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# INSECTICIDE RESISTANCE IN *CULEX PIPIENS* FROM NEW YORK

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ABSTRACT. Insecticides are the primary means to control *Culex pipiens,* an enzootic vector of West Nile virus, in the USA. To better understand how the evolution of resistance might impact control of this insect, we investigated the levels of resistance in populations collected from 2 metropolitan areas (Albany and Syracuse, NY) to 4 larvicides (methoprene, phenothrin, *Bacillus sphaericus* [*Bs*], and *Bacillus thuringiensis israelensis* [*Bti*]) and 1 adulticide (phenothrin) registered for mosquito control in New York State. High levels of resistance were found only to *Bti,* and only at 1 site (Syracuse). Resistance levels to the other insecticides were less than 10-fold. Given the large difference in *Bti* resistance between Syracuse and Albany, it appears these populations of *Cx. pipiens* do not rapidly mix, leading to localization of resistant populations.

**KEY WORDS** *Culex pipiens,* insecticide resistance, methoprene, phenothrin, *Bacillus sphaericus, Bacillus thuringiensis israelensis*

## INTRODUCTION

*Culex pipiens* L. is an important vector of several human pathogens. This species is considered especially important in the northeastern USA as an enzootic vector of West Nile virus. Control of mosquito-borne disease outbreaks relies heavily on the use of insecticides. However, the evolution of insecticide resistance can be a significant limitation to the continued use of these control agents (Georghiou 1986). In New York State, methoprene, phenothrin, *Bacillus sphaericus* (*Bs*), and *Bacillus thuringiensis israelensis* (*Bti*) are currently registered for mosquito control. Although these materials have been used for many years, no studies have been undertaken in the northeastern USA to determine if resistance is evolving to these control agents. This information is critically important to vector control agencies and is an obligatory 1st step toward the development of a resistance-monitoring strategy for this vector (NRC 1986).

In this study, we investigated susceptibility to 4 larvicides (methoprene, phenothrin, *Bs,* and *Bti*) and 1 adulticide (phenothrin) in *Cx. pipiens* collected from 2 sites in New York State. Our results indicate less than 10-fold resistance to all insecticides at both sites, except for *Bti,* where 34-fold resistance was detected at 1 site. The implications of these results to mosquito control in the northeastern USA are discussed.

## MATERIALS AND METHODS

*Strains of* Culex*:* Egg rafts and larvae were collected from catch basins within a 100-m radius in Syracuse (Onondaga County) and from catch basins at 2 sites (2 km apart) in Albany (Albany County), NY, in June 2003 and transported back to the laboratory. Information about insecticide use at these specific collection sites was not available. However, records of insecticide use by county from New York State (available from 1997 to 2001) indicate the following commercial use patterns: Syracuse, *Bti* (1998–2001), *Bs* (1999–2000), phenothrin (2000–2001), and no use of methoprene; Albany, *Bti* (2000), *Bs* (none), phenothrin (2001), and methoprene (1997–1998 and 2000–2001). No information is available for noncommercial use of these insecticides in New York State. Egg rafts were placed individually in 100-ml cups with 60 ml of distilled water and 2 ml of diet slurry (10 g of ground fish food [Tetra, Blacksburg, VA], 30 g of rabbit food [Big Red Rabbit Choice, ProPet LLC, St. Mary's, OH], and 10 g of bovine liver powder [ICN Biomedicals Inc., Aurora, OH] in 500 ml of distilled water). Each field-collected larva was identified to species by using published keys (Darsie and Ward 1981, Means 1987). Several larvae hatched from each isolated egg raft also were identified to species. *Culex pipiens* larvae (approximately 300–500) were used to establish colonies for each collection location. After colony establishment, material was removed periodically and confirmed as *Cx. pipiens* by using published diagnostic markers (Aspen and Savage 2003). Colonies were reared in the laboratory at 27°C, 80% relative humidity, and a 14:10 h light:dark photoperiod for subsequent generations. Larval diet solution (30 ml) was put into a rearing tray containing approximately 200 larvae in 1 liter of distilled water. Adult females in the colony cages were offered blood from a live restrained chicken 2–3 times per week (Cornell University Animal Use Protocol 01-56). Adults had constant access to 10% sucrose solution from cotton wicks. Females oviposited in containers filled with distilled water that were placed inside the cages. Eggs were transferred to new rearing trays and hatched within 24 h. Larvae were reared as described above. A susceptible strain (S-Lab) (Georghiou et al. 1966) was obtained from M. Raymond (Université de Montpellier II, France) and was reared and maintained in colony as described above.

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Table 1. Comparative laboratory toxicities of 4 insecticides to 4th-instar larvae of a susceptible (S-Lab) and 2 field-collected strains (Syracuse and Albany) of *Culex pipiens* from New York State, May 2003. Mortality was determined at 24 h for phenothrin and *Bacillus thuringiensis israelensis,* at 48 h *Bacillus sphaericus,* and after 10 d for methoprene.

| | Phenothrin | | | | *Bacillus thuringiensis israelensis* | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | $LC_{50}$[1] (95% CI) | $LC_{95}$[1] (95% CI) | *n* | Slope (SE) | $LC_{50}$[1] (95% CI) | $LC_{95}$[1] (95% CI) | *n* | Slope (SE) |
| S-Lab | 6.77 (6.13–7.55) | 15.3 (12.7–20.2) | 480 | 4.6 (0.5) | 0.35 (0.24–0.49) | 3.84 (1.30–11.8) | 825 | 1.6 (0.3) |
| Syracuse | 44.9 (42.2–47.9) | 113 (100–131) | 1,580 | 4.1 (0.6) | 11.4 (8.13–15.8) | 157 (66.8–377) | 1,285 | 1.4 (0.2) |
| Albany | 43.0 (38.8–49.3) | 121 (95.0–175) | 1,180 | 3.7 (0.4) | 1.98 (1.01–82.0) | 54.0 (11.5–161) | 660 | 1.1 (0.2) |

[1] In units of ppb. LC, lethal concentration.

*Insecticides:* Phenothrin (97%, *d*-(*cis-trans*)) and methoprene (98%, mix of isomers) were obtained from Chem Services (West Chester, PA). Strains of *Bs* (*Bs* 2362) and *Bti* (*Bti* IPS-80) were obtained from M. Wirth (University of California, Riverside, CA). A 2-liter flask containing 0.5 liter of media was used to culture the *Bacillus* spp. Growth media for *Bs* (Kalfon et al. 1983) contained 6.8 g of $K_2HPO_4$, 10 g of Bacto-tryptose, 2.0 g of yeast extract, 0.3 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of $CaCl_2 \cdot 2H_2O$, 0.02 g of $ZnSO_4 \cdot 7H_2O$, 0.02 g of $MnSO_4 \cdot H_2O$, and 0.02 g of $Fe_2(SO_4)_3$ in 1 liter of $H_2O$, pH 7.2. Cultures were grown while shaking at 30°C for 24–48 h. Growth media for *Bti* (Park et al. 1998) contained 1.0 g of glucose, 5.0 g of yeast extract, 3.0 g of $K_2HPO_4$, 2.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $MnSO_4 \cdot H_2O$, and 0.05 g of $CaCl_2 \cdot 2H_2O$ in 1 liter of $H_2O$. Cultures were grown while shaking at 30°C for 5 days. Cells from *Bs* and *Bti* were collected by centrifugation at 4,000 × g for 15 min, resuspended in distilled water, pelleted by centrifugation, dried under vacuum, ground into a powder, and stored at 4°C. Three independent preparations of *Bs* were pooled, thoroughly mixed, and used for all assays. Two independent preparations of *Bti* were pooled, thoroughly mixed, and used for all assays. The S-Lab strain was tested weekly to ensure that the potency of the *Bs* and *Bti* did not change.

*Bioassays:* Twenty early 4th-instar larvae of uniform size were placed in 140-ml wax-coated paper cups (Horwitz, Horseheads, NY) containing 99 ml of distilled water and 1 ml of insecticide solution for the larval bioassays. Phenothrin and methoprene were prepared in acetone (weight: volume) and *Bs* and *Bti* were prepared in distilled water (weight: volume). The *Bs* and *Bti* solutions were suspended by using a stir bar and 3-mm glass beads until the lyophilized material was completely in suspension. Control cups consisted of 99 ml of distilled water with 1 ml of acetone for phenothrin and methoprene assays. Distilled water (100 ml) served as the control in the *Bs* and *Bti* assays. Control mortality was <1%.

Mortality was determined after 24 h for phenothrin and *Bti,* and 48 h after treatment for *Bs.* Larvae that failed to move or resurface after being touched with a probe were considered dead. The methoprene bioassay cups were given food and covered with fabric (to contain emerged adults) and a perforated plastic cap (to minimize evaporation). Mortality was defined as the number of adults that failed to emerge after 10 days. Greater than 90% adult emergence was observed in the control cups after 10 days.

Phenothrin also was evaluated against adults. These bioassays were conducted in glass jars (230 ml, internal surface area of 160 $cm^2$) treated with 1 ml of insecticide solution (or 1 ml of acetone for controls), which was evenly coated on the inner walls. After acetone evaporated (30 min), 10 adult females (not bloodfed) were placed inside each jar with the top secured with fabric. Adults in the glass jars had access to 10% sucrose solution from dental wicks secured to the fabric lid. Mortality was determined as the number of ataxic adults after 48 h of exposure to the insecticide.

Each bioassay consisted of at least 6 concentrations (with a minimum of 5 concentrations giving >0% and <100% kill). Results from a minimum of 3 replications were pooled and subjected to probit analysis (Raymond 1985) based on the method of Finney (1971) with control mortality corrected by Abbott's formula (Abbott 1925).

## RESULTS AND DISCUSSION

All 4 of the larvicides were highly toxic to the susceptible strain (S-Lab) with median lethal concentration ($LC_{50}$) values (Table 1) ranging from 0.07 ppb (methoprene) to 5.34 ppb (phenothrin). The $LC_{50}$ values we obtained are similar to those reported previously for phenothrin (Kawakami 1989) and methoprene (Robert 1989), but our values for *Bs* and *Bti* were lower than published values for *Cx. pipiens* from other laboratories (Rodcharoen and Mulla 1996, Chevillon et al. 2001, Wirth et al. 2001). This is not unusual, because toxicity of bi-

Table 1. Extended.

| *Bacillus sphaericus* | | | | Methoprene | | | |
|---|---|---|---|---|---|---|---|
| $LC_{50}$[1] (95% CI) | $LC_{95}$[1] (95% CI) | *n* | Slope (SE) | $LC_{50}$[1] (95% CI) | $LC_{95}$[1] (95% CI) | *n* | Slope (SE) |
| 0.12 (0.11–0.13) | 0.45 (0.38–0.56) | 1,450 | 2.9 (0.2) | 0.07 (0.03–0.18) | 16.9 (2.40–123) | 840 | 0.7 (0.1) |
| 0.37 (0.32–0.44) | 3.33 (2.36–4.76) | 1,985 | 1.7 (0.1) | 0.54 (0.25–1.20) | 35.7 (6.45–205) | 660 | 0.9 (0.2) |
| 0.07 (0.05–0.11) | 0.42 (0.20–0.89) | 1,160 | 2.1 (0.4) | 0.24 (0.14–0.39) | 53.7 (23.2–168) | 540 | 0.7 (0.1) |

ological insecticides such as *Bs* and *Bti* can be quite variable from one laboratory to another (e.g., Rodcharoen and Mulla 1996, Chevillon et al. 2001).

Larvae from the Syracuse strain had 6.6-, 3.1-, and 7.7-fold resistance (at the $LC_{50}$) to phenothrin, *Bs,* and methoprene, respectively (Fig. 1). In addition, this strain had a relatively high level of resistance (33-fold) to *Bti*. Although variability in *Bti* susceptibility of up to 10-fold has been detected in *Culex* from Cyprus (Wirth et al. 2001), the Syracuse collection has one of the highest levels of *Bti* resistance ever reported in *Culex*. If the populations readily mix we would expect similar levels of resistance at both sites (independent of insecticide use), as has been found for highly mobile pests such as the house fly, *Musca domestica* L. (Scott et al. 1989, Kaufman et al. 2001). Given the substantially lower level of resistance found in the Albany strain, it appears that these populations of *Cx. pipiens* do not rapidly mix, leading to localization of resistant populations. This result is consistent with a study of organophosphate resistance in *Cx. pipiens* from southern France (Lenormand and Raymond 2000) and has important implications for resistance management programs. Larvae from the Albany strain showed lower levels of resistance to all insecticides, and were even (1.7-fold) more sensitive to *Bs* than the S-Lab strain. However, variation in susceptibility to *Bs* has been well documented in *Culex* (Wirth et al. 2001).

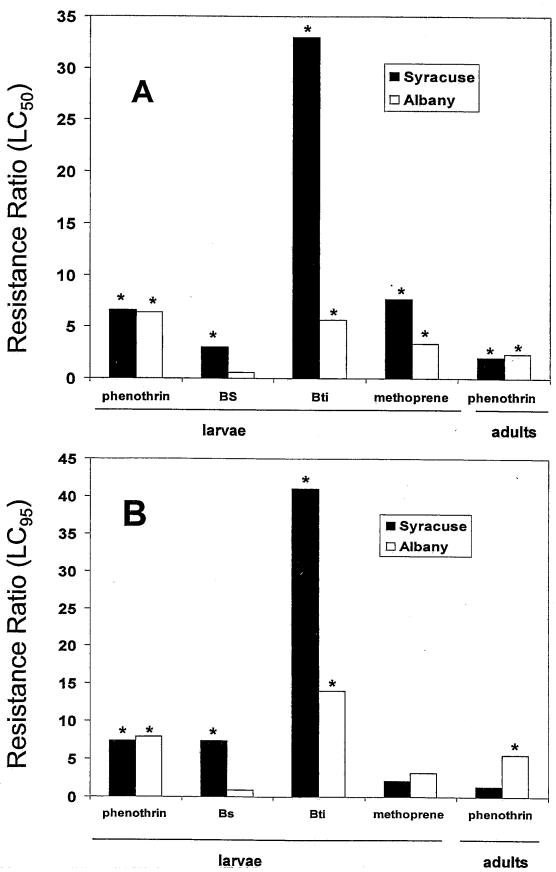


Fig. 1. Levels of resistance at (A) the median lethal concentration ($LC_{50}$) and (B) the $LC_{95}$ in larvae and adults from 2 strains of field-collected *Culex pipiens* from New York. Resistance ratios are relative to the susceptible S-Lab strain. An asterisk indicates a value significantly greater than 1.0 based on nonoverlap of 95% confidence intervals.

Phenothrin is primarily used for control of adult mosquitoes in New York State. Therefore, we examined the level of phenothrin resistance in adults from Syracuse and Albany. Although resistance ratios significantly greater than 1 were found, resistance levels were low in both strains (Fig. 1 and Table 2). Thus, even though phenothrin is potentially used against both larval and adult mosquitoes, resistance levels have not developed to more than 5.6-fold in adults and 7.9-fold in larvae.

The slopes of the concentration–response lines were similar for the Syracuse, Albany, and S-Lab strains (Tables 1 and 2), and thus the resistance ratios were similar at the $LC_{50}$ and $LC_{95}$ values (Fig. 1). This indicates limited heterogeneity in the field-collected samples to any of the insecticides tested relative to S-Lab.

Resistance levels were generally higher where more insecticide use had been reported. For example, Onondaga County had used more *Bti* and *Bs*

Table 2. Toxicity of phenothrin to adult females of a susceptible (S-Lab) and two field-collected strains (Syracuse and Albany) of *Culex pipiens* from New York State. Mortality was determined at 24 h.

| Strain | $LC_{50}$[1] (95% CI) | $LC_{95}$[1] (95% CI) | *n* | Slope (SE) |
|---|---|---|---|---|
| S-Lab | 0.007 (0.006–0.008) | 0.027 (0.020–0.039) | 540 | 2.5 (0.3) |
| Syracuse | 0.014 (0.012–0.016) | 0.037 (0.031–0.048) | 390 | 3.3 (0.4) |
| Albany | 0.017 (0.013–0.023) | 0.150 (0.070–0.360) | 750 | 1.6 (0.2) |

[1] In units of μg/cm². LC, lethal concentration.

than Albany County, and resistance levels to these insecticides were higher in Syracuse compared to Albany. However, methoprene had been used commercially only in Albany County, yet resistance levels were similar between the 2 sites. However, correlation of resistance with insecticide use is difficult for several reasons. Insecticide use information in New York was only available for commercial applicators. It is unclear at what level the insecticides we tested are used by noncommercial applicators. Lacking quantitative information about insecticide use is a limitation to our understanding of the actual selective pressure being exerted on these populations.

The relatively low levels of phenothrin resistance we detected are in stark contrast to the high levels of pyrethroid resistance (up to 940-fold for permethrin) found in *Culex quinquefasciatus* Say in Alabama and Florida (Liu et al. 2004). This is undoubtedly due to the more intensive use of pyrethroids (a function of the longer season over which mosquitoes must be controlled) for control of *Culex* spp. in Alabama compared to New York.

Given the generally low levels of resistance we detected, it appears feasible to use diagnostic concentrations of the susceptible strain $LC_{99}$ for monitoring insecticide resistance in *Cx. pipiens* in New York. Given the high level of *Bti* resistance detected in one population, it is important to widen the scope of resistance monitoring in New York, to identify populations for which resistance levels may compromise control efforts.

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