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Full Length Research Paper

Boll weevil feeding preference on squares at different ages and square shedding time of cotton cultivars

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The boll weevil is considered the major pest of cotton in the Western Hemisphere due to the large potential for direct destruction of fruiting bodies, survival on the fallow ground and dispersal ability. The purpose of this study was to evaluate the feeding preference of *Anthonomus grandis* for squares at different ages, as well as the abscission of attacked squares on five cotton cultivars. The experiment was conducted in Jaboticabal, SP, Brazil, in 2010 and 2011. Newly formed squares of the cultivars NuOPAL, DeltaOPAL, FMT-701, FMX-910 and FMX-993 were chosen and labeled for daily observations. Each evaluation was based on the registration of the age (days) at which the squares were fed to *A. grandis* and the time (days) from the first hole of boll weevil feeding until boll abscission. Results indicated a greater preference of the boll weevil for squares of the cultivars FMT-701, FMX-910 and FMX-993 than of the cultivars NuOPAL and DeltaOPAL. *A. grandis* prefers smaller two-day-old squares of the cultivars NuOPAL and FMX-910 and larger, seven-day-old bolls of the cultivars DeltaOPAL, FMT-701 and FMX-993. All feeding punctured squares fell on the ground, and the time of square abscission was independent on the age and cultivar of the square and was 1 to 2 days after the first feeding puncture.

Key words: *Anthonomus grandis*, host plant, injury, *Gossypium hirsutum*.

INTRODUCTION

Cotton is a high valued agricultural commodity for more than 8,000 years, and has long been recognized as a vital component of the global economy (Arpat et al., 2004). Cotton production provides income for approximately 100 million families and approximately 150 countries are involved in cotton import and export (Chen et al., 2007). It has been estimated to contribute US$ 15 to 20 billion to the world's economy (Benedict and Altman, 2001).

Most of the yield losses are attributed to the boll weevil *Anthonomus grandis* Boheman (1843) (Coleoptera: Curculionidae). *A. grandis* is originally from the tropic and subtropics of Mesoamerica (Burke et al., 1986). Its present distribution extends from the US Cotton Belt to Argentina (Ramalho and Jesus, 1988; Cuadrado, 2002), where it's a major pest of cotton, *Gossypium* spp. (Malvaceae) (Hedin et al., 1973). In the United States, boll weevil is one of the most economically important crop pests for nearly 100 years (Haney, 2001; Allen, 2008).

There is a boll weevil eradication program ongoing in parts of the United States (Dickerson et al., 2001), but populations are not being eradicated elsewhere in its distribution, and populations have not been truly eliminated in some eradication zones (Showler, 2005). In Brazil, a regulatory control program is underway in the State of Goiás, mid-west region of Brazil, which also relies on the synchronization of overwintering populations that infest cotton fields in the beginning of the cotton cropping (Degrande et al., 2003).

Boll weevil attack cotton reproductive structures for feeding and oviposition, causing squares and bolls

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abscission and great losses to cotton growers (Ramalho et al., 1993). Mature bolls are safe from attack because of the hardness of the rind and formation of mature, inedible lint (Walker et al., 1977; Showler, 2004, 2008). Boll weevil diapause has not been reliably occurring in subtropical and tropical habitats (Showler, 2009). Food shortage among patches of host plant was likely the greatest challenge to survival (Showler, 2009). In the subtropics and tropics, there are alternative sources of food that can sustain boll weevils in the absence of cotton (Cross et al., 1975; Benedict et al., 1991; Jones et al., 1992; Hardee et al., 1999) including the flesh of prickly pear cactus, *Opuntia* spec. and orange fruits, *Citrus sinensis* (L.) Osbeck (Showler and Abrigo, 2007).

Adult boll weevil can consume and derive some nourishment from seedling and mature cotton leaves and petioles (Showler, 2002, 2006; Esquivel et al., 2004), but cotton plant foliage does not allow egg production (Showler, 2004). After cotton square formation, *A. grandis* is attracted to cotton plant volatiles (Neff and Vanderzant, 1963; White and Rummel, 1978) and an aggregation pheromone from boll weevils already present in the field (Parajulee and Slosser, 2001).

Showler (2005) observed that large (5.5 to 8 mm in diameter) squares on the plant were most commonly used for oviposition, followed by medium (3 to 5.5 mm in diameter) squares, but use of pinhead (1 to 2 mm in diameter) and match-head (2 to 3 mm in diameter) squares was negligible. Furthermore, a diet of large squares resulted in ≥3.8-fold more gravid females that developed ≥4.8-fold more eggs than diets of match-head squares, or post-bloom (1 to 2 days old), young (5 to 10 days old), or old (3 to 5 weeks old) bolls (Showler, 2004).

Knowledge of the feeding behavior of boll weevil is essential for predictions of population development at different latitudes and in different years (Greenberg et al., 2003, 2008). Thus, the purpose of this study was to evaluate the feeding preference of *A. grandis* on squares at different ages of five cotton cultivars, as well as the time until a square drops off after first been punctured by the weevil under field conditions.

MATERIALS AND METHODS

Study location

The experiment was carried out in an experimental area of the Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (FCAV/UNESP), municipally of Jaboticabal, state of São Paulo, Brazil, during the period from November 2010 to April 2011. The university is located at latitude 21°14'05'' South, longitude 48°17'09'' West and altitude 615 m.

Description of sampling area

A total area of 100 × 54 m (0.54 ha) was marked out and divided into 20 subareas. Each subarea was divided into five blocks to each cultivar (NuOPAL (Bollgard I®), DeltaOPAL, FMT-701, FMX-910 and FMX-993), each block consisting of six rows (5.4 × 10 m), spaced at 0.9 m, representing 20 repetitions. The usable sampling area of each block, consisting of four central rows disregarding 1.0 m on either end, were sampled, forming a buffer for the sampling unit.

The experiment arrangement used was the randomized block design with split plots (5 x 8), with five cultivars, eight evaluation periods and 20 repetitions.

Preparing the sampling area, sowing the cultivars and crop treatments

The soil was prepared by plowing and harrowing, and the seeds of cotton cultivars NuOPAL (Bollgard I®), DeltaOPAL, FMT-701, FMX-910 and FMX-993 were sown. The crop treatment applied to both experimental areas was N-P-K (8-20-20) (400 kg ha^{-1}), which was made in late November, 2010.

The seeds of the cultivars used in the experiment were not pre-treated with pesticides and were sown by hand on 19th November, 2010 with a density of 14 seeds per meter and row spacing of 0.9 m. Emergence occurred 27th November, 2010 and seven days after emergence, the seedlings were thinned to an average density of 10 plants per meter.

During the experiment, no insecticides, fungicides or herbicides were applied, and weed was controlled by manual weeding throughout the development cycle in the experimental area. To control leaf-cutting ants of the genera *Atta* and *Acromyrmex*, Blitz® (fipronil) bait was locally applied 15 and 30 days after emergence (DAE) in experimental and adjacent areas (Thomazoni et al., 2010).

When the flowering of the cultivars was most intense, 63 DAE, 20 plants per cultivar (1 plant per block) were randomly selected, and on each plant, a newly formed square was chosen and labeled for daily assessments. At labeling, the plants were with the first square of the fourth sympodium visible (B4). The squares were monitored daily for eight days. After this period, the plants were with the first square of the fifth sympodium visible (B5). On the eight day, all marked squares were fed to the weevil, and the day on which the squares dropped off was recorded. Squares used for feeding were characterized by the presence of a hole, and squares used for oviposition were characterized by a wax-sealed hole (Ramiro et al., 1992).

Sampling methods

Marked squares were daily evaluated. On each evaluation day, the age (days) of the squares when they were used for *A. grandis* feeding was recorded. The number of feeding punctures on each square was counted daily.

During this period, the day on which each square fell to the ground after the first feeding punctures was observed and recorded. The number of feeding punctures per square and cultivar was also recorded at each evaluation.

A pheromone trap was installed on the experimental area to determine the pest infestation during the experiment, and was checked daily. It was collected from 16 to 23 weevils daily during the experiment.

Data analysis

The data were subjected to ANOVA using SAS (2000) and the treatment means compared by the Tukey test at 5% probability. Since the number of punctures in the squares punctured for feeding differed over the course of time, regression analysis between the number of *A. grandis* feeding punctures and square age was carried out, using Microsoft Excel® 2007 charts. In this analysis, data from up to seven-day-old squares were used due to the marked

Table 1. Mean number of feeding punctures per square caused by boll weevil, on five cotton cultivars, in eight evaluations (Jaboticabal, SP, Brazil, 2010/2011).

Cultivar (C)	Mean of feeding punctures/square
DeltaOPAL	1,00 b
NuOPAL	1,00 b
FMT-701	2,30 a
FMX-910	2,00 a
FMX-993	2,50 a
F	*10,11**
Squares age (days) (A)	
1	0,00 c
2	1.80b
3	0.75c
4	0.60c
5	0.55c
6	0.75c
7	3.00a
8	0.35c
F	2.81*
C x A	2.42*
CV (%)	21.19

Data within a column followed by the same letter are not significantly different (P = 0.05; Tukey test). *Significant at 5% probability by Tukey's test.

decrease in the number of feeding punctures on older squares.

RESULTS

Significant differences of the boll weevil were observed in relation to the number of punctures on different cultivars, square age and the interaction of cultivar with square age (Table 1). The transgenic cultivar NuOPAL and its commercial isoline DeltaOPAL were the least preferred for feeding, with on average of one feeding puncture per square. The cultivars FMT-701, FMX-910 and FMX-993 were the most preferred for feeding, with at least two feeding punctures per square, representing a significant difference between the two least and the three most preferred cultivars by the insect for feeding (Table 1).

In general, the average number of feeding punctures was highest in seven-day-old squares, showing a statistically significant preference by the weevil for squares of this age in most cultivars (Table 1). No one-day-old square of any cultivar was punctured for feeding by *A. grandis*.

Analyzing the boll weevil feeding preference on NuOPAL, a preference of the weevil for two-day-old squares was observed, when statistically the number of feeding punctures per square was highest (Table 2). On the other hand, on the conventional cultivar DeltaOPAL, a commercial isoline of NuOPAL, the insect preferred feeding on seven-day-old squares (Table 2).

The results on cultivar FMX-910 (non-Bt) were similar to that of NuOPAL, where the insects also preferred to feed on two-day-old squares (Table 2). On the other twocultivars FMT-701 and FMX-993, they preferred more developed, seven-day-old squares (Table 2).

From the day the squares are attacked, the number of feeding punctures on a square increases until the square drops on the ground (shedding). The percentage of feeding punctures on the cultivars NuOPAL and FMX-910 was greatest on younger, two-day-old squares (50 and 53.38%, respectively), while on DeltaOPAL, FMT-701 and FMX-993, the older, seven-day-old squares had most feeding punctures (30.53, 43.33 and 37.56%, respectively) (Figure 1).

In this study, a higher percentage of two-day-old squares were attacked on the cultivars NuOPAL and FMX-910 and seven-day-old squares on the cultivars DeltaOPAL, FMT-701 and FMX-993 (Figure 2). The number of attacked squares of the cultivars NuOPAL and FMX-910 was also greater than the other cultivars (Figure 2) and consequently the number of feeding punctures on two-day-old squares, while for the cultivars DeltaOPAL, FMT-701 and FMX-993, this occurred after seven days.

There were no differences in time (days) until square abscission after the first feeding puncture of *A. grandis*, and this abscission time was between one and two days (Table 3).

The results show that *A. grandis* does not attack one-day-old squares; the insect prefers squares of the cultivars FMT-701, FMX-910 and FMX-993 over the cultivars NuOPAL and DeltaOPAL; the feeding preference of the insect is for younger squares of the cultivars NuOPAL and FMX-910, and for more developed squares of the cultivars DeltaOPAL, FMT-701 and FMX-993; and regardless of the cultivar, the squares drops off due to only one feeding puncture between 1 and 2 days after being punctured for weevil feeding.

DISCUSSION

The use of squares and small bolls for boll weevil feeding is essential for reproduction, since adults fed with bolls lay fewer eggs than those fed on squares (Isley, 1928). A diet of more developed squares (seven days old), as observed for some cultivars in this study (DeltaOPAL, FMT-701 and FMX-993), increase the fertility rate 3.8 times and oviposition 4.8 times over diets based on undeveloped squares (NuOPAL and FMX-910), newly opened flowers (1 to 2 days old), or of young (5 to10 days) and old bolls (3 to 5 weeks) (Showler, 2004).

The use of larger squares (diameter of 5.5 to 8.0 mm) by the boll weevil is more common at weevil oviposition (Ramalho and Jesus, 1988; Soares et al., 1999; Showler,

Table 2. Values of the unfolding interaction analysis between cotton cultivars versus square age (days) for the mean number of feeding punctures per square, caused by boll weevil (Jaboticabal, SP, Brazil, 2010/2011).

Square age (days) (A)	Cultivar (C)					F (A)
	DeltaOPAL	NuOPAL	FMT-701	FMX-910	FMX-993	
1	0,00 bA	0,00 bA	0,00 bA	0,00 bA	0,00 bA	$0,16^{NS}$
2	0,15 bB	0,90 aA	0,10 bB	0,95 aA	0,00 bB	2,45*
3	0,05 bA	0,20 bA	0,05 bA	0,25 bA	0,05 bA	$1,32^{NS}$
4	0,10 bA	0,05 bA	0,00 bA	0,10 bA	0,10 bA	$0,64^{NS}$
5	0,10 bA	0,05 bA	0,05 bA	0,00 bA	0,10 bA	$0,98^{NS}$
6	0,20 bA	0,05 bA	0,25 bA	0,00 bA	0,25 bA	$1,57^{NS}$
7	0,95 aA	0,00 bB	1,35 aA	0,10 bB	1,35 aA	4,35*
8	0,05 bA	0,00 bA	0,10 bA	0,00 bA	0,20 bA	$1,50^{NS}$
F (C)	2,34*	2,19*	3,09*	2,36*	2,97*	-

Data within a column followed by the same lowercase letter and within a row followed by the same uppercase letter are not different significantly (P = 0.05; Tukey test). ^{NS}Non significant; *Significant at 5% probability by Tukey test.

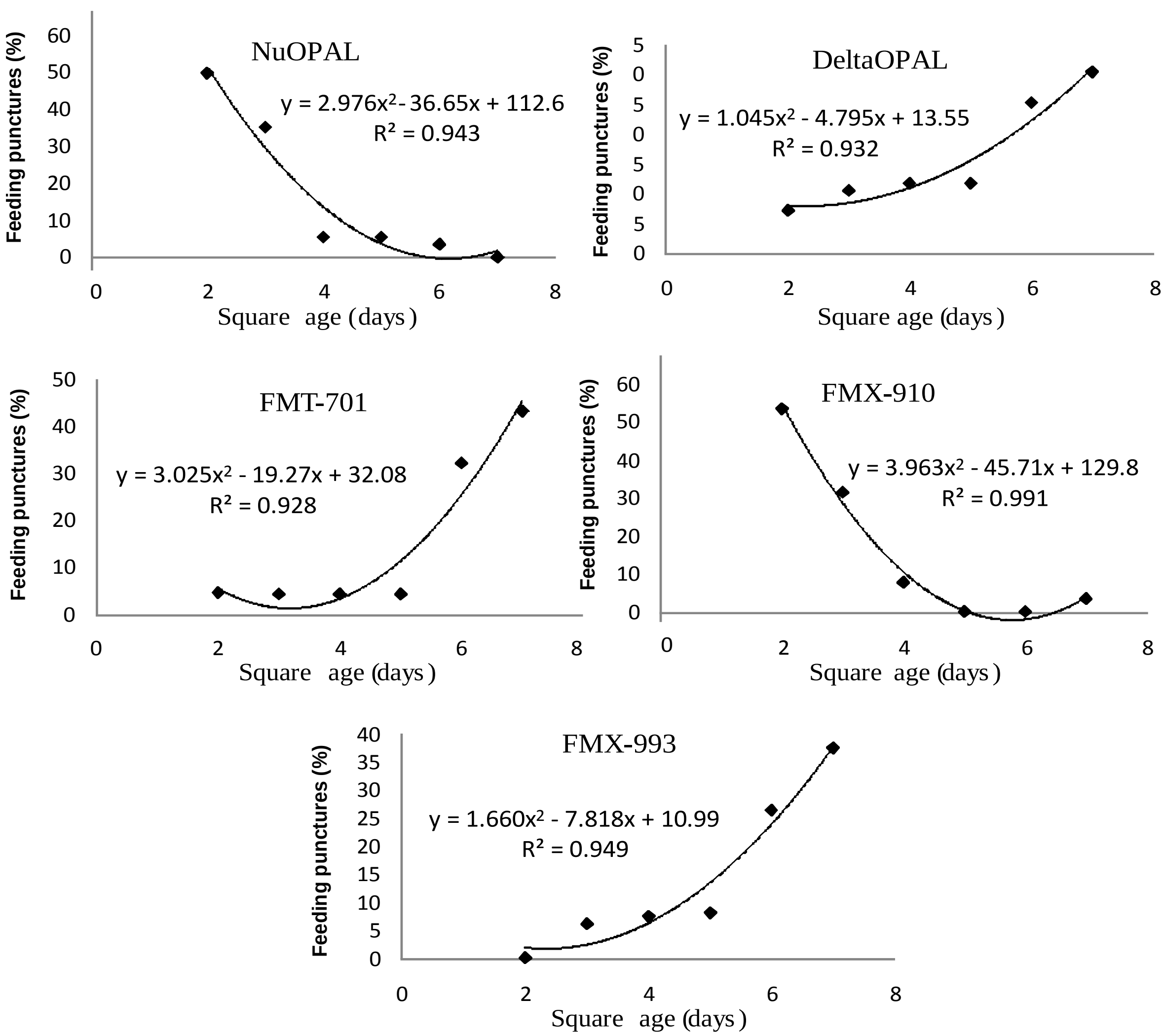

Figure 1. Relative percentage of feeding punctures caused by boll weevil on cotton squares with 1 to 7 days-old in cultivars NuOPAL, DeltaOPAL, FMT-701, FMX-910 and FMX-993 (Jaboticabal, SP, Brazil, 2010/2011).

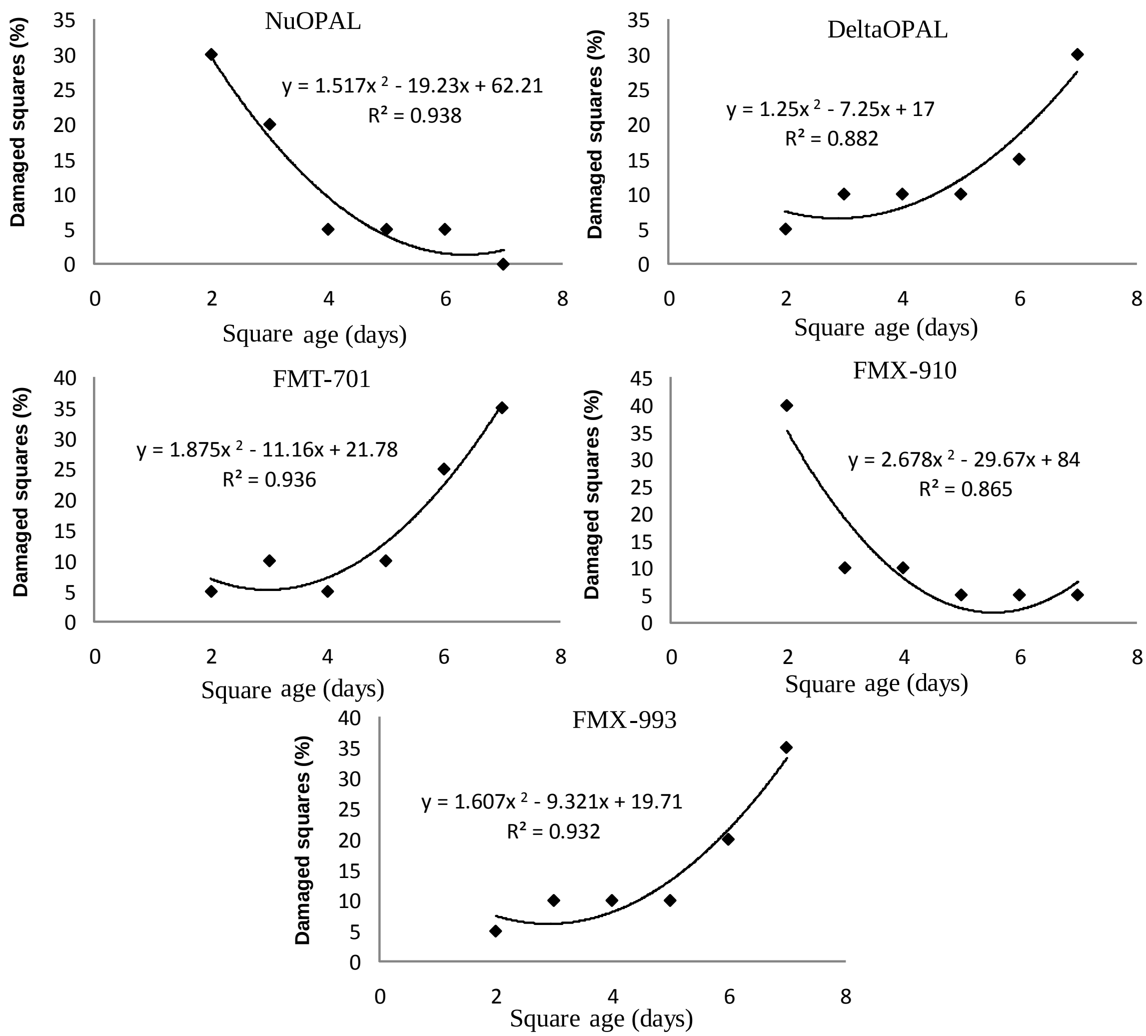

Figure 2. Relative percentage of damaged squares with feeding punctures, caused by boll weevil in cultivars NuOPAL, DeltaOPAL, FMT-701, FMX-910 and FMX-993 (Jaboticabal, SP, Brazil, 2010/2011).

Table 3. Time (days) for abscission after the first boll weevil feeding puncture on five cotton cultivars (Jaboticabal, SP, Brazil, 2010/2011).

Cultivar	Days after the initial damage for square abscission
DeltaOPAL	1.67a
NuOPAL	1.86a
FMT-701	1.71a
FMX-910	1.69a
FMX-993	1.83a
CV (%)	13.47
F cultivar	1.36^{NS}

Data within a column followed by the same letter are not significantly different (P = 0.05; Tukey test). CV– Coefficient of variation; ^{NS}– non significant.

2005). In addition, fecundity and oviposition are greater after the female feeds on developed squares (Showler, 2004).

The longevity is also higher when females boll weevils fed exclusively on the interior reproductive portions of either medium or large squares, with longevities comparable to 153.2 days average for females fed intact large squares, but the interior reproductive portions of post-bloom bolls (1 to 2 days after the petals falls) supported boll weevils for an average of only 32 days, and that declined further when weevils were fed the inner reproductive portions of older (5 to 10 days old and 3 to 5 weeks old) bolls (Showler, 2008).

The outer non reproductive ‘rind’ of match-head, medium and large squares also has nutritional benefits to boll weevils, sustaining them on average for 63, 126 and 57 days, respectively (Showler, 2008). Boll weevil longevity declines from 68 days on post-bloom boll rinds to 32 and 29 days on young and old boll rinds, respectively (Showler, 2008). While adult boll weevils can puncture the rinds of squares, regardless of size, with their mouthparts to feed on inner reproductive portions (Showler and Cantú, 2008), boll rinds are 1.4- to 2.1-fold thicker than rinds of large squares, which average 0.73 mm (Showler, 2008). Even if adult boll weevils penetrate the rinds of bolls to feed on inner reproductive portions, that food source does not offer added nutritional benefit in terms of longevity or reproduction (Showler, 2008).

Fecundity, optimized by feeding on the reproductive portion of large squares, is nevertheless achieved by nonreproductive tissue of squares and bolls alike, and more so than the reproductive portion of match-head squares (Showler, 2008). Overall, feeding on the reproductive tissue of fruiting bodies promoted more egg production than rinds, squares being nutritionally superior to bolls for that purpose. Once numbers of large squares decline or are no longer available after cut-out, which is when square populations decline and are replaced by bolls (Guinn, 1986; Cothren, 1999), reduced nutritional quality of bolls and boll weevil egg production when fed bolls is reflected by a leveling of adult weevil populations observed in commercial cotton (Showler et al., 2005).

In this study, a higher percentage of two-day-old squares were attacked on the cultivars NuOPAL and FMX-910 and seven-day-old squares on the cultivars DeltaOPAL, FMT-701 and FMX-993. These findings suggest that boll weevils are amenable to changes in the nutritional status of growing, aging cotton (Showler, 2008). Longevity was most enhanced by the reproductive portion and rind of medium squares, which sustains them to the formation of reproduction-enhancing large squares (Showler, 2004).

Despite injury to cotton squares from boll, weevil feeding does not cause square abscission (Showler and Cantú, 2008), all marked squares used for *A. grandis* feeding fell from the plants. Some aspects of feeding, oviposition and/or development of weevil eggs and larvae in cotton squares cause the abscission of these structures from the plants (Coakley et al., 1969).

Other factors aside from the activity of the boll weevil, such as rain, temperature, humidity, etc. cause abscission (shedding) of squares and bolls, reducing the productive capacity of plants in the area (Showler et al., 2005; Showler, 2006; Greenberg et al., 2008). The lack of consistency suggests that factors other than boll weevil activity were causing square abscission.

The results of this study show that the boll weevil feeds on squares of different ages. However, the squares fell on the ground one to two days after being first fed punctured, regardless of the cultivar. Thus, results obtained with only one cultivar should be better interpreted to provide accurate information on which cultivars new squares and on which more developed squares are currently preferred, underlying the monitoring of the action level or pest control.

In general, cotton fields with cultivars NuOPAL and FMX-910 should be monitored from the appearance of the first squares, that is, squares as young as two days old should be sampled, while in cultivars DeltaOPAL, FMT-701 and FMX-993, more developed, up to seven-day-old squares can be sampled. These results can be directly applied in the pest monitoring, making the action level or control of 10% of attacked squares (feeding or oviposition) in Brazil (validated by Gielfi and Busoli, 1998) more real, providing greater protection to grain yields.

The present study also points out the way for new researches concerning feeding preferences of cotton boll weevil with more cultivars under field and tropical conditions, as well as the need for future experiments on the squares abscission.

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