RAPID COMMUNICATION

Insecticide Resistance in the Bed Bug: A Factor in the Pest's Sudden Resurgence?

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ABSTRACT Infestations of the bed bug, *Cimex lectularius* L. (Heteroptera: Cimicidae), are increasing around the world at an alarming rate and have become a major public health concern. The evolution of insecticide resistance could be a primary factor in explaining this resurgence. Extremely high levels of resistance to two pyrethroid insecticides, deltamethrin and λ-cyhalothrin, relative to a susceptible colony, were detected in populations collected from human dwellings in Kentucky and Ohio. Offspring of a cross between a resistant and susceptible colony had intermediate susceptibility. Evaluations of populations from across the United States indicate that resistance to pyrethroid insecticides is already widespread. Without the development of new tactics for bed bug management, further escalation of this public health problem should be expected.

KEY WORDS bed bug, urban pest, resurgence, insecticide resistance, pyrethroids

The bed bug, *Cimex lectularius* L. (Heteroptera: Cimicidae), is a flightless blood-sucking parasite that usually feeds at night (Usinger 1966). Lesions caused by bites usually occur on exposed areas of the face, neck, and extremities, producing small clusters of erythematous papules or wheals (Thomas et al. 2004, Ter Poorten and Prose 2005). Although they are not known to be vectors of human diseases, bed bugs severely reduce quality of life by causing discomfort, anxiety, sleeplessness, and ostracism (Hwang et al. 2005). Bed bug infestations often require expensive ongoing inspections and treatments, disposal and replacement of infested beds and other furnishings, and quarantine of infested areas. In public facilities, they may result in adverse publicity and litigation by persons who are bitten (Doggett 2005, Potter 2005).

Bed bugs have a long association with humans, e.g., they were found in Egyptian tombs dating back >3,000 yr (Panagiotakopulu and Buckland 1999). Bed bugs were part of life before chlorinated hydrocarbons and other synthetic insecticides became widely used in the 1940s and 1950s. Although bed bugs never completely disappeared, they were so uncommon throughout much of the world that even pest control professionals rarely encountered them (Potter 2005). A resurgence of bed bugs has occurred in North America, Europe, and Australia over the past 10 yr (Boase 2001, Doggett et al. 2004, Potter 2005). Infestations are now common in the urban environment, including single-family dwellings, apartments, rooming houses, hotels, health care facilities, and college dormitories (Hwang et al. 2005).

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Several hypotheses have been proposed to explain the sudden resurgence of bed bugs, including increased travel to and from areas of the world where bed bugs remained common, increased exchange of second-hand furniture, a shift from premise-wide use of broad-spectrum insecticides to more selective control tactics for other urban pests, and insecticide resistance (Doggett et al. 2004, Potter 2005). Evolution of resistance is a common outcome of use of a single insecticide, or insecticides with a common mode of action, against populations for consecutive generations (Georghiou 1986). Insecticide resistance has been found in >400 species of arthropods (Roush and Tabashnik 1990), including blood-feeding insects (e.g., mosquitoes) where the loss of efficacy has led to pest resurgence and increases in rates of disease transmission (Krogstad 1996).

Our observations that some infestations were difficult to control (Potter et al. 2006) and that some field-collected bed bugs survived direct spray applications with label-rate, formulated deltamethrin (A.R. and M.F.P., unpublished data) led to this investigation. The results presented here show very high levels of resistance to two widely used pyrethroids in populations of bed bugs collected from human dwellings from across the United States.

Materials and Methods

Insects. Four colonies were initiated from infested dwellings from Lexington, KY (LEX1) and Cincinnati, OH (CIN1, CIN2, and CIN3). Dwellings were separated by at least 6.1 km. Two laboratory colonies also were established that had never been exposed to py-

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rethroids; one colony from Ft. Dix, NJ, had been collected >30 yr earlier (Bartley and Harlan 1974); and the second colony from Gainesville, FL, had been collected >20 yr earlier. An F1 generation was produced by crossing virgin female bed bugs from the Ft. Dix laboratory colony with males from CIN1. Crosses in the other direction were less successful; therefore, they were not evaluated in this study. Other insects that were used directly from the field came from Los Angeles, CA (LA1 and LA2 populations), Kissimmee, FL (KIS1), and Vienna, VA (VIE1).

Colonies of bed bugs were reared in laboratory conditions by using a parafilm-membrane feeder. Heparinized chicken blood was heated to 37°C with a circulating water bath (Montes et al. 2002). Colonies were maintained at 27°C, 70% RH, and a photoperiod of 14:10 (L:D) h.

Residual Assay. Adults from the Ft. Dix colony or recent adult descendents from the LEX1, CIN1, CIN2, and CIN3 (1:1 sex ratio; three replicates of 20 insects) were exposed for 24 h to insecticide residues on filter papers. Insects were evaluated 7–12 d after adult emergence, and they had not been fed. Based on a preliminary range test, the concentration of deltamethrin (99% purity; Chem Service, West Chester, PA) was adjusted to 4.4×10^{-5} to 1.3×10^{-2} for Ft. Dix; 1.3×10^{-2} to 3.96 for LEX1, CIN1, CIN2, and CIN3; and 4.4×10^{-4} to 3.96 mg of active ingredient (AI) per cm^2 for F1. Tested concentrations of λ-cyhalothrin (99% purity; Chem Service) ranged from 6.6×10^{-6} to 6.6×10^{-3} for Ft. Dix and from 6.6×10^{-3} to 1.32 mg/cm^2 for CIN1 (only this field-derived colony was used with this compound). An insecticide–acetone solution of 50 μl was applied to each filter paper disc (Whatman no. 2; cut to 2.27 cm^2 [1.7 cm in diameter]) and allowed to dry completely before being placed in the bottom of individual cells of 24-well cell culture plates, which were then covered. Control discs received acetone only. There was one individual bed bug per cell. Continuous exposure to the upper surface of the filter paper was ensured by the tight fit of the paper, and by a Fluon AD-1 (polytetrafluoroethylene; Northern Products, Woonsocket, RI) coating on the walls of each cell that prevented individuals from climbing off the treated surface. Temperature was maintained at 25°C after initiation of the exposure. After 24-h exposure in the culture plates, mortality was assessed by gently touching each individual with a fine paint brush and categorizing it as alive (coordinated avoidance movement) or dead (no response, usually on backs with no movement of any body parts). The few moribund individuals that were unable to maintain balance and showed uncoordinated twitching were recorded as dead in these assessments, and they did not recover.

The LC_{50} (concentration that kills 50% of individuals at 24 h) was determined for Ft. Dix and F1 by using probit analysis (Finney 1971, Minitab, Inc. 2005). LC_{50} values for other colonies could not be calculated because the highest tested concentrations resulted in little mortality. It was not practical to use higher concentrations (i.e., the highest concentration [18% (AI) in acetone, 3.96 mg/cm^2] left a visible residue on the filter paper disc; it was impractical to make more concentrated solutions). For these colonies the LC_{50} was greater than the 3.96 mg (AI)/cm^2 for deltamethrin and 1.32 mg (AI)/cm^2 for λ-cyhalothrin. Resistance ratios calculated on this basis (LC_{50} resistant colony/LC_{50} susceptible colony) underestimate the actual ratio; therefore, they are conservative.

In another experiment, 12–20 third-to-fifth instars from colonies or directly from apartments were evaluated with acetone alone or 0.13 mg/cm^2 technical grade deltamethrin (10× high labeled rate of active ingredient in commercial product). The latter treatment concentration was selected to discriminate between resistant and susceptible populations based on our earlier assays with adults. An overall difference among populations was analyzed with a chi-square test (Minitab Inc. 2005).

Results and Discussion

There was a dramatic difference in susceptibility to deltatmethrin between the Ft. Dix colony and the four field colonies from the Kentucky–Ohio area. The Ft. Dix colony suffered 100% mortality at 4.4×10^{-3} mg/cm^2 and higher tested concentrations (Fig. 1; n = 60; slope = 0.97 ± 0.09; $LC_{50} = 3.10 \times 10^{-4}$ mg/cm^2 [95% CI = 2.51×10^{-4}–3.82×10^{-4}]; $\chi^2 = 8.26$; df = 4). There was no control mortality in any of our assays. For the field-derived colonies, the highest concentration that we evaluated (3.96 mg/cm^2) killed only a few individuals (LEX1, 5%; CIN1, 1.7%; CIN2, 3.3%; and CIN3, 3.3%; n = 60 for all), and no mortality resulted from lower insecticide concentrations. The resistance ratio of these four colonies relative to the Ft. Dix colony was >12,765. The practical upper limit of solubility of deltamethrin in acetone prevented us from determining the LC_{50} for these four colonies; therefore, presentation of probit values is not appropriate. The F1 offspring of matings between CIN1 and Ft. Dix showed intermediate levels of resistance (n = 60; slope = 0.35 ± 0.034; LC_{50} = 0.46 mg/cm^2 [95% CI = 0.28–0.78]; $\chi^2 = 8.59$; df = 4; resistance ratio = 1,481). This result suggests that the genetic basis of resistance was not a single dominant-recessive gene, but it was influenced by one or more genes with incomplete dominance. In addition, that viable offspring were produced indicates that the genetic differences that influenced resistance were superimposed on a genetic background that was similar.

The results with λ-cyhalothrin paralleled those with deltamethrin (Fig. 1). The Ft. Dix colony was susceptible to λ-cyhalothrin (n = 60; slope = 0.45 ± 0.048; $LC_{50} = 2.16 \times 10^{-4}$ mg/cm^2 [95% CI = 1.39×10^{-4}–3.38×10^{-4}]; $\chi^2 = 10.63$; df = 2; no control mortality) Mortality was 100% at 6.6×10^{-3} mg/cm^2. The CIN1 colony showed no control mortality, and only 21.6% mortality at the highest concentration tested (1.32 mg [AI]/cm^2). Therefore, the resistance ratio was at least 6,123. Although the resistance ratios for deltamethrin and λ-cyhalothrin are underestimates, they seem to be of the same order of magnitude as the highest levels of

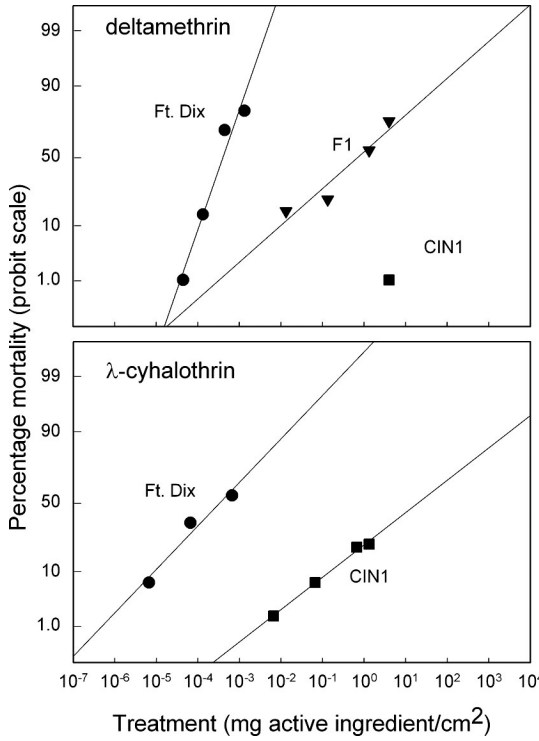

Fig. 1. Log dosage versus mortality on probit scale for adult bed bugs exposed to deltamethrin (top graph) or λ-cyhalothrin (bottom graph). Populations tested were Ft. Dix (●), a susceptible colony; Cincinnati, OH; CIN1 (■), a field-collected resistant colony, and F1 offspring of crosses between the two colonies (▼). F1 offspring were only tested with deltamethrin. For CIN1, only one data point with mortality >0% could be obtained because we were near the practical upper limit for dilution of deltamethrin in acetone. There was ≈4 orders of magnitude difference between an insecticide dose that kills the susceptible and resistant colonies for both deltamethrin and λ-cyhalothrin.

resistance seen with other species of insects (Guerrero et al. 1997, Liu and Yue 2000).

Resistance to pyrethroid insecticides is not a local phenomenon, nor is it universal. We assessed presence or absence of resistance in third-to-fifth instars from 10 populations by using a discriminating dose, and there was a clear cut and significant difference among populations (Table 1; $\chi^2 = 194$, df = 9, $P < 0.001$). Two laboratory colonies that have never been exposed to pyrethroids were susceptible (Table 1; 100% mortality at the discriminating dose). Populations collected in California, Florida, Kentucky, Ohio (three colonies), and Virginia were resistant (0% mortality at the discriminating dose). One California population collected from the same building as a resistant population was susceptible (100% mortality at the discriminating dose), indicating independent source populations, a founder effect, or rapid evolution of resistance. An alternative explanation of the latter result is that this susceptible population could have been preexposed at the collection site to some other environmental stress

Table 1. Mortality of bed bug nymphs (third to fifth instars) exposed for 24 h to a discriminating dose (0.13 mg/cm^2) of technical grade (99% active ingredient) deltamethrin (n = 20 unless otherwise noted)

Pop[a]	Origin	% mortality[b]	
		Control	Deltamethrin[c]
LAB1	Ft. Dix, NJ	0	100
LAB2	Gainesville, FL	0	100
LA1	Los Angeles, CA	0	100
LA2	Los Angeles, CA	0	0
KIS1	Kissimmee, FL	0	0
LEX1	Lexington, KY	0	0
CIN1	Cincinnati, OH	0	0
CIN2	Cincinnati, OH	0	0
CIN3	Cincinnati, OH	0	0
VIN1	Vienna, VA	0	0

[a] LAB1 maintained >30 yr without exposure to insecticides; LAB2 colony maintained >20 yr without exposure to insecticides; nymphs collected in apartments were evaluated for LA1, LA2, KIS1, and VIN1; nymphs from colonies initiated in 2005 were used for LEX1, CIN1, CIN2, and CIN3.

[b] LA2-control, n = 12; LA2-deltamethrin, n = 14; KIS1-control, n = 19.

[c] Significant difference amongst population in mortality caused by deltamethrin ($\chi^2 = 194$, df = 9, $P < 0.001$).

(such as a different insecticide). To rule out this possibility, we reared the LA1 population in the laboratory and assessed the impact of our discriminating dose. These offspring also were determined to be susceptible (100% mortality at 24 h, n = 20; no control mortality).

Evolution of resistance to insecticides is the expected outcome of their repeated use. A recent interim report by Boase et al. (2006) suggests that cypermethrin resistance is present in the United Kingdom. Because DDT resistance was reported decades ago (Busvine 1958, Mallis and Miller 1964), and cross-resistance between DDT and pyrethroid insecticides is common (Farnham 1977, Prasittisuk and Busvine 1977), resistance alleles already may have been present in populations. Failure of pyrethroids to quickly control infestations of resistant populations increases the opportunity for their spread. Spread of resistant populations is facilitated by the transport of bed bugs from one building to another and by unintended recycling of infested mattresses and furniture. Attempts to dispose of infested items may be more frequent when insecticides alone have failed to eliminate the problem. Acquisition of used furniture is a common source of new infestations (Potter 2005).

Inability to control bed bugs with pyrethroids may necessitate development of products with new modes of action, relabeling of existing efficacious products, and greater reliance on alternative tactics such as heat treatment, vacuuming, mattress encasements, or barriers. In addition, future investigations into the mechanisms of pyrethroid resistance could provide useful information to enhance existing insecticides or point to alternate compounds with different modes of action. Increased public awareness also is needed to minimize the risks of acquiring or transporting bed bugs. The options for chemical control of bed bugs

were diminished by regulatory restriction of chlorinated hydrocarbon, organophosphate, and carbamate insecticides in many countries. Resistance to pyrethroids, the largest remaining insecticide class, further limits these options. Without safe and effective alternatives, the continuing escalation of this serious pest problem seems inevitable.

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