

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) in cotton: vertical distribution of egg masses, effects of adult density and plant age on oviposition behavior

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) em algodoeiro: distribuição vertical de posturas, efeitos da densidade de adultos e da idade das plantas sobre o comportamento de oviposição

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ABSTRACT: The objective of this work was to determine the most suitable density of adults per plant, ideal plant age, and vertical distribution of eggs in different parts of the plant in a greenhouse investigation for future resistance studies and improvement of *S. frugiperda* management practices in cotton. The experiments of *Spodoptera frugiperda* oviposition in relation to adult density, relationship between cotton plant age and *Spodoptera frugiperda* oviposition, *Spodoptera frugiperda* non-preference for oviposition on cotton varieties were conducted with plants of cotton variety BRS Ita 90. The experiments were conducted with plants of cotton variety BRS Ita 90. Non-preference (antixenosis) for oviposition was studied in FiberMax 966, FiberMax 977, DeltaOpal, DeltaPenta, Acala 90, Coodetec 408, Coodetec 409, Coodetec 410, BRS Cedro, BRS Ipê, BRS Aroeira, IPR 96, IPR 20, BRS Araçá, IAC 24, and BRS Ita 90 varieties. We concluded that the fall armyworm *S. frugiperda* prefers to oviposit on plants with approximately 60 days of age, on the lower surface of leaves located in the upper third of the plant tests under greenhouse conditions. A density of at least three pairs of *S. frugiperda* adults per plant was sufficient to conduct non-preference-for-oviposition tests under greenhouse conditions. Coodetec 408, BRS Aroeira, BRS Araçá, BRS Ita 90 and DeltaPenta varieties showed non-preference-for-oviposition resistance to *S. frugiperda*. In regions with high infestations of *S. frugiperda*, it would be prudent for the cultivation the use of BRS Ita 90 cotton variety.

KEYWORDS: *Gossypium hirsutum*; host selection; plant resistance.

RESUMO: O trabalho teve por objetivo determinar, em casa-de-vegetação, a densidade adequada de adultos, a idade ideal das plantas e a distribuição vertical de ovos nas diferentes partes da planta, visando à realização de estudos de resistência e à melhoria das práticas de manejo de *Spodoptera frugiperda* em algodoeiro. Os experimentos de oviposição de *S. frugiperda* em relação à densidade de adultos, relação entre plantas de algodoeiro e oviposição de *S. frugiperda* e não preferência para oviposição de *S. frugiperda* em variedades de algodoeiro foram realizados com plantas da variedade de algodoeiro BRS Ita 90. A não preferência (antixenose) para oviposição foi acompanhada nas variedades FiberMax 966, FiberMax 977, DeltaOpal, DeltaPenta, Acala 90, Coodetec 408, Coodetec 409, Coodetec 410, BRS Cedro, BRS Ipê, BRS Aroeira, IPR 96, IPR 20, BRS Araçá, IAC 24 e BRS Ita 90. Concluiu-se que *S. frugiperda* prefere ovipositar em plantas com cerca de 60 dias de idade, na superfície inferior das folhas situadas no terço superior dos testes de plantas sob condições de estufa. Uma densidade de, pelo menos, três pares de adultos *S. frugiperda* por planta foi suficiente para realizar testes de não preferência para oviposição em casa de vegetação. As variedades Coodetec 408, BRS Aroeira, BRS Araçá, BRS Ita 90 e DeltaPenta apresentaram resistência do tipo não preferência para oviposição de *S. frugiperda*. Em regiões com altas infestações de *S. frugiperda*, seria prudente para o cultivo utilizar a variedade de algodão BRS Ita 90.

PALAVRAS-CHAVE: *Gossypium hirsutum*; seleção hospedeira; resistência de plantas.

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INTRODUCTION

The pests are one of the major limiting factors for the exploitation of cotton in Brazil (BASTOS et al., 2010), including among these the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). In recent years, this insect has grown in importance in the country's main cotton producing regions (DEGRANDE, 1998; FERNANDES et al., 2002; SANTOS et al., 2003).

The fall armyworm *S. frugiperda* is native to the tropical and subtropical regions of the Americas and it has widespread distribution in the American Continent (LUGINBILL, 1928; MOREIRA et al., 2003). In Brazil, the fall armyworm finds favorable climate and several hosts, which foster its occurrence across all regions year round (CRUZ, 1995; SOARES; VIEIRA, 1998; SANTOS, 2001). Although the fall armyworm prefers to feed on grasses such as corn, millet, wheat, sorghum, rice, and sugarcane, it also attacks plants from other families of economic importance, including cotton (ALI et al., 1989; CRUZ, 1995).

In annual agroecosystems consisting of soybean, corn, bean, and cotton, there is a continuous supply of food for polyphagous insects such as species of the genus *Spodoptera* (SANTOS, 2001). The existence of irrigated crops, especially in cerrado regions, extends the survival of insects through time, increasing the number of generations and facilitating the adaptation of insect pests. Under such situation, the moths migrate between crops consisting of related plant species, established during different seasons and among different botanical species (SANTOS, 2001; SANTOS et al., 2003).

In cotton, *S. frugiperda* oviposit in most of the plants phenological stages, more often in the lower (ALI et al., 1989), middle (ALI et al., 1989; MIRANDA, 2006) and upper thirds of the plant (MIRANDA, 2006) and preferentially on the lower surface of the leaves (PITRE et al., 1983; ALI et al., 1989; SANTOS, 1999; FERREIRA, 2003; MIRANDA, 2006), in masses that may contain from 30 to 300 eggs (KING; SAUNDERS, 1984; FREEMAN, 1999). Egg masses are held together and fixed to the substrate by a substance produced by the accessory glands of females and are covered with one or more layers of abdominal hairs (LUCCHINI, 1977; PATEL, 1981).

S. frugiperda chemical control in cotton is difficult because the egg masses are found on the lower surface of the leaves in the middle and lower thirds of the plant and also because first- and second-instar caterpillars remain near the area where eggs were laid, reducing the efficiency of sprays (SANTOS, 1998; FERNANDES et al., 2002). The need to control *S. frugiperda*, among other pests, in cotton fields is a major cause of development and expansion of chemical control within the different regions of Brazil. Thus, a new model of agriculture that recommends changing the environment as little as possible has led researchers to study new tools aimed

at improving the Integrated Management of *S. frugiperda* in cotton crop, particularly those used during the sampling procedures and other related resistance of cotton varieties pest, studied here.

Thus, the objective of this research was to determine the oviposition site preferred by *S. frugiperda* in different cotton plant parts, the most suitable adult density and the best plant age to conduct resistance tests in the greenhouse, as well as its non-preference (antixenosis) for oviposition on varieties of this crop.

MATERIAL AND METHODS

The studies reported in this paper were performed in the Entomology Laboratory of the Phytosanitary, Rural Engineering and Soils Department of the Engineering School (UNESP), Ilha Solteira, São Paulo State, in 2007, with geographic coordinates 20°25'58"S latitude and 51°20'33"O longitude.

Evaluations for adult density, plant age and oviposition distribution were conducted on plants of cotton variety BRS Ita 90. Non-preference for oviposition was studied in varieties FiberMax 966, FiberMax 977, DeltaOpal, DeltaPenta, Acala 90, Coodetec 408, Coodetec 409, Coodetec 410, BRS Cedro, BRS Ipê, BRS Aroeira, IPR 96, IPR 20, BRS Araçá, IAC 24, and BRS Ita 90.

The insects used in the experiments came from a *S. frugiperda* stock rearing started with individuals provided by EMBRAPA/CNPMS, Sete Lagoas, Minas Gerais State. The insects were reared and kept in the laboratory on artificial diet (KASTEN-JUNIOR et al., 1978), using a methodology prepared by PARRA (1986).

Cotton plants of different varieties were grown in pots (20 L) containing substrate consisting of a mixture of soil, sand, and compost at a 2:1:1 ratio. Seeding was accomplished using five seeds per pot, and a thinning operation was conducted ten days after germination, leaving one plant per pot. Fertilization was performed according to the recommendation by VAN RAIJ (1991) with daily irrigation as needed.

During the evaluation period, adults were fed a 10% honey solution supplied in a dental roll attached to the leaf petiole with a small hook and replaced daily.

***Spodoptera frugiperda* oviposition in relation to adult density**

Four *S. frugiperda* adult densities were evaluated in a non-choice experiment with ten replicates in a completely randomized design. Plants at 60 days of age were individually

protected with a hood made of tulle fabric measuring 60 cm in diameter and 120 cm in height, tied at the base of the stem. Infestations were performed on the second day after mating (observed in the laboratory) at densities of 1, 2, 3, and 4 *S. frugiperda* pairs per plant. The number of egg masses per plant was counted 48 and 72 h after infestation. Tests for densities ranging from 1 to 4 pairs of adults/plant were determined on the basis of the behavior of *S. frugiperda* which, according LUGINBILL (1928), oviposit indiscriminately, and being polyphagous, the larva has a chance to find a plant of its own to feed. Thus, it is considered that, with this number of pairs of adults tested, the probability to determine the number of couples suitable for the study of non-preference for oviposition in cotton plants is higher.

Relationship between cotton plant age and *Spodoptera frugiperda* oviposition

Cotton plants in three different phenological phases: phase B (30 to 45 days) — emergence of the first bud; phase F (60 to 75 days) — emergence of the first flower and phase C (from 90 days) — from the opening of the first capsule, were evaluated in a free-choice experiment with ten replicates, in a random block design organized as a split-plot in time. Plants, one per pot, were placed in a greenhouse within an area enclosed by tulle fabric and then subjected to infestation.

Vertical distribution of *Spodoptera frugiperda* egg masses in cotton plants

In a non-choice test, 60-day-old plants (one per pot), selected in the previous experiment, were placed in the greenhouse; each plant was protected by a hood made of tulle fabric, measuring 60 cm in diameter by 120 cm in height, tied at the base of the stem. Four pairs of *S. frugiperda* adults were released per plant on the second day after mating in the laboratory. Evaluations were made 48 and 72 h after infestation by counting the numbers of egg masses present in upper, middle and lower thirds of the plant. In this trial, a completely randomized design was adopted, with ten replicates.

Spodoptera frugiperda non-preference for oviposition on cotton varieties

In this experiment (the non-preference for oviposition of *S. frugiperda* in cotton varieties) FiberMax 966, FiberMax 977, DeltaOpal, DeltaPenta, Acala 90, Coodetec 408, Coodetec 409, Coodetec 410, BRS Cedro, BRS Ipê, BRS Aroeira, IPR 96, IPR 20, BRS Araçá, IAC 24, and BRS Ita 90 varieties were used in six replicates and randomized design. Plants with 60 days (age defined in the previous experiment), one per pot, were placed in a greenhouse, within an area covered with tulle fabric type and subjected to infestation. For each plant, four

adult couples were released, two days after mating in the laboratory. The ratings, counting the number of egg masses per plant, were taken 48 and 72 h after infestation.

Statistical analyses

The data, previously transformed to $(x + 1)^{1/2}$, were submitted to analysis of variance (ANOVA) and the means were compared by Tukey's test at 5% probability. The SisVar v. 5.0 software (FERREIRA, 2003) was employed to run the analyses and means comparison tests.

RESULTS AND DISCUSSION

The differences between mean numbers of egg masses per plant under varying *S. frugiperda* adult densities were significant (Table 1). At densities of one and two adult pairs per plant, 6.2 and 7.1 egg masses per plant were observed, respectively; however, these are statistically equal the two others. Higher means, 14.5 and 20.9 egg masses per plant, were found at densities of three and four pairs per plant, respectively, differing from the two lower densities. Three or more pairs of adults per plant can be considered an appropriate density to conduct *S. frugiperda* resistance tests with cotton varieties under greenhouse conditions.

These densities, three or four pairs, determined by counting the numbers of egg masses per plant, were higher than those cited by FERREIRA (2003), who used two mated females per plant while conducting tests with varieties BRS Ita 90 and Coodetec 404.

Considering the various cotton plant ages, the highest mean number of egg masses/plant was found in 60-day-old plants (7.89 egg masses per plant), significantly differing from the means obtained with plants at other ages (Fig. 1). Thirty- and 45-day-old plants were less oviposited (1.33 and 2.22 egg masses per plant, respectively) in relation to 75- and 90-day-old plants (3.22 and 4.78 egg masses per plant). The mean numbers

Table 1. Number (mean \pm standard deviation) of *Spodoptera frugiperda* egg masses/cotton plant, BRS Ita 90 variety, according to adult density.

Number of pairs/plant	Egg masses
1 pair	6.20 \pm 1.01b
2 pairs	7.10 \pm 1.34b
3 pairs	14.50 \pm 2.01a
4 pairs	20.90 \pm 1.52a
F	18.12**
CV	(%) 20.77
DMS	0.87

Means followed by the same letter are not different from one another by Tukey's test at 5% probability.

F: F-test; CV: Variation of coefficient and the percentage (%) is together with CV; DMS: minimum significant difference.

**Significant at 1% probability.

of *S. frugiperda* egg masses found in cotton plants, regardless of age, match those obtained by ALI *et al.* (1989), who concluded that *S. frugiperda* oviposit on all cotton plant phenological stages. FERREIRA, (2003) studied the oviposition, dispersal, and feeding behavior of *S. frugiperda* caterpillars on cotton varieties BRS Ita 90 and Coodetec 404 and used (using) two mated females per plant at 65 days of age when the plants simultaneously had flower buds, flowers, and bolls. From the results obtained in that experiment, it could be generally concluded that *S. frugiperda* prefers to oviposit on plants at 60 days of age.

Significant differences for mean number of egg masses per plant part among the three plant thirds were only observed for leaves (Table 2). Then, 52.63% of the egg masses were located in the plants' upper third; 34.21% in the lower third; and 13.16% in the middle third. In conditions of greenhouse, the results are partly similar to those found by FERREIRA, (2003), who cited the upper third of cotton plants as the preferred region for *S. frugiperda* oviposition. In contrast, the results obtained here differ, in part, from those achieved by ALI *et al.* (1989), SANTOS (1999) and MIRANDA (2006), who found a higher number of *S. frugiperda* egg masses in the plants' middle and lower thirds, when the data were collected in the field.

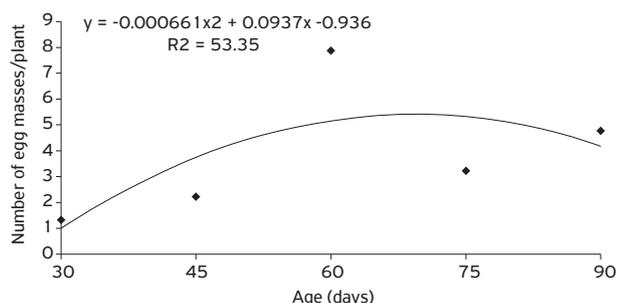


Figure 1. Relationship between cotton plant age and number of *Spodoptera frugiperda* egg masses in a free-choice test.

Regarding the distribution of eggs in different parts of the plant, no egg mass were observed on bracts, flower buds, flowers, and bolls during the evaluation period (Table 2), regardless of the position of those structures in the plant. The highest number of egg masses was found in the leaves (92.10%), preferentially on the abaxial surface. Those results confirm the data found by PITRE *et al.* (1983), ALI *et al.* (1989), FERREIRA, (2003) and MIRANDA (2006), who reported the abaxial surface of the leaves as the site preferred by *S. frugiperda* for oviposition.

Table 3. Numbers (mean \pm standard deviation) of *Spodoptera frugiperda* egg masses on different cotton varieties, 72 h after infestation, in a free-choice test.

Varieties	Egg masses
IPR 96	0.67 \pm 0.33ab
IPR 120	0.33 \pm 0.33ab
FiberMax 966	0.67 \pm 0.67ab
FiberMax 977	0.17 \pm 0.17b
Coodetec 408	0.02 \pm 0.00b
Coodetec 409	0.17 \pm 0.17b
Coodetec 410	2.17 \pm 0.70a
BRS Ipê	1.00 \pm 0.63ab
BRS Aroeira	0.00 \pm 0.00b
BRS Araçá	0.00 \pm 0.00b
BRS Cedro	0.50 \pm 0.22ab
BRS Ita 90	0.00 \pm 0.00b
DeltaPenta	0.00 \pm 0.00b
DeltaOpal	0.17 \pm 0.17b
Acala 90	0.17 \pm 0.17b
IAC 24	1.17 \pm 0.75ab
F	2.38**
CV (%)	39.24
DMS	0.76

Means followed by the same letter are not different from one another by Tukey's test at 5% probability.
F: F-test; CV: Variation of coefficient and the percentage (%) is together with CV; DMS: minimum significant difference.
**Significant at 1% probability.

Table 2. Numbers (mean \pm standard deviation) of *Spodoptera frugiperda* egg masses in different cotton plant parts, BRS Ita 90 variety.

Plant parts	Plant third		
	Lower	Middle	Upper
Leaves	2.10 \pm 0.60Ba	1.00 \pm 0.33Ca	3.90 \pm 0.85Aa
Bracts	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab
Bolls	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab
Petioles	0.20 \pm 0.13Ab	0.00 \pm 0.00Ab	0.10 \pm 0.10Ab
Flower buds	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab
Flowers	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab
Stems	0.30 \pm 0.21Ab	0.00 \pm 0.00Ab	0.00 \pm 0.00Ab
F (plant third)			4.82**
F (plant parts)			46.06**
CV (plant third) (%)			19.53
CV (plant parts) (%)			19.51
DMS			0.09

Upper case letters compare rows while lower case letters compare columns. Means followed by the same letter do not differ from one another by Tukey's test at 5% probability.

F: F-test; CV: Variation of coefficient and the percentage (%) is together with CV; DMS: minimum significant difference.

**Significant at 1% probability.

When comparing the mean number of eggs mass of *S. frugiperda* per plant, there is a preference for oviposition significantly as the higher range Coodetec 410 than in other materials (Table 3). Less oviposition was observed on FiberMax 977, Coodetec 409, Acala 90, DeltaOpal and Coodetec 408 varieties, while no oviposition was observed on plants of BRS Aroeira, BRS Araçá, BRS Ita 90, and Delta Penta varieties. Differences in oviposition behavior of *S. frugiperda* can be attributed to the morphological and chemical properties of plants of different cotton varieties studied.

The *S. frugiperda* oviposition preference results obtained on different cotton varieties (Table 3) are in agreement with those found by WIKLUND (1981), who stated that lepidopteran females show an oviposition preference hierarchy. THOMPSON; PELLMYR (1991) observed that, in a free-choice situation, the female oviposit a higher number of eggs on the preferred host plant species, a smaller number on species with a lower degree of preference and so on. Within the concept of oviposition hierarchy, BRS Ita 90 variety behaves particularly as a secondary

host, since it was oviposited by *S. frugiperda* when infested individually (Table 2), while, in the presence of other varieties, it was not oviposited at all (Table 3).

CONCLUSIONS

The fall armyworm *S. frugiperda* prefers to oviposit on plants with approximately 60 days of age, on the lower surface of leaves located in the upper third of the plant tests under greenhouse conditions.

A density of at least three pairs of adults *S. frugiperda* per plant was sufficient to conduct non-preference-for-oviposition tests under greenhouse conditions.

The Coodetec 408, BRS Aroeira, BRS Araçá, BRS Ita 90, and DeltaPenta varieties showed non-preference-for-oviposition resistance to *S. frugiperda*.

In regions with high infestations of *S. frugiperda*, it is more suitable the cultivation of the BRS Ita 90 cotton variety.

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