DOI: 10.5433/1679-0359.2022v43n5p2045

Effect of sublethal concentrations of insecticides associated with NaCl and KCl on feeding behavior and mortality of *Euschistus heros* and *Diceraeus melacanthus* (Hemiptera: Pentatomidae)

Efeito de concentracoes subletais de inseticidas associados com NaCl e KCl sobre comportamento alimentar e mortalidade de *Euschistus heros* e *Diceraeus melatacanthus* (Hemiptera: Pentatomidae)

Paulo Sergio Gimenez Cremonez^{1*}; Matheus Corseti Marcomini²; Daniela de Oliveira Pinheiro³; Pedro Manuel Oliveira Janeiro Neves⁴

Highlights _

Association of chlorides and five insecticides were tested in two major pest species. Diceraeus melacanthus salivary sheaths were reduced in corn seedlings dipped in NaCl. The LC_{25} of clorantraniliprole + NaCl is as efficient as its LC_{50} in *D. melacanthus.* The LC_{25} of spinosad + NaCl is equally efficient as its LC_{50} in *E. heros.*

Abstract .

Stink bugs are important pests of highly profitable agricultural crops worldwide, and the use of insecticides remains the main strategy for their control. The objectives of this study were to evaluate the behavioral aspects of *Euschistus heros* and *Diceraeus melacanthus* through feeding after exposure to chloride solutions (NaCl and KCl), as well as the control efficacy of insecticides in combination with salt. Two bioassays were performed with stink bugs exposed to treated substrate: i) feeding preference by salivary sheath counting in a free choice test for chloride solutions, using bean pod or corn seedling as counting substrate for newly emerged *E. heros* and *D. melacanthus* adults, respectively; and ii) comparative efficiency of five synthetic insecticides associated with NaCl or KCl in the mortality of 4th instar nymphs (N4). Pre-determined sublethal (LC_{25}) and median lethal (LC_{50}) concentrations of commercially available insecticides (Lfn), buprofezin (Bpf), pyriproxyfen (Ppx), chlorantraniliprole (Ctn) and spinosad (Spn) were used. For each species and insecticide,

¹ Postdoc Associate Researcher, Department of Entomology, College of Agricultural and Environmental Sciences, University of Georgia Tifton Campus, 2630 Rainwater Rd, Tifton 31793, GA, USA. E-mail: paulogimz@uga.edu

² Discente do Curso de Mestrado do Programa de Pós-Graduação em Agronomia, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: matheusmarcomini11@outlook.com

³ Profa Dra, Departamento de Histologia, UEL, Londrina, PR, Brazil. E-mail: daniela_pinheiro@uel.br

⁴ Prof. Dr. of Postgraduate Program in Agronomy, UEL, Londrina, PR, Brazil. E-mail: pedroneves@uel.br

^{*} Author for correspondence

the treatments were pure LC_{25} , LC_{25} + NaCl 5% m/m, LC_{25} + KCl 5% m/m, pure LC_{50} , and distilled water (dH₂O) as the control. Corn seedlings treated with 5% NaCl saline solution had reduced salivary sheaths from *D. melacanthus*. Ctn LC_{25} (0.63 mL L⁻¹) + 5% NaCl and pure Ctn LC50 (1.16 mL L⁻¹) were similar and more efficient in terms of mortality of *D. melacanthus* N4s than any other treatment. The association of Spn LC_{25} (0.37 mL L⁻¹) + 5% NaCl and Spn LC_{50} only (0.90 mL L⁻¹) was similar to that of *E. heros* N4s compared to other treatments. The insecticide concentrations used in this study have potential for stink bug pest control, thus the association of insecticides with commercially available, easy-to-find, and low-cost chloride salts may represent a good strategy for stink bug control.

Key words: Green-belly stink bug. Insecticide association. Integrated pest management. Neotropicalbrown stinkbug.

Resumo .

Os percevejos são importantes pragas de culturas agrícolas altamente rentáveis em todo o mundo, e o uso de inseticidas continua sendo a principal estratégia para seu controle. Os objetivos deste estudo foram avaliar os aspectos comportamentais de Euschistus heros e Diceraeus melacanthus através da alimentação após exposição a soluções cloradas (NaCl e KCl), bem como a eficiencia de controle de inseticidas em combinação com sal. Dois bioensaios foram realizados com percevejos expostos ao substrato tratado: i) preferência alimentar por contagem de bainhas salivares em teste de livre escolha de soluções contendo cloreto, usando vagem de feijão ou plantulas de milho como substrato de contagem para adultos de E. heros e D. melacanthus recém-emergidos, respectivamente; e ii) eficiência comparativa de cinco inseticidas sintéticos associados a NaCl ou KCl na mortalidade de ninfas de 4º estágio (N4). Foram utilizadas concentrações subletais pré-determinadas (CL25) e letais medias (LC50) de inseticidas comercialmente disponíveis lufenurom (Lfn), buprofezina (Bpf), piriproxifem (Ppx), clorantraniliprole (Ctn) e espinosade (Spn). Para cada espécie e inseticida, os tratamentos foram CL₂₅ pura, CL₂₅ + NaCl 5% m/m, CL₂₅ + KCl 5% m/m, CL₅₀ pura e água destilada (dH₂O) como controle. Plantulas de milho tratadas com a solução salina NaCl 5% apresentaram redução das bainhas salivares de D. melacanthus. Ctn CL₂₅ (0,63 mL L⁻¹) + 5% NaCl e Ctn CL₅₀ pura (1,16 mL L⁻¹) foram semelhantes e mais eficientes em termos de mortalidade de N4s de D. melacanthus do que qualquer outro tratamento. A associação de Spn CL₂₅ (0,37 mL L⁻¹) + 5% NaCl e Spn CL_{so} pura (0,90 mL L⁻¹) foi semelhante à de N4s de *E. heros* em relação aos outros tratamentos. As concentrações de inseticidas utilizadas neste estudo têm potencial para o controle de pragas de percevejos, portanto, a associação de inseticidas com sais de cloreto comercialmente disponíveis, de fácil acesso e baixo custo pode representar uma boa estratégia para o controle de percevejos.

Palavras-chave: Percevejo barriga-verde. Associação de inseticidas. Manejo integrado de pragas. Percevejo marrom-neotropical.

Introduction _

Soybean, maize, and wheat crops currently represent over 90% of the total grain production in Brazil and are of great socio-economic interest worldwide (United States Department of Agriculture - Foreign Agricultural Service [USDA-FAS], 2021). However, these crops are highly prone to economic loss due to pest attack, mainly by phytophagous stink bugs that have reached primary pest status in recent years. This process was accelerated due to the stink bug's extremely high reproductive performance and increased resistance to conventionally used, neurotoxic insecticides (Panizzi, 2013; Sparks et al., 2020).

Stink bug pests degrade part of the plant tissue by feeding on the host, and stylet retraction from the puncture site forms a characteristic salivary sheath. The count and location of these sheaths can be used for studies on feeding behavior and can be an important tool for the development of integrated pest management (IPM) practices (H. J. Huang et al., 2015; Panizzi & Lucini, 2017). Regarding the management of phytophagous stink bugs, a proposal that fits the IPM principles was primarily suggested and analyzed by Corso (1990) and revised by Niva and Panizzi (1996) and Corso and Gazzoni (1998) as a cost-effective alternative for its control. They proposed the use of a reduced dose of insecticides (e.g., 50% of the labeled dosage rate) when in combination with sodium chloride (NaCl) at a concentration of 0.5% of the final insecticide mixture. In general, the addition of these tastants to the insecticide spray can increase their efficacy and cause changes in insect behavior. Some studies have been conducted to better understand the levels of this association. Rodrigues et al. (2020) concluded that the addition of NaCl was synergistic with the effects of imidacloprid on E. heros. However, the authors also pointed out that chloride interferes positively with the reproductive performance of stink bugs. In another study, Ramos et al. (2019) evaluated the compatibility of the association of NaCl and imidacloprid with the predatory stink bug Podisus nigrispinus. They did not observe changes in mortality or sublethal effects and they concluded that the presence of chloride does not interfere with the predator; however, its use can be effective in reducing the concentration of the insecticide needed and, consequently, the applied product has less effect on natural enemies. However, many studies on the association between salts and insecticides have been conducted using commonly used products.

The use of insecticides with modes of action that differ from those of conventionally used broad-spectrum neurotoxic chemicals, such as insect growth regulators and diamides, for example, must be better studied. Due to their selective mode of action on insect-specific physiological mechanisms, these products may represent an efficient alternative for the control of pest resistant populations (Ishaaya et al., 2005; Liang et al., 2016; Sun et al., 2015), especially for possible use of integrating rotation schemes (Sparks et al., 2021). For stink bugs, previous studies have been conducted using sublethal doses of insect growth regulators (IGRs), or growth disruptors, for lower dosage effects on reproduction parameters, with reports of reduced fertility and fecundity (Cremonez et al., 2017) and internal morphological alterations in the reproductive organs of surviving adults (Cremonez et al., 2019; Matsumoto et al.,

2021). The reports are, therefore, limited for the analysis of quantitative sublethal effects, and related behavioral studies are still insipient on this subject.

Because of the great interest in the management of stink bug infesting populations in high-yield crops, such as soybean, corn, cotton, and other commodities, where the insect is a key pest, the main objectives of this study were to evaluate the feeding behavior of *E. heros* and *D. melacanthus* exposed to NaCl and KCl solutions under *in vitro* and greenhouse conditions, respectively, as well as the efficacy of selected insecticides in association with these chlorides.

Materials and Methods ____

Stink bug colony

Insects used for the study were obtained from colonies held in the Institute for Rural Development of Parana (IDR), which originated from insects collected from various locations in Parana State, Brazil (within a 200 km radius from 24°37'27" S and 51°24'20" W). The stink bugs were kept under controlled conditions ($26\pm1^{\circ}$ C, $65\pm5^{\circ}$ RH, and 14:10 h light:dark) in 10 L plastic boxes with a standard diet of insecticide-free bean pods, peanuts, and soybean seeds (Cremonez et al., 2019). Maintenance was performed under the same conditions in the controlled-environment chambers.

Chlorides on feeding behavior

For the analysis of preference and feeding behavior with chloride salt solution,

newly emerged (<12 h after emergence) adults of E. heros and D. melacanthus were fasted for a period of 48 h on a moist cotton wool. The treatments used were 5% of table salt NaCl (Cisne, São Paulo, Brazil) or 5% sodiumfree (light) table salt KCI (Synth, Diadema, Brazil) diluted in distilled water and pure distilled water as a control. The preference for stink bugs in a free choice test was assessed using organic bean pods or corn seedlings as salivary sheath counting substrates for E. heros and D. melacanthus, respectively. For each treatment, 30 mL of the mixture was applied to the pods and seedlings by using a pressurized manual sprayer with a conical nozzle (Guarany® PCP-01 1.25 L).

To determine feed preference, adults of *E. heros* were maintained under laboratory conditions using insecticide-free bean pods. The experimental unit consisted of a single insect allocated in a polystyrene crystal box arena (120 cm²) lined with filter paper and containing a treated and an untreated (check) pod placed in opposite corners. Thirty replicates were used for each experiment. For D. melacanthus, one adult was enclosed in a 15 L vase with a surface area of 120 cm² containing two corn seedlings (7 days after germination) planted in opposite corners, with one treated with salt and one as a control check. Chiffon fabric was used to cover the system and retain stinkbugs in the microcosm. Twenty replicates were used for this study. After 72 h, salivary sheaths were collected using a methodology adapted from Bowling (1979), and the bean pods and corn seedlings were collected and dipped in 1% acid fuchsine solution for 4 h, then washed with tap water, and observed under a stereoscopic microscope (25 ×) for quantification of the salivary sheaths.



In both experiments, a completely randomized design was used with two treatments: 5% NaCl or KCl 5% and distilled water. For data assessment of normality and homogeneity of variances, the Shapiro–Wilk test was performed. In positive cases, t-test for independent samples were performed and in cases of non-normality the data were submitted to the Wilcoxon–Mann–Whitney test. The saline solutions were compared with the control, but not with each other. All statistical analyses were performed using the software R v. 3.5.0 (R Core Team [R], 2021).

Association of chlorides and lethal concentrations of insecticides

Sublethal (LC $_{25}$) and median lethal (LC $_{50}$) concentrations of each insecticide were

pre-determined following the methodology described in Cremonez et al. (2017). Briefly, six base concentrations for each insecticide were defined, with the highest concentration based on the maximum label rate registered for the stink bugs or a closely related pest in soybean when not registered (e.g., the whitefly Bemisia tabaci). The next concentrations were prepared using arbitrary dilutions of the maximum concentration. Groups of ten fourth-instar nymphs were exposed to each of the concentrations previously applied to Petri dishes, and mortality was assessed seven days after initial exposure. Doseresponse curves were obtained using the protocol script in R software with the "drc" package (Ritz & Streibig, 2015). Insecticides and their relative LC_{25} and LC_{50} values are listed in Table 1.

Table 1

Predetermined values of sublethal (LC_{25}) and median lethal (LC_{50}) concentration of insecticides used
on 4 th instar nymphs of two species of stink bugs

Incasticida	IRAC group ¹	Trade mark (®) -	Euschistus heros		Diceraeus melacanthus	
Insecticide			LC ₂₅ ²	LC ₅₀	LC ₂₅	LC ₅₀
Lufenuron (Lfn)	15	Match 50 CE	4.013	8.223	2.452	5.577
Buprofezin (Bpf)	16	Applaud 250 WP	3.780	7.254	2.591	4.318
Pyriproxyfen (Ppx)	7C	Tiger 100 CE	6.208	10.124	4.856	8.093
Chlorantraniliprole (Ctn)	28	Premio 200 CS	1.256	2.270	0.632	1.157
Spinosad (Spn)	5	Tracer 480 CS	0.368	0.903	0.382	0.616

Notes: ¹Classification from Insecticide Resistance Action Committee [IRAC] (2021); ²Lethal concentration values in mL of commercial formulation L⁻¹ for Lfn, Ppx, Ctn and Spn and in g of commercial formulation L⁻¹ for Bpf.

The insecticides used for the treatments were lufenuron (Lfn, Match[®] 50 CE, Brazil), buprofezin (Bpf, Applaud[®] 250 WP, UPL Brazil), pyriproxyfen (Ppx, Tiger[®] 100 CE, Sumitomo Chemical, Brazil), chlorantraniliprole (Ctn, Premio[®] 200 CS, FMC

Brazil), and spinosad (Spn, Tracer[®] 480 CS, Dow AgroSciences Brazil). The treatments consisted of the five insecticides in four possible mixtures: pure distilled water as the control check (1), insecticide LC_{25} , insecticide LC_{25} + KCl 5%, insecticide LC_{25} + NaCl 5%, and

insecticide LC50. An additional treatment with pure distilled water was used as an untreated control, for a total of 21 treatments.

The mixtures were then applied to a Petri dish (9 cm in diameter) containing bean pods for food, with the aid of a Potter spray tower (Burkard Scientific[®]), calibrated at 68.95 kPa, and deposited at 1.0 mL of the correspondenttreatmentineach experimental unit. The dishes were then placed in a laminar flow cabinet (Veco[®] CFLV-12) for 30 min for drying.

Groups of ten 4th instar nymphs (N4) of E. heros (20±1 days) or D. melacanthus (18±1 days) were collected from the established colony and placed on a substrate-treated Petri dish, immediately supplied with the rest of the standard diet (peanuts and soybean seeds) provided ad libitum. Each dish was considered an experimental unit and placed under controlled conditions. Untreated food was replaced every two days ad libitum. Mortality was assessed on the fifth day after initial treatment exposure. The efficiency of each insecticide was calculated using the Schneider-Orelli formula based on the treatment mortality data relative to the control mortality (Püntener, 1981).

For statistical analysis, the experimental design was completely randomized with four replicates. The data were transformed using the equation $\sqrt{(x+1)}$, then submitted to an analysis of variance (ANOVA) and Tukey's test (p \leq 0.05).

Results and Discussion _

Chloride salt solution on feeding behavior

Data concerning the number of salivary sheaths observed in E. heros and D. melacanthus are presented in Table 2. The number of sheaths did not differ between the 5% NaCl-treated and control groups in E. heros fed substrates (Mann–Whitney T = 199, p = 0.3446); however, salivary sheaths were significantly higher in D. melacanthus when feeding on uncontaminated control seedlings (Mann–Whitney T = 126.5, p = 0.0234). KCl at 5% applied to the substrates did not interfere with the number of salivary sheaths for either E. heros (t-test = 0.7702, p = 0.4474) or D. melacanthus (Mann–Whitney T = 171, p = 0.2164).

Table 2

Mean number of salivary sheaths of *Euschistus heros* over bean pod substrate and of *Diceraeus melacanthus* over corn seedling substrate, treated or not with dH₂O-diluted chloride solutions

Treatment	Substrate	Salivary sheaths (mean ± SE)				
freatment	Substrate	Euschistus ł	nero ^s	Diceraeus melacanthus		
NaCl 5%	Treated	15.87 ± 1.10	ns¹	7.00 ± 0.52	0.05 ¹	
	Not treated	18.50 ± 1.41	ns.	8.95 ± 0.58		
KCI 5%	Treated	19.83 ± 1.68	ns ²	4.80 ± 0.68	ns1	
	Not treated	17.60 ± 1.18		3.85 ± 0.60		

Note: SE = standard error. ns = not significant. ¹Significance level for non-parametric test of Wilcoxon–Mann–Whitney. ²Significance level for parametric t-test for independent samples.

SEMINA – Ciências Agrárias

The presence of sheaths indicates that the stink bug has probed and, at least, penetrated the substrate with their stylets. However, some authors claim that this act does not necessarily indicate feeding activity, since ingestion may not have occurred (Zeilinger et al., 2015). In contrast, biased probing in salttreated surfaces would be expected due to the deposited chloride on the food substrate. Increasing concentrations of chlorides may act proportionately in the stimulation of gustatory neuroreceptors, as observed in Aedes aegypti L. (Diptera: Culicidae) exposed to NaCl (Sanford et al., 2013). Moreover, a study showed that a particular arrestant effect, that is, permanence of feeding, was observed in stink bugs when NaCl was associated with insecticides in soybean crop conditions (Ramiro et al., 2005). The permanence of feeding might be associated with fewer salivary sheaths over time, which was observed in the reduced probing of D. melacanthus on NaCl-treated corn seedlings. However, this reduction in probing may intrinsically indicate a putative deterrence effect. In studies of Locusta migratoria L. (Orthoptera: Acridadae), the authors concluded that this insect tends to ingest larger amounts of water to dilute the deterrence effect of saline solutions (Raubenheimer & Gäde, 1993). Salivary counting analysis alone is a quantitative method that may indicate deterrence and associated feeding behavior; however, it is inconclusive. Complementary studies using modern tools such as electropenetrography (EPG) (Lucini & Panizzi, 2018) for feeding monitoring could be performed in future studies.

The literature is rather limited with respect to the effect of KCI on feeding behavior and insecticide association in Hemiptera. However, it is known to activate the midgut α -amylases and enzymes in the salivary glands of Aelia acuminate L. (Hemiptera: Pentatomidae) (Moallemzadegan et al., 2011). Chloride salts (except MgCl² and CaCl²) mediate the activation of digestive enzymes such as α -amylases in most insects (Terra & Ferreira, 2012). In this way, the intake of salts such as NaCl and KCl for the activation of glucosidases is useful in sap sucking insects because of their importance in the breakdown of carbohydrates and consequently in growth, development, and physiological maintenance. In blood-sucking stink bugs, chlorides, such as NaCl and KCl, influence feeding activity in a concentration-dependent manner, increasing to an optimal concentration, and are credited to function in maintaining homeostatic ionic balance (Pontes et al., 2017). In fact, variations in ionic K and Na are controlled by physiological mechanisms and are consistently regulated by the hemolymph-midgut interaction during episodes of environmental or developmental changes (MacMillan et al., 2012). The feeding behavior of *D. melacanthus* was influenced by the presence of NaCl, and further studies on the molecular action of these chlorides on different physiological systems of these pests may be a topic of interest for future investigations.

Association of chlorides and lethal concentrations of insecticides

The control efficiency values were obtained based on the mortality observed for the untreated check treatment, which was less than 10% for both *E. heros* and *D. melacanthus* (Table 3). According to Yu (2014), control mortality must be lower than 20% to validate the correction; therefore, the efficiency values obtained in this study are reliable. Based on untreated and nonassociated treatment mortality data for E. heros and D. melacanthus, it was possible to observe an expected trend at the end of the evaluation period (5th day after application [5 DAA]), according to the pre-determined values of LC_{25} and LC_{50} . The association of insecticides with table salt is a topic that has been debated for many years and has good socio-economic relevance considering its low cost (Corso, 1991). Recently, some studies have focused on this association, mainly on the effectiveness of neurotoxic insecticides in E. heros control and on the selectivity towards predator stink bugs, such as Podisus nigrispinus Dallas (Hemiptera: Pentatomidae) (Ramos et al., 2019; Rodrigues et al., 2020). These results are promising and strengthen the idea of studying the association of insecticides with different modes of action with chlorines.

In general, Ctn was less toxic to both stink bugs at the concentrations used and was less effective than the other insecticides used. In a study on E. heros, it was concluded that low doses of Ctn caused a sexual hormesis effect, which may have contributed in part to their peaks of infestation in recent years in soybean crops in Brazil (Tuelher et al., 2017). In this bioassay, it was not possible to verify the positive or negative effects of the association of chlorides with low Ctn on the mortality of E. heros nymphs. However, Ctn LC₂₅ combined with a 5% NaCl solution presented a good efficiency level over D. melacanthus N4s, equaling the mortality of the higher dosage Ctn LC_{50} alone (Table 3). Ctn was found to be efficient for the control of Bagrada hilaris Burmeister (Hemiptera: Pentatomidae), being only less efficient then the neurotoxic neonicotinoid clothianidin Cremonez, P. S. G. et al.

(Josephetal., 2016). In a study on subterranean termites (Isoptera: Rhinotermitidae), Ctn also showed similar results with different forms of application in the soil, with efficient control at concentrations of 25, 50, and 100 μ g g⁻¹ (Barwary et al., 2015).

In E. heros, the efficiency values for all treatments with Spn were significantly higher than those of the control. At the end of the evaluation, Spn LC₂₅ + NaCl 5% (39.9%) and Spn LC_{50} (49.7%) were significantly similar in terms of corrected efficiency. The results obtained in the experiment with D. melacanthus indicated that the activity of Spn LC50 was similar to that of either the associated or pure Spn LC₂₅, and more efficient than the control treatment (Table 3). It is known that Spn may negatively affect the levels of natural enemies (Firake et al., 2017; Hill & Foster, 2000; Liu et al., 2007) and other beneficial insects (Mayes et al., 2003), where we can find some different results (Fernández et al., 2017; Ishaaya et al., 2005; Rimoldi et al., 2012). However, several authors have reported its efficiency in the control of several pests in urban areas (Khan, 2018; Santos et al., 2019; Valcárcel et al., 2015), stored grains (Athanassiou et al., 2008; Vayias et al., 2009), horti-fruticulture (F. Huang & Subramanyam, 2007; Stark et al., 2004), and even of major agricultural pests (Aydin & Gürkan, 2006; Pineda et al., 2007; Wang et al., 2009). There is, however, a certain caution regarding the use of this insecticide because it presents a neurotoxic mode of action (Williams et al., 2003). The validation of a biorational insecticide must go beyond its chemical composition, including its dosage, compatibility, possibility of integration with other control methods, and correct use/ application.

Table 3

Control efficiency (% ± standard error) at five days after application of 4th instar nymphs of Euschistus heros and Diceraeus melacanthus subjected to sublethal (LC25) and median lethal (LC50) concentrations of different insecticides associated or not with NaCl or KCl

Treatment	Check mortality ¹	LC ₂₅	LC ₂₅ + KCI 5%	LC ₂₅ + NaCl 5%	LC ₅₀
Euschistus heros					
Lufenuron	9.3 ± 0.6	25.6 ± 0.5 a	18.7 ± 0.4 a	27.0 ± 0.3 a	46.3 ± 0.2 b
Buprofezin	6.3 ± 0.9	29.3 ± 0.2 a	22.6 ± 0.2 a	18.7 ± 0.6 a	54.6 ± 0.7 b
Pyriproxyfen	8.8 ± 0.3	30.2 ± 0.6 a	32.9 ± 0.5 a	28.7 ± 0.4 a	65.8 ± 1.1 b
Chlorantraniliprole	3.8 ± 0.3	20.4 ± 0.3 a	23.4 ± 0.2 a	24.6 ± 0.5 a	42.8 ± 0.4 b
Spinosad	8.2 ± 0.6	27.3 ± 0.5 a	29.2 ± 0.4 a	39.9 ± 0.3 b	49.7 ± 0.3 b
Diceraeus melacanthu	S				
Lufenuron	5.0 ± 0.4	22.4 ± 0.4 a	27.7 ± 0.3 a	19.8 ± 0.3 a	30.3 ± 0.3 a
Buprofezin	10.0 ± 0.5	23.3 ± 1.5 a	23.7 ± 0.4 a	26.4 ± 0.3 a	44.4 ± 0.3 b
Pyriproxyfen	5.0 ± 0.3	19.8 ± 0.5 a	26.3 ± 0.4 a	24.5 ± 0.4 a	39.5 ± 0.3 b
Chlorantraniliprole	10.0 ± 0.0	13.9 ± 0.4 a	16.7 ± 0.6 a	37.6 ± 0.3 b	41.7 ± 0.5 b
Spinosad	5.0 ± 0.4	21.1 ± 0.4 a	24.5 ± 0.4 a	23.7 ± 0.3 a	30.3 ± 0.3 a

Note: means followed by the same letter in the row do not differ significantly from each other, Tukey test ($p \le 0.05$). ¹Untreated check mortality (dH2O) used to obtain the efficiency percentages using Schneider-Orelli's formula (Püntener, 1981). Data transformed into $\sqrt{(x+1)}$ due to null values, not-transformed percentage data shown. n = 200 each treatment for each species.

Data analysis obtained with the IGRs Lfn, Bpf, and Ppx in E. heros and Bpf and Ppx in D. melacanthus (Table 3) showed that the LC₂₅ concentrations were less efficient than the relative LC50 used, regardless of the presence of 5% NaCl or KCl in the solution. Furthermore, Lfn LC₅₀ was equally effective as Lfn LC₂₅ for *D. melacanthus*, in association or not with the chlorides used. Corso and Gazzoni (1998) showed that stink bugs did not select soybean plants sprayed with NaCl, but they stayed there for longer periods, characterizing an arrestant effect of chloride that potentialized the efficacy of monocrotophos and metamidophos. The notable efficacy of a low lethal concentration (LC₂₅) of lufenuron represents a good alternative for stink bug management, as the product is an insect growth regulator, that is, it is more selective and relatively less hazardous to the environment than neurotoxic insecticides that are currently registered and widely used for stink bug control.

Conclusions _

The use of chlorantraniliprole and spinosad at a lower concentration associated with NaCl presented the same efficiency as a higher concentration for mortality of *E. heros* and *D. melacanthus*, respectively. These results indicate that table salt association with chemical insecticides can be a strategy of IPM as a tool for the management of resistance to commonly used neurotoxic products.

Acknowledgments _____

The authors thank Dr. A. M. Meneghin from the Institute for Rural Development of Parana (IDR, former IAPAR) for the supply of insects and to the staff at the State University of Londrina. The National Council for Scientific and Technological Development (CNPq, Brazil) for MS project funding of PSGC.

References _

- Athanassiou, C. G., Kavallieratos, N. G., Chintzoglou, G. J., Peteinatos, G. G., Boukouvala, M. C., Petrou, S. S., & Panoussakis, E. C. (2008). Effect of temperature and commodity on insecticidal efficacy of spinosad dust against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrychidae). *Journal of Economic Entomology*, 101(3), 976-981. doi: 10.1093/jee/101.3.976
- Aydin, H., & Gürkan, M. O. (2006). The efficacy of spinosad on different strains of Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae). *Turkish Journal of Biology, 30*(1), 5-9. http://journals. tubitak.gov.tr/biology/vol30/iss1/2/
- Barwary, Z., Gorzlancyk, A., & Hu, X. P. (2015). Effects of concentration, distance, and application methods of Altriset (Chlorantraniliprole) on eastern subterranean termite (Isoptera: Rhinotermitidae). *Insect Science*, *22*, 451-460. doi: 10.1111/1744-7917.12122
- Bowling, C. C. (1979). The stylet sheath as an indicator offeeding activity of the rice stink bug. *Journal of Economic Entomology*,

72(2), 259-260. doi: 10.1093/jee/72.2.259

- Corso, I. C. (1990). Uso de sal de cozinha na redução da dose de inseticida para controle de percevejos da soja. (Comunicado Técnico, 45). Embrapa Soja.
- Corso, I. C., & Gazzoni, D. L. (1998). Sodium chloride: an insecticide enhancer for controlling pentatomids on soybeans. *Pesquisa Agropecuaria Brasileira, 33*(10), 1563-1571. doi: 10.1590/S1678-3921. pab1998.v33.5002
- Cremonez, P. S. G., Gouvea, S. P., Pinheiro, D. O., Falleiros, Â. M., Levy, S. M., Meneghin, A. M., Fonseca, I. C. B., & Neves, P. M. O. J. (2019). Chitin biosynthesis inhibitors in *Euschistus heros* Fabr. (Hemiptera: Pentatomidae): morphometric alterations in testes and nuclei of testicular accessory cells of adults. *Journal of Agricultural Science*, *11*(1), 410-417. doi: 10.5539/jas. v11n1p410
- Cremonez, P. S. G., Oliveira Pinheiro, D. de, Falleiros, Â. M. F., & Neves, P. M. O. J. (2017). Performance of reproductive system of *Dichelops melacanthus* (Hemiptera: Pentatomidae) subjected to buprofezin and pyriproxyfen: morphological analysis of ovarioles and testes. *Semina: Ciências Agrárias, 38*(4), 2279-2291. doi: 10.5433/1679-0359.2017v38n4Supl1 p2279
- Fernández, M. M., Medina, P., Wanumen, A., Del Estal, P., Smagghe, G., & Viñuela, E. (2017). Compatibility of sulfoxaflor and other modern pesticides with adults of the predatory mite *Amblyseius swirskii*. Residual contact and persistence studies. *BioControl*, 62(2), 197-208. doi: 10.1007/ s10526-017-9784-1

- Firake, D. M., Thubru, D. P., & Behere, G. T. (2017). Eco-toxicological risk and impact of pesticides on important parasitoids of cabbage butterflies in cruciferous ecosystem. *Chemosphere*, *168*, 372-383. doi: 10.1016/j.chemosphere.2016.10.071
- Hill, T. A., & Foster, R. E. (2000). Effect of insecticides on the diamondback moth (Lepidoptera: Plutellidae) and its parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Journal of Economic Entomology*, *93*(3), 763-768. doi: 10.1603/0022-0493-93.3.763
- Huang, F., & Subramanyam, B. (2007). Effectiveness of spinosad against seven major stored-grain insects on corn. *Insect Science*, *14*, 225-230. doi: 10.1111/j.1744-7917.2007.00148.x
- Huang, H. J., Liu, C. W., Cai, Y. F., Zhang, M. Z., Bao, Y. Y., & Zhang, C. X. (2015). A salivary sheath protein essential for the interaction of the brown planthopper with rice plants. *Insect Biochemistry* and Molecular Biology, 66, 77-87. doi: 10.1016/j.ibmb.2015.10.007
- Insecticide Resistance Action Committee (2021). The IRAC mode of action classification online. https://irac-online. org/modes-of-action/
- Ishaaya, I., Kontsedalov, S., & Horowitz, A. R. (2005). Biorational insecticides: mechanism and cross-resistance. Archives of Insect Biochemistry and Physiology, 58, 192-199. doi: 10.1002/ arch.20042
- Joseph, S. V., Grettenberger, I., & Godfrey, L. (2016). Insecticides applied to soil of transplant plugs for *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae)

management in broccoli. *Crop Protection, 87,* 68-77. doi: 10.1016/j. cropro.2016.04.023

- Khan, H. A. A. (2018). Spinosad resistance affects biological parameters of *Musca domestica* Linnaeus. *Scientific Reports*, *8*, 1-7. doi: 10.1038/s41598-018-32445-8
- Liang, J., Tang, S., & Cheke, R. A. (2016). Beverton–Holt discrete pest management models with pulsed chemical control and evolution of pesticide resistance. *Communications in Nonlinear Science and Numerical Simulation, 36,* 327-341. doi: 10.1016/j.cnsns.2015.12.014
- Liu, S. S., Li, Z. M., Liu, Y. Q., Feng, M. G., & Tang, Z. H. (2007). Promoting selection of resistance to spinosad in the parasitoid *Cotesia plutellae* by integrating resistance of hosts to the insecticide into the selection process. *Biological Control, 41*(2), 246-255. doi: 10.1016/j. biocontrol.2007.01.013
- Lucini, T., & Panizzi, A. R. (2018). Electropenetrography (EPG): a breakthrough tool unveiling stink bug (Pentatomidae) feeding on plants. *Neotropical Entomology*, *47*, 6-18. doi: 10.1007/s13744-017-0574-3
- MacMillan, H. A., Williams, C. M., Staples, J. F., & Sinclair, B. J. (2012). Reestablishmentofion homeostasis during chill-coma recovery in the cricket *Gryllus pennsylvanicus*. *Proceedings of the National Academy of Sciences*, *109*(50), 20750-20755. doi: 10.1073/pnas.1212788109
- Matsumoto, J. F., Cremonez, P. S. G., Roggia,
 S., Falleiros, Â. M. F., Levy, S. M., Neves, P.
 M. O. J., & Pinheiro, D. O. (2021). Sublethal concentration of pyriproxyfen reduces

testicular connective tissue thickness in *Euschistus heros* Fabr. (Hemiptera: Pentatomidae). Journal of Agricultural Science, 13(9), 27-35. doi: 10.5539/jas. v13n9p27

- Mayes, M. A., Thompson, G. D., Husband, B., & Miles, M. M. (2003). Spinosad toxicity to pollinators and associated risk. In G. Ware (Ed.), *Reviews of environmental contamination and toxicology* (vol. 179, pp. 37-71). Dordrecht.
- Moallemzadegan, Z., Kazzazi, M., & Hosseininaveh, V. (2011). Digestive α-amylase activity in *Aelia acuminata* L. (Hemiptera: Pentatomidae). *Archives of Phytopathology and Plant Protection, 44*(16), 1560-1571. doi: 10. 1080/03235408.2010.516080
- Niva, C. C., & Panizzi, A. R. (1996). Efeitos do cloreto de sodio no comportamento de *Nezara viridula* (L.) (Heteroptera: Pentatomidae) em vagem de soja. *Anais da Sociedade Entomologica do Brasil, 25*(2), 251-257. doi: 10.37486/0301-8059.v25i2.1126
- Panizzi, A. R. (2013). History and contemporary perspectives of the integrated pest management of soybean in Brazil. *Neotropical Entomology, 42,* 119-127. doi: 10.1007/s13744-013-0111-y
- Panizzi, A. R., & Lucini, T. (2017). Host plantstink bug (Pentatomidae) relationships. In Čokl, A., Borges, M. Stink bugs - biorational control based on communication processes (pp. 31-58). Boca Raton. doi: 10.1201/9781315120713
- Pineda, S., Schneider, M. I., Smagghe, G., Martínez, A. M., Del Estal, P., Viñuela, E.,

Valle, J., & Budia, F. (2007). Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, *100*(3), 773-780. doi: 10.1093/jee/100.3.773

- Pontes, G., Pereira, M. H., & Barrozo, R. B. (2017). Salt controls feeding decisions in a blood-sucking insect. *Journal of Insect Physiology, 98,* 93-100. doi: 10.1016/j. jinsphys.2016.12.002
- Püntener, W. (1981). *Manual for field trials in plant protection* (2nd ed.). Ciba-Geigy.
- R Core Team. (2021). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria.
- Ramiro, Z. A., Batista, A. F., & Cintra, E. R. R. (2005). Eficiência do inseticida Actara Mix 110+ 220 CE (Thiamethoxam+ Cipermetrina) no controle de percevejos-pragas da soja. *Arquivos do Instituto Biológico*, 72(2), 239-247. doi: 10.1590/1808-1657v72p2372005
- Ramos, G. S., De Paulo, P. D., Toledo, P. F. S., Haddi, K., Zanuncio, J. C., & Oliveira,
 E. E. (2019). Effects of imidaclopridsodium chloride association on survival and reproduction of the stink bug *Podisus nigrispinus. Revista de Ciências Agrícolas*, 36(E), 71-81. doi: 10.22267/ rcia.1936e.108
- Raubenheimer, D., & Gäde, G. (1993). Compensatory water intake by locusts (*Locusta migratoria*): implications for mechanisms regulating drink size. *Journal of Insect Physiology*, 39(4), 275-281. doi: 10.1016/0022-1910(93)90057-X

- Rimoldi, F., Schneider, M. I., & Ronco, A. E.
 (2012). Short and long-term effects of endosulfan, cypermethrin, spinosad, and methoxyfenozide on adults of *Chrysoperla externa* (Neuroptera: Chrysopidae). *Journal of Economic Entomology*, *105*(6), 1982-1987. doi: 10. 1603/ec12189
- Ritz, C., & Streibig, J. C. (2015). Bioassay Analysis using R. *Journal of Statistical Software, 12*(5), 1-22. doi: 10.18637/jss. v012.i05
- Rodrigues, H. S., Haddi, K., Campos, M. O., Ferreira, N. A., F^o., Guedes, R. N. C., Newland, P. L., & Oliveira, E. E. (2020).
 Synergism and unintended effects of the association between imidacloprid and sodium chloride (NaCl) on the management of *Euschistus heros. Pest Management Science*, 77, 417-424. doi: 10.1002/ps.6032
- Sanford, J. L., Shields, V. D., & Dickens, J. C. (2013). Gustatory receptor neuron responds to DEET and other insect repellents in the yellow-fever mosquito, *Aedes aegypti. Naturwissenschaften*, *100*, 269-273. doi: 10.1007/s00114-013-1021-x
- Santos, V. S. V., Silva, C. E., Oliveira, C. M., Morais, C. R. de, Limongi, J. E., & Pereira, B. B. (2019). Evaluation of toxicity and environmental safety in use of spinosad to rationalize control strategies against *Aedes aegypti*. *Chemosphere, 226*, 166-172. doi: 10.10 16/j.chemosphere.2019.03.129
- Sparks, T. C., Crossthwaite, A. J., Nauen,
 R., Banba, S., Cordova, D., Earley, F.,
 Ebbinghaus-Kintscher, U. Fujioka,
 S., Hirao, A., Karmon, D., Kennedy, R.,
 Toshifumi, N., Popham, H. J. R., Salgado,

V., Watson, G. B., Wedel, B. J., & Wessels, F. J. (2020). Insecticides, biologics and nematicides: Updates to IRAC's mode of action classification-a tool for resistance management. *Pesticide Biochemistry and Physiology*, *167*, 104587. doi: 10.1016/j. pestbp.2020.104587

- Sparks, T. C., Storer, N., Porter, A., Slater, R., & Nauen, R. (2021). Insecticide resistance management and industry: the origins and evolution of the Insecticide Resistance Action Committee (IRAC) and the mode of action classification scheme. *Pest Management Science*, 77, 2609-2619. doi: 10.1002/ps.6254
- Stark, J. D., Vargas, R., & Miller, N. (2004). Toxicity of spinosad in protein bait to three economically important tephritid fruit fly species (Diptera: Tephritidae) and their parasitoids (Hymenoptera: Braconidae). *Journal of Economic Entomology*, 97(3), 911-915. doi: 10.1093/jee/97.3.911
- Sun, R., Liu, C., Zhang, H., & Wang, Q. (2015). Benzoylurea chitin synthesis inhibitors. *Journal of Agricultural and Food Chemistry, 63*(31), 6847-6865. doi: 10. 1021/acs.jafc.5b02460
- Terra, W. R., & Ferreira, C. (2012). Biochemistry and molecular biology of digestion. In L. I. Gilbert (Ed.), *Insect molecular biology and biochemistry* (pp. 365-418). London, UK.
- Tuelher, E. S., Silva, E. H. da, Freitas, H. L., Namorato, F. A., Serrão, J. E., Guedes,
 R. N. C., & Oliveira, E. E. (2017).
 Chlorantraniliprole-mediated toxicity and changes in sexual fitness of the Neotropical brown stink bug Euschistus heros. *Journal of Pest Science*, 90, 397-405. doi: 10.1007/s10340-016-0777-0

- United States Department of Agriculture -Foreign Agricultural Service (2021). *Grain: world market and trade.* https://apps.fas. usda.gov/psdonline/circulars/grain.pdf
- Valcárcel, F., Sánchez, J. P., Tercero Jaime, J., Basco-Basco, P. I., Cota Guajardo, S., Cutuli, M. T., González, J., & Olmeda, A. S. (2015). Control of tick infestations in *Oryctolagus cuniculus* (Lagomorpha: Leporidae) with spinosad under laboratory and field conditions. *Journal of Medical Entomology*, 52(2), 207-213. doi: 10.1093/jme/tju018
- Vayias, B. J., Athanassiou, C. G., & Buchelos, C. T. (2009). Effectiveness of spinosad combined with diatomaceous earth against different European strains of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae): influence of commodity and temperature. *Journal of Stored Products Research, 45*(3), 165-176. doi: 10.1016/j.jspr.2008.11.002

- Wang, D., Gong, P., Li, M., Qiu, X., & Wang, K. (2009). Sublethal effects of spinosad on survival, growth and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Pest Management Science*, 65, 223-227. doi: 10.1002/ps.1672
- Williams, T., Valle, J., & Viñuela, E. (2003). Is the naturally derived insecticide Spinosad[®] compatible with insect natural enemies? *Biocontrol Science and Technology*, *13*(5), 459-475. doi: 10.1080/0958315031000 140956
- Yu, S. J. (2014). *The toxicology and biochemistry* of insecticides (2nd ed.). CRC Press.
- Zeilinger, A. R., Olson, D. M., Raygoza, T., & Andow, D. A. (2015). Do counts of salivary sheath flanges predict food consumption in herbivorous stink bugs (Hemiptera: Pentatomidae)?. *Annals of the Entomological Society of America*, 108(2), 109-116. doi: 10.1093/aesa/sau011