**SCIENTIFIC NOTE**

Trypanosomatid Prevalence in *Nezara viridula* (L.), *Euschistus heros* (Fabricius) and *Piezodorus guildinii* (Westwood) (Heteroptera: Pentatomidae) Populations in Northern Paraná, Brazil

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Prevalência de Tripanossomatídeos em Populações de *Nezara viridula* (L.), *Euschistus heros* (Fabricius) e *Piezodorus guildinii* (Westwood) (Heteroptera: Pentatomidae) no Norte do Paraná

RESUMO - O complexo de percevejos da soja possui um grande potencial de dano, seu controle é difícil ao final da safra por suas elevadas densidades populacionais. Trabalhos relacionados com organismos entomopatogênicos em percevejos são interessantes devido a suas possibilidades como agentes de controle e porque eles podem ser agentes causais de doenças em criação massal. A prevalência de tripanossomatideos foi determinada nas espécies mais importantes da cultura da soja, no Brasil, *Nezara viridula* (L.), *Euschistus heros* (Fabricius) e *Piezodorus guildinii* (Westwood). Os insetos foram coletados na soja e hospedeiros alternativos durante a entressafra. Gotas de hemolinfa de cada adulto foram montadas em lâminas e observadas ao microscópio; o número de percevejos com e sem flagelados foi registrado. Os flagelados encontrados nas populações de *P. guildinii* e *E. heros* foram menores que os observados em *N. viridula*. A maior prevalência dos tripanossomatideos foi observada nos espécimes de *N. viridula* coletados na soja alcançando 30% de infecção em janeiro, 2001, e 44% prevalência em fevereiro de 2003. Em *P. guildinii* as máximas prevalências foram observadas em janeiro e fevereiro de 2003 (16%) e em 27 de julho de 2003 com 20% dos espécimes infectados. Os flagelados na hemolinfa de *E. heros* foram menos freqüentes (máxima prevalência de 8,7%), sugerindo que esta espécie é menos suscetível que *N. viridula* e *P. guildinii*. Embora os níveis de prevalência sejam mais elevados que os observados para fungos entomopatogênicos, os tripanossomatideos não parecem ser eficientes reguladores das populações de percevejos de percevejos em curto prazo.

PALAVRAS-CHAVE: Inseto, percevejo, protozoa, flagellado

ABSTRACT - The stink bug complex in soybeans has a great potential to cause damage, their control has been difficult due to high population densities at the end of the season. Studies related to entomopathogenic organisms in stink bugs are interesting for their possibilities to be used as biological control agents and because they could be an etiological agent in mass rearing. We determined the prevalence of trypanosomatids in the most important species of the stink bug complex in soybean, from Brazil, *Nezara viridula* (L.), *Euschistus heros* (Fabricius) and *Piezodorus guildinii* (Westwood). Insects were collected in soybean and on alternate hosts during the intercrop season. Hemolymph drops from each adult were mounted on slides and observed under microscope; stink bug numbers with and without flagellates were recorded. The flagellates found in *P. guildinii* or *E. heros* populations were smaller than that found in *N. viridula*. The higher prevalence of the trypanosomatids was observed on soybean reaching a peak of 30% of the sampled *N. viridula* specimens in January, 2001, and 44% prevalence in February, 2003. The maximum prevalences (16%) in *P. guildinii* were observed in January and February, 2003 and on July 27, 2003 with 20% of the specimens infected. Flagellates in the hemolymph of *E. heros* were less frequent (maximum prevalence of 8.7%), suggesting that this species is less susceptible than *N. viridula* and *P. guildinii*. Trypanosomatid prevalence was higher than that observed for entomopathogenic fungi, however they seem to be inefficient control agents in short term condition.

KEY WORDS: Insect, stink bug, protozoa, flagellate
The stink bug complex in soybeans has great potential to cause damage (Corrêa-Ferreira & Panizzi 1999), and their populations can reach unusual levels, in some cases 20 to 25 individuals per soybean row meter. At the end of the growing season, stink bug control is difficult due to high population densities and some cases of insecticide resistance were recently reported (Sosa-Gómez et al. 2001). Although an egg parasitoid can be used (Corrêa-Ferreira 1993) most of the time stink bugs are controlled by a large spectrum of insecticides.

Studies related to entomopathogenic organisms in stink bugs are interesting because of their possibilities to be used as biological control agents and also because they could be a source of diseases during mass rearing. The most common naturally occurring pathogens of stink bugs are fungi [Beauveria bassiana (Bals.) Vuill., Metarhizium anisopliae (Metsch.) Sorok., Paecilomyces fumosoroseus (Wize) Brown and Smith], some RNA viruses (ssRNA small picornavirus and a dsRNA Toti-like virus) (Williamson and Wechmar 1992, 1995), and protozoan flagellates (Gibbs 1957, Batistoti 1998, Fuxa et al. 2000). However, a search for key natural control agents in stink bugs field populations has been neglected. Among the pathogens associated with soybean stink bugs, none of them cause perceivable epizootic. Sosa-Gómez & Moscardi (1998) reported high prevalence of B. bassiana on Nezara viridula (L.), in the greenhouse, an artificially protected environment, but cases like this have not been observed in the field.

Studies on 17 heteropteran species from Brazil revealed that 13 of them had trypanosomatid infections (Godoi 2000). Among the sampled specimens, two pentatomid species, Euschistus heros (Fabricius) and Piezodorus guildinii (Westwood) were taken to the laboratory and bled through a shallow puncture on the lateral angle of the pronotum respectively. The number of flagellates per microliter of hemolymph and feces. The droplet size was determined by measuring with a OSM filar micrometer eyepiece (Ramsden type) (Olympus Optical Co. Ltd., Japan).

Because we did not initially detect flagellate infections in E. heros blood samples (n = 124), we suspected that this species was not susceptible to flagellatosis of the hemolymph. For this reason, N. viridula and P. guildinii populations were collected during 2000/2001, 2002/2003, and E. heros samples were collected starting from October 11, 2002 until August of 2003.

Comparisons of flagellates prevalence among stink bug species were done with the non-parametric test of Mann-Whitney Rank sum Test from Sigmastat Statistical Software (Jandel Scientific 1994).

Trypanosomatids were very common in N. viridula and P. guildinii populations (Fig. 1). Promastigotes and long forms were predominant. Flagellates found in P. guildinii and E. heros were smaller than those found in N. viridula (Table 1). Trypanosomatids from N. viridula were 50.1 ± 2.7 µm and the flagella were 31.5 ± 1.1 µm. One specimen found in E. heros had a body length of 25.1 µm and flagellum of 14.6 µm. In P. guildinii, the trypanosomatids were 22.9 ± 3.1 µm and 18.6 ± 3.1 µm, for body length and flagellum, respectively.

During the observational periods, flagellate prevalence increased in the populations of N. viridula and P. guildinii in the summer, during the wet and hot weather (January and/or February) reaching a peak of 30% in the sampled N. viridula specimens in January, 2001, and 44% prevalence in February, 2003 (Figs. 2 and 3). Therefore, we can consider that the rain distribution during the high prevalence period, could be positively related with the infection rates (Fig. 4). N. viridula had flagellates infection ranging from 2.5% to 44%, and P. guildinii had prevalence ranging from 2.9% to 20%. The maximum prevalences in P. guildinii were observed between January 29 and February 25, 2003 (16%) and on July 27, 2003 with 20% of the specimens infected. In E. heros, flagellates were observed on only three sampling dates in October, 2002, and only at prevalence rates of 3.3%, 8.7% and 4%. The prevalence in E. heros populations was significantly lower than in N. viridula populations (P < 0.0001, T = 424.0, n = 17, N = 17, Mann-Whitney Rank Sum Test) and P. guildinii populations (P = 0.03, T = 382.5, n = 17, N = 19, Mann-Whitney Rank Sum Test).

The average volume of hemolymph droplets obtained from N. viridula was higher (2.1 µl ± 0.7 (± SEM) than those from P. guildinii and E. heros (0.8 µl ± 0.4 and 0.5 µl ± 0.1, respectively). The number of flagellates per microliter of...
hemolymph in N. viridula (2.48 flagellates/µl) and P. guildinii (2.17 flagellates/µl) was not significantly different (P = 0.69, T = 781.0, n = 19, N = 67, Mann-Whitney Rank Sum Test) but was higher in N. viridula than in E. heros (0.50 flagellates/µl) (P < 0.009, T = 715.0, n = 22, N = 67, Mann-Whitney Rank Sum Test). No significant differences between P. guildinii and E. heros were observed. The number of trypanosomatids in the hemolymph of the three hemipterans ranged from 0 to more than 50 per blood sample (Fig. 5).

Although size differences were observed, morphological aspects are not conclusive to differentiate any taxon. Godoi (2000) stated that morphometric analysis was useless to resolve differences among Genera. The taxonomy of this protozoan remains unclear at present; taxonomic studies of stink bug protozoans are necessary (Catarino et al. 2001).

D.R. Sosa-Gómez & D.G. Boucias (unpubl.) observed infection by flagellates in N. viridula samples collected from corn during the summer of 1994, in Quincy, Florida, USA. Morphologically, the trypanosomatids from N. viridula, in the current study, are similar to those found in Louisiana (Fuxa et al. 2000) and to those that we found previously, from N. viridula collected from Quincy, FL. After isolation,
we verified that the Florida flagellates reproduced very profusely in *Trichoplusia ni* cell cultures (TN368) immersed in TC100 medium (Life Technologies, Inc.), but *Spodoptera frugiperda* cells (S9) cultures were not permissive for the same trypanosomatids (D.R. Sosa-Gómez & D.G. Boucias, pers. observ.).

In the *N. viridula* samples from Quincy, FL, and in *P. guildinii* from Londrina, we found cysts attached to the flagellum (Fig. 1c). Cysts are thought to be forms of survival and propagation of trypanosomatids, and they occur in the *Blastocrithidia* and *Leptomonas* genera (Takata et al. 1996).

Although the mean size of body and flagellum in the current study does not match those of Fuxa et al. (2000), there was an overlapping of the body and flagellum length. We observed a prevalence lower than that reported by Fuxa et al. (2000) in *N. viridula*, which reached almost 95% of the stink bugs infected. The higher number observed by Fuxa et al. (2000) could be due to the sampling method, because we sampled flagellates in the hemolymph, whereas these authors diagnosed infection by dissecting the alimentary tract of each individual. The infection peaks from November until March, were observed on soybean. At present little is known about the interactions between host plant species and levels of infection.

Unclean colonies of *N. viridula* in (food contaminated with feces) mass rearing had a high prevalence of infection (D.R. Sosa-Gómez & D.G. Boucias, unpub.), possibly due to the transmission of this pathogen through the feces left on the feeding substrate. The condensation of water on soybean plants early in morning may help the locomotion of the active phase and favor transmission. This behavior could explain the high prevalence during wet weather.

The presence of flagellates in *N. viridula* hemolymph is an indicative that flagellates can transpose the gut barrier more easily that in *E. heros*. This could be due to differences in the gut wall or in the flagellates virulence. Differences in virulence among trypanosomatid isolates have been observed by Baccan et al. (2001); for example, *Leptomonas* isolated from *E. heros* caused mortality very rapidly (24h after infection) in *Veneza zonata* (Pallas) but other isolates did not. Despite the high densities of flagellates in *N. viridula* and *P. guildinii* blood droplets, the insects were alive, revealing the nature of chronic diseases against these species. Until present the effect on longevity, fertility and fecundity is unknown.

Flagellates apparently do not invade and freely circulate in the hemolymph of *E. heros* as they do in *N. viridula* and *P. guildinii*. Their detection in *E. heros* required strong pressure on the body during the bleeding procedure. Thus, it is possible that they were released into the hemocoel after rupture of the gut wall. A *Leptomonas* sp. was detected in the digestive tract of *E. heros* by Batistoti (1998).

Low levels of trypanosomatid parasites in *E. heros* compared to *N. viridula* implies a lower susceptibility of this species. The mechanisms involved in these differences of susceptibility are unknown. Baccan et al. (2001) suggest that there are several mechanisms involved in the penetration of the gut wall towards the hemocoel, some of them rendering the wall permeable to the bacteria inhabiting the digestive tract.

The importance of pathogens as regulatory agents of insect populations is becoming more evident in several host-pathogen systems. Previously, life table studies of stink bugs usually did not consider pathogens as death cause. In biology studies stink bugs present high mortality rates but nothing is known about their etiological causes (Panizzi 1987). However pathogens such as viruses have been mentioned as the cause of mortality and diminished reproductive capability in the southern green stink bug (Williamson & Wechmar 1995). Flagellates could be a causal agent partly responsible for high mortality rates and reduced life span. Additional studies are necessary, because the impact of infection by these protozoans on pentatomid biology and reproduction at population level is still unknown. Although, trypanosomatid prevalence was higher than that observed for entomopathogenic fungi, they seem to be inefficient control agents in short term condition.

Figure 3. Prevalence of flagellates on stink bugs populations from Londrina, north of Paraná State, during 2002-2003.
Figure 4. Rainfall (mm), mean temperature (°C) during 2000 to 2003. Number of days per year divided by five-day periods.
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