Neotropical brown stink bug (*Euschistus heros*) resistance to methamidophos in Paraná, Brazil

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Abstract – The objective of this work was to evaluate the soybean neotropical brown stink bug (*Euschistus heros)* resistance to methamidophos through laboratory bioassays. Bioassays were carried out using populations of adults and nymphs of *Euschistus heros* in the last instar collected in soybean crops in Paraná State. Highest frequencies of genotypes resistant to methamidophos were observed in Alvorada do Sul, Toledo and Nova Santa Rosa, PR. Populations from Bela Vista do Paraíso, Araruna, Campo Mourão, Mariluz, Cascavel, São João do Ivaí, and Mamboré were more susceptible to methamidophos. However, an increase on resistant genotypes in this locations cannot be discarded.

Index terms: *Glycine max*, chemical control, insecticide resistance, susceptibility.

Resistência de populações do percevejo-marrom (*Euschistus heros)* ao metamidofós no Paraná

Resumo – O objetivo deste trabalho foi avaliar a resistência de populações do percevejo-marrom (*Euschistus heros*) da soja ao metamidofós por meio de bioensaios em laboratório. Bioensaios foram realizados com populações de adultos e ninfas de último instar de *Euschistus heros* coletadas em lavouras de soja do Estado do Paraná. As maiores frequências de genótipos resistentes ao metamidofós foram obtidas em Alvorada do Sul, Toledo e Nova Santa Rosa. As populações de Bela Vista do Paraíso, Araruna, Campo Mourão, Mariluz, Cascavel, São João do Ivaí e Mamboré foram mais suscetíveis ao metamidofós. No entanto, o incremento incipiente de genótipos resistentes nesses locais não deve ser desconsiderado.

Termos para indexação: *Glycine max*, controle químico, resistência a inseticidas, suscetibilidade.

The neotropical brown stink bug, *Euschistus heros* (F.) (Heteroptera: Pentatomidae), is the most important pest species occurring in Brazilian soybeans. Its distribution has expanded over the last four to five years in Southern Brazil and has reached Rio Grande do Sul, where it was not as abundant as the other pentatomid species. The red banded stink bug, *Piezodorus guildinii* West., (Heteroptera: Pentatomidae) is the second most abundant species, followed by the Southern green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae) (Panizzi & Corrêa-Ferreira, 1997).

Most of the chemical applications against stink bugs are addressed to *E. heros*. Until 2004, this species was managed exclusively with chemicals using organophosphates (OPs) and endosulfan. The first mixture of neonicotinoids (imidacloprid) and pyrethroids (beta-cyfluthrin) to control stink bugs was recommended in 2004 (Tecnologias de produção de soja, 2004) and, in the following year, a new mixture of thiamethoxan plus lambda-cyalothrin was introduced into the official recommendation (Tecnologias de produção de soja, 2005). The reduction of susceptibility to OPs is possibly due to abusive use of these products for over 35 years.

Since the early 1990s, control failures with endosulfan have been observed on neotropical brown stink bug populations. In 1999, Sosa-Gómez et al. (2001) observed a slight reduction of susceptibility to OPs in populations at Pedrinhas Paulista, São Paulo, by using vial bioassays. More recently, control failures with OPs, such as methamidophos, have become frequent in Paraná State, Brazil, leading growers to increase doses by 2 to 2.4 times the recommended doses. According to Roush & Miller (1986), the diagnostic-dose bioassay could be more efficient for discriminating susceptibility from resistant populations compared to the dose response bioassay.

The objective of this work was to evaluate the soybean neotropical brown stink bug (*Euschistus heros)* resistance to methamidophos through laboratory bioassays.

Stink bugs were hand collected in soybean fields and transported to the laboratory on the same sampling date (Table 1). Geographic coordinates were recorded by a GPS with the Datum WGS84 (Garmin Etrex, Chicago, IL, USA) system. The pesticides used in the screening-dose bioassay were commercial formulations of methamidophos, Stron soluble concentrate, 600 g of active ingredient [AI] per liter, (Nufarm Indústria Química e Farmacêutica S.A., Maracanaú, Brazil).

To survey resistance status of *E. heros* populations, dose-mortality data obtained 48 hours after treatment were used. Insects from each population were topically treated with the screening dose of 1.2 micrograms of AI per adult according to the predicted LC99 values of Stron for the Bela Vista do Paraíso, PR population (Sosa-Gómez et al., 2009).

Distilled water was used as a diluent. Each treatment was replicated five times in a randomized experimental design. Adult stink bugs received two microliters applied to the pronotum by a microaplicator WD31PJ, (Burkard Manufacturing Co. Ltd. Rickmansworth, Hertfordshire, England).

In all the bioassays, a laboratory strain originally collected in Londrina, Paraná, in 2007 (23°11'45" S; 51°11'59" W) was used as the control. After treatment, insects were maintained in sterile glass vials with green beans (*Phaseolus vulgaris* L.) as food and with a wet cotton ball to provide water. Stink bugs were maintained in environmental chambers at a temperature of 25±1.5ºC, 60±10% RH and with a photoperiod of 14:10 hour (L:D).

Mortality was evaluated 48 hours after treatment and adjusted for control mortality using the formula of Henderson & Tilton (1955). Insects were considered dead if they did not show any movement after prodding. Mortality data were analyzed by ANOVA and means were discriminated by Tukey's test for multiple comparisons at 5% of probability. SAS 8.2 was used for the analysis (Freund & Littell, 1981; Zar, 1984).

Substantial differences were detected in neotropical brown stink bug susceptibility to methamidophos. Comparisons of mortality values revealed that the populations from Alvorada do Sul, Toledo and Nova Santa Rosa were less susceptible to methamidophos than the laboratory strain (Table 1). Although populations from Bela Vista do Paraíso, Araruna, Campo Mourão, Mariluz, Cascavel, São João do Ivaí and Mamboré, showed high mortalities (≥80%), the frequency of resistant genotypes appears to be increasing, because mortalities were slightly lower than in the laboratory strain.

Methamidophos has been used year after year against velvetbean caterpillars, soybean loopers, defoliator beetles and stink bugs, in a wide range of soybean regions, possibly for more than 35 years. In the year 2000, Sosa-Gómez et al. (2001) detected diminished susceptibility to methamidophos in neotropical brown stink bugs collected in Pedrinhas Paulista, São Paulo.

Today, insecticides registered against stink bugs are OPs, one cyclodiene and mixtures of neonicotinoids plus pyrethroids (Brasil, 2010). As resistance against

Table 1. Mean mortality (±SE) of geographic populations of *Euschistus heros* applied with methamidophos at a rate of 1.2 micrograms of AI per adult, after Sosa-Gómez et al. (2009)[(1)].

Municipality	Geographical Coordinates	Sampling date	Number of individuals	Mortality (%)
Alvorada do Sul, PR	22º54´13.0"S 51º14´44.9"W	Dec. 15, 2009	250	36.4±6.8a
Toledo, PR	24º43´44.0"S 53º45´56.0"W	Feb. 26, 2009	60	42.3±5.1a
Nova Santa Rosa, PR	24º32´20.0"S 53º56´24.0"W	Feb. 26, 2009	204	52.0±4.8a
Bela Vista do Paraíso, PR	23º07´03.0"S 51º11´45.5"W	Dec.7, 2009	250	76.9±3.4b
Araruna, PR	23º59´32.5"S 52º32´9.00"W	Feb. 16, 2009	120	80.0±1.9b
Campo Mourão, PR	NA[(2)]	Feb. 26, 2009	160	81.9±2.4b
Mariluz, PR	24º01´25.2"S 53º16´26.0"W	Feb. 16, 2009	220	85.5±0.9b
Cascavel, PR	24º55´20.0"S 53º29´34.0"W	Feb. 26, 2009	160	85.6±1.9b
São João do Ivaí, PR	23º57´37.0"S 51º44´71.0"W	Feb. 26, 2009	180	86.8±2.8b
Mamboré, PR	NA	Feb. 16, 2009	60	87.0±3.3b
Laboratory strain, PR	23°11´45.0"S 51°11´59.0"W	2007	160	96.0±0.2b

[(1)]Means followed by the same letter are not significantly different by Tukey test at 5% of probability. [(2)]NA, not available.

OPs and endosulfan is becoming generalized, no alternative products other than the mixture of pyrethroids plus neonicotinoids are available.

Control failures lead soybean growers to use the same insecticide repeatedly during the same season in increasing dosages. In addition, high population densities of stink bugs occurring early in the soybean season encourage growers to anticipate application by spraying before the reproductive period.

Growers frequently prefer broad spectrum insecticides instead of selective products to control soybean caterpillars, as they can use the same product for multiple purposes: control caterpillars, stink bugs and other occasional insects. Other non-recommended strategies in Integrated Pest Management programs, such as the chemical control of diapausing populations during winter or applications to control early colonizing populations during the vegetative stages, have been used (Corrêa-Ferreira et al., 2010). These strategies have intensified the problem due to the suppression of natural enemies and have caused selection pressure on stink bug populations.

Other practices favoring non-appropriate control measures include: a) early purchase of agrochemical bundles containing insecticides subject to special discounts before the growing season so that product availability encourages more applications; b) reducing the total number of applications by using mixtures (insecticides with fungicides or herbicides) to lower operational costs and to avoid wheel damage to the spraying equipment; c) large areas and rainy weather induce growers to use a routine spraying program without considering threshold levels; d) usually, the application decision is based on the presence of stink bugs without the knowledge of the real population density. Basking behavior gives the false impression that the stink bug population is high and applications are anticipated.

Mortality in 2006-2007 ranged from 92.3% to 100% (Sosa-Gómez et al., 2009). These percentages were higher than those observed in this report, suggesting a progressive increase in resistance to methamidophos and possibly to other OPs, over the last few years.

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