NEOTROPICAL BROWN STINK BUG *Euschistus heros* (Fabr., 1798) ATTACK ON BT-COTTON BOLLS CULTIVATED IN BRAZILIAN SAVANNAH

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Abstract

This work was conducted to evaluate the injury and the damage capacity of *Euschistus heros* (Fabr., 1798) adults to bolls of Bt and non-Bt cotton plants cultivated in the Brazilian savannah (22º11' S and 54º56' W). Bolls of transgenic and non-transgenic (isoline) cotton with approximately 25 mm of diameter were individually infested with *E. heros* adults. For each cotton genotype, one adult stink bug/boll was confined during five days. After the confinement period the internal and external locks bolls injury symptoms were evaluated as well the capsules seed cotton weight. Data were submitted to the analysis of variance and means compared using Student's t-test and Pearson's correlation ($P \leq 0.05$). In both genotypes, the mean numbers of locks with internal and external punctures, warts and immature and mature lint stain signs were significantly higher for infested bolls compared to non-infested ones (checks). The capsules seed cotton weight was significantly lower for infested bolls. Both non-Bt and Bt-cotton capsules seed cotton weight were significantly reduced by 24 and 13%, respectively. Internal signs of punctures and warts were moderately correlated with external punctures signs.

Introduction

As for traditional Brazilian savannah cotton production system, stink bugs (Heteroptera: Pentatomidae) are indirectly controlled by broad spectrum insecticides applied to control primarily the boll-weevil, *Anthonomus grandis* Boh., 1843, and the tobacco budworm, *Heliothis virescens* (Fabr., 1781).

In recent years, Bt-cotton varieties have received small amount of pesticides and this has led to the invasion of cotton fields by dispersing stink bugs, primary soybean pests. When cotton and soybean are cultivated at the same time and space, stink bugs, especially *Euschistus heros* (Fabr., 1798), disperse from senescing soybean to cotton plants (Bundy & McPherson, 2000; Soria et al., 2009), looking for food and shelter (Panizzi, 1997) and causing damage to yield and lint quality by feeding on ripening bolls (Greene et al., 1999; Willrich et al., 2004a;b; Bommireddy et al., 2007).

Cotton bolls attacked by stink bugs do not shed from the plants and present injury symptoms like circular dark concave spots on the epicarp and dark feeding punctures or warty growths (callous tissue) on the mesocarp (Wene and Sheets, 1964; Greene et al., 2006). The evaluation of these symptoms has been used as alternative populations scouting technique for treatment threshold of these insects in cotton fields (Bt or non-Bt) in the United States (Greene & Herzog, 1999; Musser et al., 2007; Blinka et al., 2008; Herbert et al., 2009; Toews et al., 2009).

In this study, we evaluated injury and damage capacity of adults of the neotropical brown stink bug, *E. heros*, to Bt and non-Bt cotton bolls cultivated in Brazilian savannah, in order to generate subsidies for the integrated management of this pest.

Materials and Methods

The trial was carried out from January 14th to June 17th, 2009 at the experimental area of the Agricultural Science College, Federal University of Grande Dourados, in Dourados, in the state of Mato Grosso do Sul, Brazil (22º11'

South latitude, 54º56' West longitude). An area of 180 m^2 was cultivated with the non-Bt and Bt variety (eight rows with 25 m length, 0.9 m of row centers and 10 to 12 plants/m). Insecticide and acaricide were sprayed to prevent any type of injury on the target bolls. There was a 15-days interval before the beginning of stink bugs infestations. Cotton bolls with approximately 25 mm of diameter from the NuOpal® Bollgard® variety and from its non-Bt isoline variety, DeltaOpal®, were infested with one *E. heros* adult confined for a 5-day period to evaluate the internal and external injury symptoms and the damage on seed cotton yield.

A second trial was carried out to assess the number of external punctures on the epicarp, the number of internal punctures and warty growths on the mesocarp and the number of locks with immature stained fibers. The experimental design was completely randomized with two treatments (infested and non-infested bolls with *E. heros*); 20 replications were used for the trail in which bolls of each variety (Bt and non-Bt) were destroyed to assess internal and external injury symptoms, and 16 replications in which used for the trail where external injury symptoms on bolls and capsules were evaluated.

The experimental unit was constituted by a boll with 25 mm of diameter that was selected randomly on the 86th day after seedling from the first position of any fruiting branch, within the six central rows of each variety, using a cardboard template. Bolls (one/plant) with 25 mm of diameter were selected because they are considered more susceptible to the stink bugs attack (Bacheler & Mott, 2005; Greene et al., 2006). They were marked and infested with one field-collected *E. heros* adult. Stink bugs were confined with the bolls using a similar cage used by Greene et al. (1999) for 4 days. The non-infested bolls were also caged. Bolls were dissected and evaluated for internal and external injury symptoms in the laboratory.

For the statistical analysis, original data from the assessed parameters on the bolls and capsules of each genotype (Bt and non-Bt) were transformed in square root of (x+0.5), except for bolls diameter, locks number, and seed cotton yield. Data were submitted to analysis of variance ($P \leq 0.05$) and means compared using Student's t-test ($P \leq 0.05$). As complementation, a Pearson's correlation analysis ($P \leq 0.05$) for each genotype was made between injury symptoms types and seed cotton yield, utilizing the transformed data. All analyses were performed using SAS®.

Results and Discussion

For the Bt and for the non-Bt varieties, the number of external and internal punctures, respectively on the epicarp and mesocarp of cotton bolls, after *E. heros* attack, was statistically higher on the infested bolls compared to the non-infested bolls; the same pattern was observed for the number of locks with immature and mature stained fibers, and the number of hard locks (Tables 1 and 2).

In particular, the number of internal punctures on the mesocarp showed higher values than the number of external punctures. These results are similar to those obtained with 5th instar nymphs of *N. viridula* and adults of *E. servus*, in which it was observed that there was a higher number of internal punctures on the mesocarp in relation to number of external punctures on the epicarp of cotton bolls (Greene & Herzog, 1999; Willrich et al., 2004a). These authors stated that internal injury symptoms are more reliable indicators of the stink bug presence on cotton fields, and puncture symptoms on the epicarp underestimate the injury and damage capacity of these bugs on bolls.

Table 1. Mean number (±SE) of external and internal punctures, internal warts and locks with immature stained fibers of Bt and non-Bt cotton bolls infested with *E. heros* adults (n=20). Dourados, MS, Brazil. Growing season 2008/2009.

Symptom(1)	Bt bolls		Non-Bt bolls	
	Infested	Non-infested	Infested	Non-infestad
NEP	0.97(±0.12)*(2)	0.77(±0.05)	1.07(±0.12)*	0.80(±0.05)
NIP	3.98(±0.61)*	0.70(±0.00)	3.14(±0.54)*	0.73(±0.03)
NW	1.82(±0.21)*	0.84(±0.10)	1.80(±0.31)*	0.70(±0.00)
NLISF	1.38(±0.11)*	0.80(±0.05)	1.21(±0.11)*	0.70(±0.00)

(1) NEP = Number of external punctures, NIP = Number of internal punctures, NW = Number of warts, and NLISF = Number of locks with immature stained fibers. (2) * = Significant between infested and non-infested by Student's *t* test ($P \leq 0.05$); means obtained with the original data transformed in square root of (x+0.5).

Table 2. Mean number (±SE) of external punctures, hard locks and locks with mature stained fibers of Bt and non-Bt cotton bolls infested with *E. heros* adults (n=16). Dourados, MS, Brazil. Growing season 2008/2009.

Symptom[(1)]	Bt bolls		Non-Bt bolls	
	Infested	Non-infested	Infested	Non-infested
NEP	0.94(±0.11)*[(2)]	0.70(±0.00)	1.36(±0.17)*	0.70(±0.00)
NHL	1.22(±0.13)*	0.73(±0.03)	1.46(±0.14)*	0.79(±0.06)
NLMSF	1.52(±0.16)*	0.82(±0.09)	1.70(±0.12)*	0.79(±0.06)

[(1)] NEP = Number of external punctures, NHL = Number of hard locks, and NLMSF = Number of locks with mature stained fibers. [(2)] * = Significant between infested and non-infested by Student's *t* test ($P \leq 0.05$); means obtained with the original data transformed in square root of (x+0.5).

This is confirmed by the positive strong correlation observed between the number of punctures and warts on the mesocarp, and the number of locks with immature stained fibers in both varieties (Bt and non-Bt), despite the moderate significant correlation between the number of punctures on the epicarp and the number of punctures and warts on the mesocarp, not to mention the number of locks with immature stained fibers (Table 3).

Blinka et al. (2008) obtained similar results on the correlation between external and internal injury symptoms of stink bugs injuries on Bt-cotton bolls; these authors stated that it is reasonable to use this parameter as a scout technique to stink bug treatment threshold determination, once the correlation between external and internal punctures increase according to the number of external injury increase.

The number of external punctures presented negative correlation with the seed cotton yield, and it was not statistically significant (Table 4). This reinforces that the number of punctures on the cotton bolls epicarp, due to *E. heros* attack, does not represent the real damage caused in the seed cotton yield from capsules derived from attacked bolls. However, it is suggested that depending on the number of sampled bolls, the external lesions evaluation could be used as a reasonably accurate method to decide whether to use or not a stink bug control measure on cotton (Toews et al., 2009).

E. heros adult causes cotton fibers discoloration, similar to what is observed for *N. viridula* adults that can affect negatively the values of fineness, strength, uniformity, and fiber length, besides increasing the amount of stained fibers, affecting negatively the production quality (Bommireddy et al., 2007). The number of hard locks on the

Table 3. Pearson's correlations matrices between internal and external injury symptoms caused by the *E. heros* adults attack on Bt and non-Bt cotton bolls (n=40). Dourados, MS, Brazil. Growing season 2008/2009.

Bt bolls			
Symptoms[(1)]	Pearson's coefficient (r)[(2)]		
	NEP	NIP	NW
NIP	0.426**		
NW	0.451**	0.763**	
NLISF	0.348*	0.729**	0.707**
Non-Bt bolls			
Symptoms	Pearson's coefficient (r)		
	NEP	NIP	NW
NIP	0.554**		
NW	0.593**	0.758**	
NLISF	0.604**	0.899**	0.740**

[(1)] NEP = Number of external punctures, NIP = Number of internal punctures, NW = Number of warts, and NLISF = Number of locks with immature stained fibers. [(2)] Pearson's coefficient obtained with the original data transformed in square root of (x+0.5); ** = Significant ($P \leq 0.01$).

Table 4. Pearson's correlations matrices between seed cotton yield and external injury symptoms caused by the *E. heros* adults attack on Bt and non-Bt cotton bolls (n=40). Dourados, MS, Brazil. Growing season 2008/2009.

Bt bolls			
Parameters[(1)]	Pearson's coefficient (r)		
	SCY	NEP	NHL
NEP	-0.269^{NS}		
NHL	-0.648**	0.477**	
NLMSF	-0.434*	0.342^{NS}	0.734**
Non-Bt bolls			
Parameters	Pearson's coefficient (r)		
	SCY	NEP	NHL
NEP	-0.138^{NS}		
NHL	-0.716**	0.425*	
NLMSF	-0.387*	0.536**	0.661**

[(1)] SCY = Seed cotton yield, NEP = Number of external punctures, NHL = Number of hard locks, and NLMSF = Number of locks with mature stained fibers. [(2)] Pearson's coefficient obtained with the original data transformed in square root of (x+0.5); ** = Significant ($P \leq 0.01$), * = Significant ($P \leq 0.05$), and NS = Non-significant ($P \leq 0.05$).

infested cotton bolls was significantly higher than that for non-infested (Table 2). Similarly, the percentage of capsules with some or all locks partially open (hard locks) was significantly higher when cotton bolls were confined with *N. viridula* adults (Willrich et al., 2004c). The hard locks not only affect the potential of the plants' yield directly but they also harm the production quality (Bélot & Vilela, 2006).

E. heros adult was able to reduce significantly the cotton seed yield of both varieties Bt and non-Bt, respectively, by 13 and 24% (Figure 1). On the other hand one 5th instar nymph of *N. viridula* confined with a 13-day-old cotton bolls for 7 days showed a seed cotton yield reduction of 59%, detecting that these nymphs caused more injuries on 8-9-day-old cotton bolls than adults and even 2nd, 3rd or 4th instars nymphs (Greene et al., 1999).

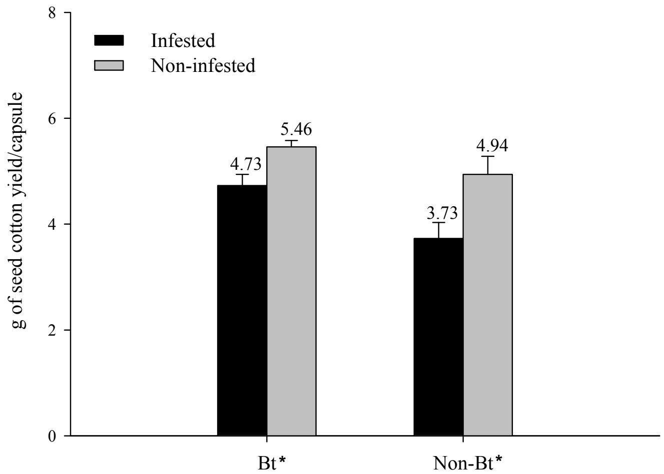

Figure 1. Mean (±SE) of seed cotton yield from capsules derived from Bt and non-Bt cotton bolls infested or not with *E. heros* adults (n=16). Dourados, MS, Brazil. Growing season 2008/2009.
* = Significant between infested and non-infested by Student's *t* test ($P \leq 0.05$) for each genotype.

Although seed cotton yield between different genotypes was not compared, it is possible that the Bt variety presented certain tolerance to adults *E. heros* attack, even if these two varieties have the same genome, except for the *cry1Ac* gene.

The damages caused by stink bugs on cotton varied according to varieties, species, developmental stages of species, population densities, plant phenological stage, and cotton bolls age and/or size (Greene et al., 1999; Siebert et al., 2005; Ward, 2005; Roberts et al., 2006; Greene et al., 2006; Herbert et al., 2009). Therefore, more research on bioecological and economic stink bugs aspects are necessary to consolidate stink bugs control strategies, mainly for *E. heros*, in the Bt and non-Bt Brazilian savannah cotton production systems, considering the Integrated Pest Management (IPM) context and using alternative scouting techniques, as well as the external and internal injuries symptoms evaluation on cotton bolls.

Conclusions

E. heros adult reduced significantly seed cotton yield of Bt and non-Bt cotton bolls.

E. heros adult attack on Bt and non-Bt cotton bolls caused lint stains.

Hard locks formation was significantly higher on capsules derived from Bt and non-Bt cotton bolls infested by *E. heros* adults compared to non-infested capsules.

E. heros attack caused circular dark concaves spots on the epicarp and dark feeding punctures and/or warty growths (callous tissue) on the mesocarp of Bt and non-Bt cotton bolls.

The number of punctures on cotton bolls epicarp (external injury symptoms) do not represent the real damage caused on seed yield of capsules derived from Bt and non-Bt cotton bolls attacked by *E. heros* adults.

The internal injury symptoms (punctures and callous tissue) observed on cotton bolls are reliable indicators of *E. heros* presence on Bt and non-Bt cotton fields.

Injury symptoms evaluation on the epicarp and mesocarp of cotton bolls can be used as a scouting technique of *E. heros* populations on Bt and non-Bt cotton fields.

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