

Lambda-Cyhalothrin Resistance in the Lady Beetle *Eriopsis connexa* (Coleoptera: Coccinellidae) Confers Tolerance to Other Pyrethroids

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ABSTRACT Pyrethroid insecticides are widely recommended to control insect defoliators but lack efficacy against most aphid species. Thus, conserving aphid predators such as the lady beetle *Eriopsis connexa* (Germar) is important to pest management in crop ecosystems that require pyrethroid sprays. In a greenhouse, early fourth-instar larvae and 5-day-old adults from susceptible (S) and resistant (R) *E. connexa* populations were caged on lambda-cyhalothrin-treated cotton plants, after which survival and egg production (for those caged at adult stage) were assessed. In the laboratory, similar groups were subjected to dried residues and topical treatment with one of eight pyrethroids (alpha-cypermethrin, bifenthrin, deltamethrin, esfenvalerate, fenpropathrin, permethrin, zeta-cypermethrin, and lambda-cyhalothrin), the organophosphate methidathion, or water and wetting agent. After caging on treated cotton terminals, 66% of the R-population larvae survived to adulthood, compared with 2% of those from the S-population. At 12 d after caging at adult stage under the same conditions, 64% of the females from the R-population survived and laid eggs, compared with 100% mortality and no oviposition for the S-females. In trials involving dried insecticide residues, gain in survival based on the survival difference (percentage for R-population minus percentage for S-population) across all tested pyrethroids varied from 3 to 63% for larvae and from 3 to 70% for adults. In trials involving topical sprays of the tested pyrethroids, survival differences ranged from 36 to 96% for larvae and from 21 to 82% for adults. Fenpropathrin and bifenthrin were the least and most toxic, respectively.

KEY WORDS Coccinellidae, resistance, integrated pest management, cotton pest pyrethroid

Introduction

Integration of predators and insecticides for pest control in crop ecosystems has been a core component of integrated pest management, but due to incompatibilities it has rarely been achieved (Tabashnik and Johnson 1999). Some form of obtaining integration is improving the ecological and physiological selectivity of pesticides to natural enemies. The ecological selectivity deals with forms of reducing the contact of the insecticide with the natural enemy, while the physiological selectivity refers to characteristics exhibited by the natural enemy resulting in less toxicity of the insecticide to the natural enemy compared with the target pest (Croft 1990). One way to obtain this last form of selectivity is using natural enemies exhibiting resistance to insecticides (Torres 2012).

Nowadays despite advances in pest control methods, pesticides are still one of the most used tools worldwide for managing pest populations in row crop ecosystems.

The pesticides, however, are responsible for many negative impacts, especially against the beneficial organisms cohabiting the crop ecosystem. In many crop ecosystems, such as cotton, naturally occurring predators provide control of pest species without additional costs for producers (Whitcomb and Bell 1964; Torres and Ruberson 2005, 2007).

Among the registered insecticides in Brazil, >70% include pyrethroids as active ingredient (AGROFIT - Sistemas de Agrotóxicos Fitossanitários 2014). In Brazil, lambda-cyhalothrin is one of 14 pyrethroids recommended for use on cotton fields and accounts for about 16% of overall pesticide (only deltamethrin is used more, with about 17% of the market). Pyrethroid insecticides are known to be of low selectivity (Croft 1990), and thus affect not only pest species but their natural enemies as well.

Natural enemies generally are less prone to develop resistance to pesticides than insect herbivores for various biological, ecological, and operational reasons (Georghiou and Taylor 1986). However, selection for resistance can also occur in insect natural enemies (Ruberson et al. 2007; Pathan et al. 2008, 2010; Sayed et al. 2010; Kumral et al. 2011; Rodrigues et al. 2013a,b). Among predatory species, resistance has been reported in the lacewing *Chrysoperla carnea* Stephens from Pakistani cotton fields (resistance to

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pyrethroids and organophosphates; Pathan et al. 2008, 2010; Sayyed et al. 2010); and the lady beetles *Coleomegilla maculata* (De Geer) from U.S. cotton fields (resistance to organophosphates; Head et al. 1977, Graves et al. 1978), *Hippodamia convergens* (Guérin-Méneville) from U.S. cotton fields (resistance to lambda-cyhalothrin; Ruberson et al. 2007, Rodrigues et al. 2013c), *Stethorus gilvifrons* Mulsant from Turkish apple orchards (resistance to bifenthrin; Kumral et al. 2011), and *Eriopis connexa* (Germar) from Brazilian cabbage fields (resistance to lambda-cyhalothrin; Rodrigues et al. 2013a, 2013b).

This last lady beetle species, *E. connexa*, is the focus of this study. A neotropical species, it is commonly associated with soft-bodied arthropod pests, especially aphids, in several crops (Gyenge et al. 1998, Sarmiento et al. 2007, Rodrigues et al. 2013a). Because of its potential utility as an aphid predator, it has been introduced into the United States for use against the Russian wheat aphid (Reed and Pike 1991). A larva of *E. connexa* can consume >100 aphids or mites per day depending on prey size and >2000 prey items during the adult stage (Gyenge et al. 1998, Oliveira et al. 2004).

Preservation and maintenance of lambda-cyhalothrin-resistant *E. connexa* populations in crop ecosystems will require knowledge about their responses to other potential pesticides commonly applied to those same crops. Therefore, this study evaluated the survival of larvae and adults of *E. connexa* from resistant and susceptible populations in three contexts: 1) when caged on cotton plants treated with lambda-cyhalothrin; 2) when exposed to leaf discs treated with one of eight pyrethroids as compared with an organophosphate or plain water plus the surfactant; and 3) after direct topical treatment with these same chemicals.

Two hypotheses were investigated: 1) the lambda-cyhalothrin-resistant population survives application of this broad-spectrum insecticide when caged on treated plants, and successfully reproduces; and 2) resistance to this pyrethroid confers tolerance to certain other commercially available pesticides belonging to the same insecticide group.

Materials and Methods

The experiments were conducted in the Laboratory of Biological Control and Insect Ecology and in the greenhouse of the Universidade Federal Rural de Pernambuco (UFRPE), Recife-PE, Brazil. The greenhouse environmental conditions were monitored at 30-min intervals using a Datalogger Hobo (Onset Computer Corp., Bourne, MA). The average conditions in the greenhouse was $30.2 \pm 2^\circ\text{C}$, $68.2 \pm 8.9\%$ relative humidity (RH), and a photoperiod of 11.9:12.1 (L:D) h. In the laboratory, conditions were maintained at a temperature of $25 \pm 1^\circ\text{C}$, $\sim 70\%$ RH, and a photoperiod of 12:12 (L:D) h.

Insects. Characteristics of the susceptible and resistant populations (herein designated as S- and R-populations) of *E. connexa* have been described by Rodrigues et al. (2013b). The R-population was

maintained for 11 generations under artificial selection after the initial field report of a 36.7-fold increase in resistance ratio (Rodrigues et al. 2013b) using lambda-cyhalothrin (Karate Zeon 50 CS; lambda-cyhalothrin 5% m/v—50 g/liter; Syngenta S.A., São Paulo, Brazil) at the rate of 0.35 mg of a.i./ml of the commercial formulation.

In the generation F₁₂, a new dose-response curve was determined using lambda-cyhalothrin at technical grade (99.5%; Chem Service, West Chester, PA) diluted into acetone. Based on the results of this new curve, the LD₅₀ was calculated to be 0.35 g a.i./liter, and this value was used in the following generations until a new curve was determined. As of this writing (January 2014), the R-population has completed 42 generations in the laboratory, with an LD₅₀ of 0.62 g a.i./liter. The S-population has been reared without exposure to any insecticide.

Adults of each population were reared using 1-liter plastic containers, in which the lid had an opening covered with organdy fabric to allow ventilation. Pieces of paper towels were offered as oviposition substrata and to increase surface area inside the containers to minimize possible egg predation by cannibalistic adults. Egg masses were collected daily and transferred to 500-ml containers. Later, 2-day-old larvae were reared to adult emergence using 80-ml transparent plastic cups with three larvae per cup. The larvae were fed with *Anagasta kuehniella* (Zeller) eggs ad libitum until pupation. Pieces of wrinkled paper towel ($\sim 4\text{ cm}^2$) were placed inside the cups to reduce cannibalism and as pupation substrata, although pupation could occur on different parts of the cup.

Survival and Reproduction of Resistant and Susceptible *E. connexa* on Plants Treated with Lambda-cyhalothrin. Susceptible and resistant fourth-instar larvae or adults were from populations reared in the laboratory for F₁₇ and F₂₂, and for F₂₁ and F₂₄ generations, respectively. These were deployed upon greenhouse-grown, potted cotton plants (variety BRS Rubi). For lambda-cyhalothrin treatments, a solution of the commercial formulation Karate Zeon 50 CS (5% w/v—50 g/liter SC [soluble concentrated]; Syngenta, Brazil) was dripped onto the plant leaves. The pesticide was diluted at a rate of 4.0 ml of Karate per liter of water, a dose equivalent to the highest recommended field rate of 20 g of lambda-cyhalothrin per hectare in Brazil (AGROFIT - Sistemas de Agrotóxicos Fitossanitários 2014). Control plants were given equivalent amounts of dripped water. For both larvae and adults, four treatment groups were established, designated as R+L, S+L, R-L, and S-L, where R stands for resistant population, S for susceptible population, and L for lambda-cyhalothrin, respectively.

Approximately one hour after insecticide application, the plants were transferred to the greenhouse. For the larval experiment, each upper stratum (5–6 nodes) of 50- to 70-d-old plants was enclosed within a nylon mesh cage (60 by 40 cm²). At the lower end of the cage a 30-cm-long cotton fabric sleeve was secured around the plant's main stem. A zippered 50-cm-long lateral opening allowed access to the plant. The plants were

then infested with cotton aphids *Aphis gossypii* Glover (Hemiptera: Aphididae) one week before, and at the moment of the experiment there were colonies scattered through the plant terminals. An artificial diet composed of honey and yeast (50:50) was applied on the top four leaves of the cotton plants and sprinkled with *A. kuehniella* eggs.

Ten larvae initiating the fourth-instar (<24 h) were released per plant or cage and considered as one replication. A total of 50, 60, 50, and 70 larvae were used in the treatments R+L, R-L, S+L, and S-L, respectively. Two hours after release, the larvae were evaluated for knockdown effect. Larval mortality was recorded after 24 h; larvae were considered dead when they were unable to move. The surviving larvae were kept in the same cages for 7 d to allow time for all larvae to molt to pupae. Pupae were transferred singly to Petri dishes for rearing to adult emergence in the laboratory. Mortality of larvae and total adult emergence were recorded for each replicated treatment.

The experiment with adults used individual beetles of both sexes up to 5 d old. For this experiment, 25-d-old cotton plants similarly infested with cotton aphids were used. The whole plant was enclosed using the previously described cages. Eight days after aphid infestation, an artificial diet composed of honey and yeast (50:50) was applied on the top two expanded leaves; this was repeated every 2 days. Eggs of *A. kuehniella* were sprinkled over the diet on the leaves every two days to guarantee plentiful food. On each plant or cage, five pairs of adult beetles (5 males and 5 females) were released. The experiment was replicated six times for the R+L and R-L and five times for the S+L and S-L treatments. Adult survival and the numbers of eggs produced were recorded daily for 12 d.

Survival of Lambda-Cyhalothrin-Resistant and Susceptible *E. connexa* Larvae and Adults after Treatment with Other Pyrethroids. These bioassay experiments exposed both fourth-instar larvae and adults of S- and R-populations of *E. connexa* either to one of eight pyrethroid insecticides (alpha-cypermethrin, bifenthrin, deltamethrin, esfenvalerate, fenpropathrin, permethrin, lambda-cyhalothrin, zeta-cypermethrin) or to either the organophosphate methidathion as a positive control or and water + surfactant

as a negative control (Table 1). These insecticides were applied at the highest recommended field application rate for Brazilian cotton (AGROFIT - Sistemas de Agrotóxicos Fitossanitários 2014).

Trials included two exposure protocols, via dried residue and via direct topical treatment. The dried residue bioassay employed the leaf dipping method. The insecticide dilution was prepared by diluting the commercial product in distilled water plus Will Fix at 0.05%, which served as surfactant. Fully expanded cotton leaves were harvested and washed in tap water. Leaf discs were cut out from these leaves with the aid of a 9-cm-diameter cylindrical cutter and dipped for 5 s into the appropriate insecticide dilution. The control treatment consisted of distilled water plus Will Fix at 0.05%. The leaf discs were then placed on a laboratory bench over paper toweling for 2 h to remove excess moisture before being transferred to Petri dishes (90 by 15 mm²) lined previously with filter paper moistened with 1 ml distilled water. Eggs of *A. kuehniella* were sprinkled over the leaf discs to provide food for the beetles.

The treatments (insecticides and controls) consisted of six replications each with five insects of both sex per replication. The procedure was similar in the bioassays with larvae and adults. The insects were released into the Petri dishes over the leaf discs and allowed contact with the dried insecticide residue for 24 h. The material was maintained under conditions of 25 ± 1°C, 65 ± 10% RH, and a photoperiod of 12:12 (L:D) h. After 24 h, surviving insects were transferred to new clean Petri dishes and provided prey and water. Knockdown and mortality were assessed and recorded 2 and 48 h after exposure to the insecticide. Lady beetles were considered dead when either a larva was unable to move at least the length of its body or an adult was unable to turn upright after being placed on its dorsum. The surviving larvae were reared to adults and the percentages of adult emergence calculated.

For the topical treatment protocol, appropriate dilutions of the respective pyrethroid insecticides or the positive (methidathion) or negative control (water + Will Fix at 0.05%) were sprayed directly upon fourth-instar larvae and adults of both S- and R-populations. Six replications per treatment were set up, each consisting of five insects of both sex placed into Petri

Table 1. Highest recommended field application rates for the commercially available insecticides that were used in the bioassays in this study

| Insecticides | Active ingredients | Highest field rate recommended (g a.i./ha) ^a | Target pests |
|-------------------|------------------------------------|---|---------------------------------|
| Suprathion 400 EC | Methidathion ^{OP} | 400 | <i>Anthonomus grandis</i> |
| Karate Zeon 50 CS | Lambda-cyhalothrin ^{P-II} | 20 | <i>Heliiothis virescens</i> |
| Decis 25 EC | Deltamethrin ^{P-II} | 10 | <i>Heliiothis virescens</i> |
| Fastac 100 | Alpha-cypermethrin ^{P-II} | 20 | <i>Heliiothis virescens</i> |
| Fury 400 EC | Zeta-cypermethrin ^{P-II} | 50 | <i>Alabama argilacea</i> |
| Danimen 300EC | Fenpropathrin ^{P-II} | 120 | <i>Pectinophora gossypiella</i> |
| Sumidan 150 SC | Esfenvalerate ^{P-II} | 30 | <i>Anthonomus grandis</i> |
| Talstar 100 EC | Bifenthrin ^{P-I} | 60 | <i>Spodoptera frugiperda</i> |
| Valon 384 EC | Permethrin ^{P-I} | 124.8 | <i>Heliiothis virescens</i> |

The targeted pests for each insecticide when used on cotton are also shown.

^a Source: AGROFIT, 2014.

^{OP}, organophosphate; P-I, pyrethroids of type I (absence of the cyano group in the molecule); P-II, pyrethroids of type II (presence of the cyano group in the molecule).

dishes (90 by 15 mm²) lined with filter paper of the same diameter. Immediately after they were placed in the Petri dishes, the insects were sprayed using an electric power Airbrush set (Paasche Airbrush Co., Harwood Heights, IL) under 15 lb pol⁻² pressure. The volume of the insecticide applied was regulated to 1 ml per Petri dish to obtain a homogeneous covering. At 2 h after treatment, surviving beetles were transferred to a new set of clean Petri dishes. Knockdown and mortality were recorded at this time and 48 h later.

Statistical Analyses. For the experiments in which beetles were placed on lambda-cyhalothrin-treated plants, mortality and adult emergence data for the larval experiment, and the accumulated number of eggs produced during 12 d of the adult experiment, were subjected to analysis of variance (PROC ANOVA) after being tested for normality (Kolmogorov–Smirnov) and homogeneity of variance (Bartlett's test), and the data transformed into square roots ($x+0.5$). Prior to analysis, the accumulated number of eggs produced was calculated per live female per cage, except for females in the S+L treatment that all died within 24 h without oviposition. Treatment means were separated by Tukey HSD test at a 0.05 significance level. The number of dead adult beetles during the 12 d of evaluation across all treatments was utilized to calculate the survival curves using the Kaplan–Meier method, and the survival means were compared through the Log-Rank's test using PROC LIFETEST of SAS. Further, the accumulated number of eggs produced as function of evaluation time was calculated using the regression analysis performed by PROC REG of SAS selecting the equation with significant parameters ($P < 0.05$) and the greatest adjusted coefficient of correlation (r). All analyses were carried out using the statistical package SAS (SAS Institute 2002).

In both the dried residue and the topical exposure experiments, the knockdown evaluation 2 h after beetle exposure to the insecticides was used only to confirm insecticide contamination, and subsequent analyses were based solely upon mortality data. Analyses of mortality data were based upon a three-way experimental design $8 \times 2 \times 2$ (insecticides, insect stages, and populations), whereas analyses of data on larval maturation to adulthood were based upon a two-way experimental design (insecticides and populations). Mortality and emergence data were subjected to tests for normality (Kolmogorov–Smirnov) and homogeneity of variance (Bartlett), and when needed, the data were transformed into square roots ($x+0.5$) to satisfy the ANOVA assumptions.

To reduce the volume of mortality data (e.g., larvae and adults, dried and topical treatments) to be presented, a new measure of major interest, gain in survival (defined as percent survival of R-population minus percent survival of S-population), was calculated for each replication across all insecticides and predator stages in this study. The mean gain in survival and respective 95% confidence intervals were then calculated for each replication within the experimental treatment. Statistical significance for the mean gain in survival data was determined using the overlap rule for

95% CI of the insecticide differences (Di Stefano 2005). When the 95% CI bars of the gain in survival cross the x-axis, there are no differences for survival between R- and S-populations, whereas 95% CI bars that do not cross the x-axis indicate a significant gain in survival for the resistant population. Further, means of survival gain across tested insecticides were separated by Tukey HSD test at a 0.05 significance level.

Results

Survival and Reproduction of Resistant and Susceptible *E. connexa* on Plants Treated with Lambda-cyhalothrin. The differential survival of fourth-instar *E. connexa* larvae caged on treated and untreated plants was highly significant ($F_{df} = 3, 19 = 42.36, P < 0.0001$), as was the emergence of adults for harvested pupae originating from these larvae ($F_{3, 19} = 101.49, P < 0.0001$). The survival of larvae within 24 h of exposure was 100, 100 and 94% for the treatments R–L, S–L, and R+L, respectively, but only 35.7% in the S+L treatment group. The emergence of adults from R-larvae released on treated plants (R+L) was significantly lower (66%) than for larvae released on untreated plants (R–L [95%] and S–L [88%]). However, only 2% of S-larvae in the S+L treatment group reached adulthood (Fig. 1).

The adults also exhibited survival differences between resistant and susceptible populations and between untreated and lambda-cyhalothrin-treated plants (Log-Rank, $\chi^2 = 217.48, df = 3, P < 0.0001$). Beetles released in cages enclosing untreated plants survived similarly during the 12 d of observations (R–L and S–L; Log-Rank, $\chi^2 = 1.57, df = 1, P = 0.2100$). On the other hand, when adults were released on treated plants there was variation in survival between treatments set up with R- and S-adults (R+L and S+L; Log-Rank, $\chi^2 = 22.65, df = 1, P < 0.0001$), and between untreated and treated plants with R-adults (R+L and R–L; Log-Rank, $\chi^2 = 12.61, df = 1, P = 0.0004$). After 12 d, 64% of the R-adults caged on treated cotton plants were alive, whereas all S-adults died before day 3 (Fig. 2 above). In fact, only 1.3 and 1.6% of S-adults were alive at day 2 and 3 after release, respectively. In contrast, at 12 d after release upon untreated plants, 88% of the R-adults and 95% of the S-adults were still alive.

The total mortality of the females and males in the treatment S+L precluded oviposition (Fig. 2 above). The cumulative mean number of eggs produced varied in the other three treatments ($F_{df} = 2, 14 = 15.70, P = 0.0003$). The R-females in the treatment R+L produced significantly lower numbers of eggs compared with females from either R- or S-populations caged on untreated plants (Fig. 2 below). The egg production as function of time after exposure or not to lambda-cyhalothrin (L) is described by linear equations of regression to all three treatments (R+L: $y = -1.679 + 0.685x; r^2 = 0.29, F = 9.23, P = 0.006$; R–L: $y = 5.168 + 1.22x; r^2 = 0.32, F = 10.10, P = 0.0043$; and S–L: $y = -3.949 + 2.767x; r^2 = 0.72, F = 36.95, P < 0.0001$).

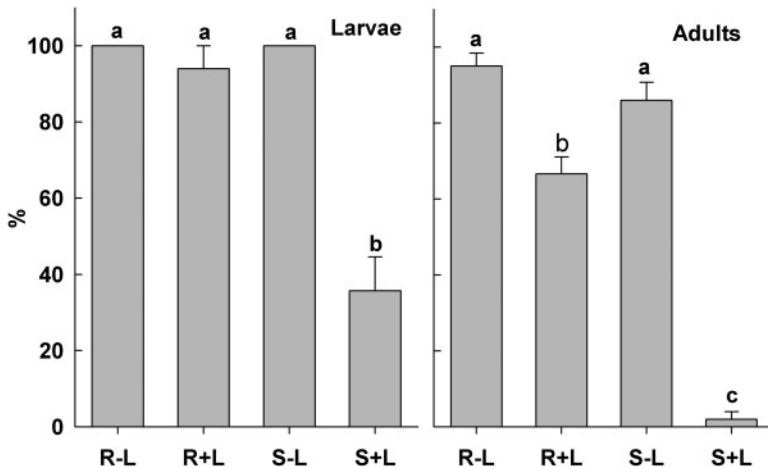


Fig. 1. Mean (+SE) percent survival of fourth-instar larvae after 24 h of exposure in the different treatments (left) and percent adult emergence (right) from susceptible (S) and resistant (R) populations of *E. connexa* caged on lambda-cyhalothrin-treated (+L) and untreated (-L) cotton plants. Values with the same small letter do not differ significantly (Tukey HSD test at 0.05 significance level).

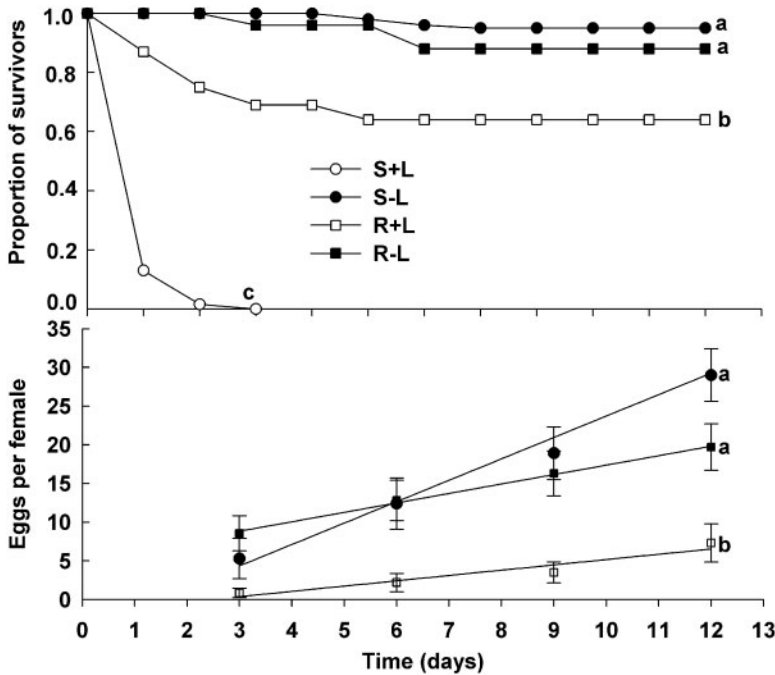


Fig. 2. Survivorship (above) and regression lines for accumulated egg production (below) of resistant (R) and susceptible (S) populations of *E. connexa* over 12 d after exposure to cotton plants untreated (-L) and treated (+L) with lambda-cyhalothrin at maximum recommended field application rates. All females of S+L treatment died before laying eggs. Same letters indicate no significant difference between survivorship curves (Log-Rank test [above]) and between means of accumulated egg production (Tukey HSD tests [below] at 0.05 significance level).

Survival of Lambda-Cyhalothrin-Resistant Larvae and Adults of *E. connexa* Treated with Different Pyrethroids. All larvae and adults from both R- and S-populations in the negative control (water + Will Fix) group survived, whereas the positive control methidathion caused 100% mortality for larvae

and adults from both populations and experimental treatments.

In the dried residue experiment, overall mortality was 8.1% for individuals from the R-population compared with 49.2% for individuals from the S-population ($F_{1,190} = 118.39, P < 0.0001$). Considering only life

stage of the lady beetles, overall mortality of larvae was lower (21.4%) than that of adults (35.8%; $F_{1, 190} = 8.79$, $P = 0.0034$). Regarding the larval and adult responses to dried residue, we noticed that after being in contact with the treated surface, larvae walked with their abdomen elevated and thus might pick up less compounds to be detoxified. However, whereas larvae of the S-population exhibited lower mortality compared with adults ($F_{1, 94} = 13.60$, $P = 0.0004$), the larvae and adults of the R-population exhibited similar mortality ($F_{1, 94} = 1.89$, $P = 0.1725$). Irrespective of the lady beetle age and populations, results varied among the eight pyrethroids tested ($F_{7, 184} = 8.25$, $P < 0.0001$), with mortality rates ranging from 15.8 to 32.9%; fenpropathrin and bifenthrin were at the extremes (6.7 and 65%, respectively).

In the topical treatment experiments, population lambda-cyhalothrin resistance status was the only main effect that was statistically significant. Disregarding the type of pyrethroid insecticide and the life stage of the lady beetle studied, the overall mortality was only 7.9% for individuals from R-population compared with 87.5% for individuals from S-population ($F_{1, 190} = 787.12$, $P < 0.0001$). On the other hand, larvae and adults exhibited the same mortality pattern as a function of the population ($F_{1, 190} = 1.53$, $P = 0.2176$) and across the insecticides ($F_{7, 184} = 1.31$, $P = 0.2486$).

The difference in survival to adulthood between R- and S-larvae after exposure to the insecticides was significantly higher for the R-population (Fig. 3). For beetles exposed to dried residue, the average gain in emergence was 70.8% for R-larvae and varied across all tested pyrethroid insecticides ($F_{8, 45} = 7.46$, $P < 0.0001$; Fig. 3). Likewise, for beetles exposed to topical treatment the gain in emergence averaged 41.1% and varied across the pyrethroids ($F_{8, 45} = 8.40$, $P < 0.0001$).

However, among the eight pyrethroids, the gain in emergence was not significant for permethrin (Fig. 3).

The gain in survival of adults and larvae in the topical treatments was positive and significant across all pyrethroids tested, while survival was not observed to the organophosphate methidathion (Fig. 4A). Permethrin for adults and bifenthrin and fenpropathrin for larvae exhibited smaller differences ($F_{8, 45} = 4.40$, $P = 0.0021$) due to lower mortality of both populations treated with these compounds. Likewise, dried residue exposure treatment was numerically positive across all pyrethroids, but not statistically significant for adults or larvae exposed to fenpropathrin or for larvae exposed to permethrin (Fig. 4B). In these three cases, the mortality of both R- and S-populations was lower than with other pyrethroids tested ($F_{8, 45} = 11.23$, $P < 0.0001$), resulting in positive gains, but not enough to be different statistically. Again, survival was not observed to the organophosphate methidathion.

Discussion

Resistance to insecticides in natural enemies is poorly studied. Hence, few data exist upon which to base an informed discussion as to the best ways to sustainably match the need to control crop pests while simultaneously promoting the survival and increase of beneficial insects. In this study, we tested whether a population of the neotropical lady beetle *E. connexa* known to be resistant to lambda-cyhalothrin would be able to survive and reproduce when caged on lambda-cyhalothrin-treated cotton plants, and whether its resistance to lambda-cyhalothrin would confer tolerance to other pyrethroid insecticides. The results support both hypotheses. Females from the R-population survived a field rate application dose of

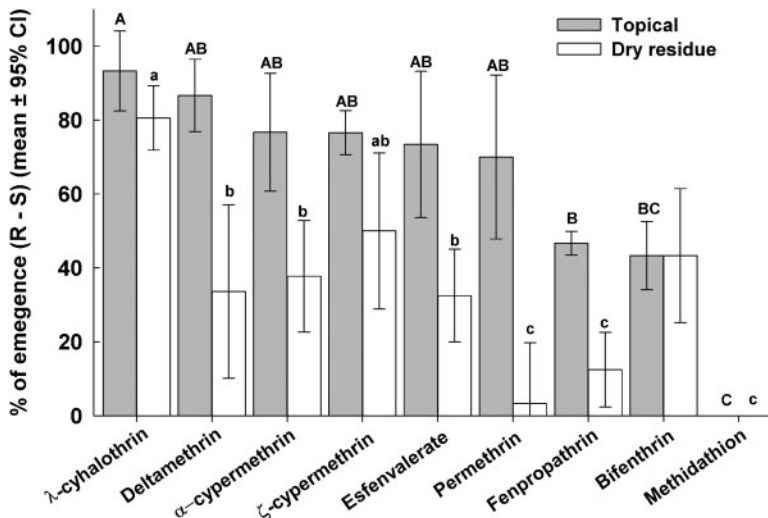


Fig. 3. Gain in adult emergence (percent emergence for resistant [R] minus percent emergence for susceptible [S] populations) for *E. connexa* larvae exposed to an organophosphate or various pyrethroids either through direct topical application or exposure to dried residue. When the 95% CI bars do not cross the x-axis, it indicates a significant gain in survival for the resistant population, while the same capital or small letters indicate no significant difference between insecticides through topical or dry residue treatments, respectively (Tukey HSD tests at 0.05 significance level).

lambda-cyhalothrin and initiated oviposition whereas all females from the S-population died from exposure to the same dose level (Fig. 2). A positive gain in emergence of R-larvae and survival of larvae and adults exposed to the eight different pyrethroid insecticides tested also was found (Fig. 3-4).

In a previous study (Ferreira et al. 2013), we showed that R-females recovering from knockdown suffered a cost in egg production, with about 50% less fecundity compared with S-females. Despite the reduction in fecundity (which began to recover after 6 d; Fig. 2), the important finding here is that R-females exposed to lambda-cyhalothrin were able to survive and to produce offspring, a situation that did not occur with similarly exposed S-females. These offspring will be important to sustain the population's presence in the field after insecticide exposure.

Pyrethroid insecticides are widely recommended to control insect defoliators, but most of them lack efficacy against aphid species. This may be a reason for

outbreaks of cotton aphids commonly observed after application of pyrethroid insecticides (Broza 1986, Hardin et al. 1995, Kidd et al. 1996, Kidd and Rummel 1997, Deguine et al. 2000, Godfrey et al. 2000). Thus, the conservation of aphid predators such as *E. connexa* has practical implications for pest management in crop ecosystems requiring pyrethroid sprays to control defoliators. Despite variation in response to topical and dried residue treatments of larvae and adults of *E. connexa*, there was high positive gain in survival for the R-population across all pyrethroids tested (Fig. 4).

In applying a judicious cotton pest management approach in Brazil, pyrethroids are recommended primarily for late season sprays for three reasons: 1) they are efficient against important worms attacking fruit structures; 2) early applications can result in mite and aphid outbreaks; and 3) reducing the number of pyrethroid-based sprays within a growing season is seen as a proactive program for resistance management. Thus, to have minimal impact on populations of *E. connexa*

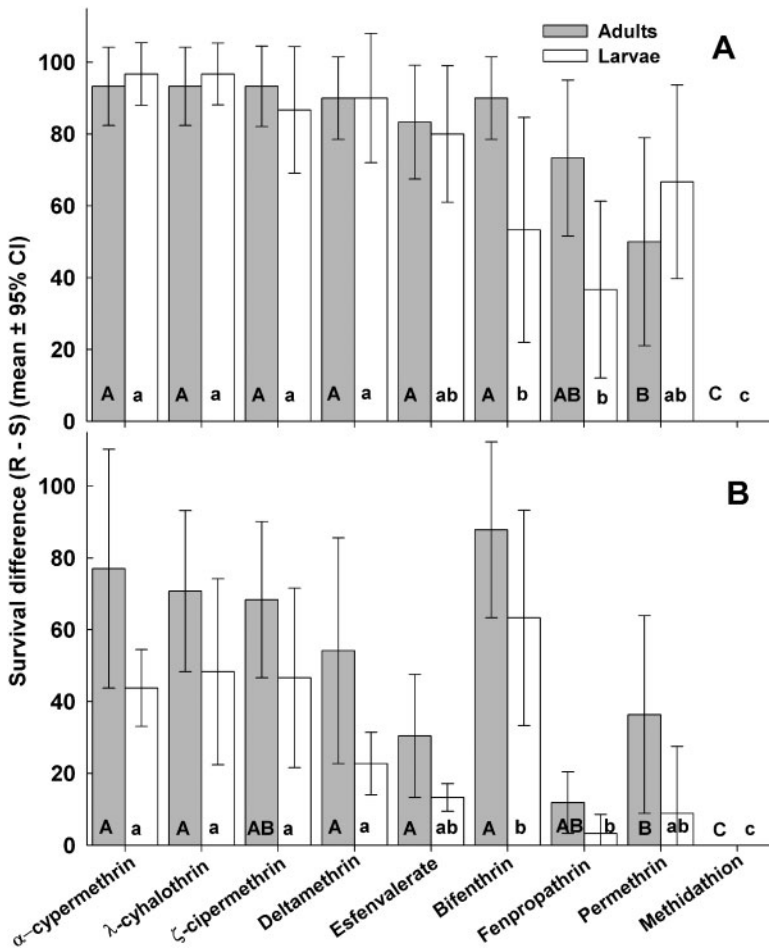


Fig. 4. Survival gain (percent survival for resistant [R] minus percent survival for susceptible [S] populations) for adults and larvae of *E. connexa* assayed against different pyrethroids and an organophosphate through (A) topical and (B) dried residue exposure. When the 95% CI bars do not cross the x-axis, it indicates a significant gain in survival for the resistant population, while the same capital or small letters indicate no significant difference between insecticides for adults and larvae, respectively (Tukey HSD tests at 0.05 significance level).

and other beneficial species in early- and mid-season cotton fields, other insecticides should be recommended. Based on our results, the R-population tested was highly susceptible to the broad-spectrum organophosphate methidathion, indicating general susceptibility to this group of insecticides. In fact, **Rodrigues et al. (2014)** observed inhibition of general esterases by the organophosphates S, S, S-tributylphosphorotrithioate (DEF) and methyl-paraoxon which can inhibit acetylcholinesterases, and type-B carboxylesterase that are involved in the pyrethroid lambda-cyhalothrin resistance.

Choice of insecticides for early- and mid-season use on cotton should look for selectivity through ecological and physiological mechanisms. For example, early protection of seeds and cotton seedlings can be obtained by treating seeds with systemic pesticides, and later foliar sprays belonging to the growth regulator group (IGRs) can be used. Possibilities for managing defoliators (especially worms) include commercial formulations of *Bacillus thuringiensis* Berliner (Bts), and products of the new group of diamide insecticides (e.g., Chlorantraniliprole) that have been considered to be soft insecticides (**Brugger et al. 2010**, **Larson et al. 2012**, **Martinou et al. 2014**). For sap-sucking pests such as cotton aphids and whiteflies, which are common during early season cotton crops in Brazil and elsewhere, pymetrozine and pyriproxifen can be recommended; these are also marketed as having low impact on natural enemies (**Torres et al. 2003**, **Liu and Stansly 2004**, **Bastos and Torres 2006**, **Bozisk 2006**).

The differential responses of larvae and adults to the pyrethroids we tested may relate to insecticide chemistry, stereochemistry of the molecule (**Sattelle and Yamamoto 1988**), and to differences in behavior and detoxification mechanisms. Pyrethroids are esters formed by the association of acid and alcohol moiety (**Elliott and Janes 1978**), and can be differentiated by the absence (type I) or presence (type II) of a cyano group in the molecule (**Khambay and Jewess 2010**). Pyrethroids representing both groups were used in our study. Type I pyrethroids are considered to be of relatively minor toxicity owing to the absence of the cyano group (**Khambay and Jewess 2010**); however, both permethrin and bifenthrin caused high mortality of beetles from both R- and S-populations in our study. The beetles' responses to type II pyrethroids, including lambda-cyhalothrin, varied in our tests. Unfortunately, there is no information regarding lambda-cyhalothrin detoxification in R-larvae of *E. connexa*, although adults of the R-population are known to exhibit metabolic detoxification mechanisms with carboxylesterase type B involvement (**Rodrigues et al. 2014**).

Because both adult and larval beetle life stages are continually present in a variety of agro-ecosystems in the landscape, and are not plant specific, whenever possible lowest impact insecticides are to be recommended to facilitate conserving these natural enemies in crop plant pest management programs. Among the insecticides we tested, the pyrethroid fenprothrin caused relatively low mortality for both larvae and adults of both S- and R-populations. On the other

hand, bifenthrin was found to be the most toxic among the tested pyrethroids for larvae and adults of both beetle populations.

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