Development of a Larval Bioassay for Susceptibility of Cat Fleas (Siphonaptera: Pulicidae) to Imidacloprid

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ABSTRACT Strategies for controlling cat fleas, *Ctenocephalides felis felis* (Bouché), have undergone dramatic changes in the past 5 yr. With the advent of on-animal treatments with residual activity the potential for the development of insecticide resistance increases. A larval bioassay was developed to determine the baseline susceptibility of field-collected strains of cat fleas to imidacloprid. All four laboratory strains tested showed a similar level of susceptibility to imidacloprid. Advantages of this bioassay are that smaller numbers of fleas are required because flea eggs are collected for the test. Insect growth regulators and other novel insecticides can also be evaluated. Using a discriminating dose, the detection of reduced susceptibility in field strains can be determined with as few as 40 eggs.

KEY WORDS cat flea, *Ctenocephalides felis felis*, larval bioassay, flea eggs, imidacloprid

INTEGRATED PEST MANAGEMENT (IPM) approaches to cat flea control have been advocated for at least 5 yr (Rust and Dryden 1997) with a view to conserving the new generation of insecticides used for this purpose. These are a valuable resource in veterinary practice as, when used therapeutically, they not only have high curative efficacy but prevent new flea infestations from establishing for a period of weeks. It is important in the management of flea bite hypersensitivity that a high level of flea control is maintained because no other effective therapeutic approaches are currently available (Carlotti and Jacobs 2000). Thus, it is likely that flea control will continue to be largely dependent on the intelligent use of these new chemical classes. One important aspect of conserving this valuable asset is the ability to monitor the susceptibility of populations to these insecticides and to determine when alternative treatments may be necessary. Insecticide resistance has typically been determined with adult fleas using various modifications of the World Health Organization filter paper exposure test (Collart and Hink 1986, El-Gazzar et al. 1986, Rust and Dryden 1997, reviewed by Bossard et al. 1998) and recently, by topically applying insecticides (Moyses and Gfeller 2001). Many factors may affect contact exposure assays including relative humidity (Rust 1993), solvents (Rust 1993), CO_2 exposure (El-Gazzar et al. 1988), and the difficulty of collecting and shipping fleas from field sites. To conduct adult bioassays it is generally necessary to maintain the flea strain on artificial feeding systems or live hosts such as cats until there are sufficient numbers to test. With adult bioassays large numbers of adult fleas are required to obtain satisfactory probit lines. Certain insecticides such as insect growth regulators (IGRs) do not have contact activity against adult cat fleas in tests such as the WHO bioassay. However, larval bioassays are attractive because eggs can be easily collected and shipped, insecticides that are active against adults and larvae can be evaluated and sufficient numbers of eggs can be collected from field infestations, thereby avoiding laboratory maintenance of each strain. Chamberlain et al. (1988) developed a technique to determine the susceptibility of late instar oriental rat flea, *Xenopsylla cheopis* (Rothschild), to various organophosphate, carbamate and potential insect growth regulator (IGR) compounds. Zakson-Aiken et al. (2000) exposed cat flea larvae to pretreated media. These techniques offer a

This study was conducted according to the "Guide for the Care and Use of Laboratory Animals" promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, 1996 and protocols adopted by each institution.

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promising alternative to testing cat flea adult susceptibility.

The objective of this study was to develop a larval bioassay that could be used to determine the sensitivity of cat fleas to imidacloprid using a minimal number of insects, and eliminating the need to maintain field strains on laboratory hosts or use artificial feeding systems.

Materials and Methods

Insects. Four laboratory strains of cat fleas, *Ctenocephalides felis felis* (Bouché) (UCR, KSU, Auburn, Monheim) were maintained on separate cats according to a procedure modified from Metzger and Rust (1996). UCR is the flea strain maintained for about 19 yr at the University of California, Riverside. The other laboratory strains include KSU (Kansas State University), Auburn (Auburn University), and Monheim (Bayer Animal Health laboratories in Monheim, Germany). Cat flea eggs were collected from trays underneath cats supporting each strain. The eggs and debris were passed through a series of four sieves, 10, 16, 20, and 60 mesh, with the eggs being retained on the 60-mesh screen.

The eggs were placed on UCR larval flea rearing medium (1 part nutritive medium [0.15 dried beef blood (America's Laboratories, # NK3027034 SD Hemoglobin Powder, Omaha, NE)] to 0.75 ground dog chow by weight to 0.1 inactive baker's yeast [Red Star Bio Products –Nuttrex 55, Milwaukee, WI]) to three parts silica sand [30 mesh] by volume) and held at 80%RH and 26°C. Larvae of all four strains completed development in 11–13 d and the cocoons and larval medium were passed through a 16-mesh sieve to separate the cocoons. Adults began emerging 16–18 d after egg collection. To maintain the strains on each cat, ≈30 males and 30 females were placed on each cat every 2 wk.

Larval Media Studies. To determine if solvents affected the larval rearing media, 0.2 ml aliquots of various solvents were applied to 2 g of larval rearing media in 76 by 20 mm plastic vials (Sarstedt, Newton, NC) and allowed to dry for at least 4 h before being transferred to glass petri dishes (5 cm diameter by 1.5 cm). Twenty cat flea eggs (UCR strain) were placed on the media and the dishes and eggs were placed in a chamber maintained at 80% RH and 26 ± 2°C. After 28 d, the number of adults that emerged was determined. The data were analyzed with a one-way analysis of variance (ANOVA) and means separated with Tukey's honestly significant difference (HSD) (Jandel 1995).

Larval Bioassays. To determine the activity of imidacloprid against larval cat fleas, immature fleas were exposed to larval rearing media treated with serial dilutions of imidacloprid (99.9% technical, Bayer Animal Health, Monheim, Germany). Two grams of media were placed in the bottom of each plastic Sarstedt vial and treated with 2 ml of the serial dilutions of technical imidacloprid in acetone (0.003, 0.0015, 0.001, 0.0005, 0.0003, 0.0001, 0.00005, 0.00001, and 0.000005%), providing treated media ranging from 30 to 0.05 ppm. The mixture was stirred and the media was allowed to dry for at least 4 h. The treated media was poured into glass petri dishes (5 cm diameter by 1.5 cm); it is important to use glass petri dishes instead of plastic to reduce static and prevent eggs and media from flying out of or clinging to the sides of the petri dishes.

To determine the number of flea eggs that hatched, eggs were cemented to the upper inner surface of the petri dish. A thin streak of glue (UHUStic, Saunders, Winthrop, ME) was applied to the glass with a moistened paint brush. Eggs and debris were put into a porcelain crucible dish. The crucible was tipped on its side and the eggs rolled forward, separating them from the dried fecal blood and other debris. Eggs were carefully placed in the petri dish lid and rolled onto the tacky surface with a fine camel hair brush (size 00000). Attachment of the eggs was verified by touching the eggs with a fine camel's-hair brush. Once the glue dried, the eggs remained attached to the petri dish lid. As the eggs hatched, the larvae fell down in the media. This method (the so called 'Advantage Monitoring method') permitted an accurate count of the number of eggs that hatched and separated the eggs from the adult fecal blood collected under the cats, ensuring that immatures fed only on treated media. The petri dish lids were transferred over the media and placed into incubators maintained at 26 ± 2°C and 80% RH. The number of hatched eggs was counted at day 5.

The media and cocoons were passed through a 16-mesh screen at day 12 and the number of cocoons counted. The cocoons were placed in a 2.5 cm diameter by 4.5 cm plastic snap cap vial and a 5.5 cm diameter disk of Whatman filter paper (Whatman, Hillsboro, OR) was placed over the top and secured with a snap cap lid. The vials and cocoons were returned to a chamber maintained at 26 ± 2°C and 80% RH.

All larval bioassays were conducted at UC Riverside. The number of adults that developed was counted on day 28. Adult emergence data were analyzed by probit analysis with POLO program (Robertson and Preisler 1992).

Results and Discussion

Of the solvents applied to larval rearing media, acetone, water, hexane, and methylene chloride produced the least amount of larval mortality (Fig. 1). Larval rearing media treated with 100% EtOH or MeOH produced significant larval kill. It is likely that alcohols affect the nutritional quality of the yeast because larvae did develop on dried fecal blood treated with alcohols (M.K.R., unpublished data). Silverman and Appel (1994) showed that yeast was an important component of larval rearing medium, increasing the number of larvae maturing and decreasing the developmental time. Acetone was selected as a convenient solvent in this study.

Four laboratory strains of cat fleas (Auburn, Monheim, KSU and UCR) were tested (Table 1). The LD_{50}s ranged from 0.21 to 0.70 ppm, but none of the

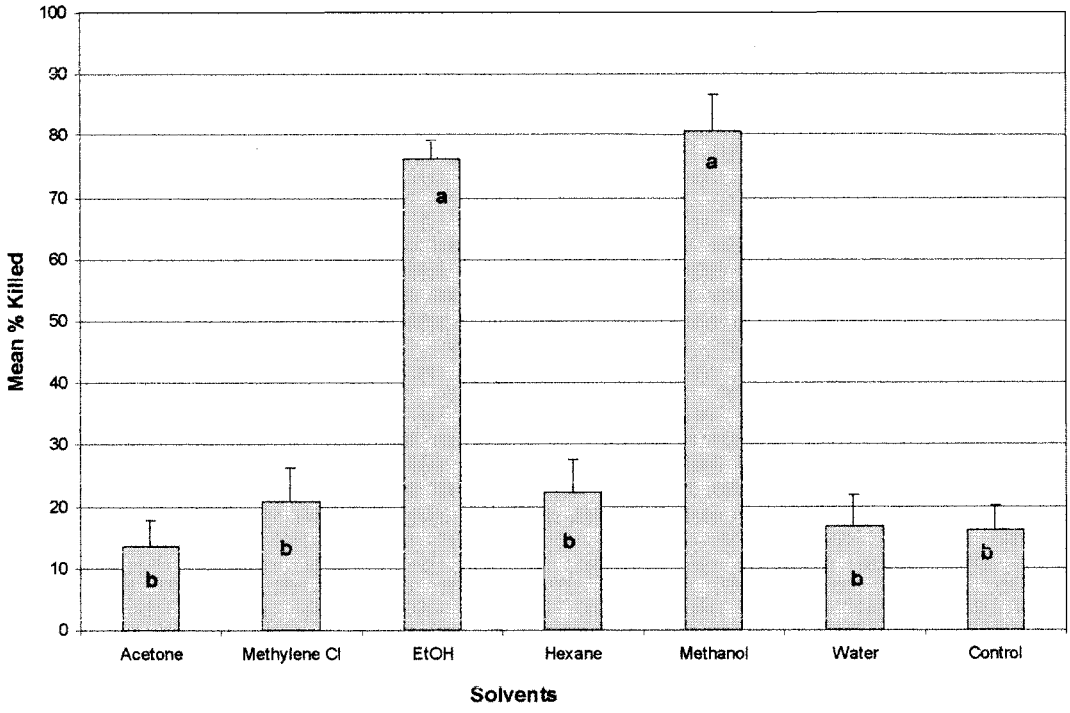

Fig. 1. Development of cat flea larvae in media treated with various solvents ($\bar{x} \pm$ SEM).

strains had the same slope and intercept. The Monheim, UCR, and KSU strains had parallel slopes (2.2–3.1), whereas the Auburn strain's slope was about twice as steep (4.9). The Auburn strain had considerably less variability in response to imidacloprid. Monheim and UCR were the most susceptible to imidacloprid. The LD_{95} ratios compared with the UCR strain ranged from 1.08 to 1.81, indicating that there were differences among these laboratory strains but no significant tolerance or insecticide resistance. All strains were susceptible to imidacloprid.

The larval bioassay provided a convenient method of determining susceptibility of *C. felis* to imidacloprid. By counting the number of hatched eggs an accurate count of larvae being exposed to the treated media can be determined. It is not unusual for as many as 30% of the eggs to fail to hatch even in laboratory strains. Cat flea larvae will eat both eggshells and intact eggs making it extremely difficult to estimate the number of larvae that hatch from eggs (Lawrence and Foil 2000). Consequently, it becomes important to accurately determine the number of eggs failing to hatch or eaten, especially when testing small numbers of flea eggs. The difference of just several eggs can have a significant effect on determining the probit lines. Eggs hatch within 1–2 d and larvae fall into the media reducing the likelihood of cannibalism by late instars.

The larval bioassay will more easily permit testing of field-collected strains. Eggs can be collected off pets by grooming them over large trays. Aliquots of 10–20 field-collected eggs can be exposed to a discriminating dose of imidacloprid such as 3 ppm to determine susceptibility, eliminating the need to maintain and rear cat flea strains in the laboratory. Strains of fleas that survived exposure to 3 ppm imidacloprid would be considered suspicious and the adults from the untreated control would be transferred to hosts for additional testing.

Table 1. Susceptibility profiles of four laboratory strains of cat fleas, *C. f. felis*, to larval media treated with imidacloprid

Strains	*n*	Slope ± SE	LD_{50} (95% CI)	LD_{95} (95% CI)
Auburn	274	4.9 ± 1.13	0.70 (0.508–0.844)	1.50 (1.207–2.371)
KSU	443	2.3 ± 0.30	0.40 (0.208–0.578)	2.04 (1.254–6.132)
Monheim	378	3.1 ± 0.64	0.36 (0.185–0.481)	1.22 (0.899–2.672)
UCR	352	2.2 ± 0.33	0.21 (0.127–0.286)	1.13 (0.795–2.007)

Data analyzed with POLO program (Robertson and Preisler 1992).

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