DOI: 10.1111/afe.12552

ORIGINAL ARTICLE

cultural and Forest

Sex-dependent body mass and respiratory responses on Euschistus heros individuals resistant to imidacloprid

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Funding information

Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Numbers: 308576/2018-7, 427304/2018-0; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: Finnance Code 001; Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Grant/Award Number: APQ 03771-18

Abstract

- 1. In insect-resistant populations, adaptive fitness is intimately related to the physiological responses of males and females to different stressors.
- 2. By using laboratory-selected imidacloprid-resistant individuals of the Neotropical brown stink bug Euschistus heros, one of the most prevalent pests in soybean fields in the Neotropical region, we evaluated whether resistance to imidacloprid (synergized by piperonyl butoxide–PBO) would differentially affect the longevity, body mass and respiratory responses of E. heros males and females.
- 3. We used an imidacloprid-susceptible E. heros strain (ImiSusc), whose individuals were reared under controlled and imidacloprid-free conditions for over a decade, to select the PBO-synergized imidacloprid-resistant strain (ImiPBOSel).
- 4. After eight generations using PBO-synergized imidacloprid, our results revealed that resistant individuals exposed only to dry residues of imidacloprid (tarsal contact) could survive at concentrations as high as 168 μ g a.i./cm², which is equivalent to 40-fold the label recommendation for field applications. Furthermore, ImiPBOSel males and females exhibited higher longevity, higher body mass and elevated respiratory activity than ImiSusc individuals.
- 5. Altogether, the findings described here showed that imidacloprid resistance can select E. heros individuals with better metabolic capacities, which can complicate the management of this pest under field conditions.

KEYWORDS

neonicotinoids, Neotropical brown stink bugs, piperonyl butoxide synergism, soybean insect pests

INTRODUCTION

Neonicotinoid insecticide usage is the most common method for controlling the phytosuccivorous stink bug Euschistus heros (F.) (Hemiptera: Pentatomidae), which is one of the most prevalent pests of soybean fields in the Neotropical region (Panizzi & Lucini, 2016; Sosa-Gómez et al., 2020; Tuelher et al., 2016). The adoption of this control strategy has caused neonicotinoid substances to be integrated

(alone or in combination with other molecules) into virtually all insecticide formulations registered for controlling stink bugs in the Neotropical region (MAPA, 2022), which threatens the efficacy of the strategy. Reductions on the strategy efficacy might derive from the indiscriminate use and overuse of neonicotinoids, as it has favoured the selection of resistant individuals (Castellanos et al., 2019; Somavilla, da Gubiani, et al., 2020; Somavilla, Reis, et al., 2020; Sosa-Gómez et al., 2020; Steinhaus et al., 2022; Tibola et al., 2021; Tuelher

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et al., 2018), and led to hormetic (i.e., stimulatory) effects on the behavioural and physiological responses of sublethally exposed insects (Haddi et al., 2016; Rodrigues et al., 2021; Santos, Campos, et al., 2016; Tuelher et al., 2017). The selection of resistant individuals and the undesirable hormetic effects, that is, when mild stress protects and improves animal performance (Berry & López-Martínez, 2020; Cutler et al., 2022; Guedes et al., 2016), may have contributed to *E. heros* population outbreaks in Neotropical regions.

Among neonicotinoids, imidacloprid is one of the largest marketable insecticides worldwide (Casida, 2018; Jeschke et al., 2011, 2013) and one of the most commonly used for the control of *E. heros*. However, an increasing number of reports of imidacloprid resistance have been demonstrated in Brazilian populations of *E. heros* (Somavilla, da Gubiani, et al., 2020; Somavilla, Reis, et al., 2020; Steinhaus et al., 2022; Tibola et al., 2021; Tuelher et al., 2018). Imidacloprid exerts its toxicity primarily by causing irreversible disruption of the functions of nicotinic acetylcholine receptor (nAChR) subtypes expressed in the insect nervous system (Jeschke & Nauen, 2008; Oliveira et al., 2010, 2011; Salgado, 2016; Salgado & Saar, 2004), although its actions at secondary targets also play pivotal roles in its efficacy (Castellanos et al., 2021; Perez Campos et al., 2021; Stamm et al., 2011; Yuan et al., 2021).

In addition to its lethal action, imidacloprid interactions with primary or secondary targets can lead to stimulatory (hormetic) or detrimental sublethal consequences that may modify the adaptive responses of these organisms (Cutler et al., 2022; Desneux et al., 2007; Guedes et al., 2016), which can accelerate or delay the selection of resistant individuals and influence the management of insect pests. Other factors, such as sex-related differential responses (Haddi et al., 2016; Santos et al., 2018; Santos, Santos, et al., 2016), and insecticide resistance mechanisms such as increased metabolism (Castellanos et al., 2019) appear to influence the evolution of imidacloprid resistance in *E. heros*. There are some recent investigations (Soares et al., 2018; Zucchi et al., 2019) suggesting the occurrence of two *E. heros* lineages with strong genetic diversity, but the potential contribution of these lineages in terms of insecticide susceptibility have not been addressed yet.

Early detection of individuals with high imidacloprid resistance can increase the success rates of integrated management plans for E. heros populations, as it would allow the adoption of practices that would postpone the selection of resistant populations. Recent investigations (Castellanos et al., 2019) have shown the involvement of cytochrome P450 monooxygenases in laboratory-selected imidacloprid-resistant E. heros strains, and we evaluated whether synergism with piperonyl butoxide (PBO, a metabolic modulator used to inhibit the insect monooxygenase system) would accelerate the selection of E. heros resistant to imidacloprid. We further assessed the ability of resistant individuals selected through tarsal contact to cope with imidacloprid-treated diets, as these molecules can also be applied by using the plant systemic transmission. Finally, we investigated the potential consequences of high imidacloprid resistance on longevity and adaptive responses (body mass and respiratory responses) of E. heros males and females.

MATERIALS AND METHODS

Insect strains and selection for resistance to synergized imidacloprid

We used one *E. heros* imidacloprid-susceptible strain (*ImiSusc*), whose individuals have been reared in an insecticide-free environment for more than 10 years. The initial *ImiSusc* colony consisted of approximately 2000 eggs of a laboratory colony reared under controlled conditions at the Semiochemical Laboratory at EMBRAPA Genetic Resources and Biotechnology (Brasília, DF, Brazil). The imidaclopridresistant strain (*ImiPBOSel*) was selected from *ImiSusc* for 26 consecutive generations using a concentration range from 1.26 µg a.i./cm² (at the beginning of selection procedure) to 168 µg a.i./cm² (at the last two consecutive generations prior to the use of PBO), which is equivalent of 0.30- to 40-fold the field label rate. The concentration of 168 µg a.i./cm² was the highest concentration that allowed an equal cover of all the internal glass vials.

Selection followed the procedure described by Castellanos et al. (2019). Briefly, adult insects (less than 2 days old) were exposed to a solution containing a commercial formulation of imidacloprid (waterdispersible granules at 700 g/L of active ingredient (a. i.); Bayer Crop Science, São Paulo, SP, Brazil). We used distilled water to dilute the formulated product up to the desired concentrations. Such concentrations were prepared every time that we conducted exposure experiments. Two millilitre of the desired imidacloprid-containing solutions was used to coat the inner walls of 250 ml transparent glass vials (EME Equipment, Paulicéia, SP, Brazil). The vials were kept under rotation, by means of a heavy-duty rotator (Roto-Torque model 7637, ColeParmer, Vernon Hills, IL, USA) to allow uniform distribution and drying of the insecticide solution as previously suggested elsewhere (Castellanos et al., 2019; Perez Campos et al., 2021; Rodrigues et al., 2021; Snodgrass et al., 2005; Willrich et al., 2003). The drying period was of approximately 3 h, at controlled conditions (temperature $25 \pm 2^{\circ}$ C; humidity 70% \pm 5%), and the 2 ml solution was sufficient to assure that all internal vial walls were covered at least five times. After drying, 10 unsexed adults (less than 2 days old) were transferred to each vial, and the mortality was assessed after 48 h. Surviving insects were transferred to insecticide-free plastic boxes to reproduce and generate new individuals for the next selection. The same method was repeated for all generations with increasing insecticide concentrations based on the mortality rate of the parental generation.

From the 19th generation of the *ImiPBOSel* strain, designated hereafter as the FO generation of each strain, the selection for resistance was conducted by exposing the insects to piperonyl butoxide (PBO) to increase the probability of select resistant individuals with different resistance mechanisms, as this compound can inhibit enzymes (e.g., insect monooxygenase system—especially the cytochrome P450 monooxygenases) that break down imidacloprid molecules. The synergism experiments followed procedures previously described (Haddi et al., 2015; Ribeiro et al., 2003) with slight modifications. Briefly, we applied 1 ml of the PBO (1 mg/ml)-containing

solution to uniformly coat the inner surface of the transparent glass vials and allowed to air dry (1 h). Once dry, 10 unsexed adults (less than 2 days old) were transferred to each vial and left in contact with the PBO (2 h) before exposure to dry imidacloprid residues, as described above. These PBO concentration and exposure period did not cause mortality in preliminary assays with E. heros, as demonstrated to other insect types (Haddi et al., 2015; Ribeiro et al., 2003). The imidacloprid concentrations and number of newly emerged (less than 48 h old) adults used once we started the previous exposure to PBO were as follows: $F_1 = 42 \ \mu g \ a.i./cm^2$ (100 insects) and 63 $\mu g \ a.$ i./cm² (200 insects); $F_2 = 63 \,\mu g$ a.i./cm² (200 insects); $F_3 = 63 \,\mu g$ a. i./cm² (460 insects); $F_4 = 84 \ \mu g$ a.i./cm² (470 insects); $F_5 = 126 \ \mu g$ a. i./cm² (1260 insects); $F_6 = 168 \ \mu g \ a.i./cm^2$ (760 insects); $F_7 = 168 \ \mu g$ a.i./cm² (760 insects) and $F_8 = 168 \,\mu g$ a.i./cm² (580 insects). The selection to imidacloprid with E. heros adults exposed previously to PBO started from F_1 .

Both strains were mass-reared under controlled conditions $(25 \pm 1^{\circ}C, 60\% \pm 20\%$ relative humidity, and 14 h photophase) in plastic boxes $(19 \times 22 \times 10 \text{ cm})$ lined with paper. A mixture of fresh green bean pods (*Phaseolus vulgaris*), soybean seeds (*Glycine max*), raw shelled peanuts (*Arachis hypogaea*) and sunflower seeds (*Helianthus annuus*) was offered as feed ad libitum (Borges et al., 2008; Silva et al., 2008), and renewed at 72 h intervals, when cages were cleaned, and when the laid eggs were collected.

Survival bioassays

Recently emerged adult insects (less than 2 days old) were starved for 24 h before exposure to imidacloprid at 4.2 μ g a.i./cm² (equivalent to the field label rate) or 12.6 μ g a.i./cm² (equivalent to threefold the field label rate). Contact exposure to imidacloprid involved tarsal interactions with dried residues on the inner sides of glass vials and food previously treated with imidacloprid solutions, as previously described by Castellanos et al. (2021). Briefly, we immersed 5 cm long pieces of green bean pods in imidacloprid-containing solutions for 3 s and left them to air dry. Each glass vial received a single bean pod and 10 newly emerged (less than 2 days old) unsexed adults to constitute the experimental unit. Each treatment (4.2 and 12.6 μ g a.i./cm²) contained 10 replicates, and the control green bean pods were immersed in distilled water only.

The mortality of exposed insects was initially evaluated at 1 h intervals over the first 12 h and then at 12 h intervals. After each 72 h period, the treated green pods were replenished and the glass vials were replaced with new ones previously coated with insecticide. The survival experiment was terminated when the insects in each treatment reached 80% mortality.

Respiration rate and body mass bioassays

Respirometry bioassays were conducted using a TR3 CO₂ Analyser (Figure S1, Sable Systems International, Las Vegas, NV, USA) following

ImiPBOSel

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FIGURE 1 Response to piperonyl butoxide (PBO)-synergized imidacloprid selection in a strain (*ImiPBOSel*) of *Euschistus heros*. Data are presented as means \pm standard error (SE). F₀ corresponds to the 19th imidacloprid-resistant generation selection occurring in the absence of PBO. The horizontal line indicates when the PBO-synergized imidacloprid selection begun (F₁ generation) and the bars with different colours indicated variations on the imidacloprid concentration.

previously described methods (Haddi et al., 2015; Oliveira et al., 2005). The average respiratory rate was individually recorded for newly emerged (less than 2 days old) female and male adults from each strain (*ImiSusc* and *ImiPBOSel*). Briefly, the insects were placed individually in 25 ml chambers that were connected to a closed system for 90 min before CO_2 -free air was injected into the flasks for 2 min at a flow rate of 600 ml/min. The air current directed the CO_2 produced by insect respiration to an infrared reader connected to the system, which allowed the quantification of CO_2 (µmol) production per hour. We measured the respiratory activity of a minimum of newly emerged (less than 2 days old) 25 males and 25 females of each strain. A flask without insects was used as a control. Three replicates were used for each strain and sex.

We also measured the body mass of the newly emerged (less than 2 days old) adult insects after the respiratory analysis using an analytical balance (Sartorius BP 210 D, Göttingen, Germany).

Statistical analyses

Survival results of the bioassay were analysed using the Kaplan–Meier estimator (log-rank method) in Sigma Plot 12.5 (Systat Software, San Jose, CA, USA). The insects that remained alive at the end of the bioassay were censored. Differences among median survival times (LT_{50} values) were tested using the log-rank test, followed by pairwise comparisons using the Holm-Sidak test (p < 0.05). Using the GLM procedures available in SigmaPlot 12.5, the respiratory and body mass results were subjected to a two-way (sex and strain) analysis of

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Imidacloprid-resistant Euschistus heros individuals better survived the combined tarsal and ingestion imidacloprid exposures



FIGURE 2 Estimated probabilities of *Euschistus heros* individuals of imidacloprid-susceptible (*ImiSusc*) or PBO-synergized imidaclopridresistant (*ImiPBOSel*) strains to survive when continuously exposed (by tarsal contact and contaminated diets) to imidacloprid at two different concentrations (4.2 and 12.6 g a.i./cm²). The symbols at the end of each curve indicate the censored data. LT_{50} = estimated time to kill 50% of the exposed strains. Survival curves labelled with different lower-case letters denote significantly different from each other at p < 0.001(log-rank test).

variance (two-way analysis of variance [ANOVA]). When applicable, comparisons between sexes within each strain type were conducted by applying a Student's *t*-test or Mann–Whitney *U*-test (i.e., if the normality and homogeneity of variance assumptions were not met) using SigmaPlot 12.5.

RESULTS

Selection for PBO-synergized imidacloprid resistance

Our results revealed that previous exposure to PBO contributed to increased speed in the selection of imidacloprid-resistant individuals (Figure 1). The exposure of PBO-synergized imidacloprid for eight consecutive generations (F_1 - F_8 generations) required increased imidacloprid concentrations of 42–168 µg a.i./cm² over the generations to cause mortality levels below 50% in the *ImiPBOSel* F_8 generation (Figure 1).

Survival bioassays

The *ImiPBOSel* adults exhibited higher abilities to survive continuous exposure (via contact and ingestion pathways) to imidacloprid at both concentrations (4.2 and 12.6 μ g a.i./cm²) than those of imidacloprid-susceptible individuals (Figure 2). While 100% of mortality was achieved within 192 h (4.2 μ g a.i./cm²) and 48 h (12.6 μ g a.i./cm²) for *ImiSusc* individuals that faced imidacloprid exposures, a maximum of 80% of mortality was achieved for *ImiPBOSel* individuals at the highest (12.6 μ g a.i./cm²) imidacloprid concentration. Furthermore, the estimated median survival time (LT₅₀) after exposure to a

concentration of 4.2 µg a.i./cm² of imidacloprid was significantly ($\chi^2 = 545.3$; df = 2; p < 0.001) less for *ImiSusc* (LT₅₀ = 6.0 [5.5-6.5] h) than for the imidacloprid-resistant strain (LT₅₀ = 264.0 [240.2-287.8] h) (Figure 2). Similarly, the LT₅₀ value (4.0 [3.9-4.1 h]) estimated for *ImiSusc* individuals at an imidacloprid concentration of 12.6 µg i.a./cm² was significantly ($\chi^2 = 749.2$; df = 2; p < 0.001) lower than that (LT₅₀ = 252.0 [206.7-297.3] h) estimated for *ImiPBOSel* insects.

Respiration rates and body masses

Our results showed that the selection of PBO-synergized imidacloprid modified the respiratory rate of the *ImiPBOSel* individuals in comparison to those of *ImiSusc* strain (Table 1). While *ImiPBOSel* females exhibited a respiratory rate of $38.6 \pm 0.97 \,\mu$ l of CO₂ insect⁻¹ h⁻¹, the *ImiSusc* females exhibited a significantly (t = -4.787; p < 0.0001) lower value ($32.5 \pm 0.89 \,\mu$ l of CO₂ insect⁻¹ h⁻¹) (Figure 3a). Males of the *ImiPBOSel* strain also exhibited a significantly higher (t = -1.276; p = 0.0204) respiratory activity ($36.3 \pm 1.05 \,\mu$ l of insect CO₂⁻¹ h⁻¹) in comparison to *ImiSusc* males ($29.3 \pm 0.95 \,\mu$ l of insect CO₂⁻¹ h⁻¹) (Figure 3a). Imidacloprid resistance selection differentially affected the respiratory abilities (t = 2.355; p = 0.0227) of the male and female *ImiPBOSel* strains (Figure 3a). No significant differences (t = 1.634; p = 0.11) were recorded between males and females of the *ImiSusc* strain (Figure 3a).

Our results revealed significant differences in body mass measurements between sex and *E. heros* strains (Table 1, Figure 3b). *ImiSusc* females (7.1 ± 4.0 mg) were significantly (U = 280.0; p = 0.010) heavier than *ImiSusc* males (5.8 ± 0.8 mg) (Figure 3b). However, no significant differences (U = 386.5; p = 0.71) were recorded for the body masses of males (7.3 ± 1.8 mg) and females

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TABLE 1	Statistical values for the Euschistus heros respiratory activity and body mass analysis as a function of sex (male and female) and					
strain (ImiSusc and ImiPBOSel) types (Figure 3).						

	Respiratory activity			Body masses			
Source of variation	df	F	p-value	df	F	p-value	
Strain	1	10.2	<0.001*	1	33.4	<0.001*	
Sex	1	1.6	0.21	1	6.6	0.012*	
$Strain \times Sex$	1	1.2	0.30	1	0.1	0.71	
Residue	116	-	-	116	-	-	

*denotes statistical significance at a P value < 0.05.



FIGURE 3 Respiratory activities (A) and body masses (B) of *Euschistus heros* males and female of strains that are either susceptible (*lmiSusc*) or resistant (*lmiPBOSel*) to PBO-synergized imidacloprid. (A–B) Horizontal histograms represent the mean observed data \pm standard error (SE) and those grouped by the same vertical bar indicate no significant (two-way ANOVA with Scheffe's post-hoc analysis, p > 0.05) differences in relation to the strain types. The asterisks denote differences (p < 0.05) between sex within each *E. heros* strain type.

(7.6 ± 2.1 mg) of the *ImiPBOSel* strain (Figure 3b). The PBO-synergized imidacloprid selection resulted in higher body masses for males (U = 91.5; p < 0.001) and females (U = 221.5; p < 0.001) of *ImiPBOSel* strain when compared to their *ImiSusc* counterparts (Figure 3b).

DISCUSSION

Here, we demonstrated that previous exposure to PBO potentiated the selection of imidacloprid-resistant *E. heros* individuals. Our findings revealed that exposure to imidacloprid-treated diets enhanced imidacloprid potency against resistant *E. heros* individuals selected by the tarsal contact exposure pathway. Furthermore, we demonstrated that *E. heros* males benefited more from imidacloprid-resistance selection, as resistant (*ImiPBOSel*) males significantly increased their respiratory activities, similar to *ImiPBOSel* females, and showed high body mass gain rates in comparison with those recorded for *ImiPBOSel* females. The rate exhibited by *ImiPBOSel* males counteracted the body mass differences in imidacloprid susceptible (*ImiSusc*) males and females.

Neonicotinoid resistance can be mediated by changes in the target site, especially in nAChR subtypes (Oliveira et al., 2011; Salgado, 2016; Salgado & Saar, 2004), and the activities of enzymes involved in metabolic detoxification (Bass et al., 2015; Matsuda et al., 2020). In a previous study with two E. heros strains (including ImiLabSel, from which our ImiPBOSel strain originated), imidacloprid resistance was mainly dependent on increased activities of cytochrome P450 monooxygenases (Castellanos et al., 2019). Our present efforts highlight the involvement of other resistance mechanisms in the ImiPBOSel strain, as the application of PBO (an inhibitor of the monooxygenase system) speeds up the selection of imidacloprid resistant individuals, leading ImiPBOSel individuals to resist exposure to imidacloprid concentrations as high as 40-fold the field rate recommendation. The existence of multiple resistance mechanisms may contribute to the increasing number of reports indicating that imidacloprid-containing commercial formulations do not adequately control E. heros populations (Somavilla, Reis, et al., 2020, Somavilla, da Gubiani, et al., 2020; Sosa-Gómez et al., 2020; Steinhaus et al., 2022; Tibola et al., 2021; Tuelher et al., 2018).

Although further investigations are still required before drawing firm conclusions, it is worth noting that recent population genomic analyses have proposed the involvement of other physiological pathways (e.g., odorant and GABA receptors) as a potential driving force for the natural selection of two *E. heros* lineages in Brazilian soybean fields (Soares et al., 2018; Zucchi et al., 2019). However, how these mechanisms contribute to the selection of imidacloprid-resistant *E. heros* individuals remains undetermined, as a detailed toxicological investigation addressing the imidacloprid or other insecticide toxicity against these two lineages have not been provided yet. The potential contribution of odorant receptors in imidacloprid potency has been demonstrated in other insect species (Gao et al., 2020; Li et al., 2021; Liu et al., 2020), but this does not appear to be the case for GABA receptors, as molecules (e.g., ethiprole) targeting GABA receptors Royal Entomolo Society

were only recently allowed to be used in the management of Brazilian *E. heros* populations (MAPA, 2022). Thus, such contributions of GABA receptors, if any, to imidacloprid resistance in *E. heros* may be indirect and potentially derived from adaptive advantages mediated by the selection of less costly alleles or gene modifiers (ffrench-Constant and Bass, 2017; Kliot & Ghanim, 2012; Pang et al., 2021).

Our results showed that tarsal contact exposure selects imidacloprid resistant E. heros individuals, but imidacloprid potency was significantly increased when the insects were continuously exposed to insecticide-contaminated diets. This may indicate that ingestion can facilitate the imidacloprid molecules to reach their primary targets when compared to tarsal contact exposure, especially since previous contact exposure to PBO would inhibit the detoxifying system of the insect. However, this may reinforce the relevance of imidacloprid actions to secondary targets involved in digestion (Castellanos et al., 2021; Catae et al., 2018; Martínez et al., 2019; Perez Campos et al., 2021). ImiPBOSel individuals survived significantly longer than their susceptible counterparts (ImiSusc) in feed-treated bioassays, which provides further evidence to verify that tarsal contact selection is sufficient to select imidacloprid resistant individuals, as previously described (Castellanos et al., 2019; Snodgrass et al., 2005; Sosa-Gómez et al., 2009; Takeuchi & Endo, 2012).

Several life-history traits (e.g., individual size, behavioural patterns, energy/resources allocations) can be sex biased in insects. For instance, male insects are usually smaller and play different adaptive roles than females (Field et al., 2015; Stillwell et al., 2010). Thus, it is reasonable to expect that sex-dependent physiological responses would shape the adaptive success of insects facing insecticidal challenges (Andreazza et al., 2020; Cremonez et al., 2019; Feng et al., 2019; Fujii et al., 2020; Haddi et al., 2016, 2017). Here, although the continuous selection for PBO-synergized imidacloprid resistance led to providing both E. heros males and females with increased respiratory activities, previous investigations have shown that only E. heros males exhibited reduced respiratory activities when sublethally exposed to imidacloprid (Haddi et al., 2016). Sex-dependent differences in respiratory responses to imidacloprid challenges, as described here and in comparison, to those described in Haddi et al. (2016), may reflect challenge intensity rather than sex itself. Considering that increased respiration rates are generally associated with oxidative stress (Murphy, 2009), it is reasonable to expect that more intense (i.e., multiple generational selections for resistance) would differentially modify the physiological pathways used to ameliorate less intense challenges (e.g., a single exposure to an insecticide concentration estimated to kill only 1% of the tested organisms, LC₁). Recent investigations of imidacloprid-resistant E. heros individuals (Castellanos et al., 2019; Perez Campos et al., 2021) demonstrated alterations in the activities of metabolizing enzymes, which can serve as an indirect proxy for the increased respiratory activities described here.

Sex-dependent differences can also occur in relation to the method of accumulation or mobilization of energy reserves when challenged with insecticidal exposure (Cremonez et al., 2019; Oliveira et al. 2007). The findings described here reinforce this hypothesis, as

the selection for imidacloprid resistance revealed heavier *E. heros* males than females. Although the basis for such differences may require further investigation, it has been shown that *E. heros* males and females differ in the amount of macro-elements in the hemo-lymph when challenged by insecticides (Cremonez et al., 2019), and other hemipteran pests show large differences in the storage and mobilization of fundamental biomolecules (Scheys et al., 2019, 2020). Such sex-related parameters can be differentially affected by the intensity and type of insecticidal challenge, which is unbalanced by the physiological trade-offs (e.g., energetic investments for reproduction or reserve accumulation) that lead to differential weight gains in males and females, but this needs further attention.

In summary, our findings reveal a relevant and complex scenario for the management of E. heros as the inevitable selection of individuals resistant to imidacloprid (and other neonicotinoids) may favour the selection of individuals with better capacities for accumulating energy reserves (higher body masses) and improved respiratory activities. An expanding scientific literature (Agathokleous et al., 2022; Berry & López-Martínez, 2020; Cutler et al., 2022; Hernández et al., 2020) urge the adoption of sublethal thresholds into the insecticide regulatory risk assessments, as these approaches being part of a more realistic risk simulations, including for the agroecosystems where combination of practices need to be integrated in order to postpone undesirable effects of such sublethal exposures. As suggested by this recent literature, the selection of resistant individuals with increased adaptive performance (e.g., survival abilities, respiratory capacities) can complicate the integrated management of E. heros, as such increased adaptive performance can be readily used to metabolize other insecticide molecules. Despite further investigations still required, the increasing number of reports for resistance to neonicotinoids and for molecules (i.e., ethiprole) that have been used only in the recent years for controlling E. heros may reflect an increasing proportion of individuals with adaptive performance able to mitigate insecticides-mediated stresses.

AUTHOR CONTRIBUTIONS

Kamilla E. X. Azevedo: Investigation, formal analysis, writing—original draft. Giovana R. Cunha: Investigation, formal analysis. Javier G. Mantilla-Afanador: Investigation, formal analysis, software, funding acquisition, writing-reviewing and editing. Lorenzo B. Ferraza: Investigation, formal analysis. Nathaly L. Castellanos: Investigation, writing-reviewing and editing. Khalid Haddi: Investigation, conceptualization, writing-reviewing and editing. Graziela D. A. Lima: Investigation, conceptualization, investigation, funding acquisition, software, supervision, writing-reviewing and editing. Conceptualization, software, supervision, writing-reviewing and editing.

ACKNOWLEDGEMENTS

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001); the Brazilian National Council of Scientific and Technological Development (CNPq); and the Minas Gerais State Foundation for Research Aid (FAPEMIG).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Components of the TR3 CO_2 analyser system. (A) Glass respiratory chambers. (B) CO_2 analyser system. (C) Multiplexed respiratory system. (D) Unit for controlling the mass flow

How to cite this article: Azevedo, K.E.X., Cunha, G.R., Mantilla-Afanador, J.G., Ferrazza, L.B., Castellanos, N.L., Haddi, K. et al. (2023) Sex-dependent body mass and respiratory responses on *Euschistus heros* individuals resistant to imidacloprid. *Agricultural and Forest Entomology*, 25(2), 303–311. Available from: https://doi.org/10.1111/afe.12552