

Multiple Factors Mediate Insecticide Toxicity To A Key Predator For Cotton Insect Pest Management

Denner Manthay Potin

Universidade Federal Rural de Pernambuco

Anderson Vinnicius Arruda Machado

Universidade Federal Rural de Pernambuco

Paulo Roberto Ramos Barbosa

Universidade Federal dos Vales do Jequitinhonha e Mucuri

Jorge Braz Torres (✉ jorge.torres@ufrpe.br)

Universidade Federal Rural de Pernambuco <https://orcid.org/0000-0002-0717-6493>

Research Article

Keywords: Pesticide toxicology, Biological control, Insect behaviour, Toxicity bioassay, Chemical control

Posted Date: October 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-857349/v1>

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Version of Record: A version of this preprint was published at Ecotoxicology on February 7th, 2022. See the published version at <https://doi.org/10.1007/s10646-022-02526-6>.

Abstract

Mortality of agricultural pests caused by arthropod predators is a valuable ecosystem service for crop production. The earwig, *Euborellia annulipes* (Lucas), attacks different pest species in various crop ecosystems, including larvae and pupae of the boll weevil, *Anthonomus grandis grandis* (Boh.). Despite such biological control, cotton pest management remains heavily dependent on synthetic insecticides. In this study, multiple factors were assessed to measure the selectivity of insecticides used against sap-sucking and chewing cotton pests for two *E. annulipes* populations. Nymphs and adults of *E. annulipes* were exposed to the insecticides in two ways: ingestion of contaminated prey, and contact with dried residues on either inert surfaces or treated plants bearing prey. Pymetrozine, chlorantraniliprole, and spinetoram had little effect on the predator regardless the tested earwig population, life stage, or the route of exposure. Cyantraniliprole affected the predator in some life stages and through some types of contact. Pyriproxyfen was harmless to adult earwigs, but prevented normal development of nymphs to adults. Chlorfenapyr, indoxacarb, lambda-cyhalothrin, chlorpyrifos, dimethoate, and malathion were harmful to the predator regardless earwig life stage or method of exposure. The negative impact was diminished when exposure occurred on plants with predator allowed to shelter in the soil. The results indicate that insecticide selectivity outcome is a multi-factor driven by the insecticide, predator life stage and the predator's behavior. Therefore, testing different predator life stages via several routes of exposure, without denying the insect the opportunity to engage in its normal behaviors can provide better estimates of insecticide selectivity.

Introduction

Natural enemies provide valuable ecosystem services by killing arthropod pests in crop ecosystems. For example, in Brazilian cotton field, some 58 arthropod species can reach the economic threshold and require curative control (Silvie et al. 2013), but many other herbivorous arthropod species are kept under natural control by abiotic and biotic factors, including the action of natural enemies. The conservation of natural predators in the cotton ecosystem is widely recognized as an important part of integrated pest management (IPM) of cotton pests (Bordini et al. 2021; Deguine et al. 2008; Luo et al. 2014; Machado et al. 2019; Naranjo et al. 2004), and it should be encouraged as much as possible.

Among the many potential cotton pests in Brazil, the boll weevil, *Anthonomus grandis grandis* (Boh.), is the most damaging species and is the hardest to control. Cotton fields in Brazil cover around 1.4 million hectares each year, with boll weevils causing significant losses and being responsible for most of the pest control expenditures in the crop. Both larvae and adult boll weevils damage the cotton's reproductive structures, i.e., flower buds and bolls (Neves et al. 2013; Showler and Cantu 2005). Adult females oviposit inside cotton fruiting structures but preferentially flower buds. About one week after oviposition, infested flower buds fall to the ground (Coakley et al. 1969), while infested bolls remain on the plants (Neves et al. 2013). When infestations reach the action threshold of 3-5% of the flower buds attacked, insecticide application begins and applications are repeated at 5-day intervals for the following 20-25 days to ensure control of the emerging adults (Bélot et al. 2016; Miranda and Rodrigues 2015). Following this schedule

of applications for boll weevil control is estimated to cost US \$360 per hectare (Bélot et al. 2016), which when added to the yield losses caused by the pest is a serious loss of profits for growers.

In addition to the boll weevil, various sucking and chewing pests attack the roots, leaves, and reproductive structures of cotton, either alone or together (Silvie et al. 2013). Such multi-species infestations usually require applications of broad-spectrum insecticides, such as organophosphates, carbamates, pyrethroids, phenylpyrazoles, or neonicotinoids. Despite being effective against many pest species, these insecticides are often kill important natural enemies found in cotton fields (Barros et al. 2018; Kim et al. 2018; Machado et al. 2019; van Hamburg and Guest 1997), pointing to a need for environmentally safer insecticides. Previous studies have shown that well designed monitoring plans and use of selective insecticides can enhance biological control of cotton pests (Bordini et al. 2021; Machado et al. 2019; Naranjo et al. 2004; Torres and Bueno 2018). As a result of such practices, the number of insecticide applications per cropping season can be reduced, and direct economic and environmental benefits can be achieved. Key to this process is to improve the action of natural enemies as mortality factors of different pest species, especially the boll weevil, a key pest of cotton.

The larvae and pupae of boll weevils inside abscised flower buds can be attacked by epigeal predators such as ants (Fillman et al. 1983) and the ring-legged earwig, *Euborellia annulipes* (Lucas) (Ramalho and Wanderley 1996). In addition to attacking the immature stages of boll weevil, *E. annulipes* also preys on cotton aphids, eggs from various arthropod, mealybugs, and newly hatched caterpillars, among others. This predator lives in the ground and feeds at night (Bharadwaj 1966; Klostermeyer 1942), which reduces its risk of being topically contaminated during insecticide applications. However, while foraging in the plant's canopy for prey at night, earwigs may be exposed to the dried residues of insecticides or eat contaminated prey. From the perspective of conservation biological control, these are potential impacts need to be assessed. With that aim and to help find more environmentally friendly insecticides for use in cotton, we assessed the impact of 12 widely used insecticides on nymphs and adults from two populations of *E. annulipes* when earwigs were exposed through different routes.

We tested the hypothesis that an insecticide's selectivity is determined by several factors acting together. Firstly, insecticides with different modes of action have different impacts on the ring-legged earwig, and these impacts may vary among the predator's life stages, and the particular predator population. Thus, we evaluated the survival of nymphs and adults of two populations of *E. annulipes*, through two methods of exposure (1) consumption of contaminated prey and (2) consumption of contaminated prey, either on inert surfaces or on treated plants bearing natural prey.

Materials And Methods

Insect population sources and rearing methods

Colonies of two populations of *E. annulipes* were established in the Laboratory of Biological Control of the "Universidade Federal Rural de Pernambuco (UFRPE)", Recife, Pernambuco State, Brazil. The first one (hereafter 'AR') was initiated from specimens previously reared at the Entomology Laboratory of the

“Universidade Federal da Paraíba (UFPB)”, Areia, Paraíba, Brazil. This population has been reared in the laboratory for more than 50 generations without exposure to insecticides. The second population (hereafter ‘PD’) was initiated in October 2018 from nymphs and adults collected from an open field located close to a chicken house in Paudalho County, Pernambuco State, Brazil (07° 55′ 44.62” S, 35° 02′ 29.65” W). By the time the experiments were performed, this population was in the 4th–6th laboratory generations without exposure to insecticides. Insect rearing and all experiments were carried out under controlled conditions of 25 ± 1 °C temperature, 12:12 L:D photoperiod, and air relative humidity »60%.

Ring-legged earwig nymphs and adults were reared inside transparent plastic containers (13 × 20 × 7 cm in Ht × L × Wd) covered with a tight lid in which ventilation openings (»4 cm diam) were cut and then covered with fine mesh screen. Because this predator spends the day hidden in the ground, we kept the rearing containers on a shelf covered by a black curtain to avoid direct light over the 12h light period. In addition, 8–10 layers of a double sheet toilet paper were added at the bottom of the containers as a hiding and oviposition substrate. The paper sheets were moistened every two days with tap water and were replaced once a week. Following Silva et al. (2009), the insects were fed *ad libitum* with a dry diet prepared from chicken feed (35%), wheat bran (26%), yeast (22%), powdered milk (15%), and the antimicrobial Nipagin (4%) (Ueno Fine Chemicals Ind, Ueno, Japan). To avoid contact by the diet with the moistened paper sheets, the diet was placed in plastic caps (10 × 3 mm in diam × Ht), and it was replaced whenever necessary.

Each rearing container held 40 adults in a 4♂:1♀ ratio. Female earwigs exhibit parental care; consequently, whenever egg groups were seen, they were transferred individually along with their females to Petri dishes (8 × 1.5 cm in diam × Ht) containing moistened paper sheets. The females were kept in these dishes until the resulting nymphs were 3-days old. After that, the females were returned to their original containers, while the new nymphs were transferred in batches of 50-60 same-age individuals to new rearing containers.

Insecticides

We tested 12 insecticides recommended to control key pests of cotton via a series of applications over the cotton-growing season (Fig. 1S, and Table 1S). We classified the early-season pests as aphid and whiteflies. Mid-season pests were various foliar-feeding caterpillars (Lepidoptera), and the late-season pests were lepidopteran bollworms, the boll weevil, and various stinkbugs. For insecticide-resistance management rotation of materials with different modes of action was taking in consideration. In addition, in selecting pesticides to test in our experiments, we also considered available information about the impact of on other natural enemies (Barros et al. 2018; Crosariol Netto et al. 2014; Kim et al. 2018; Machado et al. 2019).

The insecticides chosen were tested using their commercial formulations and at the maximum field rate (Table 1S) registered in Brazil to be used in cotton (AGROFIT 2021), mixed to tap water in a spray volume of 150 liters per hectare. To facilitate adhesion of the insecticide residues on Petri dishes and plant

surfaces, we added the surfactant Halten (Arysta LifeScience do Brasil, Pirapora, SP) at rate of 0.05% of the solution, which was also used as the control treatment in all experiments.

Exp. #1. Nymphal and adult exposures to dried residues

Application of insecticides was done with an Airbrush set (Paasche Airsbush Co, Harwood Heights, IL, USA) adjusted to 15 lbs/pol (34.47 Pa) to obtain uniform cover of the inside of the Petri dishes (8 × 1.5 cm in diam × Ht) applying 2 mL of the control or insecticide solution. Both lids and bottoms of the Petri dishes were sprayed with the test solutions (1 mL of solution per piece) and left to air-dry for about 2 h inside an exhaust chamber Nalgon 3700 (Nalgon Equipamentos Científicos, Itupeva, São Paulo, Brazil) before placing the test earwigs inside the dishes.

This bioassay followed a factorial randomized design of 2×2×13 treatments: two ring-legged earwig populations (AR and PD), two life stages (nymph and adult), and 13 treatments (12 insecticides and one control treatment) with four replicates per treatment. Each replicate included 10 3rd-instar nymphs (48-72 h since the 2nd-nymphal molt) or 10 adult earwigs (3- to 5-d-old). Dry diet and moistened cotton pads were provided separately in caps of 1.5 mL microcentrifuge tubes to prevent contamination of the food or water supply.

Acute toxicity was determined by recording the number of dead insects 72 h after their introduction into treated dishes. In addition, chronic toxicity was assessed daily by recording the number of dead insects maintained in the same contaminated or control dishes either for 20 days (adults) or, for nymphs, all insects died or molted into adults.

Exp. #2. Ingestion of contaminated prey

The ingestion assay (Exp. #2) was carried out by providing contaminated *Ephestia kuehniella* (Zeller) eggs to nymphs and adult earwigs. First, an aliquot of 500 µL of each control or insecticide solutions was applied on 0.5 g of frozen moth eggs uniformly distributed into clean glass Petri dishes (8.0 cm diam). After air-drying for »2 h inside an exhaust chamber (Nalgon 3700, Nalgon Equipamentos Científicos, Itupeva, São Paulo, Brazil), the treated eggs were carefully dislodged with a soft hairbrush and transferred to caps of microcentrifuge tubes of 1.5 mL. Each cap received 15 mg or 20 mg of treated eggs previously weighed on an electronic balance with 0.0001 g precision (FA-2104N, Bioprecisa, Curitiba, Paraná, Brazil), and these eggs were placed inside clean dishes holding either a single 2-day-old 3rd-instar nymph (15 mg of eggs) or one 3-d-old adult female earwig (20 mg of eggs). To encourage consumption of contaminated moth eggs, both nymphs and adult earwigs were starved for 24 h before testing. After 24 h, the remaining eggs were weighed to estimate food consumption with corrections by discounting the natural weight loss obtained from a sample not offered to the predator and maintained under the same physical conditions. The surviving earwigs were transferred to new Petri dishes along with rearing dry diet. Adult survival was assessed daily for 20 d, while nymphs were observed until they either died or molted to adults.

This bioassay followed a factorial design of 2×2×13 treatments: two ring-legged earwig populations, two life stages, and 13 treatments (12 insecticides and one control treatment) with 20 replicates per treatment. Each replicate was represented by either a single 3rd-instar nymph or one adult female earwig.

Exp. #3. Mixed exposure to dried residues on treated plants and contaminated prey

When ring-legged earwigs forage at night, they may be exposed to insecticide residues on treated plants and may ingest contaminated prey. To simulate those exposures, we evaluated the earwigs' survival and the level of predation they achieve when caged on treated cotton plants bearing moth eggs as prey. Results of Exps. #1 and #2 described previously indicated that the 12 insecticides tested showed different rates of acute toxicity to *E. annulipes*. Thus, for this Exp. #3, we selected five insecticides that caused either low (pymetrozine), intermediate (indoxacarb and thiamethoxam), or high mortality (lambda-cyhalothrin and malathion) to the earwigs. Our findings in Exps. #1 and 2 also showed that, of the two earwig populations tested, the PD population was more susceptible to thiamethoxam and lambda-cyhalothrin insecticides than the AR population, and for that reason, we used the more susceptible PD population in Exp. #3.

Cotton plants (cv. IMA2106 GL) for Exp. #3 were grown in 2 L plastic containers filled with a mixture of soil and humus (3:1). To set up the trial, 25-d-old cotton plants were used, and on each plant we attached a piece of Bristol blue board card (2 × 1 cm in L × Wd) that held 40 mg of *E. kuehniella* eggs. The egg cards were attached with instant glue (Amazonas Professional, Quimican, São Paulo, Brazil) to the underside of the fully upper cotton leaf on each test plant. The 40 mg of treated or untreated (control) eggs were divided into four portions of 10 mg each, with the eggs being attached with 20% Arabic gum diluted into water. The plants with attached prey eggs were treated with the selected insecticides at their maximum field rates (Table 1S) by using a 1.25 L hand-sprayer (Guarany, São Paulo) regulated for a flow pressure of »2.8 Kgf/cm², with spray liquid applied to the drip point.

After insecticide application, the plants were allowed to air dry for »2 h and then were transferred to the laboratory. To facilitate the observations of the earwigs, a layer (»1 cm thick) of light colored, moist sand was applied over the soil in each pot. One adult earwig female was then released on the sand layer, and the cotton plant was covered with a 2.7 L transparent plastic pot cage (20.5 × 30 cm in diam × Ht) with two side vents (»16 cm² each) cut into the cage and sealed with fine mesh screening to allow air exchange.

Exp. #3 comprised six treatments (5 insecticides and one control treatment) with 19 to 23 replicates of each. Each cage was a replicate, each containing one female adult earwig and a treated cotton plant bearing moth eggs as prey. To encourage prey consumption, adult earwigs were starved for 24 h before testing. The predation rate was rated by the consumed egg (each portion = 25% of total) and it was recorded 24, 48, 72, 96, and 120 h after releasing the earwigs into the cages. This consumption rate measured over time also allowed the estimation of the percentage of earwigs that foraged in the plant

canopy in each evaluation interval. The earwig survival was recorded in the last evaluation time (120 h), when the cages were removed and the sand layer was carefully inspected for dead earwigs.

Statistical analysis

The percentage data on earwig survival from all experiments, as well as the time required by nymphs to complete development after exposure to dried residues on inert surfaces or ingestion of contaminated prey were checked for normality (Shapiro-Wilk's test, PROC UNIVARIATE) and homoscedasticity (Bartlett's test, PROC GLM). Survival data required $\arcsin \sqrt{x/100}$ transformed to fit the assumptions of ANOVA.

Data from bioassays with earwig exposure to dried residues on inert surfaces or via ingestion of contaminated prey were subjected to three-way ANOVA (PROC GLM, SAS Institute, 2002). Survival means were separated by the Scott-Knott's test ($\alpha = 0.05$) using SISVAR statistical package, but means of nymphal developmental times were separated by Tukey's HSD test ($\alpha = 0.007$ after Bonferroni correction for $\alpha/\text{number of means in comparisons}$) across all insecticides. Comparisons of means between earwig populations and predator life stages were made with Student t-tests (PROC TTEST, SAS Institute, 2002). Furthermore, to assess the chronic impact of tested insecticides, survival curves of nymphs and adult earwigs from each population were calculated by the Kaplan-Meier Survival Analysis followed by a pairwise comparison using the Log-Rank test ($\alpha = 0.05$) performed with SigmaPlot 12.5 (Systat Software Inc., 2013). We also tested the hypothesis of differential insecticide susceptibility between the two earwig populations studied. For survival means, statistical significance was determined by the non-overlap of the means' 95% confidence intervals (Di Stefano 2005). The null hypothesis of no difference in susceptibility was taken to be 95% CI survival bars that included the zero value (i.e., cross the axis).

Survival data from the mixed exposure bioassay were subjected to one-way ANOVA, with means separated by Tukey HSD's test ($\alpha = 0.008$ after Bonferroni correction $\alpha/\text{no. of means}$). , predation rate data were subjected to one-way repeated measures ANOVA (PROC GLM, SAS Institute, 2002) for six treatments (five insecticides plus control) and the first and last evaluation intervals (24 h and 120 h after caging the female earwigs). Thereafter, means were separated by Tukey HSD test (SAS Institute 2002).

Results

Across all bioassays carried out, both earwig populations and life stages exhibited few cases of differences in the susceptibility to the tested insecticides (Fig. 1). Adults from the AR population were less susceptible than the PD population when exposed to lambda-cyhalothrin or thiamethoxam through either dried residues or ingestion of contaminated prey. However, indoxacarb dried residues allowed greater adult survival for the PD population than the AR population, whereas nymphs from the AR population survived better than nymphs from PD population when exposed to indoxacarb.

Exp. #1. Nymphal and adult exposures to dried residues

The three-way ANOVA for survival of the ring-legged earwig exposed to dried-residues of insecticides testing for predator population ($F_{1,156} = 2.90, P = 0.0901$), predator life stage ($F_{1,156} = 2.2, P = 0.1359$), and treatments (insecticides and control), resulted in significant effect only for insecticide treatments ($F_{12,156} = 431.90, P < 0.0001$), among these main sources of variation. The average survival was 54.4% and 52.3% for individuals from populations AR and PD, respectively. Likewise, the survival of nymphs and adults was 54.3% and 52.4%, respectively, irrespective of population and insecticides. Across the two- and three-factor interactions, only those interactions involving the insecticide treatments were significant: predator population and treatments ($F_{12,156} = 5.10, P < 0.0001$), predator life stage and treatments ($F_{12,156} = 8.51, P < 0.0001$), predator life stage, treatment, and population ($F_{12,156} = 7.90, P < 0.0001$). Based on that, the three-way statistical model was reduced to a one-way model considering only the treatments as a source of variation, thus enhancing the degrees of freedom of the error. The insecticides significantly affected the earwig survival ($F_{12,195} = 185.06, P < 0.0001$; Fig. 2), and hence we grouped them with respect to their toxicity levels. Pyriproxyfen, pymetrozine, chlorantraniliprole, cyantraniliprole, and spinetoram were classified as low-impact, allowing predator survival of >90%, which was similar to control. In contrast, chlorfenapyr, chlorpyrifos, dimethoate, and malathion caused high mortality of the earwig (survival <3%), and these products were classified as a high-impact group. The remaining insecticides, i.e., thiamethoxam, indoxacarb and lambda-cyhalothrin, allowed 20–60% earwig survival and were classified as of intermediate impact.

The chronic toxicity evaluation (survival or molting into adult across 20 d exposure) in Exp. #1 showed different results (Fig. 3). Over 70% of adult earwigs from both AR and PD populations survived exposure to chlorantraniliprole, pymetrozine, spinetoram, and pyriproxyfen residues, which was significantly greater than the ~27% survival after exposure to cyantraniliprole (Fig. 2). The remaining insecticides caused 100% mortality of both earwig populations. With respect to nymphs, over 78% of both populations exposed to pymetrozine or spinetoram residues and the nymphs of the PD population exposed to chlorantraniliprole, successfully molted into adults within 30–40 d (Fig. 3). Survival of nymphs in these treatments was similar to the control treatment and significantly greater than the rate observed for the AR population exposed to chlorantraniliprole (Fig. 3). Regardless of the population, nymphs exposed to pyriproxyfen did not reach adulthood, although they lived for up to 80 d. All remaining insecticides caused 100% nymphal mortality.

Within treatments that allowed survival of nymphs until the adult molt, the time required to complete nymphal stage varied by earwig population ($F_{1,221} = 30.0, P < 0.0001$), insecticides ($F_{1,221} = 12.0, P < 0.0001$), and interaction of these factors ($F_{3,221} = 11.7, P < 0.0001$). Nymphs from the PD population exposed to spinetoram or pymetrozine residues delayed their development about 2 d in comparison to nymphs from the AR population (Table 1). Furthermore, nymphs from the AR population exposed to chlorantraniliprole residues delayed their development in comparison to nymphs exposed to pymetrozine and spinetoram residues (Table 1).

Exp. #2. Ingestion of contaminated prey

Ring-legged earwigs from the AR and PD populations consumed similar quantities (average = 5.6 and 5.4 mg, respectively) of contaminated prey during a 24 h confinement period ($F_{1,985} = 0.9$, $P = 0.3402$), with lack of significant effect predator population and treatment interaction ($F_{12,985} = 1.6$, $P = 0.084$). However, contaminated prey consumption was significantly affected by earwig life stage ($F_{1,985} = 474.9$, $P < 0.00001$), treatments ($F_{1,985} = 43.4$, $P < 0.0001$), and by the interactions of predator population and treatments ($F_{1,985} = 159.9$, $P < 0.0001$), life stage and treatments ($F_{1,985} = 159.9$, $P < 0.0001$), and predator population, life stage and treatments ($F_{12,985} = 2.0$, $P = 0.016$). Prey consumption by nymphs was similar to control values for eggs treated with chlorfenapyr, spinetoram, lambda-cyhalothrin, pyriproxyfen, indoxacarb, and chlorantraniliprole, but was reduced for prey treated with chlorpyrifos, pymetrozine, thiamethoxam, cyantraniliprole, malathion, and dimethoate (Fig. 4 - nymphs). For adult earwigs, the highest and the lowest prey consumption rates were observed in the spinetoram and thiamethoxam treatments, respectively, with the other compounds resulted in intermediate consumption levels (Fig. 4 – adults).

The developmental time (days to molt into adult) of 3rd-instar nymphs preying on contaminated prey was affected by earwig population ($F_{1,166} = 222.7$, $P < 0.0001$), insecticide treatments ($F_{5,166} = 38.1$, $P < 0.0001$), and the interaction of these factors ($F_{5,166} = 23.2$, $P < 0.0001$). Nymphs from the PD population that consumed prey treated with chlorantraniliprole, cyantraniliprole, lambda-cyhalothrin, pymetrozine, or those eating the untreated (control treatment) took longer to reach adulthood compared to those from AR population in these same treatments (Table 1). Among the insecticides that allowed the PD population nymphs to molt into adults, indoxacarb and lambda-cyhalothrin caused developmental delays (Table 1). In contrast, nymphs from the AR population were most affected by eating prey contaminated with cyantraniliprole, chlorfenapyr, and spinetoram (Table 1).

Survival of nymphs (that molted into adults) and of adult earwigs did not vary as a function of the population. However, survival of nymphs after the consumption of contaminated prey was affected by life stage, insecticide treatment, and the interaction of these factors. Consumption of prey contaminated with pyriproxyfen, thiamethoxam, chlorpyrifos, dimethoate, or malathion caused $\geq 97\%$ mortality to nymphs irrespective of population. The same high level of mortality was also observed for the PD population after eating chlorfenapyr-contaminated prey and for the AR population after eating indoxacarb-contaminated prey (Table 1, Fig. 5). Nevertheless, the lowest level of nymphal mortality was observed with pymetrozine and spinetoram, which were similar to controls (Fig. 5). Furthermore, nymphs of the PD population were more affected than nymphs of the AR population following consumption by lambda-cyhalothrin, chlorfenapyr, and cyantraniliprole-contaminated prey. Yet, nymphs of the AR population suffered high mortality from eating prey contaminated with cyantraniliprole (Fig. 5).

Mortality of adult earwigs from the PD population, when followed for 20 days was 20% for pymetrozine, spinetoram, pyriproxyfen, chlorantraniliprole, lambda-cyhalothrin, and the control, whereas mortality were 100% for malathion, chlorpyrifos, dimethoate, cyantraniliprole, and thiamethoxam. For indoxacarb and chlorfenapyr, mortality was intermediate (Fig. 6). Similarly, for the AR population, there was $< 20\%$

mortality following exposure to spinetoram or the control, but exposure to malathion, chlorpyrifos, dimethoate, or indoxacarb caused 100% mortality, with the remaining insecticides causing intermediate levels of mortality (Fig. 6).

Exp. #3. Mixed exposure to dried residues on treated plants and contaminated prey

The survival of adult females of *E. annulipes* that were simultaneously exposed to treated cotton plants and contaminated prey for five days was significantly affected by the insecticide treatments ($F_{5,128} = 32.26$, $P < 0.0001$, Fig. 6). The greatest and the lowest survival were observed in the thiamethoxam and malathion treatments, respectively, whereas the control, pymetrozine, lambda-cyhalothrin, and indoxacarb showed intermediate rates of survival.

Repeated measures ANOVAs detected an effect of time on the level of prey consumption (24 h vs. 120 h) (Wilks' lambda = 0.71, $F = 40.53$, $P < 0.0001$, $DF_{num} = 1, den = 100$), the insecticide treatments [$F_{5,100} = 8.20$, $P < 0.0001$ (24 h); and $F_{5,100} = 2.49$, $P = 0.006$ (120 h)], and the interaction of these factors (Wilks' lambda = 0.72, $F = 7.90$, $P < 0.0001$, $DF_{num} = 5, den = 100$). In the 24h-evaluation period, >85% of the predators in the control and indoxacarb treatments had foraged on the plant canopy and consumed ca. 82% of the available prey, which was significantly greater than that observed in the thiamethoxam, pymetrozine, and malathion treatments, followed by lambda-cyhalothrin, for which the percentage of predators foraging on the plant was lowest (Fig. 7). By the last 120h-evaluation, the prey consumption level and the percentage of predators that foraged on the plants for the indoxacarb and malathion treatments were not different that the first assessment, as mentioned before. Nevertheless, a significant increase of the percentage of predators that foraged on the plants and consumed prey was observed over the time in the pymetrozine and lambda-cyhalothrin treatments. For thiamethoxan, the increase in these parameters over the trial was very slight.

Discussion

Knowledge of the relative susceptibility of particular natural enemies to specific insecticides, across classes with different modes of action, is useful in guiding growers to use more environmentally safe materials. This information allows extension personnel to recommend products with selectivity that will protect natural enemies but also allows growers to pick materials that will aid in avoiding development of insecticide-resistant by the pest species. Our results showed that the susceptibility of the ring-legged earwig, *E. annulipes*, differs among insecticides that are recommended against various sap-sucking and chewing cotton pest species. Overall, neurotoxic insecticides were highly toxic to the predator, but insecticides that target the allosteric modulator nicotinic receptor (i.e., spinetoram) or the chordotonal organ channel modulator (i.e., pymetrozine) should have low to moderate impact of the predator's survival based on our findings. The highly toxic insecticides in the group of materials we tested included acetylcholinesterase inhibitors (malathion, chlorpyrifos, and dimethoate), a nicotinic acetylcholine receptor competitive modulator (thiamethoxam), a voltage-dependent sodium channel blocker (indoxacarb), a sodium channel modulator (lambda-cyhalothrin), and an oxidative phosphorylation

inhibitor (chlorfenapyr). In contrast, the juvenile hormone mimic (pyriproxyfen), along with the ryanodine receptor modulators (chlorantraniliprole and cyantraniliprole) generally had low impact on earwig survival, although the level did vary with life stage and the route of chemical exposure.

The relative harmlessness of spinetoram and pymetrozine to both nymphs and adults of the ring-legged earwig may reflect some level of physiological selectivity. Spinetoram is a semi-synthetic spinosyn that causes hyperexcitation and insect paralysis due to muscle fatigue (Sparks et al. 2001). In contrast to our results with earwigs, spinetoram was reported to be highly toxic to adults of *Chrysoperla johnsoni* Henry and *Chrysoperla carnea* (Stephens) (Amarasekare and Shearer 2013), as well as to *Orius armatus* (Gross) and *Orius laevigatus* Fieber (Broughton et al. 2014; Kim et al. 2018). Spinetoram, on the other hand, was harmless to the lady beetle *Eriopis connexa* (Germar) at the recommended field rate for cotton (Costa et al. 2020). With respect to pymetrozine, it affects the chordotonal organ channels, blocking feeding by sap-sucking insects and resulting in starvation (Kristinsson et al. 1994). In our trial, this insecticide initially seemed to affect the earwigs' prey consumption, but this effect became less evident over time and exposure did not affect *E. annulipes*' survival. Similar findings have been reported for various other predators as well (Barros et al. 2018; Machado et al. 2019; Torres et al. 2003; Talebi et al. 2008), reinforcing the idea that pymetrozine is a selective insecticide useful in IPM programs.

With some exceptions, neurotoxic insecticides are non-selective (Barbosa et al. 2018; Barros et al. 2018; Bozsik 2006; Campos et al. 2011; Machado et al. 2019; Mills et al. 2016; Torres et al. 2003), which agrees with our findings for malathion, chlorpyrifos, and dimethoate. These compounds caused high mortality of *E. annulipes* irrespective of the population, life stage, or route of contamination. The results for malathion of our mixed exposure experiment showed that most earwigs moved around on treated plants and consumed contaminated prey, allowing only a few survivors that escaped contact with the insecticide. In contrast, most female earwigs survived exposure to treated plants and ingestion of contaminated prey for lambda-cyhalothrin, thiamethoxam, and indoxacarb. The reduced impact of these non-selective insecticides under these conditions indicates that some insecticides that are physiologically non-selective may become compatible with natural enemies depending on the predator developmental stage and its behavior. Under field conditions, predators may avoid contact with insecticides contact through various types of ecological selectivity or behaviors. Ring-legged earwigs build nests and forage primarily on the ground (Klostermeyer 1942), both of which are behaviors considered important for a natural enemy of the immature stages of boll weevil, which are found inside fallen, infested flower buds. Even when the earwigs are on the plant canopy, they gain partial protection by hiding on the buds and bolls, which are enclosed by the bracts, while they hunt for prey in the night. These results highlight the need to consider not only an insecticide's mode of action, but also the natural enemy's foraging behaviors when estimating the insecticide's likely degree of selectivity. Such influences of foraging patterns are one reason why laboratory assays designed to ensure maximum exposure of a predator to insecticide residues may not reflect the field outcome (El-Wakeil et al. 2013).

As in the present study, other studies have found chlorfenapyr to be toxic to various predator species (Barros et al. 2018; Elzen 2001; Kim et al. 2018), including another earwig species (Campos et al. 2011;

Redoan et al. 2013). Chlorfenapyr kills by disrupting the proton gradient across mitochondrial membranes, which impairs ATP production and leads to death (Yu 2014). Based on our test results, it seems that the contamination route may be important given that *E. annulipes* survived better when it consumed chlorfenapyr-contaminated prey than when it contacted dried residues. Similar results were reported for *Dorus luteipes* (Scudder) preying upon treated eggs of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Redoan et al. 2013). Detoxification by salivary and digestive enzymes following ingesting (but not after dermal exposure) may explain this outcome (Yu 2004).

Pyriproxyfen, regardless of the earwig population or the route of exposure, showed compatibility with adult ring-legged earwigs, as in previous studies (Machado et al. 2019; Medina et al. 2003). However, this juvenile hormone agonist prevented earwig nymphs from becoming adults, as has been observed for nymphs (Ishaaya and Horowitz 1995) and larvae (Barbosa et al. 2018). Many affected species experience increased stage duration (slow development) or extra instars (Vennard et al. 1998). The ring-legged earwig nymphs exposed to pyriproxyfen lived for more than twice as long as control nymphs, but failed to molt into adults. Because immature natural enemies exposed to pyriproxyfen rarely reach the adult stage, this insecticide cannot be considered as environmentally safe from the perspective of long-term conservation biological control.

The diamide pesticides we tested – chlorantraniliprole and cyantraniliprole – had little effect on *E. annulipes*, which is consistent with the perception that pesticides in this group mainly affect caterpillars and sap-sucking species (Barry et al. 2014). Although chlorantraniliprole did not cause significant mortality of the ring-legged earwig, it reduced prey consumption by adults. According to Smagghe et al. (2013), workers of *Bombus terrestris* (L.) also exhibited reduced pollen consumption and lethargic behavior when fed pollen contaminated by chlorantraniliprole. Dinter et al. (2010) found similar effects on *B. terrestris* and *Apis mellifera* L., but these species' behaviors returned to normal 48 and 72 h, respectively, after pesticide contact ended. It therefore seems likely that chlorantraniliprole caused lethargy in *E. annulipes*, reducing prey consumption but not survival. In contrast to chlorantraniliprole, cyantraniliprole caused significant mortality of ring-legged earwigs, specially nymphs. This diamide has a structural modification caused by the presence of chlorine in the 5th position of the phenyl moiety in the cyano group (Hughes et al. 2004), which appears to expand its activity against several additional pest species (Selby et al. 2013). Our results corroborate the lower compatibility of cyantraniliprole with natural enemies compared to other diamides (Amarasekare and Shearer 2013; Jiang et al. 2019; Machado et al. 2019; Mills et al. 2016). However, cyantraniliprole's effect on *E. annulipes* varied with the life stage, route of exposure and, in a lesser extent, population.

Overall, the differences in the survival found in the present study were due to the earwigs' life stage and route of exposure, and in minor extension to the population tested. Natural enemy populations may exhibit different survival response to insecticides leading a misinterpretation regarding the selectivity when the tested insects have evolved resistance (Barbosa et al. 2016; Costa et al. 2018; Luna et al. 2018). Overall, the long lab-rearing colony AR and the recently field-collected PD populations exhibited minor differences generating robustness to our data. In respect to earwig life stage differential response,

generally, adult insects survived better than immatures, and exposure to dried residues was more harmful than consumption of contaminated prey. For example, >30% earwig nymphs fed on cyantraniliprole contaminated prey reached the adult stage, but none survived if exposure to dried residues. On the other hand, adults exposed to cyantraniliprole-dried residues and contaminated prey exhibited 28 and 45% survival across 20 days, respectively. Only three of 12 insecticides presented as dried residues, and six as contaminated prey allowed nymphs to survive to the adult stage. This outcome may be explained by the morphology of the earwig's integument and composition. Further, lipophilic nature of the insect integument limits the penetration of many insecticides into the body (Winteringham 1969). Our results show that studies testing only a single life stage or a single route of contamination may not produce a proper comprehension of the selectivity of insecticides.

In conclusion, our results showed that regardless of the route of contamination, the life stage and population, the ring-legged earwig would not be affected by pymetrozine, chlorantraniliprole, or spinetoram, and hence these insecticides can be safely recommended against cotton pests because they are compatible with the conservation of earwigs and likely other natural enemies. Furthermore, the insecticide cyantraniliprole may be safely used on cotton IPM depending on the life stage of the predator and on the route of contamination, while pyriproxyfen is safe for adults only. The insecticides chlorfenapyr, indoxacarb, and lambda-cyhalothrin exhibited moderate impact when earwig females were allowed to nest on the ground, but chlorpyrifos, dimethoate, and malathion were harmful for *E. annulipes*, regardless of any of the studied factors.

Despite the relevance of these results to cotton IPM, other insecticides are also available for use in cotton that were not tested (Fig. 1S). Insecticides belonging to the same chemical group and sharing a similar mode of action may cause a similar level of toxicity to the tested predator, but this could vary. Among insecticides that we did not test, data would be particularly useful for ethiprole, diafenthiuron, spiromesifen, and sulfoxaflor because they exhibit different modes of action from the insecticides tested here. Also, these compounds are often used to control different pest species that are not controlled by Bt-cotton, such as whitefly and aphids (sulfoxaflor), whitefly and mites (spiromesifen), whitefly, aphids and mites (diafenthiuron), and boll weevil (ethiprole). Third, these compounds have been classified as harmless to moderately harmful for key natural enemies of cotton pests (Barbosa et al. 2017; Colares et al. 2017; Singh et al. 2016; J.B. Torres, unpublished).

Declarations

Acknowledgements

The authors acknowledge the "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) as source of research funding (No. 420815/2018-0) and the research grant No. 303445/2020-3 for JBT, and to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) through the program CAPES PROEX-PPGEA. Also, to Roy Van Driesche, from University of Massachusetts, we are

thankful for corrections and suggestions addressed on an earlier draft of this manuscript improving it deeply.

Author contribution

DMP: Investigation, analysis, writing. **AVAM:** Investigation, writing. **PRRB:** Conceptualization, writing. **JBT:** Conceptualization, investigation, writing, funding acquisition. All authors read, corrected, and approved the final version.

Compliance with Ethical Standards

Conflict of Interest:

The authors declare they have no conflicts of interest.

Ethical approval:

This article does not contain any studies with human participants performed by any of the authors.

Informed consent

The authors recognize that the paper is being submitted for publication in the *Ecotoxicology*, and assure that this paper has not been submitted, accepted, or published in another journal.

Funding:

The authors declare they have no competing financial interests.

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Table

Table 1 Time (mean \pm SE) (days) required by two populations (PD and AR) of third instar ring-legged earwig nymphs, *Euborellia annulipes*, to complete development after exposure to insecticide dried residues on inert surfaces or fed contaminated moth eggs for 24 h. (Values in parentheses are sample sizes, as numbers of individuals tested).

Insecticides ¹	Insecticide dried residues		Ingestion of contaminated prey	
	Paudalho (PD) (n = 40)	Areia (AR) (n = 40)	Paudalho (PD) (n = 20)	Areia (AR) (n = 20)
Chlorantraniliprole	34.7 ± 0.27 a (22)	35.1 ± 0.56 a (30)	34.6 ± 0.46 b* (17)	32.1 ± 0.22 b (15)
Chlorfenapyr	- ²	-	-	34.2 ± 0.46 a (9)
Control	34.5 ± 0.25 a (32)	34.5 ± 0.08 ab (36)	33.0 ± 0.40 b* (19)	30.1 ± 0.20 cd (17)
Cyantraniliprole	-	-	35.3 ± 0.76 b (6)	34.3 ± 0.33 a (6)
Indoxacarb	-	-	41.8 ± 0.99 a (11) ²	-
Lambda-cyhalothrin	-	-	40.3 ± 0.75 a* (12)	31.6 ± 0.26 bc (10)
Pymetrozine	35.5 ± 0.25 a* (27)	33.8 ± 0.32 b (35)	33.8 ± 0.39 b* (19)	29.5 ± 0.15 d (19)
Spinetoram	34.6 ± 0.08 a* (32)	32.1 ± 0.29 c (15)	33.7 ± 0.34 b (19)	33.0 ± 0.27 ab (19)
Pyriproxyfen, thiamethoxam, chlorpyrifos, dimethoate, malathion ^b				

¹ Means followed by different letters were significantly different within the column (Tukey HSD, $\alpha = 0.05$ or $\alpha = 0.007$); and (*) indicates difference between populations (t-test, $\alpha = 0.05$) exposed to the same route of contamination; and ²(-) indicates insufficient data for analysis ($n < 3$).

Figures

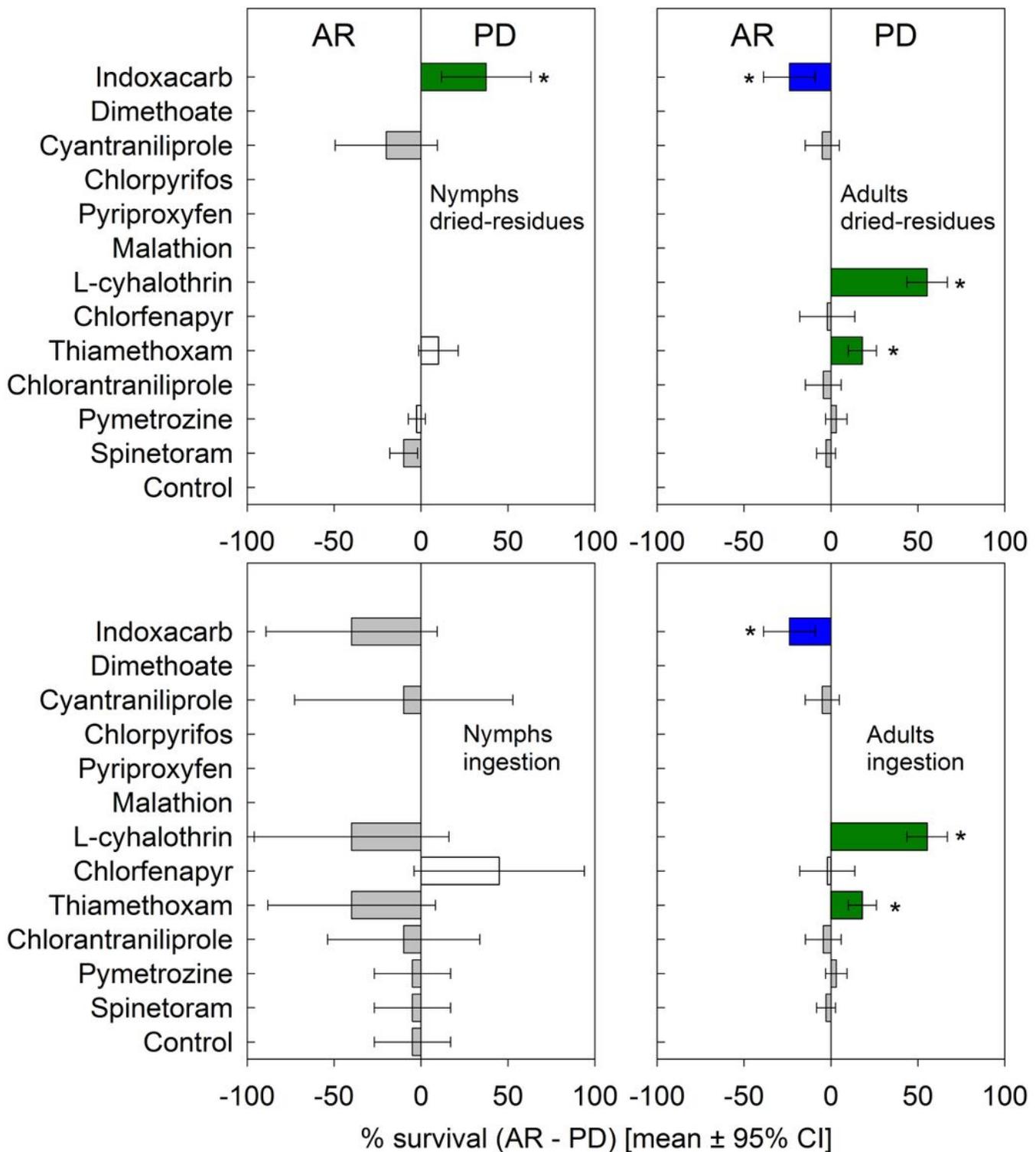


Figure 1

Survival (percentage + SE) of pooled data for nymphs and adults and two populations of ring-legged earwigs, *Euborellia annulipes*, 72 h after exposure to dried-residues of 12 insecticides recommended for cotton pest control (lack of life stage and population effect through three-way ANOVA). Bars bearing different letters indicate survival difference by Scott-Knott's test ($\alpha = 0.05$).

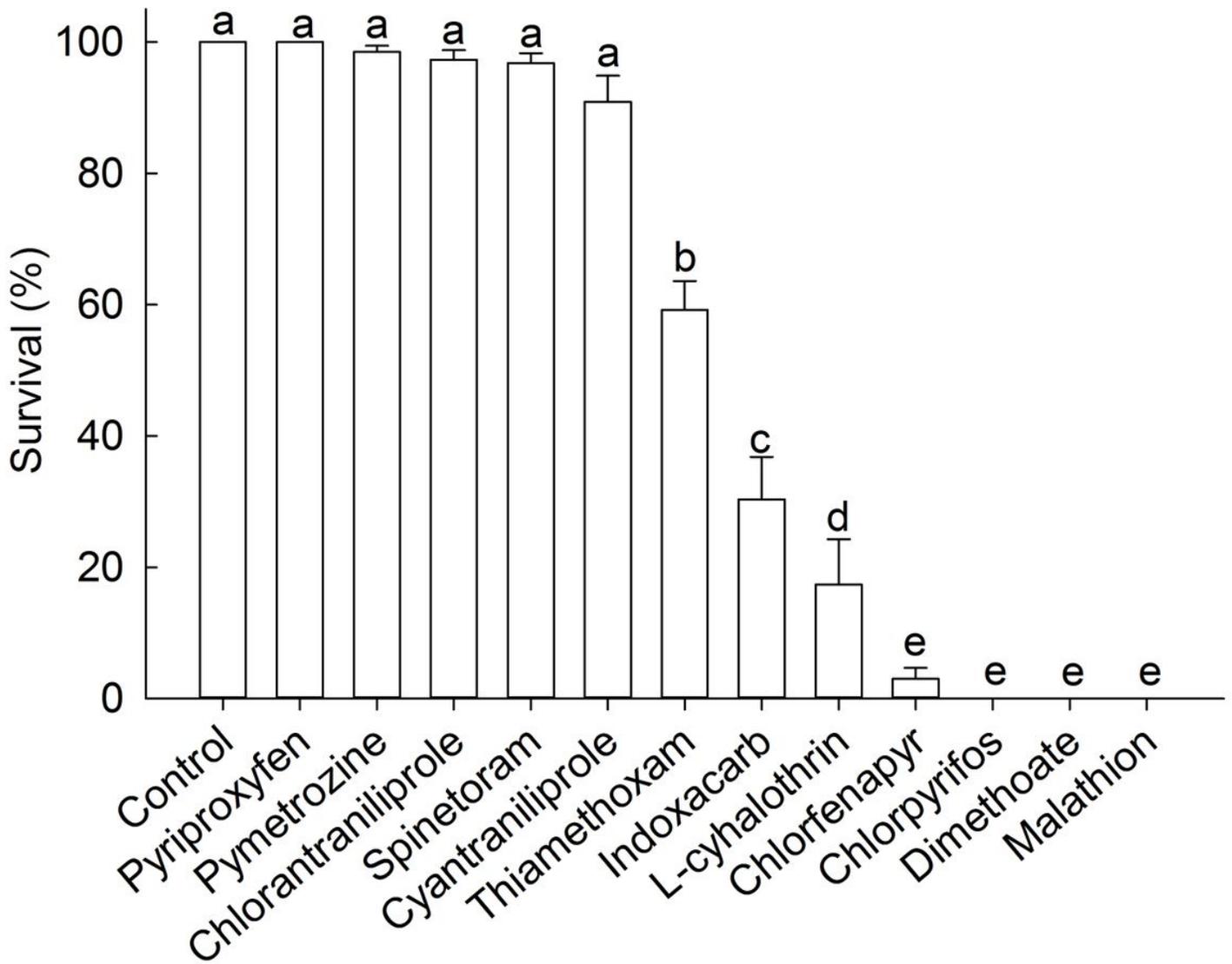


Figure 2

Survival curves of nymphs and adults of ring-legged earwigs, *Euborellia annulipes*, from two populations [Paudalho (PD) and Areia (AR)] exposed to dried residues of 12 insecticides recommended for cotton pest control. PD-nymphs: $\chi^2 = 768.51$, $P < 0.0001$; PD-adults: $\chi^2 = 553.05$, $P < 0.0001$; AR-nymphs: $\chi^2 = 641.59$, $P < 0.0001$; and AR-adults: $\chi^2 = 706.39$, $P < 0.0001$. Lines followed by similar letters indicate similar survival curves by Log-Rank's test pairwise comparisons ($\alpha = 0.05$).

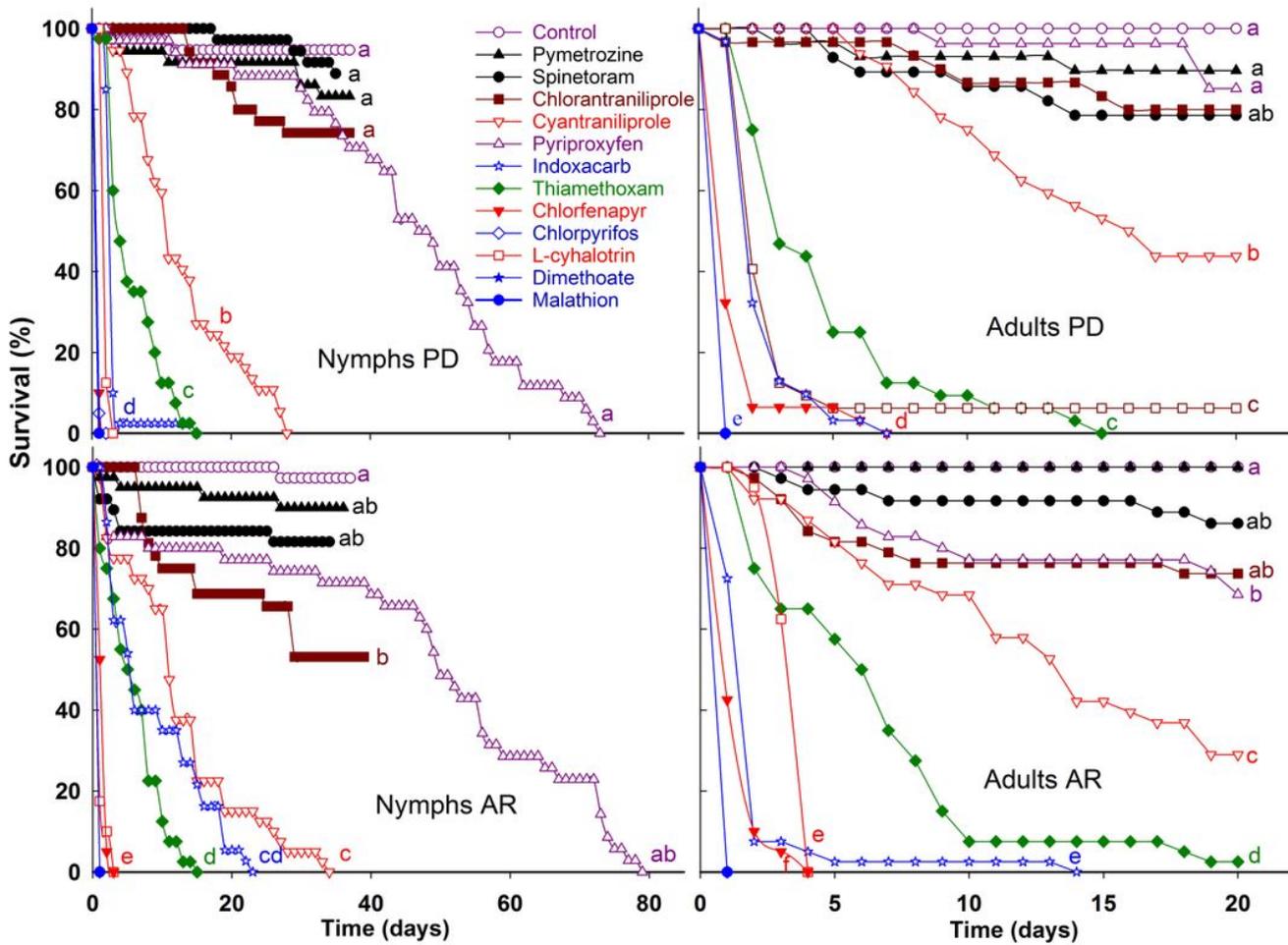


Figure 3

Consumption [mean + SE (mg)] of prey (*Ephestia kuehniella* eggs) treated with different insecticides by third instar nymphs (top) and adults (bottom) of *Euborellia annulipes* during 24 h. Lower case letters on the right side in each figure indicate similar treatment mean groups by Scott-Knott's test ($\alpha = 0.05$), and bars bearing (ns) indicates lack of difference between nymphs and adults of ring-legged earwigs by t-test ($\alpha = 0.05$) for the specific insecticide.

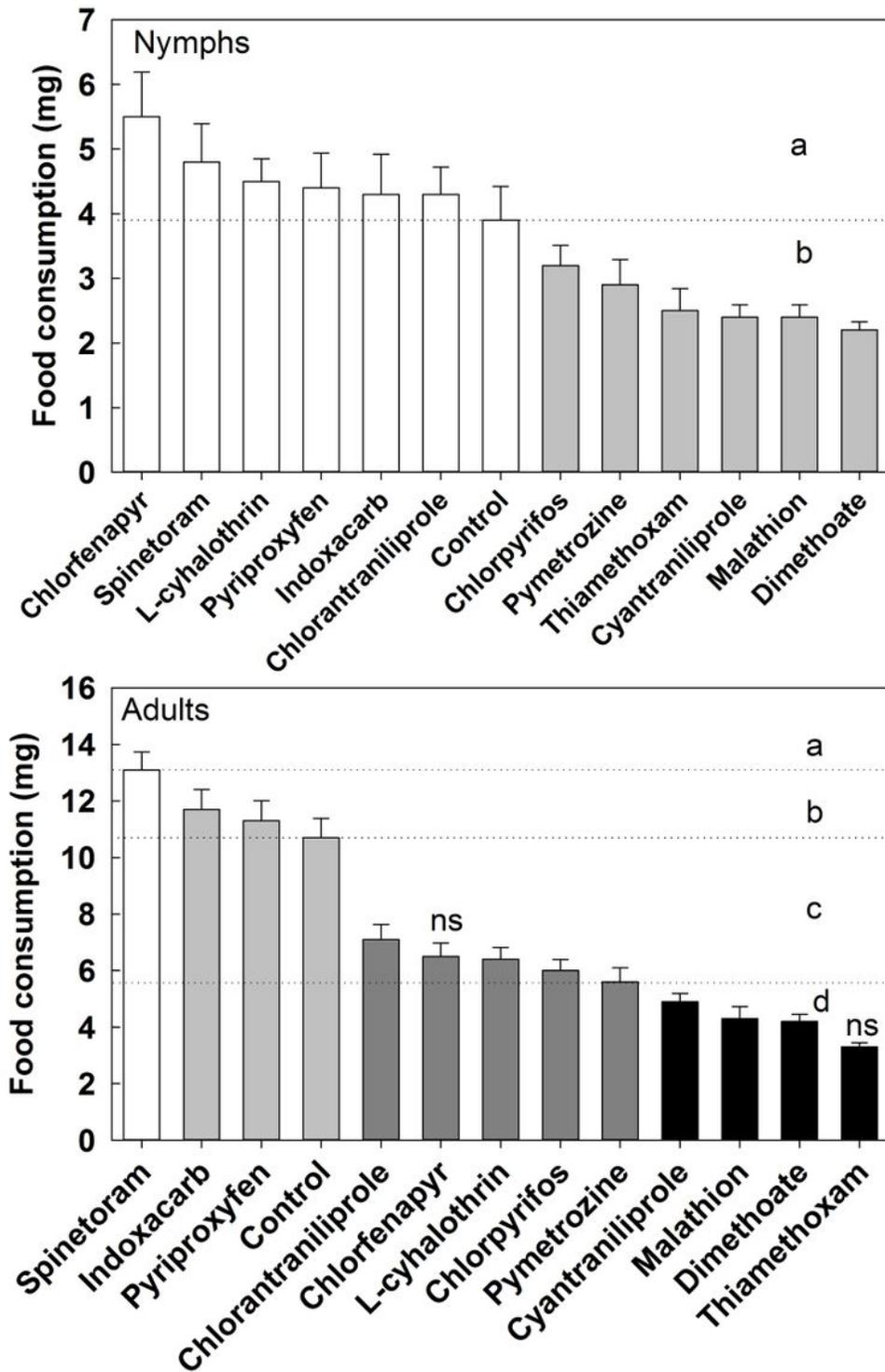


Figure 4

Survival curves of nymphs and adults of ring-legged earwigs, *Euborellia annulipes*, from two populations [Paudalho (PD) and Areia (AR)] after exposure to contaminated prey treated with 12 insecticides recommended for cotton pest control. PD-nymphs: $\chi^2 = 349.38$, $P < 0.0001$; PD-adults: $\chi^2 = 285.26$, $P < 0.0001$; AR-nymphs: $\chi^2 = 388.01$, $P < 0.0001$; and AR-adults: $\chi^2 = 364.27$, $P < 0.0001$. Lines followed by similar letters indicate similar survival curves by Log-Rank's test pairwise comparisons ($\alpha = 0.05$).

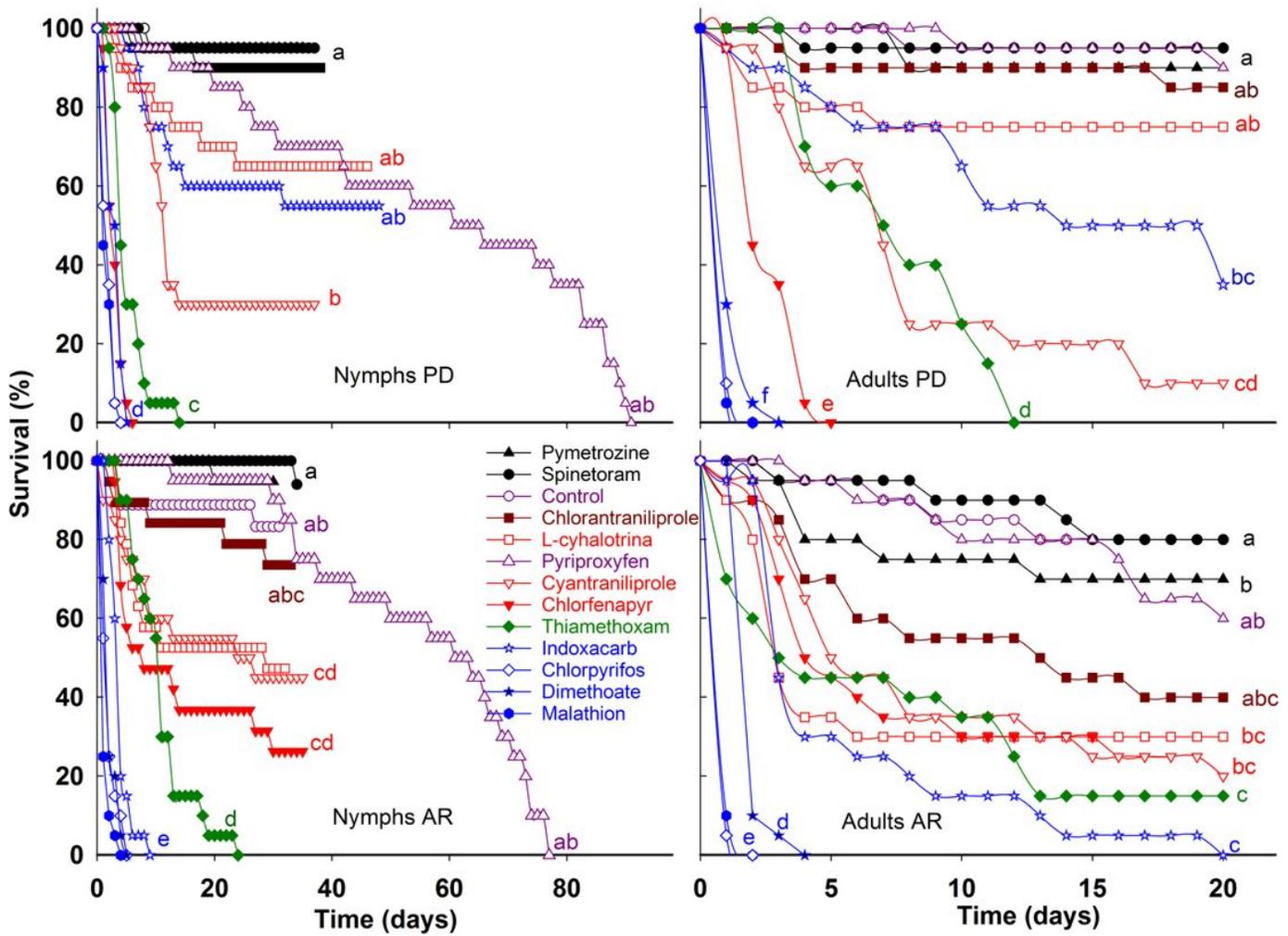


Figure 5

Survival (percentage + SE) of female *Euborellia annulipes* simultaneously exposed to treated cotton plants and contaminated prey. Bars bearing different letters indicate difference in survival means by Tukey HSD's test ($\alpha = 0.008$).

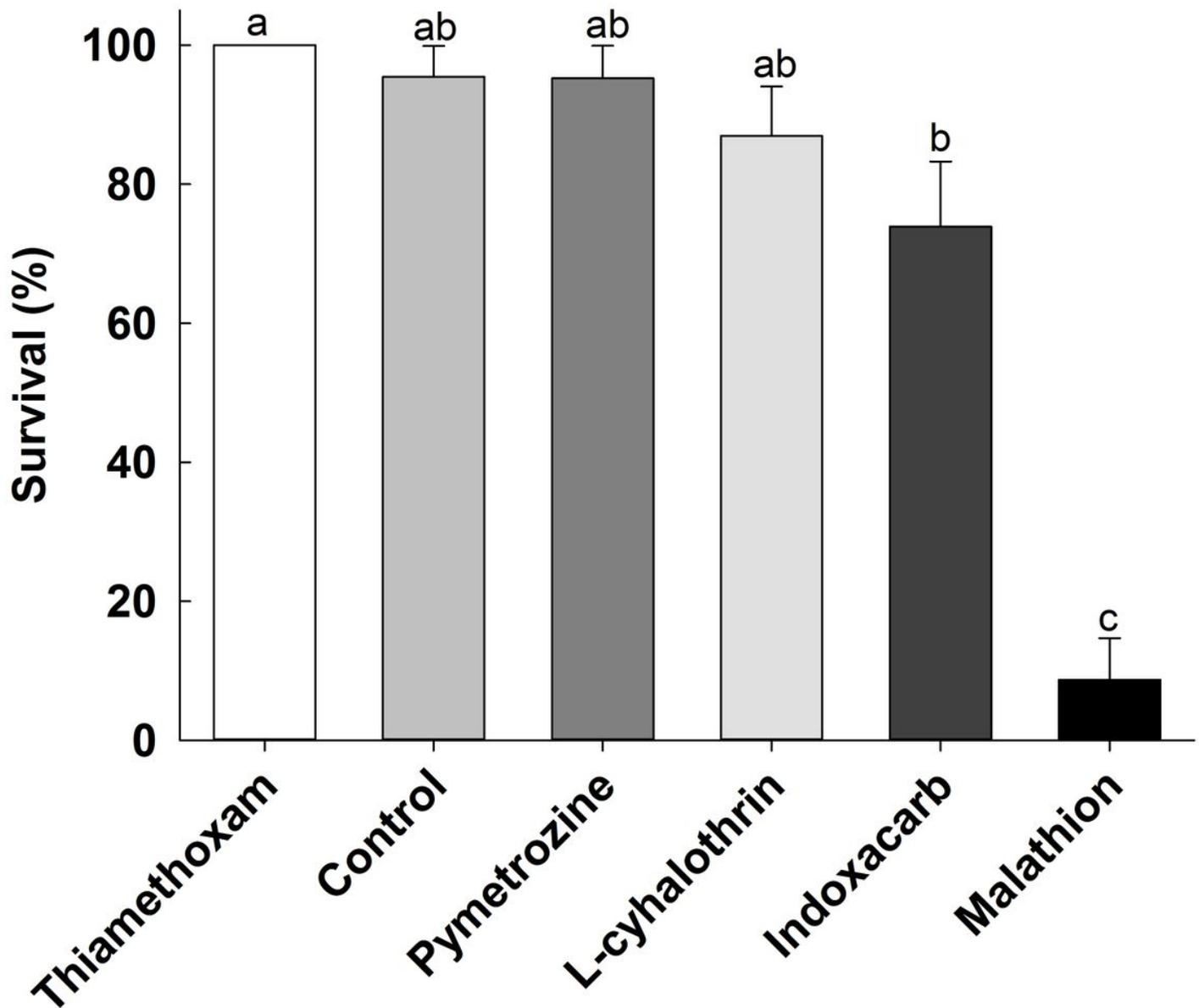


Figure 6

Percentage of contaminated and uncontaminated prey consumed by female adults of ring-legged earwig, *Euborellia annulipes*, from the PD population caged with treated cotton plants. Mean symbols with different letters indicate differences in prey consumption across treatments within each evaluation interval (24 h or 120 h), while (*) indicates significant differences for each treatment between the evaluation intervals (24 h vs 120 h) by Tukey HSD's test ($\alpha = 0.008$). Values between parentheses stand for the percentage of predators foraging on the plant canopy at each evaluation interval.

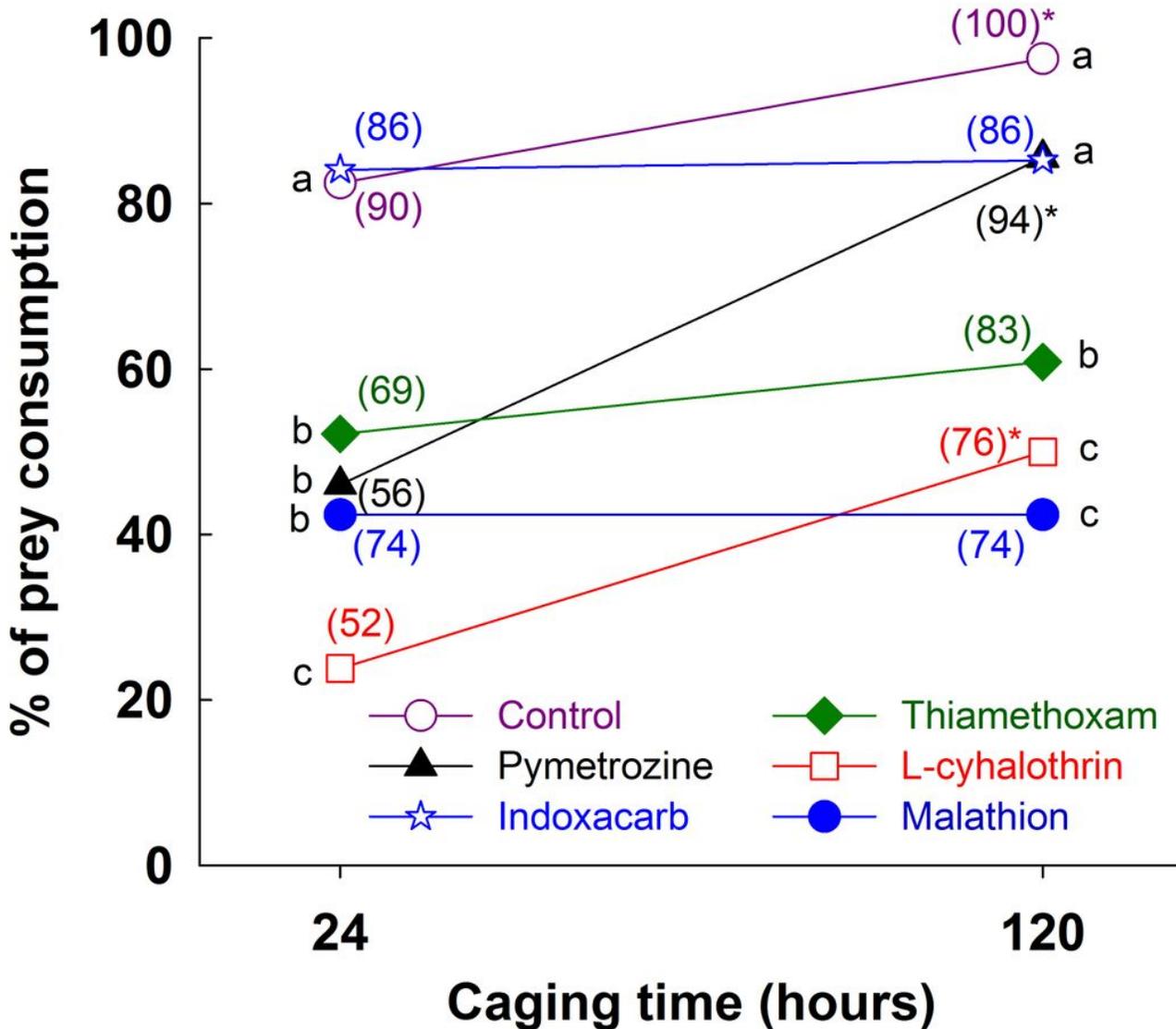


Figure 7

Magnitude of the susceptibility response (survival differences between populations \pm 95% CI) for nymphs and adults from two populations [Areia (AR) and Paudalho (PD)] of the ring-legged earwig, *Euborellia annulipes*, exposed to insecticide-dried residues or ingestion of contaminated prey treated with insecticides used for cotton pest control. *Positive mean differences (right side – green color) stand for greater survival of the AR population; while negative mean differences (left side – blue color) stand for greater survival of the PD population, with 95% CI bars not crossing 0.

Supplementary Files

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