to feed at the 4-6 leaf stage. He indicated that up to the 10-leaf stage feeding occurs preferably among the whorl of young leaves, where the worms remain until the end of their development. After the 12-14-leaf stage, the worms migrate to the tassel and the middle region of the plant, during tasselling and ear formation, remaining in the leaves and ears. This egg laying and larva distribution pattern was also observed for *Ostrinia nubilalis* (Hüb.) by Shelton et al. (1986) and Labatte (1991), who

TABLE 3. MEAN NUMBER AND PERCENTAGE OF *SPODOPTERA FRUGIPERDA* EGG MASSES PARASITIZED BY *TRICHOGRAMMA* SPP. AT THREE DIFFERENT STAGES AND ON THREE DIFFERENT PARTS OF THE CORN PLANT, DURING THE 1998 - 2000 GROWING SEASONS IN PIRACICABA, SÃO PAULO STATE.

Phenological stage	n	Number of parasitized egg masses <sup>1</sup>	Percentage parasitism
4-6 leaves	21	7.0 ± 3.01a	43.7 ± 11.29a
8-10 leaves	42	2.9 ± 1.14a	18.4 ± 4.67a
12-14 leaves	36	2.8 ± 1.15a	27.7 ± 6.52a
Plant region			
Lower	33	2.0 ± 0.44a	26.0 ± 5.39a
Middle	38	1.5 ± 0.13a	18.6 ± 3.52a
Upper	29	0.6 ± 0.05b	15.4 ± 5.33a

<sup>1</sup>means followed by the same letter do not differ by the F test among phenological stages or the PLSD (Protected Least Significant Difference) and F tests, respectively among plant regions ( $\alpha = 0.05$ ).

TABLE 4. PARASITISM OF *SPODOPTERA FRUGIPERDA* EGGS BY *TRICHOGRAMMA* SPP. AT DIFFERENT PHENOLOGICAL STAGES OF THE CORN PLANT DURING THE 1998 - 2000 GROWING SEASONS IN PIRACICABA, SÃO PAULO STATE.

Phenological stage	n	Total egg masses	Total eggs	Average number of parasitized eggs <sup>1</sup>	Average parasitism percentage <sup>2</sup>
4-6 leaves	8	114.0	18,752.0	51.8 ± 21.85a <sup>3</sup>	2.2 ± 0.91a
8-10 leaves	16	243.0	58,364.0	33.2 ± 12.66a	0.9 ± 0.60a
12-14 leaves	15	116.0	31,293.0	38.8 ± 16.05a	1.8 ± 0.57a

<sup>1,2</sup>Data transformed into (x)-0.1 and 1/√ x, respectively.

<sup>3</sup>Means followed by the same letter do not differ by the F test (alpha = 0.05).

stated that the phenological stage of the corn is the main factor affecting the pest's behavior.

The decreased number of egg masses observed in our study as the plant grew older coincides with the population fluctuation of *S. frugiperda* adults and larvae observed by Mitchell et al. (1984) and Gutiérrez-Martínez et al. (1989). Surveys conducted by Gutiérrez-Martínez et al. (1989) showed that the first adults appear right after plant emergence, with the highest frequency from the 10th through the 41st day of emergence, when the plant is more susceptible, decreasing considerably up to the 72nd day, when fewer attacks on the corn crop occur. This phase also coincides with the period of greatest production losses, which may reach 19% when the plant achieves the 8-10 leaf stage (Cruz & Turpin 1982).

Although the number of egg masses decreased with plant age, the number of eggs found on the 8-10 and 12-14 leaf stages was higher than at the 4-6-leaf stage (Table 4). Meneses et al. (1991) also observed a high frequency of egg masses with three layers and an average of 385.6 eggs/egg mass in rice. Sifontes et al. (1988) found *S. frugiperda* with a higher number of layers and eggs in egg masses, and a higher scale density on older rice plants.

Studies by García & Sifontes (1987), Toonders & Sánchez (1987) and Hoffmann et al. (1995) revealed that *Trichogramma* spp. have difficulty in parasitizing *S. frugiperda* eggs, which is in accordance with our results. García & Sifontes (1987) reported that 46.9% of 47 egg masses parasitized by *Telenomus* and 4.3% parasitized by *Trichogramma* spp. together with *Telenomus*. Moreover, they considered only the number of egg masses parasitized and neglected the number of eggs parasitized, which obviously would give an even lower percentage of parasitism. Toonders & Sánchez (1987), on the other hand, counted the number of parasitized eggs, and observed that natural parasitism by *Trichogramma* spp. varied from 0 to 10% in samples examined in six different fields and when 30,000 parasitoids were released in 1.5 ha of corn the parasitism rate was only 4%.

The differences found in the degree of parasitism observed among the three parts of the plant (upper, middle and lower) does not indicate a pref-

erence of *Trichogramma* spp. for parasitizing the host eggs in the lower and middle portions, rather it may be associated with the oviposition behavior of *S. frugiperda* which overall oviposited more in these regions (average 5.37, 5.64 and 3.29 egg masses for the lower, middle and upper regions, respectively), allowing an increase in the number of parasitized eggs, although there were no differences in the percentage of parasitism (Table 3). These results are in accordance with those reported by Wang et al. (1997), who observed a greater preference of *T. ostrinia* for parasitizing *O. nubilalis* eggs on the lower and middle regions of the corn plant, as these are the sites with the greatest concentration of the host's egg masses. However, a greater availability of eggs in a given region of the plant does not always result in increased parasitism by *Trichogramma*. For example, Romeis et al. (1998, 1999) observed that *Trichogramma chilonis* Ishii parasitized more *Helicoverpa armigera* eggs on pigeonpea leaves than on flowers and pods, even though more *H. armigera* eggs were available on the latter structures. These differences, in this case, can be explained by the presence of trichomes, which damage the parasite.

Records of parasitism by *Trichogramma* on *S. frugiperda* eggs are scarce and usually indicate low intensities, as found by García & Sifontes (1987) in the Sancti Spiritus region of Cuba, with 4.3% parasitized egg masses, similar to our data.

Knowledge about changes in the oviposition behavior of *S. frugiperda* at the various phenological stages could help in the planning of field-sampling methods, location of ovicide applications, and releases of *Trichogramma* spp. in augmentative biological control. However, doubts remain on the effectiveness of using *Trichogramma* to control this lepidopteran pest as natural infestation rates are quite low. This problem needs to be addressed before this parasitoid can be used as a viable alternative to chemical controls.

#### ACKNOWLEDGMENTS

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship grant which enabled this research, and Agricultural Engineer Ranise Barbosa for identifying the *Trichogramma* species.

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