SYMPTOMS, INJURIES, YIELD REDUCTION AND QUALITY LOSS OF COTTON ATTACKED BY THE NEOTROPICAL BROWN STINK BUG *Euschistus heros* (F.) (HEMIPTERA: PENTATOMIDAE)

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Abstract

The NBSB (neotropical brown stink bug) *Euschistus heros* (F.) has became important cotton pest in Brazil as a result of nearby soybean population dispersion and reduced use of broad spectrum insecticides. The objective of this study was to assess the effect of different population levels of laboratory reared NBSB adults on cotton lint yield and quality. Under greenhouse conditions, cotton plants of NuOpal® Bollgard® were caged at the cutout stage with 0, 2, 4, 6 and 8 NBSB adults per plant for a four day period. The experimental design used was the completely randomized with five replications. After the infestation, internal and external injuries to bolls symptoms were evaluated and at maturity, bolls were harvested and ginned. Fiber quality was measured by HVI methodology. Data were subjected to analyses of variance ($P \leq 0.05$) followed by regression analyses ($P \leq 0.10$). A linear regression model showed the increase of the mean number of boll locks with internal punctures and warts increased as insect population increased. The mean number of boll locks with immature stained lint was higher at 4, 6 and 8 NBSB adults per plant treatments, and fitted a quadratic regression model. Seed and lint cotton yield per plant were linearly reduced to 28 and 36% at the 8 NBSB adults per plant infestation level. The fiber micronaire and yellowness indexes were respectively reduced and increased linearly with the increase in infestation levels. This paper reports an overview of injuries and symptoms caused by NBSB on cotton.

Introduction

The importance of stink bugs (Heteroptera: Pentatomidae) as a cotton pest has increased in Brazil. This has occurred as a result of reduced use of broad spectrum insecticides due to the success of boll weevil [*Anthonomus grandis* (B.)] suppression programs and the adoption of Bt cotton cultivars (Soria et al 2009, 2010a). A similar phenomenon has been reported in Southeast and Mid-South of the United States (Haney 1996, Greene et al 2001).

Among the three main stink bugs species that infests cotton crops in Brazil [NBSB (neotropical brown stink bug) *Euschistus heros* (F.), BGSB (black green stink bug) *Edessa meditabunda* (F.) and SGSB (southern green stink bug) *Nezara viridula* (N.)], the NBSB is the prevailing species (Soria et al 2010c). In addition, the migration of NBSB from neighboring soybean fields, plays an important role to the infestation of this pest to cotton crops (Panizzi 1997, Soria et al 2009, 2010c).

Even though the knowledge about the injuries and losses capability of some stink bugs species, such as *N. viridula* and BSB (brown stink bug) *Euschistus servus* (Say), are well known (Wene & Sheets 1964, Barbour et al 1990, Greene et al 1999, Willrich et al 2004a, 2004b, 2004c; Siebert 2005, Bommireddy et al 2007), there are few studies reporting the injuries and damage of stink bugs to cotton in Brazil (e.g. NBSB *E. heros* and BGSB *E. meditabunda*) (Soria et al 2010a, 2010b). Given that injuries in cotton can differ among stink bugs species and that NBSB is a new emerging pest problem for cotton in Brazil, studies about its injury potential are needed to develop its treatment thresholds under Brazilian conditions.

Furthermore, the adoption of Bt cultivars by cotton growers, which increases every growing season, can make increase the damage of stink bugs to Brazilian cotton crops. Thus, the objective of this work was to assess the capability of different population levels of NBSB adults on green boll damage, seed cotton yield and fiber quality of Bt cotton plants grown in a greenhouse. The information will be used to determine NBSB treatment threshold for Brazilian cotton crops.

Materials and Methods

Two experiments were carried out under greenhouse conditions (22°11' S and 54°56' W) at the Laboratory of Applied Entomology and Biotechnology of the Federal University of Grande Dourados (UFGD), in Dourados, MS, Brazil, from December, 2009 to May, 2010. In the first experiment, Bt-cotton plants were infested with NBSB adults at the cutout stage for four days to assess internal and external injuries symptoms on bolls. The second experiment was similar to the first, but bolls remained on the plants to assess cotton yield and fiber quality indexes at the end of plants cycle. All bolls had a quarter-size (approximately 25 mm of diameter) on average.

Bt-cotton plants (NuOpal® Bollgard®) were cultivated in 15 l pots (two plants.pot^{-1}) using a mixture of soil and soil conditioner (Ribumin®) in a 10:1 ratio. The soil was previously collected to a depth of 0.40 m, sieved and limed with dolomitic lime, based on soil analysis. The soil pH was corrected to the recommended levels for cotton production in Brazil (Embrapa 2001). Forty days after liming, the soil was fertilized with NPK (00-20-20) fertilizer (4 g.pot^{-1}), expecting to provide adequate nutrients for plant development.

Six seeds of NuOpal® Bollgard® cotton were sown per pot, thinning the density to two plants per pot three days after emergence. All pots were fertigated with water-soluble fertilizers (Yogen-5® and Sett®) periodically to maintain plant development through the experimental period. The plants were sprayed with specific insecticides to control whitefly *Bemisia tabaci* (Genn.) B-biotype and cotton aphid *Aphis gossypii* (Glov.) infestations, as well with fungicides to control *Ramularia areola* Atk. as needed.

When plants reached the cutout stage, NBSB adults with approximately 10-d of age reared under laboratory conditions (24±2°C, 70±10% of RH and 14 h of photoperiod) with natural diet (green bean pods [*Phaseolus vulgaris* L.], soybean seeds [*Glycine max* (L.)], peanut seeds [*Arachis hypogaea* L.] and ligustro berries [*Ligustrum lucidum* Ait.]), were infested for four days. The NBSB adults were left in a starvation period of 48 h under field environmental conditions before infestation.

A tulle fabric cage (1.00 × 1.80 m) was used to confine the NBSB adults with the cotton plants. The cages covered up the two plants of each pot and had its bottom tied at the pot. In each cage, NBSB males and females were used in a 1:1 ratio to avoid possible differences in injuries capacity between stink bugs males and females, with daily replacement of insects in case of mortality. Completely randomized experimental design was used for both experiments with five replications per treatment. A replication consisted of a pot with two plants. The treatments consisted of five different NSBS infestation densities: 0, 2, 4, 6 and 8 adults.plant^{-1}.

Before the infestation period, all the bolls from the plants of the first experiment were collected, taken to the laboratory and dissected to assess external and internal injury symptoms. The injury symptoms assessed were dark spots on bolls locks epicarp, warts and punctures signs on bolls locks mesocarp, and percentage of locks with immature stained lint. Yield (seed and lint) and HVI fiber quality parameters (length, micronaire, strength, uniformity and yellowness) were obtained from the plants of the second experiment.

Mean data of internal and external injuries, cotton seed and lint yield per plant, and fiber quality indexes were submitted to an analysis of variance ($P \leq 0.05$), with posterior regression analyses ($P \leq 0.10$), using PROC GLM and PROC REG of SAS® (SAS Institute 2008).

Results and Discussion

In our study, the four days period of infestation was enough to provide significative appearance of internal injuries symptoms on the mesocarp of the bolls. Internal signs of punctures ($F = 17.18$; df = 4, 20; $P < 0.001$) and warts ($F = 4.58$; df = 4, 20; $P = 0.0087$) presented, respectively, a quadratic and a linear increase, putting in evidence the enhancement of NBSB attack on the cotton bolls as the infestation increases (Fig 1; graphs A and B).

Previous studies have shown that there is a relationship between internal punctures and warts caused by the attack of stink bugs on cotton bolls, and that these two types of symptoms should be used to confirm stink bugs injuries to cotton crops (Greene et al 2001, 2006; Willrich 2004b).

Soria et al. (2010a) found a significant Pearson’s correlation of 0.76 between internal punctures and warts on NuOpal® Bollgard® cotton bolls that were infested for four days with one feral NBSB, reporting that the number of internal punctures per boll lock was 3.98.

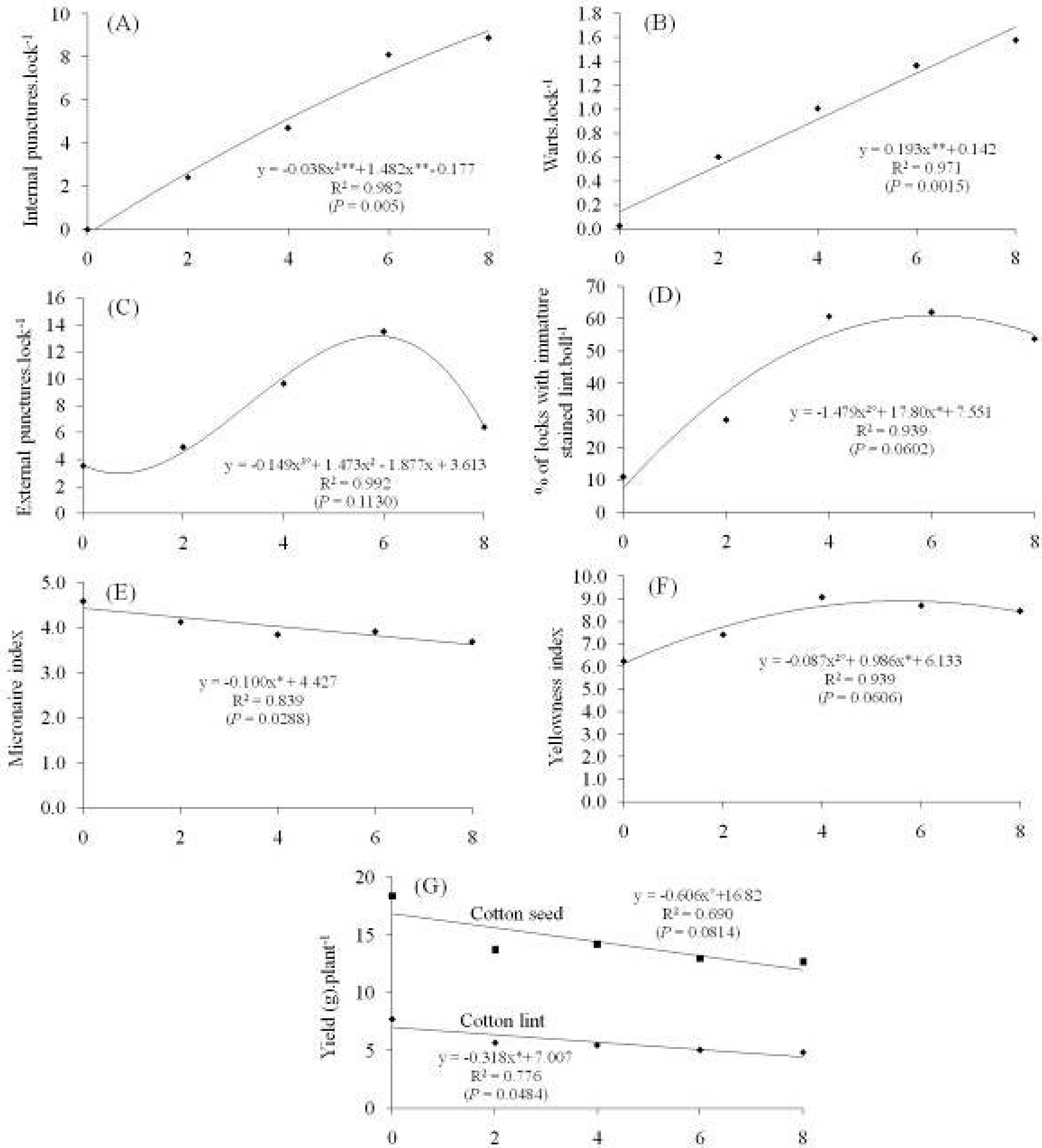

Fig 1. Average of injury symptoms (A, B, C and D), fiber quality indexes (E and F) and yield (G) of cotton plants infested with 0, 2, 4, 6 and 8 adults of NBSB per plant under greenhouse conditions (n=5). Dourados, MS, Brazil. Growing season 2009/2010. (* $P = 0.01$; ** $P = 0.05$; ° $P = 0.10$)

This number of internal punctures was similar to our observations (3.92) at the infestation level of 3 stink bugs/plant. However, the number of warts per lock obtained in this work at the infestation level of 3 stink bugs/plant (0.71) was numerically different to the observations of Soria et al. (2010a), who reported a value of 1.82.

This shows the probability of the growth of warts on the mesocarp after a stink bug attack can be lead by other factors than those related with the injury caused by the stink bug feeding process on the cotton bolls. It is known that feral stink bugs can carry microorganisms such as pathogens and symbionts (Medrano et al 2007, 2009a, 2009b;

Prado et al 2006, Prado and Almeida 2009), being convenient not to discard that these organisms would be responsible for the intensification of injuries symptoms caused by stink bugs on cotton bolls, mainly those that have relationship with plant physiology, e.g. warts growth (callous tissues) and bolls deformation.

The response for the NBSB attack by assessing external signs of punctures (F = 5.32; df = 4, 20; P < 0.0044) was fitted to a cubic model (Fig 1; graph C). The data showed an increase at the second level of infestation (2 stink bugs/plant), followed by a decrease in the 8 stink bugs/plant level. This phenomenon occurred in spite of the observed increase in internal injuries symptoms.

This unstable pattern and the low statistical significance of the model (P = 0.1130) reinforces the fact that the assessment of external punctures on bolls is not a reliable indicator of stink bugs damage to cotton crops. Dissection of bolls and assessment of internal injuries are needed to confirm stink bugs attack (Bundy et al 2000, Willrich 2004b).

The percentage of locks with immature stained lint (F = 16.59; df = 4, 20; P < 0.0001) has presented a quadratic pattern, maintaining a similar response in the last three levels of infestation (4, 6 and 8 stink bugs/plant), ranging from 55 to 60% (Fig 1; graph D). The detection of discolored fibers when assessing immature bolls for internal stink bugs injuries symptoms indicates that the stink bugs pierces reached the seeds in the boll, once these stains are formed because the cotton seeds exudes fluids in response to the injury.

When stink bugs are acting as vectors, carrying boll rot pathogens, the incidence of immature stained lint can be higher due to its growth capacity inside the bolls, causing rotting of bolls and consequent losses in yield and fiber quality (Medrano 2009a). However, despite the detection of immature stained lint is considered a reliable indicator of stink bugs damage in cotton crops, it cannot show the real losses caused by the stink bugs attack, since the yield and cotton quality measured in this study were negatively affected to increases in levels of infestation of NBSB (Fig 1; graph E, F and G).

Among the quality indexes analyzed, the micronaire (F = 3.04; df = 4, 20; P = 0.0412) and yellowness (F = 5.68; df = 4, 14; P = 0.0062) were significantly affected (Fig 1; graphs E and F). Using the regression models obtained at the maximum level of infestation (8 stink bugs/plant), the decrease of micronaire can be in an order of 18% and the increase of yellowness in an order of 27%. Bommireddy et al. (2007) obtained similar responses assessing the impact of the SGSB attack on micronaire and yellowness indexes from lint cotton of infested bolls.

In Brazil, Cruz Júnior (2004) obtained 12% of losses in the micronaire index from cotton lint ginned of bolls infested with SGSB and RSB (redbanded stink bug) *Piezodorus guildinii* (West.). These differences are probably due to the stink bugs species type, time of attack, phenological stage of the plants (bolls age) and variety studied (Greene et al 1999, Willrich et al 2004a, 2004b; Ward 2005, Soria et al 2010a, 2010b). However, we found that the decrease in micronaire and increase in yellowness indexes are dependent of the NBSB population density, indicating that they have to be considered when determining NBSB treatment threshold.

Quality indexes are important to the textile industry and its losses can depreciate cotton despite satisfactory yields. Particularly, the yellowness index showed the same pattern of the percentage of immature stained lint, indicating agreement and some degree of relationship between these two parameters.

The yield parameters decreased linearly as the NBSB infestation level increases, presenting, respectively, 7 and 9% less production of cotton seed (F = 3.19; df = 4, 20; P = 0.0351) and lint (F = 4.01; df = 4, 20; P = 0.0151) at the minimum level of infestation (2 stink bugs/plant), and 28 and 36% at the maximum level of infestation (8 stink bugs/plant) (Fig 1; graph G). Soria et al. (2010) reported that one adult of NBSB was capable of causing losses of approximately 13% in cotton seed production of a NuOpal® Bollgard® quarter-sized boll, which is considered the most susceptible boll size to a stink bug attack. Willrich et al. (2004) found that the cotton seed yield loss of plants infested with BSB (brown stink bug) *Euschistus servus* (Say) depends on the time of flowering due to the plants compensation and bolls tolerance capacity to the BSB attack, being the first three weeks of flowering more critical to the attack.

Although in our study the plants were infested in the cutout stage, the bolls plants had a quarter-size on average, which can represent the cotton plants status in the weeks of flowering that they are more susceptible to stink bugs attack. Thus, the results of this study can be used to determine the treatment threshold for the NBSB in Brazilian cotton crops by extrapolating data, predicting the quality and yield losses by assessing internal injuries symptoms on bolls mesocarp and NBSB density, according to the IPM.

Conclusions

Internal signs of punctures and warts on bolls mesocarp increase as the NBSB (neotropical brown stink bug) *Euschistus heros* (F.) infestation increases.

External signs of punctures on bolls epicarp do not follow a stable pattern of increase as the NBSB infestation increases.

The assessment of external signs of punctures on bolls epicarp are not a reliable way to estimate NBSB infestation in cotton crops, being opposed to the assessment of internal injuries symptoms on boll mesocarp for its purpose.

The percentage of locks with immature stained fibers of bolls that are attacked by NBSB has limited increases that can range from 55 to 60%, not accompanying the increase on the infestation level of NBSB.

The cotton plants seed and lint production are linearly decreased as the NBSB infestation increases, occurring losses from 28 and 36% in cotton seed and lint, respectively.

The cotton quality indexes micronaire and yellowness are linearly affected by the NBSB infestation growth, reaching an 18% micronaire reduction and an increase of 27% on fiber yellowness.

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