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Research

Ecotoxicology

Susceptibility of Boll Weevil (Coleoptera: Curculionidae) to Ethiprole, Differential Toxicity Against Selected Natural Enemies, and Diagnostic Concentrations for Resistance Monitoring

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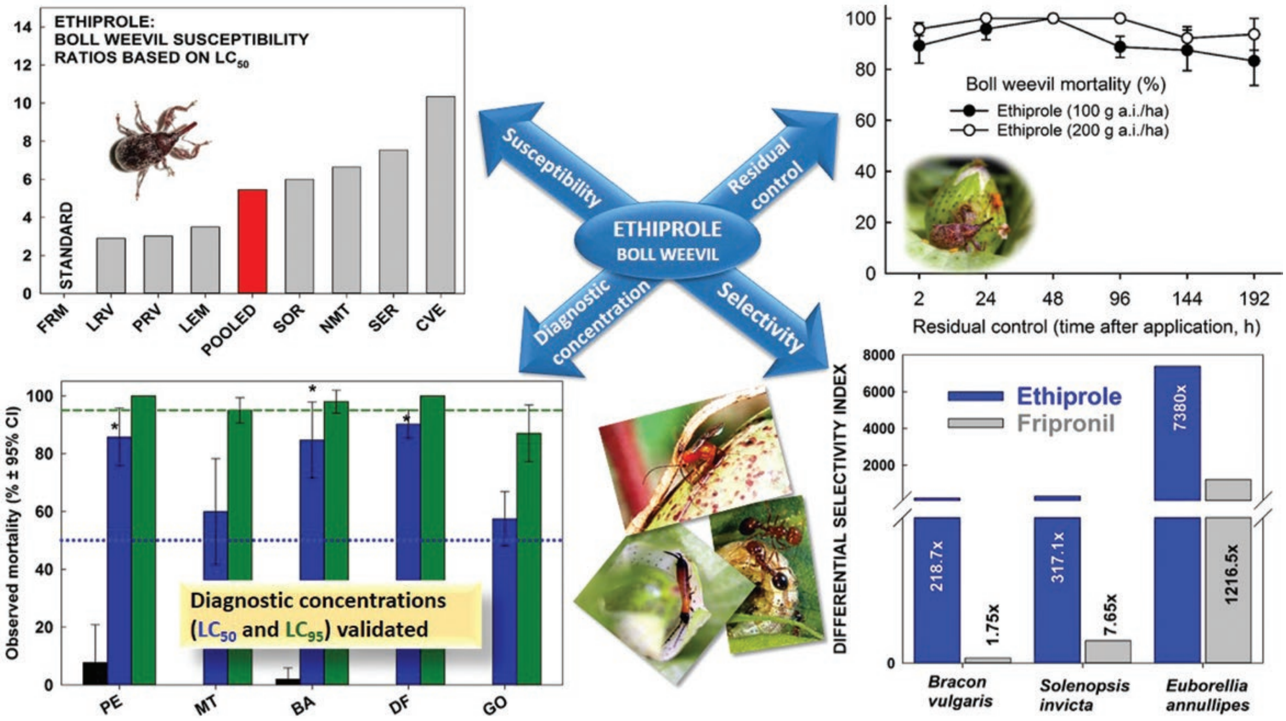
Abstract

Synthetic insecticide application is one tactic for reducing boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), infestations during the cotton, *Gossypium hirsutum* L., reproductive stage. We assessed the susceptibility of the boll weevil and its natural enemies to ethiprole (mode of action 2B), a phenylpyrazole insecticide, and diagnostic concentrations of ethiprole indicative of boll weevil susceptibility. Differences in the lethal concentrations of ethiprole were calculated with susceptibility ratios based on LC_{50} ranging from 2.89- to 10.34-fold relative to a natural susceptible population. The lowest and the highest recommended field rates of ethiprole, 100 and 200 g a.i./ha, produced residues that caused 83.3% and 93.7% mortality of weevils caged with cotton leaves from field-treated plants for 8 d. We found that ethiprole was less toxic than fipronil to the boll weevil parasitoid *Bracon vulgaris* Ashmead (Hymenoptera: Braconidae) and to the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), while fipronil was highly toxic to both. Adult earwigs, *Euborellia annulipes* Lucas (Dermaptera: Anisolabididae), were relatively tolerant to ethiprole and fipronil at the highest field rates. Pooled LC_{50}-and LC_{95}-concentrations of ethiprole calculated from studied populations were used as diagnostic for boll weevil mortality, and the outcome fitted to the expected mortality for boll weevil populations from different locations serving for further control failure assessment. Ethiprole appears to be suitable for boll weevil control with low impact on natural enemy communities.

Graphical Abstract



Key words: ecotoxicology, *Anthonomus grandis grandis*, *Bracon vulgaris*, *Euborellia annulipes*, *Solenopsis invicta*

Fipronil and ethiprole are currently the only two commercially available phenylpyrazole (mode of action [MoA] 2B) insecticides; they block GABA-activated chloride channels, causing hyperexcitation and convulsions of the target species (Cole et al. 1993). Fipronil was first marketed in 1993, and registered in Brazil ten years later against cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), cotton leafworm, *Alabama argillacea* (Hübner), and boll weevil, *Anthonomus grandis grandis* Boheman (MAPA 2020).

In Brazil, ethiprole was registered in 2015 against termite, *Heterotermes tenuis* (Hagen), and root spittlebug, *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae), in sugarcane, *Saccharum officinarum* L. In 2017, registration was expanded to include coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytida e), rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae), red-banded green stinkbug, *Piezodorus guildinii* (Westood) (Hemiptera: Pentatomidae), brown stinkbug, *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae), and boll weevils. Aspects of boll weevil susceptibility and that of important natural enemies, however, have not been reported.

Ethiprole differs slightly from the earlier phenylpyrazole, fipronil, by an ethylsulfinyl substituent replacing the trifluoromethylsulfinyl moiety, but retaining its insecticidal potency (Caboni et al. 2003). The low lipophilic activity of ethiprole compared to fipronil might affect its toxicity. Compounds with lower lipophilicity are more water soluble (McDougal and Boeniger 2002), with lower affinity for insect integument, potentially reducing its rate of penetration. Differential rates of penetration can result in physiological selectivity among species (Winteringham 1969).

Because boll weevils develop inside cotton fruiting structures (Coakley et al. 1969, Showler 2012), and their dispersal within the plant canopy is limited and cryptic, they become protected from topically applied insecticides (Showler and Scott 2004, Arruda et al. 2021). Thus, compounds with long lasting residues, such as fipronil, have a higher probability of adult weevil exposure and subsequent control (Arruda et al. 2021). Insecticides applications begin when 3–5% of the flower buds are attacked, and the applications are repeated at 5-d intervals for 20–25 d to control of the emerging adults (Bélot et al. 2016; Miranda and Rodrigues 2015). This intensive management results in estimated cost of US $360 per hectare, in Brazil (Bélot et al. 2016), which when added to the yield losses caused by the pest is a serious loss of profits for growers. Nonetheless, preferred insecticides with extended chemical residues involve exposure of natural enemies that can attack all weevil life stages (Cross and Chesnut 1971, Fillman and Sterling 1983, Fernandes et al. 1994, Ramalho and Wanderley 1996, Santos et al. 2013).

Flower buds and young bolls (5–8 d after anthesis) harboring boll weevil larvae abscise and fall to the ground where the larvae and pupae complete development (Showler and Cantú 2005, Showler 2008, Neves et al. 2013), while older infested bolls are retained by the plant and produce deformed open bolls (Showler and Robinson 2005, Showler 2006, Neves et al. 2013). Boll weevil larvae, pupae, and adults are attacked by natural enemies, including the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Fillman and Sterling 1983, Fernandes et al. 1994, Ramalho and Wanderley 1996), the ring-legged earwig, *Euborellia annulipes* Lucas (Dermaptera: Anisolabididae) (Ramalho and Wanderley 1996, Lemos et al. 2003) and the parasitoid, *Bracon vulgaris* Ashmead (Hymenoptera: Braconidae) (Nunes and Fernandes 2007, Wanderley et al. 2007, Santos et al. 2013). In this study, we tested the susceptibility of regional boll weevil populations to ethiprole and determined the appropriate diagnostic concentration indicative of boll weevil mortality to ethiprole. Furthermore, ethiprole toxicity for three natural enemies of boll weevil was determined.

Materials and Methods

Insecticides

Commercial formulations of fipronil and ethiprole (Fipronil Nortox 800 WG; Nortox Co., Arapongas, Paraná, Brazil, and Curbix 200 SC; Bayer CropScience, São Paulo, Brazil), respectively, were used in this study. The products were diluted in tap water (pH 6.15 from week measures [ranging from 5.8 to 6.5]) and added 0.05% of the surfactant Halten (Arysta Lifescience do Brasil, Pirapora, São Paulo, Brazil).

Insects

Boll weevil adults and its parasitoid were obtained from infested cotton fruiting structures collected from cotton field cultivated in the Semiarid in Frei Miguelinho Co., Pernambuco state, Brazil (-7.91917500 S, and -35.86266667 W). This is a small cotton field that has received few insecticide applications over the years with malathion, or any pyrethroids, against lepidopteran larvae, when needed. Thus, weevils from this location have been used as a reference population for insecticide susceptibility here and in other studies (Rolim et al. 2019,2021).

Boll weevils were also collected in the Cerrado areas representing seven populations infesting commercial cotton fields in Mato Grosso and Bahia states for susceptibility bioassays, and from other five different locations (see specific section) for validation of an ethiprole diagnostic concentration for boll weevil mortality.

Field-collected infested flower buds and bolls were stored in plastic trays (25 × 30 × 10 cm L × Wd × Ht) placed inside Plexiglass cages (50 × 40 × 50 cm L × Wd × Ht) with one opening on each side of 15 cm diameter that was covered by an anti-aphid screen to allow ventilation inside the cage until adult emergence. Emerged boll weevils were collected twice each day and fed cotton plant terminals, flower buds, and a honey: yeast mixture (1:1) (Rolim et al. 2019).

Adults of the parasitoid *B. vulgaris* were obtained from field-collected cotton bolls infested with boll weevils in the locale of Frei Miguelinho, Pernambuco state, Brazil. The bolls were placed in the same kind of cages used to obtain adult boll weevils. Emerge adults were collected twice each day using a hand-held aspirator, then placed in glass vials (1.3 × 6 cm in diam × Ht) at the rate of 8 to 10 adults per vial, which were sealed with polyvinyl chloride film (Wyda Pratic, São Paulo, Brazil). The film was punctured three to four times with an entomological pin (no. 0) for ventilation. Adult *B. vulgaris* were fed pure honey offered in drops inside the vial walls. The vials were kept in the laboratory at 25 ± 1°C, 12:12 h (L:D) photoperiod, and 60–65% relative humidity.

Solenopsis invicta were field-collected ≈2 h before the bioassay began from a single colony located at the Universidade Federal Rural de Pernambuco, Campus of Recife, Pernambuco state, Brazil (-8.01861111 S, and - 34.94527778 W). We used individuals from a single colony to reduce potential variation in response to the insecticides. Workers and soldiers were collected directly from the nest, using a hand-held aspirator, and placed inside 50 ml centrifuge tubes (Olen, KASVI Imp., São José dos Pinhais, Paraná, Brazil). Before collection, drops of pure honey were smeared inside the tubes as food. The vials containing the ants were stored inside a Styrofoam box in darkness to reduce stress until the bioassay.

Euborellia annulipes adults were obtained from our laboratory colony, established in 2018 with nymphs and adults collected from noncultivated fields in Paudalho County, Pernambuco State, Brazil (-7.92888889 S, and -35.04138889 W). The colony was maintained using a dry diet prepared from 35% chicken feed, 24% wheat bran, 22% yeast, 13% powdered milk, and 4% of the antimicrobial Nipagin (Ueno Fine Chemicals, Ueno, Japan) after Silva et al. (2009).

Bioassays

All bioassays were conducted with adult boll weevils and natural enemies exposed to dried insecticide residues on green cotton leaf tissue allowing comparisons across species (Jepson 1989). We used treated-leaf discs (≈8 cm diam) and untreated-leaf discs as controls. The treated leaf discs were obtained by immersion into insecticide dilutions for 10–20 s and air-dried for 2 h inside an exhaust chamber Nalgon mod. 3700 (Nalgon Equipamentos Científicos, Itupeva, São Paulo, Brazil). For concentration–response bioassays, preliminary assays were conducted on each insect species to determine the concentration range that produced mortality >0% and ≈100% to calculate the lethal mean concentrations using Probit analysis (Finney 1971). The outcome is an

Table 1. Susceptibility of adults from eight boll weevil (*Anthonomus grandis grandis*) populations to dried residues of ethiprole on cotton leaves

Population	*n* (df)[1]	Slope ± SE	LC_{50}[2] ($FL_{95\%}$)	LC_{90}[2] ($FL_{95\%}$)	SR_{50}[3] ($FL_{95\%}$)	$\chi^{2\ P\text{-value}}$
Pernambuco State						
Frei Miguelinho	149 (4)	1.21 ± 0.27	2.71 (1.18–4.25)	30.76 (16.71–120.83)	-	$3.23^{0.5044}$
Bahia State						
Luiz E. Magalhães	198 (3)	1.31 ± 0.37	11.37 (0.55–29.59)	107.48 (51.39–209.75)	3.49 (1.63–7.48)	$2.58^{0.4598}$
Mato Grosso State						
Lucas do Rio Verde	150 (6)	2.51 ± 0.47	7.84 (5.11–10.59)	25.39 (18.08–45.60)	2.89 (2.03–4.12)	$3.43^{0.7531}$
Primavera	188 (5)	1.25 ± 0.21	8.18 (5.06–12.10)	86.23 (47.66–246.73)	3.02 (1.68–5.42)	$0.94^{0.9673}$
Sorriso	150 (6)	2.97 ± 0.45	16.27 (11.38–22.10)	43.90 (31.57–70.72)	6.00 (4.12–8.75)	$9.47^{0.1484}$
Nova Mutum	145 (6)	2.37 ± 0.36	18.05 (13.12–23.90)	62.69 (44.19–109.44)	6.65 (4.47–9.89)	$4.63^{0.5917}$
Serra	160 (4)	1.07 ± 0.17	20.40 (11.88–32.36)	287.26 (152.04–405.40)	7.53 (2.96–19.11)	$1.90^{0.7531}$
Campo Verde	150 (6)	2.29 ± 0.30	28.02 (21.04–37.43)	104.05 (71.46–185.04)	10.34 (6.81–15.70)	$8.95^{0.2562}$
Pooled						
Diagnostic concn.	1141 (12)	1.57 ± 0.08	14.79 (12.72–17.05)	96.68 (80.25–119.91)	5.46 (3.96–8.66)	$16.07^{0.1878}$

[1] Number of tested insects and degree of freedom (df); [2] LC = lethal concentrations and their respective 95% fiducial limits rated as mg a.i./L; [3] SR = susceptibility ratio; and χ^2 test used to test goodness-of-fit to Probit model.

Table 2. Relative susceptibility of boll weevil (*Anthonomus grandis grandis*) and three natural enemies to phenylpyrazoles, evaluated after exposure to dried residues on treated cotton leaves in the laboratory

Insecticide/ Tested species	*n* (df)	Slope(± SE)	LC_{50}[3](95% FL)	LC_{90}[3](95% FL)	$\chi^{2\ P\text{-value}}$	DSI_{50}[4](95% FL)
Ethiprole						
Anthonomus grandis grandis	149 (4)	1.21 ± 0.27	2.71 (1.18–4.25)	30.76 (16.71–120.83)	$3.23^{0.5044}$	-
Bracon vulgaris	222 (5)	1.12 ± 0.15	592.94 (380.04–888.12)	8,181.0 (4,416–21,822)	$4.74^{0.4408}$	218.79 (53.74–896.86)
Solenopsis invicta	322 (5)	2.32 ± 0.22	849.79 (701.5–1,018.0)	3,017.0 (2,362–4,187)	$2.75^{0.7379}$	313.57 (204.10–481.38)
Euborellia annulipes	252	-	>20,000[1]	-	-	>7,380.07
Fipronil						
Anthonomus grandis grandis	191 (4)	1.19 ± 0.29	0.32 (0.08–0.58)	3.74 (2.01–15.61)	$4.07^{0.3956}$	-
Bracon vulgaris	142 (4)	2.31 ± 0.38	0.56 (0.39–0.74)	2.01 (1.43–3.56)	$2.51^{0.6431}$	1.75 (0.14–0.30)
Solenopsis invicta	432 (3)	0.69 ± 0.08	2.45 (1.54–4.29)	167.58 (58.91–467.80)	$2.85^{0.4414}$	7.65 (3.20–13.57)
Euborellia annulipes	196 (3)	2.32 ± 0.28	389.30 (299.95–498.57)	1,387.0 (1,010–2,194)	$5.64^{0.1304}$	1,216.56 (991.36–1,425.71)

[1]Ten concentrations were tested from 500 to 20,000 mg a.i./L with a maximum mortality rate of 10% at the highest tested concentration. [2]*N* = number of tested insects and degree of freedom (df); [3]LC = lethal concentrations and their respective 95% fiducial limits rated as mg a.i./L; χ^2 tests were used to test goodness-of-fit to Probit model; and [4]DSI = differential selectivity index (LC_{50} for tested natural enemy/LC_{50} for boll weevil).

individual binary response (live and dead) rated as the number of dead by the total number of individuals tested per concentration. The whole bioassay, including all concentrations, is repeated to check the parallelism of the response (Robertson and Preisler 1992) and to compose the total number of insects evaluated with no less than 20 individuals per concentration and 120 per bioassay (*n* values in Tables 1 and 2). These, bioassays were carried out separately for each species, and at 25 ± 1.5°C, 12:12h (L:D) photoperiod, and ≈60% air relative humidity.

Boll Weevil Bioassays

Adult mature boll weevils 5–8-d-old were caged on cotton leaf disc plus two flower buds without bracts. The boll weevils were from the Frei Miguelinho population, exposed to 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg a.i./L of fipronil and 1.56, 3.12, 6.25, 12.5, 25.0, and 50 mg a.i./L of ethiprole residues.

Populations from Mato Grosso and Bahia were only tested against ethiprole. Seven boll weevil populations from these locations were exposed to ethiprole concentrations ranging from 3.12 to 400 mg a.i./L, plus the untreated controls. These bioassays were carried out in the Experiment Center for Diffusion of Technologies of the "Instituto Mato-grossense do Algodão", Campo Verde, MT, Brazil; while the bioassays involving natural enemies and residual boll weevil control were conducted at the Universidade Federal Rural de Pernambuco, Recife, Pernambuco state, Brazil.

Mortality was recorded 48 h after confinement by transferring adult weevils to a clean Petri dish. The Petri dish was previously warmed up to 35°C (Hot Plate, Fisatom mod. 752A, Rio de Janeiro, Rio de Janeiro, Brazil) to overcome the insect's thanatosis behavior. Adults that did not walk were considered dead; while live weevils walked to the border of the Petri dish.

Diagnostic Concentration of Ethiprole Against Boll Weevil

Control failures might be associated to various factors including insecticide resistance, and diagnostic concentration serves to resistance selection monitoring. Weevils collected from our experimental plots in the Universidade Federal Rural de Pernambuco (Recife-PE), and Cerrado areas [Brasília-DF, Luiz Eduardo Magalhães-BA, Chapadão-GO, and a mix of populations from Mato Grosso (hereafter named as Mato Grosso-MT)] were tested against the pooled LC_{50}- and LC_{95}-concentrations determined in the previous bioassay. Three treatments were carried out exposing adult weevils to dried-residues of ethiprole corresponding to the calculated LC_{50} (14.8 mg a.i./L), LC_{95} (96.7 mg a.i./L), and untreated controls. Each treatment was run with 5 to 8 replications represented by Petri dishes lined with insecticide treated or untreated control leaf discs holding 5 to 12 weevils per replication according to the available number of insect per population to be assayed.

Natural Enemies Bioassays

Toxicity of ethiprole and fipronil to boll weevil natural enemies, *B. vulgaris*, *S. invicta*, and *E. annulipes*, were determined. Adults 24–36-h-old of the parasitoid, *B. vulgaris*, were used. Tested concentrations were 25, 100, 200, 300, 1,500, 3,000, and 6,000 mg a.i,/L of ethiprole, and 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 mg a.i./L of fipronil. Cotton leaves for both the untreated- and insecticide-treated treatments received drops of pure honey to stimulate parasitoid foraging. One leaf was rolled and placed inside glass test vials (13 × 60 mm in diam × Ht). Using a light source, 10–12 unsexed parasitoids were allowed to walk from the rearing vials into the test vials per concentration, with whole bioassay repeated three times. The vials were closed with organdy fabric secured by a rubber band (Mercur, Santa Cruz do Sul, Rio Grande do Sul, Brazil).

Workers and soldiers of the red imported fire ant, *S. invicta*, were transferred to Petri dishes lined with one treated-leaf disc (≈8 cm diam) or untreated-leaf disc as control. Preliminary tests determined the concentrations 296, 444, 666, 1,000, 3,000, 6,000, and 9,000 mg a.i./L of ethiprole, and 0.05, 0.15, 0.45, 1.33, 4, and 12 mg a.i./L of fipronil to be tested. A piece of ≈0.1 g of the honey: yeast mixture (1:1) was placed over the leaf disc as ant diet in all treatments followed by 20–24 field-collected ants released inside each Petri dishes per concentration with whole bioassay repeated three times. The dishes were placed inside a plastic tray (40 × 30 × 10 cm in L × Wd × Ht) covered by a paper card larger than the tray to produce shade over the Petri dishes with the ants.

The bioassay with the earwig, *E. annulipes*, was carried out with 7–8 adult earwigs, 5–6 d old, confined with one untreated- or insecticide-treated cotton leaf disc placed in Petri dishes per concentration, and provided with ≈0.5 g of the rearing dry diet (Silva et al. 2009). A total of 10 concentrations (500, 750, 1,000, 1,500, 2,500, 3,000, 5,000, 10,000, 15,000, and 20,000 mg a.i./L) of ethiprole were tested, but without recording mortality, even at the highest tested concentration. In contrast, the concentrations 60, 200, 400, 1,200, and 1,600 mg a.i./L of fipronil caused mortality enough to calculate the LC values.

The mortality of *B. vulgaris* and *E. annulipes* was recorded 48 h and *S. invicta* 24 h after the beginning of their exposures. The vials containing the parasitoids were opened, and the parasitoids dislodged into a white tray. Those parasitoids unable to fly during 1 min observation period, even when being touched with a brush, were declared dead. The earwigs were placed upside down in a clean Petri dish, with live individuals turning upright quickly and walking due to the negative phototropism behavior. The criterion for mortality was the inability of the earwig to upright itself and walk. The Petri dishes confining the red imported fire ant were opened inside a white plastic tray, and those ants that did not walk to the border of the Petri dish, even after taking out the leaf discs, were considered dead. In this bioassay, we used 24 h period of confinement considering the social behavior of ants. By doing that, we hold survival, in the control groups, greater than 97%.

Survival of Boll Weevil Natural Enemies Exposed to Field Rates of Phenylpyrazoles

Adults of *B. vulgaris*, *E. annulipes*, and *S. invicta* were confined on cotton leaves collected from plants in a field treated with the lowest (LFR), the highest (HFR) field rates and three times the HFR (3xHFR) labeled of ethiprole and fipronil. Adults of each natural enemy were caged separately on untreated- and insecticide-treated field-collected cotton leaves using the same procedure used in the previous bioassays. Cotton plants, var IMA2106GL, grown in the experimental plot of the Crop Protection Experiment Field of the Universidade Federal Rural de Pernambuco, Recife, Pernambuco state, were treated at 60 d old with 100, 200, and 600 g a.i./ha of ethiprole, and 12, 80, and 240 g a.i./ha of fipronil corresponding to the treatments LFR, HFR, 3xHFR, respectively, plus the controls. The insecticides were applied through a backpack sprayer (Model Jacto XP 10L, Pompéia, São Paulo, Brazil) with empty cone spray nozzle model JD12 and adjusted for a flow rate of 200 l per hectare with spray pressure ≈2.8 Kgf/cm^2.

The uppermost fully expanded leaves from untreated- and insecticide-treated cotton plants were harvested 2–3 h after insecticide application and taken to the laboratory, where leaf discs of ≈8 cm diam. were prepared and used to line Petri dishes of the same diameter (for earwig and fire ants) or as discs rolled inside glass vials (for boll weevil parasitoid). Following the same procedure used in the previous bioassays, adults of natural enemies were confined with insecticide-treated and untreated cotton leaves at a rate of 7 earwigs, 10–12 parasitoids, and 20–40 ants per replication with five replications per treatment.

Mortality was tallied 48 h later, except fire ants, for which mortality was recorded 24 h after caging as previously described. All mortality data were then transformed into survival rates for analysis.

Residual Toxicity of Ethiprole to Boll Weevil and Its Parasitoid

This bioassay evaluated the effect of residues of ethiprole when applied on cotton plants in the field at the lowest (LFR) and the highest (HFR) field rates. Mortality was assessed against the field-dried residues on leaves of field plants 2, 24, 48, 96, 144, and 192 h for boll weevils, and 2, 24, 48, 96, 144 h for *B. vulgaris* after field application.

The experiment consisted of two ethiprole rates (LFR and HFR) and untreated controls. For each treatment × evaluation interval, there were five replications with 5 weevils or 10–12 parasitoids each. The exposure methods and mortality assessment were similar to previous bioassays for boll weevil and its parasitoid.

Statistical Analysis

In all bioassays where recorded mortality in the control treatments were zero, correction for natural mortality was disregarded. In the bioassays with the boll weevil parasitoid and the red imported fire ant, control mortality varied from 1.7 to 3.2%. For these species, we used Abbott's formula to correct for control mortality (Abbott 1925).

Lethal-concentration (LC) values for each species of the natural enemy and each boll weevil population, as well as all associated 95% fiducial limits (FLs), were estimated by Probit analysis (Finney 1971) using the Proc Probit function of SAS (SAS Institute 2002). In addition, we calculated the LC values for the pooled group of all weevil populations. The susceptibility ratio (SR_{50}) across tested boll weevil populations for ethiprole and the SR_{50} values for both insecticides (ethiprole and fipronil) and the three natural enemy species were also calculated. Thus, the SR_{50} values and their 95% fiducial limits (FLs) calculated were considered significantly different when these intervals did not include value 1.0 (Robertson and Presley 1992).

The mortality for weevils exposed to the mean lethal concentrations (LC_{50} and LC_{95}) and the expected mortality (50% and 95%) were tested considering the honesty hypothesis of equality using the Proc Freq of SAS and the χ^2 test ($\alpha = 0.05$) aiming to validate a diagnostic concentration for weevils' mortality.

The mortality rate of the boll weevil and survival of natural enemies from exposure to field rates of each insecticide were checked for normality and homoscedasticity using Shapiro–Wilk and Lavene tests, respectively (Proc Univariate and Proc Anova of SAS), and arcsin square root of (×/100) transformed to fit the assumptions for analysis of variance. Furthermore, the earwig survival data were submitted to a one-way ANOVA (Proc Anova) with means separation performed by Tukey HSD's test using alpha-value corrected by the number of means in comparisons ($\alpha/n = 0.007$). Due to the overdispersion of the results obtained with the parasitoid and the red imported fire ant (100% adult mortality when exposed to fipronil tested field rates), the results did not fit ANOVA assumptions. Therefore, means were compared by Kruskal–Wallis test (Proc Npar1way), and the means of treatments were compared pairwise to the control treatment by Dunnett's test ($\alpha = 0.05$).

Ethiprole rates (LFR and HFR) and the evaluation dates were compared for boll weevil mortality and survival of the parasitoid. The data were arcsin square root of (×/100) transformed to fit the assumptions for ANOVA, and tested with one-way ANOVA, with two treatments and six and five evaluation dates (for boll weevil and parasitoid, respectively) as repeated measures data (SAS Institute 2002), with each evaluation date being compared between the insecticide rates by Fisher's test (df = 1, $\alpha = 0.05$).

Results

Susceptibility Boll Weevil and its Natural Enemies to Phenylpyrazoles

Boll weevil populations from Campo Verde and Frei Miguelinho were the most and the least tolerant to ethiprole, with the last used as the standard population for susceptibility comparisons. Thus, the SR_{50} ratio ranged from 2.89- to 10.34-fold across the boll weevil populations (Table 1). The field recommended rates of ethiprole and fipronil (200 and 80 g a.i./ha diluted into 200 l of water; equal to 1,000 and 400 mg a.i./L) exceeded the LC_{90} values and were expected to kill >90% of any field boll weevil tested population. Furthermore, the LC_{50} and LC_{90} indicate that fipronil is ≈8.5- and ≈8.2-fold more toxic to boll weevils than ethiprole.

The 95% FL upper band for the LC_{90} from more tolerant weevils to ethiprole (Serra and Campo Verde) resulted in 405.4 and 185.04 mg a.i./L, respectively (Table 1). Likewise, the ethiprole concentrations needed to reach the LC_{90} level (and its 95% FL) for the pooled set of populations from the Cerrado areas were 96.68 and 119.91 mg a.i./L, respectively. These results are lower than the recommended field rates of ethiprole.

Diagnostic Concentration of Ethiprole Against Boll Weevil

Adult boll weevils from five populations showed ≥ 50% mortality when exposed to the LC_{50} concentration of ethiprole (Fig. 1). Likewise, the confinement of weevils against LC_{95} concentration residues resulted in observed mortality statistically similar to the expected 95% mortality. These findings confirm that either LC_{50} or LC_{95} concentrations would detect any control failures in the absence of further resistance development.

Natural Enemies Bioassays

Bracon vulgaris was significantly more susceptible to fipronil than ethiprole (Table 2). The LC_{50}- and LC_{90}-values calculated for *B. vulgaris* with ethiprole were grater 218- and 265-fold compared to the boll weevil, showing that the compound was less toxic to the natural enemy (Table 1). On the other hand, these same ratios with fipronil turned out as 1.75- and 0.53-fold indicating that fipronil was highly toxic to the parasitoid.

There was significant difference in the susceptibility of *S. invicta* between ethiprole and fipronil (Table 2), with lower LC_{50}- and LC_{90}-values for fipronil compared to ethiprole. These LC values with fipronil were also lower for *S. invicta* relative to boll weevil. Fact that the differential selectivity index (DSI_{50}) was 313.57-fold with ethiprole but only 7.65-fold with fipronil (Table 2).

Adult earwigs exposed to ethiprole exhibited low mortality across the tested concentrations in both the preliminary and final bioassays. Across preliminary bioassays testing 500, 750, 1,500, and 3,000 mg a.i./L, only four deaths were recorded out of 98 exposed earwigs. Further, the other six tested concentrations, all greater than the highest recommended field rate of ethiprole: 2,500, 3,000, 5,000, 10,000, 15,000, and 20,000 mg a.i./L, resulted in mortality rates from zero to 10%. Therefore, these data did not fit the calculation of lethal concentrations even using ethiprole rates 20-fold than the highest recommended field rate. Based on this information, we estimated that the DSI is >7380.07-fold compared to toxicity to adult boll weevils for ethiprole (Table 2).

Fipronil was more toxic to the earwig than ethiprole (Table 2). Despite that, the upper band of the 95% FL found for the LC_{90}-value for boll weevil was 15.61 mg a.i./L compared to 2194 mg a.i./L for the earwig, suggesting lower toxicity of fipronil to earwig than for boll weevil. This difference in susceptibility resulted in a DSI of 1216.56-fold in favor of the earwig (Table 2).

Survival of Boll Weevil Natural Enemies Exposed to Field Rates of Phenylpyrazoles

Exposure of *B. vulgaris* to residues of fipronil or ethiprole on leaves collected from cotton plants treated in the field showed difference in survival ($H = 30.35$, $P < 0.001$, df = 6, Fig. 2). Adult of *B. vulgaris* did not survive fipronil exposure but exhibited survival of 54.5%, 40.5%, and 23.3% to LFR, HFR, and 3xHFR of ethiprole, respectively.

Solenopsis invicta did not survive exposed to fipronil and exhibited reduced survival exposed to residues of ethiprole rates relative to the control ($H = 29.25$, $P < 0.001$, df = 6, Fig. 2). About 40%, 18.2%, and 9.8% of the red imported fire ant survived at LFR, HFR, and 3xHFR of ethiprole, respectively, compared to 98.4% survival in the control treatment.

Euborellia annulipes survived exposure to either insecticide across all tested rates. Despite that, significantly lower survival was observed for fipronil in the HFR (60%) and 3xHFR (18%) treatments, compared to the control (99.5%) ($F = 63.83$, df = 6, 28, $P < 0.001$, Fig. 2). Adult earwigs had high survival (>94%) for

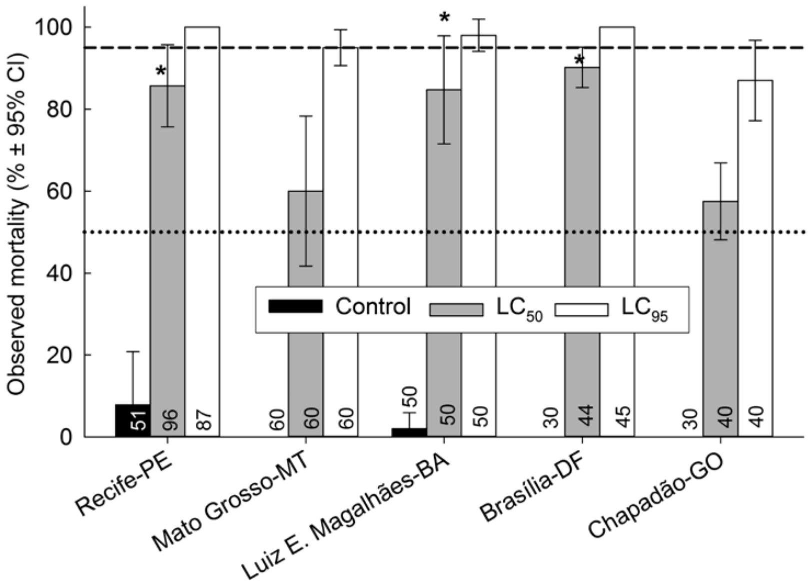


Fig. 1. Mortality of adult boll weevils (*Anthonomus grandis grandis*) (± 95% confidence limits) from control and diagnostic concentrations (LC_{50} and LC_{95}) of ethiprole. Dashed and dotted lines stand for 95 and 50% expected mortality; while, numbers inside bars stand for the number of weevils tested. Bars bearing asterisks indicate that the obeserved and expected mortality rates differ by χ^2 test ($\alpha = 0.05$).

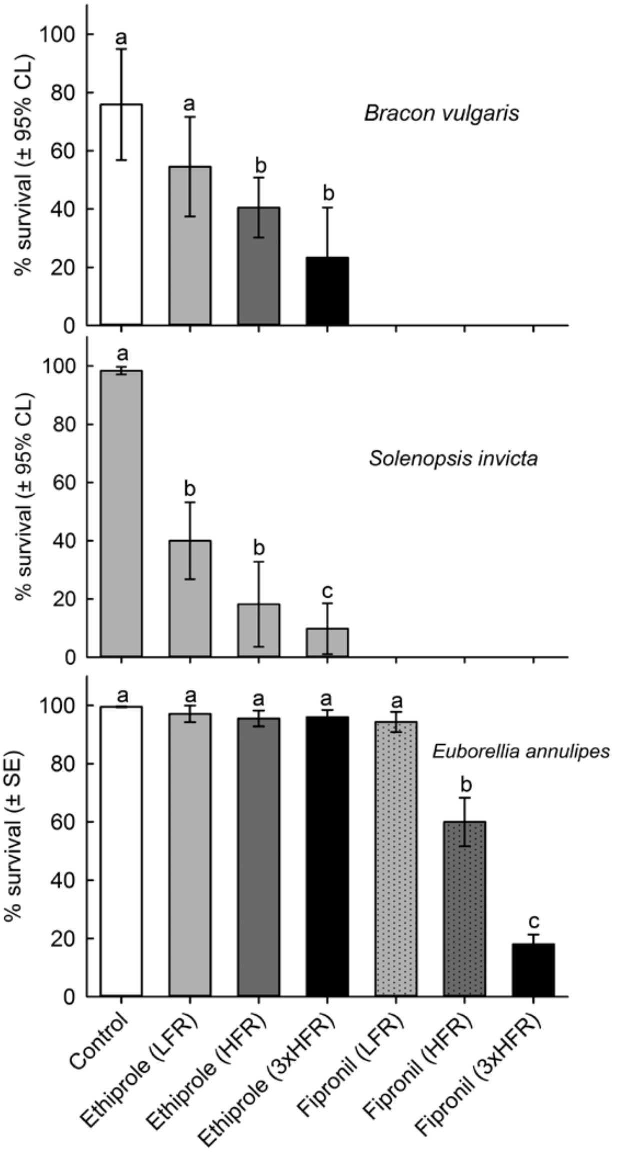


Fig. 2. Mean survival of *Bracon vulgaris* and *Euborellia annulipes* adults 48 h after confinement and *Solenopsis invicta* 24 h after caging on cotton leaves collected from field treated-plants with phenylpyrazoles insecticides, at the lowest field rate (LFR), the highest field rate (HFR), and three times the highest field rate (3xHFR) of ethiprole and fipronil. Bars with different letters differ significantly from others [means of survival ± 95% confidence limits for *B. vulgaris* and *S. invicta* were analyzed by Kruskal–Wallis followed by Dunnett's test; while, means of survival ± SE for *E. annulipes* differ from others by one-way ANOVA followed by Tukey HSD test ($\alpha = 0.007$)].

ethiprole at all three tested rates (LFR, HFR, and 3xHFR) and only in the LFR for fipronil (Fig. 2).

Residual Toxicity of Ethiprole to Boll Weevil and Its Parasitoid

The survival of the *B. vulgaris* did not differ between LFR and HFR of ethiprole across the five evaluation time points (Wilks' lambda = 0.001, $F_{1,4} = 0.89$, $P = 0.716$; Fig. 3 top). However, the rate of toxicity of the ethiprole residues to *B. vulgaris* declined significantly over time after application (Wilks' lambda = 0.076, $F_{4,16} = 231.46$, $P < 0.001$, Fig. 3) with both LFR and HFR of ethiprole. The survival rates were 45.5% and 59.4% and significantly lower for fresh residues (2 h after application) of ethiprole at both LFR and HFR, and significantly greater (84.1–94.3%) for the last two evaluated intervals (94 and 144 h after application) in these same application rates.

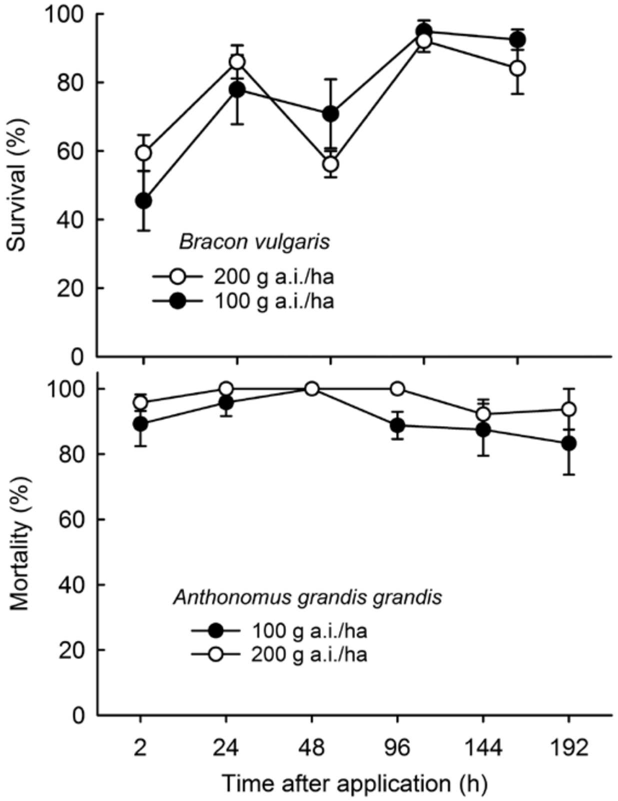


Fig. 3. Survival of the parasitoid *Bracon vulgaris* (top), and mortality of the boll weevil (*Anthonomus grandis grandis*) (bottom), when confined on cotton leaves collected from field treated-plants with the lowest and the highest field rates of ethiprole recommended against boll weevil. Symbols identified by different letters compare means (± SE) within each ethiprole rate across evaluation dates by Tukey HSD's test ($\alpha = 0.05$); while, no difference was found at each interval post-application between ethiprole rates.

Mortality of boll weevils caged on leaves collected at various post-application intervals with ethiprole did not differ between the LFR and HFR (Wilks' lambda = 0.076, $F_{1,3} = 2.23$, $P = 0.263$) or across evaluation intervals (Wilks' lambda = 0.004, $F_{5,15} = 1.67$, $P = 0.202$; Fig. 3 bottom). Average mortalities using the LFR and HFR of ethiprole were 89.3% and 95.7%, and 83.3% and 93.7% at the first (2 h) and at the last (192 h) evaluations, respectively.

Discussion

Ethiprole was toxic to boll weevil, irrespective of tested populations, and safer for its natural enemies than fipronil. Ethiprole showed lower toxicity against *B. vulgaris*, *S. invicta*, and *E. annulipes*, allowing survivors when used at field rates. Caboni et al. (2003) reported that ethiprole is less lipophilic than fipronil, which may reduce the penetration of the insect cuticle (Winteringham 1969). The log Kow (kow, n-octanol/water partition coefficient) of ethiprole is 2.9 (USEPA 2011) compared to 4.0 for fipronil (Tomlin 2000); consequently, movement of ethiprole through the insect tegument would like to be less efficient than for fipronil.

Regarding the ethiprole toxicity to boll weevils, the upper 95% fiducial limits (FLs) for LC_{90} values varied from 45.6 to 405.4 mg a.i./L for boll weevils collected from Lucas do Rio Verde and Serra localities. The observed LC_{90} upper band range was lower than the recommended field rate of ethiprole (500 mg a.i./L), suggesting that adequate control, would be achieved. Furthermore, the LC values from our experiments served to define a baseline for a future resistance monitoring if field control failures might be due to insecticide resistance or other factors. Boll weevil survival greater than that expected when treated with LC_{50} or LC_{95} concentrations of ethiprole will indicate resistance selection. These diagnostic concentrations have not been made available until now, based on the literature for phenylpyrazoles insecticides and the boll weevil.

In Brazil and elsewhere, boll weevil control relies on several control practices, from mandatory stalk destruction to sequential insecticide application after field infestation (Cross 1973, Showler 2012). Insecticide application, however, is the only viable practice to reduce adult weevils established in cotton fields through the whole cotton reproductive stage, which ranges from 40 to 120 d. Despite that, insecticide recommendations are centered on few insecticides with broad-spectrum action prevent proper rotation of materials with a different mode of action (MOA) as a recommended practice to slow the development of insecticide resistance (Sparks and Nauen 2015). In addition, the lack of registered insecticides with selective action makes it difficult to find suitable insecticides able to conserve natural enemies within the cotton crop (Barros et al. 2018, Torres and Bueno 2018). Despite many insecticide formulations available for use against boll weevil in Brazil (>100 commercial products) (MAPA 2020), about half are formulated with pyrethroids with a similar MOA (i.e., 3A). Other available products are formulated with three organophosphates (1B), three neonicotinoids (4A), two carbamates (MOA 1A), and the tested two phenylpyrazoles (MOA 2B). This diversity of MOAs would be sufficient to allow for adequate insecticide rotation or alternation programs for many other pest management programs. However, boll weevil requires 15–25 applications per season (Miranda and Rodrigues 2015, Bélot et al. 2016). This intensive use of insecticides aims to kill adults from sequential emergence and to limit new generations (Showler 2006, 2012, Arruda et al. 2021).

The variation in susceptibility to ethiprole indicates a risk for resistance development. Weevil populations from Serra and Campo Verde produced SR_{50} values of 7.53- and 10.34-fold relative to the most susceptible population (Frei Miguelinho). Therefore, our findings suggest careful attention concerning monitoring ethiprole and fipronil in these areas. Thus, these data provide support for further monitoring to detect possible changes in the susceptibility of boll weevil against ethiprole and fipronil.

Effective insecticides against boll weevil require extended residuals. In addition, they will benefit cotton IPM when having a low impact on natural enemies. These features correspond to what we found with ethiprole compared to the currently nonselective recommended materials. The levels of residual control of boll weevil on cotton plants treated with ethiprole at the LFR and HFR extended for one week (Fig. 2). This level of residual control is comparable to fipronil (Arruda et al. 2021); and longer than malathion, carbosulfan, and thiamethoxam (Rolim et al. 2019, Arruda et al. 2021). An additional benefit of ethiprole for boll weevil control is ≈50% survival of *B. vulgaris* when tested against 0 d old residues and over 80% when tested against 4-d-old residues.

The earwig *E. annulipes* survived when exposed to dried residues of both ethiprole and fipronil at field rates (Fig. 2). Fipronil at the HFR level allowed >60% adult earwig survival, and ethiprole did not cause earwig mortality at a concentration 20-fold than the HFR. The levels of mortality of earwigs at HFR and 3xHFR due to fipronil, would be likely diminished under field conditions. Earwigs prey upon boll weevil larvae and pupae inside flower buds that have fallen to the ground. Therefore, earwigs might escape the prolonged contact with fipronil dried-residues on plant foliage. On the other hand, fipronil was highly toxic to both boll weevil parasitoid and red imported fire ant compared to the boll weevil itself (Table 2) at field rates (Fig. 1). The observed toxicity of fipronil to ants is not surprising considering that it has been used to red imported fire ant control (Collins and Callcott 1998); causing extended toxicity than pyrethroids (Jiang et al. 2014). It is also used in baits to control various other species of ants (Sakamoto et al. 2019).

Resistance of boll weevil to pyrethroids has been reported lately in Brazil (Rolim et al. 2021). The use of pyrethroids in insect pest management can lead to aphid and whitefly outbreaks due to destruction of their predators and parasitoids (Barros et al. 2018, Torres and Bueno 2018), which also occurs with many of the other long residual broad-spectrum insecticides (Torres and Bueno 2018). For such reason, research to find new insecticides with a lower impact on beneficial insects and control of boll weevil benefits cotton pest control. Examples are ethiprole (this study) and spinosyns (Rolim et al. 2019). In addition to the natural enemies, we examined other groups relevant for insect pest management include the lady beetles such as *Eriopis connexa* (Germar) (Coleoptera: Curculionidae) that suppress outbreaks of sucking insects. One hundred adults of *E. connexa*, confined for 48 h on cotton leaves containing dried-residue of ethiprole at the LFR and HFR levels, exhibited 98% survival compared to 0% survival exposed to fipronil (not published). Furthermore, ethiprole is relatively safe for lady beetle species, including *Stethorus japonicus* H.Kamiya (Coleoptera: Coccinellidae) (Masui 2010), *Serangium japonicum* Chapin (Coleoptera: Coccinellidae) (Ozawa and Yama 2016), *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) (Abbade Neto 2017), and the assassin bug, *Rhynocoris marginatus* Fabricius (Hemiptera: Reduviidae) (Patel 2020).

In summary, our results characterized the susceptibility of boll weevil adults to ethiprole across several populations and highlighted the variability among populations. This variability must be monitored as ethiprole becomes more widely used. Therefore, the pooled LC_{50} or LC_{95} concentration from our study seems suitable for such monitoring to detect resistance development. Ethiprole residues from the lowest and the highest recommended field rates remained active against adult weevils on plants up to 8 d. Furthermore, ethiprole was not toxic to *E. annulipes* and had moderate toxicity for *B. vulgaris* and *S. invicta* compared to fipronil. Therefore, ethiprole can improve insecticide rotation options to slow insecticide resistance development in boll weevil populations across Brazil's cotton-growing regions.

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