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# IMPACT OF NONCONVENTIONAL SELECTION BY INSECTICIDES ON SUSCEPTIBILITY OF THE SOUTHERN HOUSE MOSQUITO, *CULEX QUINQUEFASCIATUS*

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ABSTRACT. Mosquitoes are an important target of pest control as they vector pathogens that are associated with many debilitating human diseases. Given that mosquitoes have been selected with insecticides for over 100 years, much is known about the development of insecticide resistance associated with targeted application against populations of these insects. However, off-target selection by applications of insecticides in agricultural and residential sites also impacts development of insecticide resistance and is understudied. Similarly, the impact of selecting one life stage of mosquitoes on the insecticide susceptibility of different life stages is largely unknown. Here, we show that susceptibility to chlorantraniliprole, which is applied in rice (*Oryza sativa*) and sugarcane (*Saccharum officinarum*) fields in Louisiana, decreased (5.7- to 12-fold) in populations of *Culex quinquefasciatus* collected from near these fields compared with a reference field strain. In addition, application of bifenthrin by commercial application on an individual residence increased the resistance frequencies to bifenthrin in 5 nearby residential sites. These increased frequencies of resistance, as measured with diagnostic concentrations of bifenthrin, were highly correlated ($R^2 = 0.92$) between larvae and adults, suggesting that selection of adults also confers resistance to larvae. Finally, esterase activities and bifenthrin susceptibilities were moderately correlated ($R^2 = 0.4$ for larvae and 0.52 for adults), suggesting that multiple mechanisms (including metabolism by esterases) were associated with the observed resistance. Results from this study suggest that nonconventional selection by insecticides is a variable to consider when developing management strategies for populations of *Cx. quinquefasciatus*.

**KEY WORDS** Esterase, mosquito, nonconventional, off-target effects, resistance, selection

## INTRODUCTION

Development of resistance from frequent use of chemical insecticides has compromised efforts to suppress mosquito populations. Use of insecticides is one of the most common and effective strategies for abatement, which is especially important when there is a high risk of mosquito nuisance and associated diseases (Rose 2001). However, application of insecticides selects pest populations by allowing initially rare individuals that express resistance phenotypes to survive exposure and pass their resistance traits to offspring (Mallet 1989, McKenzie and Batterham 1994). Thus, insecticide resistance results from heritable traits that decrease the susceptibility of insect populations towards insecticides and is a direct consequence of exposure to insecticides in the field (Crow 1957).

Whereas selection from direct, targeted spray of insecticides on insect populations is common and well studied (Crow 1957, Georghiou 1972, Liu et al. 2004, Davari et al. 2007, Cuamba et al. 2010, Edi et al. 2012, Casimiro et al. 2014), selection on nontargeted insect populations from unintentional exposure also impacts development of insecticide resistance and is understudied. For example, irrigated agricultural fields are a favored breeding ground of mosquitoes, which may be unintentionally selected with agrochemicals. This off-target exposure may impact the insecticide susceptibility of these populations, ultimately reducing the efficacy of insecticides used in mosquito control. Earlier studies have reported development of resistance against different insecticides in such nontargeted populations of the black fly, *Simulium slossonae* Dyar and Shannon (Montagna et al. 2012), and house fly, *Musca domestica* L. (Khan 2020). In addition, the impact of indirect exposure to organochlorine and pyrethroid insecticides on insecticide susceptibility in nontargeted populations of *Anopheles gambiae* Giles from agricultural fields has been described (Diabate et al. 2002, Chouaïbou et al. 2008, Yadouleton et al. 2009). Similarly, often intensive, residential spray of insecticides is another potential source of unintentional exposure to nontargeted mosquito populations. Repeated residential use of insecticides to suppress mosquitoes not only impacts the susceptibility of targeted populations but also may influence the insecticide susceptibility of neighboring off-target populations. To our knowledge, no previous studies have examined the effect of insecticide sprays at individual residences by commercial pest control applicators on the insecticide susceptibility of neighborhood mosquito populations.

Finally, few studies have examined the impact of selection on one life stage of mosquitoes on susceptibility in other life stages. Both larvicides and adulticides are used in mosquito control programs, providing intense selection on both life stages. However, the impact of selection of the adult

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on susceptibility of larval mosquitoes (and vice versa) is largely unknown. The life stage–specific sensitivity to cyhalothrin has been measured in the house fly (Fu-xing et al. 2002), where larvae were over 100-fold more resistant than adults. In contrast, Brewer et al. (1990) found that larval and adult susceptibilities to fenvalerate and methomyl were correlated in field-collected beet armyworms, *Spodoptera exigua* (Hubner). In mosquitoes, differential expression of genes encoding different detoxification enzymes was found between larval and adult *An. gambiae* (Strode et al. 2006), but the impact of this difference on insecticide susceptibility was not measured.

The knowledge of stage-specific susceptibility towards insecticides is critical for formulating life stage–specific strategies to manage insecticide resistance. Adult (but not larval) populations of mosquitoes are generally selected with ester-containing insecticides such as organophosphates and pyrethroids, and esterases are often associated with resistance to these insecticides in adult insects (Hemingway and Ranson 2000). Esterases are a group of major detoxifying enzymes that hydrolyze organophosphates and pyrethroids into less-toxic products, and have been studied extensively as a major mechanism of insecticide resistance (Hemingway and Karunaratne 1998, Hemingway 2000, Xu et al. 2005, Gordon and Ottea 2012, Gong et al. 2022).

In this study, the role of off-target selection from the application of an agrochemical (i.e., chlorantraniliprole) on susceptibility of *Culex quinquefasciatus* Say was examined. Chlorantraniliprole and malathion susceptibilities of *Cx. quinquefasciatus* collected from rice *Oryza sativa* L. and sugarcane *Saccharum officinarum* L. fields were measured. In addition, we examined the impact of insecticide application at an individual residence on resistance frequencies of mosquitoes collected from other, unsprayed residences along a transect in the same neighborhood. Finally, we measured and compared bifenthrin susceptibilities in larval and adult mosquitoes collected at the residential sites, and measured contribution of esterase activities as a mechanism of insecticide resistance in the two life stages.

## MATERIALS AND METHODS

### Chemicals

Malathion (99.5%), bifenthrin (99.5%), and chlorantraniliprole (99.5%) were purchased from Chem Services (West Chester, PA). 1-Naphthyl acetate (α-NA; ≥98%), Fast Blue B salt (approximately 95%), Brilliant Blue G-25 (ultrapure), sodium phosphate monobasic monohydrate (≥98%), sodium phosphate dibasic heptahydrate (98%), sodium dodecyl sulfate (99%), acetone (99.5%), and phosphoric acid (ACS grade) were purchased from Millipore Sigma (Burlington, MA). Hydrochloric acid (99.7%), phosphoric acid (85%), potassium chloride (ACS grade), and sodium hydroxide (ACS grade) were purchased from Fisher Scientific (Kansas City, MO). Bovine serum albumin (Biotechnology grade) was purchased from Amresco (Solon, OH). Absolute ethanol (ACS/UPS grade) was purchased from Pharmco-Aaper (Brookfield, CT). Yeast powder was purchased from Solgar (Leonia, NJ), and beef liver powder was purchased from Now Foods (Bloomingdale, IL).

### Insects

Sebring-S (SEB-S), a reference-susceptible lab strain of *Cx. quinquefasciatus*, with no known history of exposure to insecticides, was originally colonized in 1988 at the US Department of Agriculture Research Station in Gainesville, FL (Sbrana et al. 2005), and has been reared in the Medical Entomology Lab at Louisiana State University (LSU) since 2015. A reference-susceptible field strain of *Cx. quinquefasciatus* was collected near the LSU Lake (LSU LAKE-S; Table 1) with no recorded history of exposure to insecticides. Field populations of *Cx. quinquefasciatus* were collected from agricultural sites where chlorantraniliprole was applied in fields of rice (as a seed treatment; RICE) and sugarcane (as a foliar spray; CANE-1 and CANE-2) against stem-boring pests. Field populations of *Cx. quinquefasciatus* were also collected from residential sites along a linear transect on the same street. The sprayed residential site (0-0) was treated repeatedly with bifenthrin against mosquito populations by a commercial pest applicator (Table 1). The study was initiated during a period of active commercial spraying of bifenthrin (August 2021), and residences were resampled in November 2021, when commercial spraying had ceased. A 3rd sampling was made the following year in June (2022). A total of 5 nonsprayed residential sites were also sampled: 2 were located north (i.e., N-1 and N-2; 80 and 128 m away from 0-0, respectively) and 3 were located south (i.e., S-1, S-2, S-3; 72, 127, and 215 m away from 0-0, respectively) of the sprayed residential site (0-0). The total distance between the sites located from the far north to the far south of the residential street was 343 m. Egg rafts were collected from all field sites using black plastic containers containing yeast powder (2 g) and aged, distilled water (4 liters). Field-collected populations of *Cx. quinquefasciatus* were used in the same generation in which they were collected. Larval and adult *Cx. quinquefasciatus* were reared under conditions of constant photoperiod (14 h light and 10 h dark) and RH (70%). Larvae were fed daily a mixture of liver powder and yeast (1:1), and adults were provided with 10% sugar solution. Once per generation, adult females (7–10 days old) were fed defibrinated chicken blood (Rockland Immuno-

Table 1. Mosquito populations with collection date, site, and pesticide used in the study.

| Mosquito population | Collection site | GPS coordinates | Parish | Collection date | Sprayed chemical |
|---|---|---|---|---|---|
| SEB-S | Laboratory | | | | Unsprayed |
| LSU LAKE-S | Residential | 30°24′33″N 91°10′35″W | East Baton Rouge | 2021—August and November 2022—June | Unsprayed |
| CANE-1 | Sugarcane field | 30°27′59″N 91°17′44″W | West Baton Rouge | 2020—August | Chlorantraniliprole |
| CANE-2 | Sugarcane field | 30°16′4″N 91°6′29″W | Iberville | 2021—August | Chlorantraniliprole |
| RICE | Rice field | 30°14′23″N 92°20′45″W | Acadia | 2021—August | Chlorantraniliprole |
| 0-0 | Residential | 30°25′56″N 91°9′52″W | East Baton Rouge | 2021—August and November 2022—June | Bifenthrin |
| N-1 | Residential | 30°25′59″N 91°9′52″W | East Baton Rouge | 2021—August and November 2022—June | Unsprayed |
| N-2 | Residential | 30°26′1″N 91°9′52″W | East Baton Rouge | 2021—August and November 2022—June | Unsprayed |
| S-1 | Residential | 30°25′54″N 91°9′52″W | East Baton Rouge | 2021—August and November 2022—June | Unsprayed |
| S-2 | Residential | 30°25′52″N 91°9′53″W | East Baton Rouge | 2021—August and November 2022—June | Unsprayed |
| S-3 | Residential | 30°25′49″N 91°9′53″W | East Baton Rouge | 2021—August and November 2022—June | Unsprayed |

chemical, Pottstown, PA) using a membrane feeding system (Hemotek, Blackburn, UK).

### Biological assays

Adult susceptibility of *Cx. quinquefasciatus* to insecticides was examined using a topical bioassay. Adult females (3–7 days old) were anesthetized with $CO_2$ for less than 1 min then treated with 0.5 μl of acetone solutions containing malathion or bifenthrin on the thoracic dorsum using a syringe fitted with a repeating dispenser (Hamilton, Reno, NV). A full range of doses were used to measured malathion susceptibility in adults, whereas a diagnostic concentration was calculated and used to measure bifenthrin susceptibility in adults (i.e., 10× $LD_{50}$ obtained from log dose probit line using the SEB-S strain of mosquito). The control group of insects was treated with acetone only. Treated adult females were then placed in waxed paper cups (270 ml capacity) fitted with a fine mesh top and fed by placing a 10% sucrose-soaked cotton ball on top of the mesh. Treatment cups were incubated as described above.

Mortality was recorded 18 h after treatment and was defined by an inability of treated insects to right themselves after being flipped onto their dorsa. Larval susceptibility to insecticides was examined in a Pyrex glass dish filled with 100 ml of aged water containing early-4th instars, into which a 1-ml aliquot of malathion, bifenthrin, or chlorantraniliprole was added. A full range of concentrations was used to measure malathion (0.003–0.12 ppm) and chlorantraniliprole (0.4–15 ppm) susceptibilities in larvae of *Cx. quinquefasciatus*, whereas a diagnostic concentration (i.e., 10× $LC_{50}$ obtained from log dosage probit line using the SEB-S strain of mosquito) was used to measure bifenthrin susceptibility in larvae. Control and treated mosquitoes were held in environmental chambers as described above. Mortality was recorded 18 h after treatment and was defined by an inability to move after being poked by a pipette tip. Control mortality for all biological assays was below 5% in all experiments and was corrected using Abbott's formula (Abbott 1925).

### Measurement of esterase activity

Esterase activity towards α-NA was measured using the colorimetric assay described by Gomori (1952) as modified by Van Asperen (1962) and Grant et al. (1989) in polystyrene 96-well flat bottom microplates (Costar, Cambridge, MA). Mosquitoes (5 larvae or adults; 3–7 days old) were homogenized in ice-cold 1.15% KCl using 10 strokes of an all-glass mortar and pestle then centrifuged at 4°C for 10 min at 15,000 × *g*. The supernatant from this spin was used as an enzyme source immediately after preparation. A stock solution of α-NA (30 mM in acetone) was diluted 100-fold with buffer (0.1 M sodium phosphate, pH 7.4), and reactions were started by the addition of 200 μl of this solution (0.22 mM final concentration) to 20 μl of either enzyme homogenate (12–15 μg protein) or buffer (control). Reactions were stopped after 10 min by addition of 50 μl Fast Blue B dye (2.18 mM final concentration). Optical densities of reactions were measured at 570 nm using a Thermomax microplate reader (Molecular Devices, Palo Alto, CA), and converted to micromoles per minute using an experimentally derived extinction coefficient for α-naphthol (2.6 $\mu M^{-1}$ 270 μl). Protein concentrations

Table 2. Chlorantraniliprole and malathion susceptibilities of *Culex quinquefasciatus* adults and larvae.

| | | Larvae | | | Adults | | |
|---|---|---|---|---|---|---|---|
| Insecticide | Population | $LC_{50}$ (95% CI)[1] | RR[2] | Slope (SE) | $LD_{50}$ (95% CI)[3] | RR | Slope (SE) |
| Chlorantraniliprole | SEB-S | 0.57 (0.50–0.63) A | 1.00 | 2.78 (0.23) | ND[4] | — | — |
| | LSU-LAKE | 1.59 (1.30–1.92) B | 2.80 | 2.27 (0.32) | ND | — | — |
| | CANE-1 | 4.46 (3.68–5.18) C | 7.82 | 3.11 (0.44) | ND | — | — |
| | CANE-2 | 3.25 (2.40–4.20) C | 5.70 | 1.99 (0.33) | ND | — | — |
| | RICE | 6.90 (5.60–9.20) D | 12.1 | 2.42 (0.34) | ND | — | — |
| Malathion | SEB-S | 0.02 (0.01–0.02) A | 1.00 | 2.18 (0.35) | 3.36 (2.78–3.87) a | 1.00 | 3.32 (0.47) |
| | LSU-LAKE | 0.03 (0.02–0.04) B | 1.50 | 3.38 (0.62) | 4.64 (3.88–5.48) b | 1.38 | 2.95 (0.44) |
| | CANE-1 | 0.06 (0.05–0.07) C | 3.00 | 5.11 (0.94) | 9.80 (8.89–10.80) c | 2.92 | 5.54 (0.90) |
| | CANE-2 | 0.05 (0.04–0.07) C | 2.50 | 5.36 (1.24) | 9.22 (8.38–10.10) c | 2.74 | 6.05 (0.93) |
| | RICE | 0.06 (0.05–0.07) C | 3.00 | 4.43 (0.81) | 9.65 (8.71–10.70) c | 2.87 | 5.32 (0.80) |

[1] Parts per million (ppm) of insecticides (95% confidence interval). In comparisons within life stage, values followed by the same letter are not significantly different ($P > 0.05$), based on nonoverlap of confidence intervals.
[2] Resistance ratio (RR) = $LD_{50}$ or $LC_{50}$ of field-collected/$LD_{50}$ or $LC_{50}$ of SEB-S.
[3] Nanograms (ng) of malathion (95% confidence interval). In comparisons within life stage, values followed by the same letter are not significantly different ($P > 0.05$), based on nonoverlap of confidence intervals.
[4] ND, not determined.

were measured using the method of Bradford (1976) with bovine serum albumin as the standard.

### Statistics

All statistical analyses were conducted in R (Team 2013). Data from bioassays were subjected to probit analysis using the function LCprobit from the package ecotox, and resulting $LD_{50}$ values were used to compare insecticide susceptibilities to malathion and chlorantraniliprole. Values were considered significantly different if resulting 95% confidence intervals failed to overlap. In addition, percent mortalities following exposure to diagnostic concentrations of bifenthrin were compared among mosquitoes collected from sprayed and nonsprayed residential sites using the analysis of variance (aov) function. Tukey's multiple pairwise comparison (Tukey HSD function; $P < 0.05$) was made to compare resistance frequencies among different residential sites. Linear regression (lm function) was used to determine relationships between esterase activities and percent mortality resulting from exposure to diagnostic concentrations. Resistance ratios (RR) were based on $LD_{50}$ or $LC_{50}$ values and were calculated as: RR = $LD_{50}$ or $LC_{50}$ of field-collected/$LD_{50}$ or $LC_{50}$ of SEB-S.

## RESULTS

### Susceptibilities of *Cx. quinquefasciatus* collected from agricultural sites

The $LC_{50}$ values for chlorantraniliprole calculated for larvae collected from 3 agricultural sites were significantly higher than those of the reference susceptible-laboratory (SEB-S) and reference-field populations (LSU LAKE-S; Table 2). The RR measured were highest in RICE (12.1-fold) and lowest in CANE-2 (5.70-fold). Resistance in mosquito populations collected from the 3 agricultural sites was lower to malathion and ranged from 2.50- to 3.0-fold (larvae) and 2.74- to 2.92-fold (adults). The RR measured with larvae were minimal for both chlorantraniliprole (2.80-fold) and malathion (1.50-fold) at the field-reference (LSU-LAKE) site. Similarly for adults, the resistance ratio of malathion was low (1.38-fold at the field-reference site.

### Effect of residential sprays on susceptibility of *Cx. quinquefasciatus*

Percent mortalities from diagnostic concentrations of bifenthrin in larval and adult *Cx. quinquefasciatus* collected from all the residential sites were significantly lower ($P < 0.05$) than that measured in the reference field strain, LSU LAKE-S (Fig. 1). Additionally, bifenthrin susceptibility in larvae and adults from all the residential sites were highly correlated ($R^2 = 0.92$; Fig. 1, inset). Susceptibility was variable among the sites, and there was no clear trend between bifenthrin susceptibility and distance from the sprayed site (0-0). However, there were differences in insecticide susceptibility in residential sites in different seasons (Fig. 2): bifenthrin susceptibilities of adult populations from all residential sites were generally lower in August 2021 (immediately following insecticide application) than in November 2021 or June 2022. For example, after bifenthrin application in August 2021, mortality following application of a diagnostic concentration of bifenthrin ($10\times LC_{50}$) at the sprayed site was only 8% and was significantly lower than 3 nonsprayed residential sites (N-1, S-1, and S-3). In contrast, the average percent mortalities of the sprayed and 2 nonsprayed residential sites (N-2 and S-2) were not significantly different ($P > 0.05$). At all sites, resistance frequencies were significantly reduced in November 2021 compared with August 2021 and, except for the S-1 and S-2 sites, did not

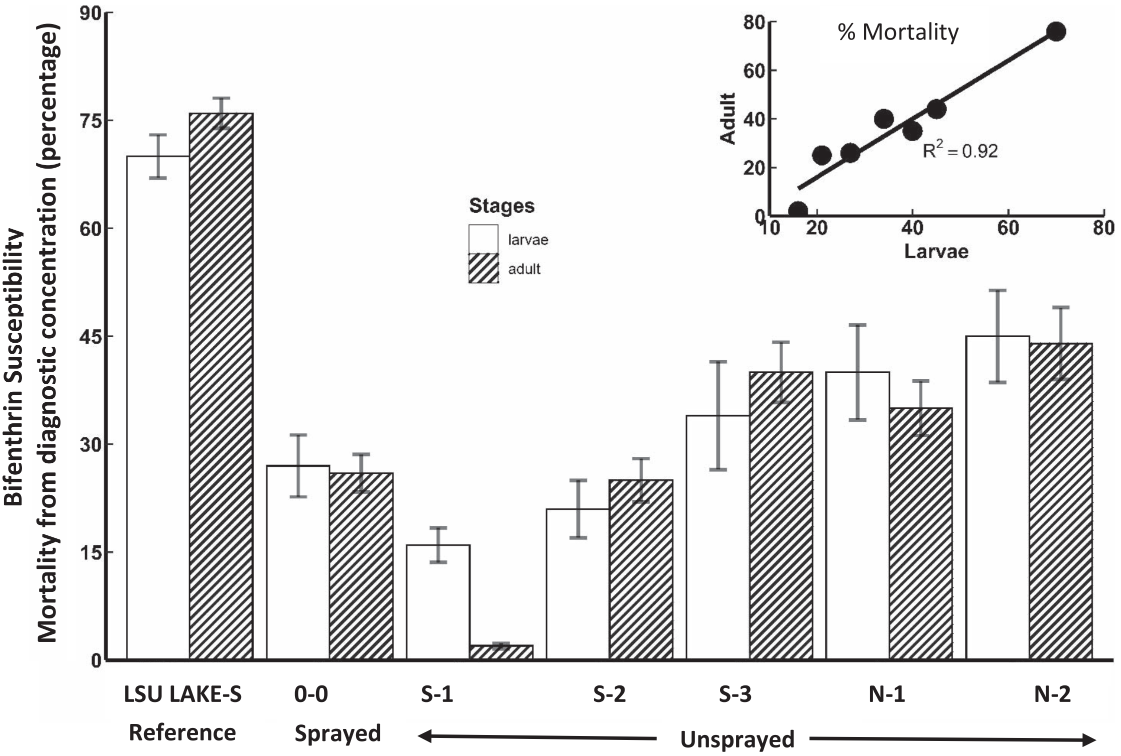


Fig. 1. Bifenthrin susceptibility in larval and adult populations of *Culex quinquefasciatus* collected from residential sites during June 2022. Mortality was measured following treatment with 10 times the $LC_{50}$ (larvae) or $LD_{50}$ (adult) of bifenthrin calculated from assays with SEB-S. Bars represents average percent mortality (± SD) from 3 different collections of *Cx. quinquefasciatus*. Inset: Larval versus adult mortality.

vary significantly between November 2021 and June 2022.

### Esterase activities of *Cx. quinquefasciatus* from residential sites

Esterase activities were moderately correlated with bifenthrin susceptibilities in both larval ($R^2 = 0.40$) and adult ($R^2 = 0.52$) populations of *Cx. quinquefasciatus* collected from residential sites (Fig. 3). Furthermore, esterase activities of larvae were correlated with those of adults ($R^2 = 0.75$; Fig. 3, inset).

## DISCUSSION

Insecticide resistance is widespread and well studied in populations of mosquitoes (Liu 2015, Coleman et al. 2017). While most studies describe effects on susceptibility in populations of mosquitoes targeted directly by insecticide application, less traditional sources of selection, such as insecticide runoff from agricultural fields, have been less well studied despite potential impacts on susceptibility of mosquito populations in areas adjacent to sprayed fields. In the current study, susceptibility to chlorantraniliprole (which is not used in mosquito abatement) was reduced in populations adjacent to both rice and sugarcane fields, where this insecticide is applied to manage stem-boring pests. Chlorantraniliprole susceptibility in the population collected from the rice field was lower compared with that in sugarcane, a possible reflection of different degrees of selection resulting from different methods of chlorantraniliprole application. In Louisiana sugarcane fields, chlorantraniliprole is generally used as a foliar spray (Wilson et al. 2022), whereas in rice fields, it is used as a seed treatment (Stout et al. 2011, Sidhu et al. 2014), which generally increases intensity of selection by chlorantraniliprole in larvae residing in the aquatic environment of rice fields. Water management practices in the rice field can readily result in the leaching of seed treatments into nearby water sources (Gupta et al. 2008, Vela et al. 2017), which increases exposure of invertebrate communities in the aquatic environment. In previous studies, populations of mosquitoes in agricultural fields have reduced susceptibilities to dichloro-diphenyl-trichloroethane and deltamethrin (Overgaard 2006, Ranson et al. 2009, Yadouleton et al. 2011, Fodjo et al. 2018). As expected, malathion resistance in both larval and adult *Cx. quinquefasciatus* collected from agricultural sites was minimal,

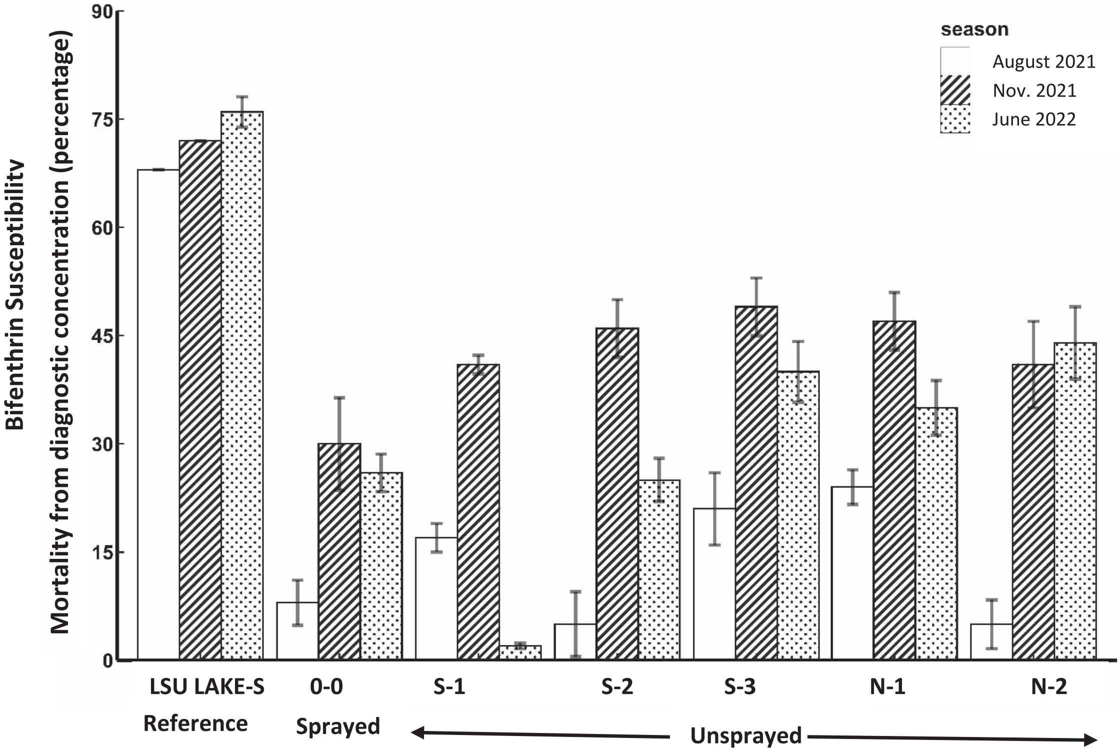


Fig. 2. Bifenthrin susceptibility in adult *Culex quinquefasciatus* collected from residential sites following treatment with 10 times the $LD_{50}$ of bifenthrin calculated from assays with SEB-S. Bars represents average percent mortality ($\pm$ SD) from 3 different collections of *Cx. quinquefasciatus*.

as malathion is not used in agricultural fields and selection pressure is low.

Insecticide application at individual residences is another nontraditional source of selection that impacts susceptibility of nontarget mosquito populations. In this study, application of bifenthrin at an individual residential site by a commercial pest applicator affected the susceptibility of *Cx. quinquefasciatus* throughout the neighborhood. Resistance frequencies of bifenthrin in both larval and adult *Cx. quinquefasciatus* at all residential sites were significantly higher compared with the reference-field (LSU LAKE-S) site. There was no clear pattern between resistance frequencies and distance from the sprayed site. However, resistance frequencies of bifenthrin varied in different seasons of the year and were lower in adult populations collected in November 2021 and June 2022 compared with August 2021, which might be due to reduced frequencies of bifenthrin application in November 2021 and June 2022. Frequency of bifenthrin resistance at the sprayed site was significantly higher than 3 unsprayed residential sites in the summer collection (August 2021) but lower in N-2 and S-2. There are several factors that affect exposure of populations of mosquitoes to applied insecticides, such as wind speed and direction, spray methods, and distance from point of application (Schleier et al. 2012, Rinkevich et al. 2017, Desmarteau et al. 2020). In a semi–field study, Rinkevich et al. (2017) suggest that distance is one of the most important environmental factors that affect exposure and selection of mosquitoes and honey bees *Apis mellifera* L. with different groups of insecticide used in mosquito abatement programs. Similarly, genetic mixing among populations of *Cx. quinquefasciatus* at the sampled residential sites might have contributed to variable frequencies of bifenthrin resistance in unsprayed residential sites (Service 1997). Alternatively, it is possible that the same population of *Cx. quinquefasciatus* was being sampled from all 6 residential sites, although the variability measured in resistance frequencies across the study site might suggest otherwise.

An additional, important factor that contributes to the chemical management of insects is the impact of selection on different life stages of insect pests. In the current study, larval and adult susceptibilities to bifenthrin were correlated. Because bifenthrin is generally used against adult mosquitoes in abatement programs (Hougard et al. 2002, Qualls et al. 2012), selection is most intense on this life stage. However, bifenthrin susceptibility in larval populations was decreased as well and was correlated with bifenthrin susceptibility in adults.

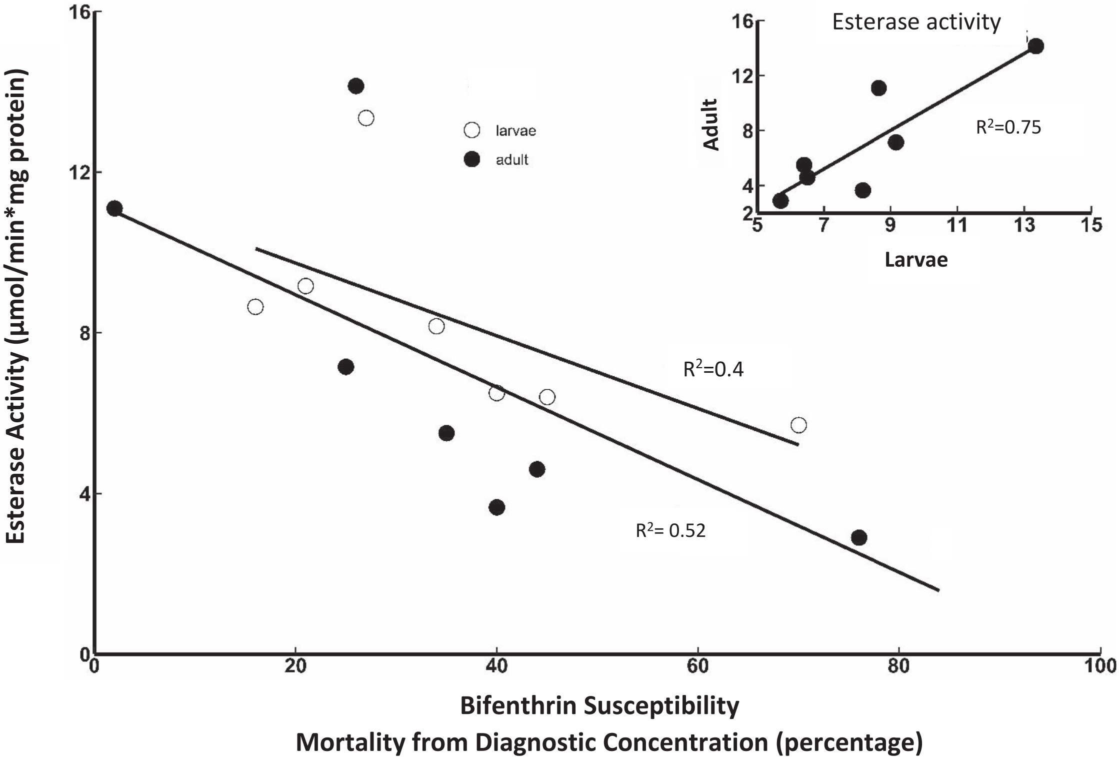


Fig. 3. Esterase activities and bifenthrin susceptibilities of larval (open circle) and adult (closed circle) populations of *Culex quinquefasciatus* collected from residential sites. Mortality was measured following treatment with 10 times the $LC_{50}$ (larvae) or $LD_{50}$ (adult) of bifenthrin as calculated from assays with SEB-S. Inset: Esterase activities in larvae versus adults.

Esterases hydrolyze ester-containing insecticides (including bifenthrin) have been shown in previous studies to be associated with insecticide resistance to organophosphate and pyrethroid insecticides (Raymond et al. 1993, Rider et al. 1998, Jackson et al. 2013, Wei et al. 2020). In the current study, esterase activities were moderately correlated with bifenthrin susceptibilities in both larval and adult populations of *Cx. quinquefasciatus* ($R^2 = 0.4$ and 0.52 for larvae and adults, respectively), which suggests involvement of other resistance mechanisms such as altered target sites (Lopez-Monroy et al. 2018) or enhanced metabolism by other enzymes (Riveron et al. 2013). It should be noted that target sites differed among the insecticides studied here: the ryanodine receptor for chlorantraniliprole, acetylcholinesterase for malathion, and voltage-sensitive sodium channel for bifenthrin. In addition, the contribution of other detoxifying enzymes such as P450s or glutathione-*S*-transferases was not studied and cannot be excluded. Finally, esterase activities in larvae and adults were correlated suggesting that expression of the esterase gene(s) was not life stage specific.

In conclusion, nonconventional sources of selection impact the development of insecticide resistance on nontargeted pest populations. Similarly, in the populations studied here, selection on adult *Cx. quinquefasciatus* affects insecticide susceptibility of the larval stage. Results from this study may be useful for developing management strategies for populations of *Cx. quinquefasciatus*.

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