

Emergence, Survival, and Fecundity of Adult Cat Fleas (Siphonaptera: Pulicidae) Exposed as Pupae to Juvenile Hormone Mimics

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ABSTRACT Cat flea, *Ctenocephalides felis felis* (Bouché), adults exposed to sprays of methoprene, pyriproxyfen, or fenoxycarb as cocooned pupae emerged ≈1 d earlier than adults from water-treated control pupae. Mortality of adult fleas, after exposure to juvenile hormone mimics as pupae, was increased over that of controls. Females had higher mortality than males within the first 48 h of feeding. Fecundity of females exposed as pupae to juvenile hormone mimics was not different from that of controls. Early emergence of preemerged adults from treated cocoons is discussed along with reasons for higher female susceptibility to juvenile hormone mimics.

KEY WORDS *Ctenocephalides felis*, methoprene, pyriproxyfen, fenoxycarb

CAT FLEA, *Ctenocephalides felis felis* (Bouché), control has changed dramatically over the past 7 yr with the use of insect growth regulators (IGRs) and neurotoxic insecticides that can be administered to host animals. However, even with these highly effective treatments, elimination of cat flea infestations in indoor environments requires 2–4 mo to accomplish. This is because immature fleas within the indoor environment are unaffected by products applied only to the host animal. A thorough application of an insect growth regulator (IGR) to the floor and furniture indoors can eliminate infestations 2–3 wk sooner by killing eggs and larvae. However, little can be done to kill pupae located deep in protected areas of furniture, carpet, cracked floors, and baseboards, because they are difficult to reach with insecticidal sprays. This period of flea emergence from pupae unaffected by indoor insecticide treatment has been termed the “pupal window” by Dryden (1991).

Much research has concentrated on factors that lead to high tolerance of flea pupae to insecticide applications and on ways to eliminate them. Dryden and Reid (1996) demonstrated that pupae are susceptible to neurotoxic insecticides and that the silk cocoon and debris that cover the pupae increase the efficacy of a given dose of insecticide, possibly by wicking the chemical to the developing flea. In subsequent studies, we examined the toxicity of IGRs on pupae and found that they were highly tolerant to residues of juvenile hormone mimics, but died pre-

maturely after eclosion (unpublished data). Because juvenile hormone mimics are currently key elements of the indoor treatment strategy for cat fleas, it is important to know what long-term posttreatment effects they have on adult fleas exposed to them as pupae. Therefore, the objective of this study was to determine emergence, survival, and fecundity of adult fleas exposed to juvenile hormone mimics as pupae.

Materials and Methods

Fleas. Cat fleas were from the Kansas1 colony, established in 1990 with fleas from naturally infested dogs and cats collected at an animal shelter in Manhattan, KS. The colony is maintained on cats as hosts, as described by Dryden (1989).

Treatment of Pupae. To test the effect of juvenile hormone mimics on emergence of adult cat fleas, cocooned pupae (2–4 d old) were collected from their rearing medium using a #10 standard testing sieve (W. S. Tyler, Mentor, OH). Four groups of 50 pupae each were placed in a single layer on the bottom of a glass petri dish and sprayed (nozzle model # 050P, Gilmour, Somerset, PA; height = 45 cm) with distilled water or with methoprene (Precor EC [emulsifiable concentrate], Sandoz, Des Plaines, IL; 2.12 mg [AI]/m²); pyriproxyfen (Nylar EC, MGK, Minneapolis, MN; 2.76 mg [AI]/m²); or fenoxycarb (Torus WP, [wetttable powder] Novartis, Greensboro, NC; 2.42 mg [AI]/m²) at the recommended label rate. Each group of pupae was then transferred to a flat-bottom glass culture tube (25 by 150 mm, Pyrex, Corning, NY), which was covered with Parafilm “M” (American National Can, Greenwich, CT) and placed in an environmental chamber (28°C, 85% RH, and a photoperiod of 0:24 [L:D] h) until adult emergence. Emerging adults from each culture tube were counted and sex determined daily.

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Mortality and Fecundity of Adult Fleas Treated as Pupae with Juvenile Hormone Mimics. Cocooned pupae, 2–4 d old, were removed from their rearing medium by sifting and placed (8.2–10.9 g) onto the bottom of a glass petri dish in a single layer. The pupae were sprayed with juvenile hormone mimics using the protocol described above, but were left in the petri dishes after treatment. After development in an environmental chamber (described above), adults were collected with an aspirator at 24-h intervals and counted by sex. When a ratio of 1:4 (M:F) was obtained from a single collection, 15–50 fleas were placed into a flea feeding chamber (Thomas et al. 1996). Chambers were attached to 6 domestic cats along the side just posterior to the foreleg and against the ribs. Each cat had 2 feeding chambers, 1 containing adults treated as pupae with water and the other with adults treated as pupae with 1 of the 3 juvenile hormone mimics. After 48 h, the chambers were removed. Surviving adults and eggs were collected and counted. The adults were returned to the feeding chamber, which was placed on the same cat from which it originally came. Thereafter, this procedure was repeated every 24 h for 4 d.

Collected eggs were placed into a petri dish and covered with rearing medium (Dryden 1989); this dish then was placed in an environmental chamber set at the same conditions described above. After 5 d, the medium was sifted with a #20 Standard Testing Sieve (W. S. Tyler) to remove any larvae. The larvae were counted and returned to the medium in the environmental chamber for 9 more days, allowing time for the larvae to pupate (Dryden 1989).

Pupae were removed from the rearing medium using a #10 Standard Testing Sieve (W. S. Tyler). They were counted and placed in a 7-dram plastic vial (Fisher, St. Louis, MO), which was returned to the environmental chamber for 14 d. The vials were then frozen, and the number of emerged adults was counted.

Statistical Analysis. The mean time to emergence was analyzed using general linear model (GLM) and mean separations with a Waller–Duncan test (Waller and Duncan 1969, SAS Institute 1988). Adult mortality

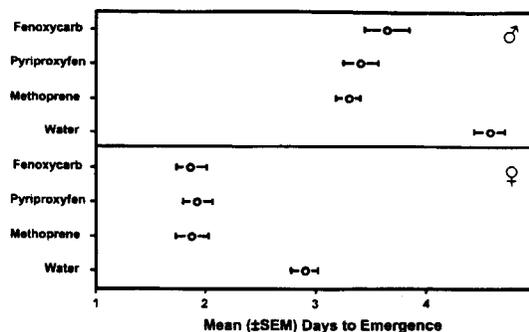


Fig. 1. Mean time to emergence (\pm SEM) for 50 cat flea cocooned pupae exposed to water, methoprene (2.12 mg [AI]/m²), pyriproxyfen (2.76 mg [AI]/m²), or fenoxycarb (24.2 mg [AI]/m²) ($n = 3$).

was analyzed with GLM and *t*-test (SAS Institute 1988). Numbers for egg production, egg hatch, pupation, and eclosion were divided by the number of females alive at the time of collection. The values from juvenile hormone mimics treatments then were subtracted from values for their corresponding water treatment. Results were analyzed with GLM and *t*-test.

Results

On average, female cat fleas in the control groups eclosed 1.7 d earlier than males ($F = 249.26$; $df = 1, 16$; $P = 0.0001$). Eclosion was earlier for both sexes when cocooned pupae were exposed to juvenile hormone mimics ($F = 40.80$; $df = 3, 16$; $P = 0.0001$; Fig. 1). The mean emergence time for female pupae treated with water was 2.9 d, whereas females from pupae treated with methoprene, pyriproxyfen, or fenoxycarb all eclosed at 1.9 d. The same trend was true for male pupae exposed to juvenile hormone mimics; emergence times were 4.6 d for the water treatment and 3.3, 3.4, and 3.7 d for methoprene, pyriproxyfen, and fenoxycarb treatments, respectively.

Adult mortality throughout the 6 d confinement on cats was higher after juvenile hormone mimic expo-

Table 1. Mortality (%) among adult cat fleas exposed to JHMs as cocooned pupae, then confined in feeding chambers on cats for 6 d ($n = 3$)

Item	Treatment (JHM)	Control (Water)	SEM	<i>t</i>	<i>P</i>
Methoprene (2.12 mg [AI]/m ²)					
Total	45.8	4.3	10.4	0.10	0.058
Female	50.0	3.0	12.1	0.12	0.059
Male	28.9	11.1	9.7	0.10	0.210
Pyriproxyfen (2.76 mg [AI]/m ²)					
Total	48.4	1.3	3.9	12.2	0.007
Female	49.2	1.7	2.5	19.0	0.003
Male	45.7	0.0	10.9	4.1	0.053
Fenoxycarb (24.2 mg [AI]/m ²)					
Total	41.0	0.0	15.3	4.7	0.04
Female	46.4	0.0	23.9	3.4	0.08
Male	20.0	0.0	11.5	1.7	0.23

Each replication consisted of 15–50 fleas in a ratio of 1:4 (M:F). JHMs, juvenile hormone mimics.

Table 2. Cumulative daily percent control (\pm SEM) of adult female and male fleas exposed to JHMs as cocooned pupae, during confinement in feeding chambers on cats for 6 d ($n = 3$)

Sex	Days after placement on cats				
	2	3	4	5	6
Methoprene (2.12 mg [AI]/m ²)					
Females	45.5 (4.3)	45.5 (4.3)	45.5 (4.3)	45.5 (4.3)	48.3 (3.6)
Males	0.0 (0.0)	0.0 (11.1)	0.0 (6.7)	7.1 (9.7)	20.0 (19.8)
Pyriproxyfen (2.76 mg [AI]/m ²)					
Females	43.8 (15.2)	43.8 (15.2)	43.8 (15.2)	43.8 (15.2)	46.4 (13.8)
Males	20.0 (11.6)	20.0 (11.6)	23.3 (12.0)	34.4 (2.9)	45.6 (11.0)
Fenoxycarb (24.2 mg [AI]/m ²)					
Females	39.7 (14.0)	43.1 (12.4)	44.7 (12.1)	45.6 (12.0)	50.0 (14.5)
Males	14.4 (9.9)	20.0 (11.6)	20.0 (11.6)	20.0 (11.6)	20.0 (11.6)

Percent control was calculated using Abbott's (1925) formula. Control mortality was <11.2%. Each replication consisted of 15–50 fleas in a ratio of 1:4 (M:F). JHMs, juvenile hormone mimics.

sure during the pupal stage ($P < 0.06$, Table 1). Females in all juvenile hormone mimic treatment groups had $\approx 47\%$ greater mortality than the water-treated controls. Males exposed to juvenile hormone mimics did not have higher mortality than those treated with water, except for the pyriproxyfen treatment ($P = 0.053$).

Most of the adult control occurred during the first 48 h after placement on cats (Table 2). Females had little increase in mortality from days 2 to 6, the highest being 10.3% in the fenoxycarb treatment. Males had greater increases in mortality, 32.5 and 25% for methoprene and pyriproxyfen, respectively.

The juvenile hormone mimics had no significant effects on fecundity of the surviving adults or on survival of their offspring, although some trends were evident (Table 3). Egg production was consistently higher in the water treatment (≈ 9 more eggs per female per day from days 2 to 6). Mortality during development from the egg to the adult stage was higher in the juvenile hormone mimic-treated fleas, with most of the mortality attributed to death in the egg stage. Larval mortality was consistently though

not significantly greater in the water than in the juvenile hormone mimic treatments (mean = 5.5%). No differences in adult mortality or fecundity occurred among methoprene, pyriproxyfen, and fenoxycarb treated fleas (Table 4).

Discussion

All the juvenile hormone mimics decreased the mean days to emergence of both adult male and female cat fleas by ≈ 1 d. This may be important in the prevention of cat flea reinfestations by shortening the length of time preemerged adults remain in their cocoons. After eclosion, cat fleas can remain in the cocoon for extended periods as preemerged adults. Temperature, relative humidity, and vibration all affect this stage, but under the proper conditions, it can last for months, and in some populations, up to a year (Olsen 1982, Silverman and Rust 1985, Metzger and Rust 1997). The decrease in the length of the preemerged adult stage after juvenile hormone mimic treatment could be the result of the degradation of metabolic reserves. Meola et al. (1996) showed that adult fleas

Table 3. Fecundity of cat fleas exposed to JHMs as cocooned pupae then confined in feeding chambers on cats for 6 d ($n = 3$)

Variable/Female	<i>n</i>	Treatment (JHM)	Control (Water)	SEM	<i>t</i>	<i>P</i>
Methoprene (2.12 mg [AI]/m ²)						
No. eggs/day	3	7.29	16.1	3.8	2.3	0.15
% egg to adult mortality	3	68.0	48.6	28.4	-0.69	0.56
% egg mortality	3	60.1	34.0	24.7	-1.1	0.38
% larva mortality	3	0.0	7.3	6.5	1.1	0.78
% pupa mortality	3	13.4	15.1	5.4	0.32	0.56
Pyriproxyfen (2.76 mg [AI]/m ²)						
No. eggs/day	3	7.53	16.5	4.4	2.3	0.15
% egg to adult mortality	3	51.6	32.0	9.9	-0.69	0.56
% egg mortality	3	40.0	16.8	9.4	-1.1	0.40
% larva mortality	3	1.0	5.6	3.2	1.1	0.39
% pupa mortality	3	18.3	12.7	5.5	0.32	0.78
Fenoxycarb (24.2 mg [AI]/m ²)						
No. eggs/day	3	6.89	16.6	6.0	1.62	0.25
% egg to adult mortality	3	73.0	33.5	23.3	-1.7	0.23
% egg mortality	3	63.1	19.8	25.3	-1.7	0.23
% larva mortality	2	0.20	4.8	5.4	1.1	0.46
% pupa mortality	2	30.8	12.2	2.9	-0.85	0.55

Each replication consisted of 15–50 fleas in a ratio of 1:4 (M:F). Values were calculated using data from day 2–6, because fleas do not begin oviposition until 24–48 h after placement on cats (Thomas et al. 1996). JHMs, juvenile hormone mimics.

Table 4. Results of GLM test for differences in adult cat flea survival and fecundity during confinement on cats for 6 d following treatment with methoprene (2.12 mg [AI]/m²), pyriproxyfen (2.76 mg [AI]/m²), and fenoxycarb (24.2 mg [AI]/m²) as cocooned pupae (df = 2, 3)

Variable	F	P
% male and female mortality	0.38	0.71
% female mortality	0.01	0.99
% male mortality	1.87	0.27
Eggs ^a	0.11	0.90
% egg to pupa mortality ^a	0.98	0.45
% egg mortality ^a	1.37	0.35
% larva mortality ^a	0.54	0.63
% pupa mortality ^a	1.37	0.38

Each replication consisted of 15–50 fleas in a ratio of 1:4 (M:F). Values from the treatment were subtracted from the corresponding control (water), and the result was used for the ANOVA.

^a Variables calculated on a per female basis.

exposed to pyriproxyfen die from degradation of internal tissues, especially the midgut epithelium and fat body. Silverman and Rust (1985) demonstrated that the preemerged adult stage can be shortened by poor nutrition and dehydration of larvae before pupation. If juvenile hormone mimics cause degradation of the fat body in treated pupae, then the short preemerged adult stage measured in this study may be caused by the same reasons Silverman and Rust (1985) demonstrated for poorly nourished larvae (i.e., lack of metabolic reserves to sustain life without feeding).

Adult cat fleas are able to excrete or metabolize juvenile hormone mimics, allowing them to survive if they are not exposed continuously. Meola et al. (1996) showed that adult fleas exposed continuously in an artificial feeding system to pyriproxyfen-treated dog hair died at a constant rate over 12 d. In our study, adult mortality was restricted to the first 48 h after fleas were removed from the juvenile hormone mimics, and no changes occurred in the fecundity of surviving adults. These results are consistent with earlier experiments where exposure of adult fleas to pyriproxyfen and methoprene for 24 h inhibited viable egg production for 94 and 88 h, respectively (Palma et al. 1993).

Most of the adult mortality in our experiments within the first 48 h was attributed to death of females. This increased susceptibility of females has also been observed in adult mosquitoes exposed to sublethal doses of methoprene as larvae (Sawby et al. 1992). One explanation for the greater susceptibility of females to juvenile hormone mimics could be premature production of vitellogenin. Juvenile hormone in the adult insect is important in the production and deposition of yolk (Nijhout 1994). Normally, egg production will not start until after the female has taken her 1st blood meal (Dryden 1989). Therefore, female cat fleas may not have the fat and protein reserves to sustain yolk production without a blood meal. The fact that most females died in the first 2 d after exposure to juvenile hormone mimics could mean that surviving females were able to feed enough to avoid starvation. Mortality among males and females after feeding may be attributed to a toxic effect of the juvenile hormone

mimics apart from the hormonal mode of action normally seen in immature fleas.

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