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

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Introduction

As for traditional Brazilian savannah cotton production system, stink bugs (Heteroptera: Pentatomidae) are indirectly controlled by broad spectrum insecticides applied so as 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, looking for food and shelter and causing damage to yield and lint quality by feeding on ripening bolls. Cotton bolls that are 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. 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.

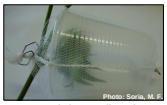
Objectives

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

Euschistus heros (adult)

Boll with ≈ 25 mm diameter used for the *E. heros* infestation.



Cage used for confine the *E. heros* adult with the cotton boll.

E. heros adult caged with a ≈ 25 mm diameter (Bt or non-Bt) cotton boll.

- 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 (22°11' South latitude, 54°56' West longitude).
- An area of 180 m2 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 sprays were made to prevent any type of injury on the target bolls afterwards, there was a 15-day 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.They were marked and infested with one field-collected *E. heros* adult. The 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 for internal and external injury symptoms and evaluated 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

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)ns[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)ns[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).

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.

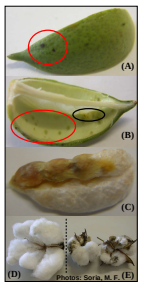
Bt bolls			
Symptoms[1]	Pearson's coefficient (r)[2]		
	NEP	NIP	NW
NIP	0.426**		
NW	0.451**	0.763**	
NLISF	0.348*	0.729**	0.707**
Non-Bt bolls			
Symptoms	Pearson's coefficient (r)		
	NEP	NIP	NW
NIP	0.554**		
NW	0.593**	0.758**	
NLISF	0.604**	0.899**	0.740**

[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	-0.269ns[2]		
NHL	-0.648**	0.477**	
NLMSF	-0.434*	0.342ns	0.734**
Non-Bt bolls			
Parameters	Pearson's coefficient (r)		
	SCY	NEP	NHL
NEP	-0.138ns		
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 ($P \leq 0.05$).



External punctures on the epicarp (A), internal punctures and warty growth on the mesocarp (B), immature stained fibers (C), capsule without any damage (D) and capsules with mature stained fibers and hard locks (E).

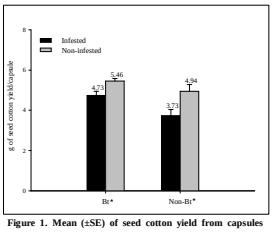


Figure 1. Mean (±SE) of seed cotton yield from capsules derived from Bt and non-Bt cotton bolls infested or not with *E. heros* adults (n=16). Dourados, MS, Brazil. Growing season 2008/2009.
*=Significant between infested and non-infested by Student's *t* test ($P \leq 0.05$) for each genotype.

- 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. Internal injury symptoms are considered 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.
- 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). The number of external punctures presented negative correlation with the seed cotton yield, and it was not statistically significant (Table 4). 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.
- *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). Although seed cotton yield between different genotypes was not compared, it is possible that the Bt variety presented certain tolerance for 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 change between varieties, species, developmental stages of species, population densities, plant phenological stage, and cotton bolls age and/or size. In this way, more researches are necessary to consolidate stink bugs control strategies, mainly for *E. heros* species, in the Bt and non-Bt Brazilian savannah cotton production systems.

Conclusions

- ✓ *E. heros* adult is able to reduce significantly seed cotton yield of Bt and non-Bt cotton bolls.
- ✓ *E. heros* adult attack on Bt and non-Bt cotton bolls cause lint stains.
- ✓ Hard locks formation is 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 causes circular dark concaves spots on the epicarp and dark feeding punctures or warty growths 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.

References are available upon request.