Susceptibility of Black Soldier Fly (Diptera: Stratiomyidae) Larvae and Adults to Four Insecticides

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ABSTRACT Dosage-mortality regressions were determined for black soldier fly, *Hermetia illucens* (L.), larvae fed cyromazine or pyriproxifen treated media. Cyromazine LC_{50}s for larvae dying before becoming prepupae ranged from 0.25 to 0.28 ppm with dosage-mortality regression slopes between 5.79 and 12.04. Cyromazine LC_{50}s for larvae dying before emergence ranged from 0.13 to 0.19 ppm with dosage-mortality regression slopes between 3.94 and 7.69. Pyriproxifen dosage-mortality regressions were not generated for larvae failing to become prepupae since <32% mortality was recorded at the highest concentration of 1,857 ppm. LC_{50}s for larvae failing to become adults ranged from 0.10 to 0.12 ppm with dosage mortality-regression slopes between 1.67 and 2.32. Lambda-cyhalothrin and permethrin dosage-mortality regressions were determined for wild adult black soldier flies and house flies, *Musca domestica* L., and for susceptible house flies. Our results indicate that the wild house fly, unlike the black soldier fly, population was highly resistant to each of these pyrethroids. Regression slopes for black soldier flies exposed to λ-cyhalothrin were twice as steep as those determined for the wild house fly strain. Accordingly, LC_{50}s for the black soldier fly and susceptible house fly were 10- to 30-fold lower than those determined for wild house flies. The differential sensitivity between wild black soldier flies and house flies might be due to behavioral differences. Adult house flies usually remain in animal facilities with the possibility of every adult receiving pesticide exposure, while black soldier fly adults are typically present only during emergence and oviposition thereby limiting their exposure.

KEY WORDS Diptera, Stratiomyidae, *Hermetia illucens*

SUPPRESSION OF HOUSE FLIES, *Musca domestica* L., in poultry facilities can be accomplished with biological, cultural, and chemical methods (Axtell and Arends 1990). The black soldier fly, *Hermetia illucens* (L.), can suppress house fly populations by 94–100% and reduce manure accumulation by 42–56% (Sheppard 1983). Additionally, an estimated 55 metric tons of black soldier fly prepupae with 44% dry matter possibly can be harvested in 5 mo from a 100,000 hen caged-layer house (Sheppard et al. 1994). This system requires no external energy inputs and the prepupae can be used for livestock feed or other products (Sheppard et al. 1994). The black soldier fly is distributed throughout the tropical and temperate regions of the world, extending north into the southeastern United States (James 1935, 1947), where it is active from April to November (Sheppard et al. 1994).

The use of insecticides can disrupt the life cycle of beneficial nontarget insects, resulting in an increase of pest numbers (Van Driesche and Bellows 1996). For example, Axtell and Edwards (1970) determined that suppression of soldier flies in poultry facilities resulted in a resurgence of the resident house fly population.

This study was conducted to determine the response of black soldier fly larvae and adults to insecticides commonly used for house-fly control in poultry facilities. This information could facilitate the use of pesticides and the black soldier fly together in an integrated pest management (IPM) program for poultry facilities. The first objective of our study was to examine the effects of two insect growth regulators, cyromazine and pyriproxifen, on the larval development of the black soldier fly. Our second objective was to determine the susceptibility of wild black soldier fly and house fly populations to λ-cyhalothrin and permethrin. We compared the susceptibility of the two wild populations to results for susceptible house flies, which originated from a Cornell culture initiated 20 yr before this study.

Materials and Methods

Larval and adult flies used in the experiments were from colonies maintained at the Coastal Plain Experiment Station, Tifton, GA. Black soldier fly larvae used in experiments with insect growth regulators were from a colony initiated with flies collected in June 1999 from a poultry facility in Alma, GA. Wild soldier flies

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Table 1. Toxicity of cyromazine and pyriproxifen following treatment of CSMA diet fed to *H. illucens* larvae

Compound	Stage	Trial	Slope ± SE	LC_{50} (95% CI)	*n*	χ^2
Cyromazine	Pupae	1	12.04 (1.44)	0.26 (0.24–0.27)	27	9.48
		2	8.14 (0.73)	0.25 (0.24–0.26)	27	9.48
		3	5.79 (1.11)	0.28 (0.23–0.35)	27	12.44
	Adult	1	7.69 (1.89)	0.19 (0.16–0.23)	27	9.48
		2	3.94 (0.74)	0.13 (0.09–0.16)	27	9.48
		3	4.17 (1.00)	0.18 (0.14–0.21)	27	9.48
Pyriproxifen	Pupae	1	0.53 (0.84), NS	NS	42	9.48
		2	−1.37 (1.70), NS	NS	42	9.48
		3	0.63 (0.77), NS	NS	42	9.48
	Adult	1	2.32 (1.00)	0.12 (0.06–0.17)	42	11.07
		2	1.73 (0.50)	0.12 (0.05–0.47)	42	7.81
		3	1.67 (0.21)	0.10 (0.07–0.13)	42	9.49

Significance set at $P < 0.05$; NS, not significant.

and house flies used in the pyrethroid experiments were from colonies originating from black soldier fly prepupae collected in December 1999 and adult house flies in March 2000 from a poultry facility located in Hoboken, GA. Susceptible house flies were from a colony originating from the Department of Entomology at Cornell University (Tomita et al. 1995). Voucher specimens from each colony were placed in the University of Georgia Museum of Natural History.

Insect growth regulators were tested using the following procedures. Black soldier fly larvae were exposed to nine concentrations of cyromazine (Larvadex liquid, Norvartis, Greensboro, NC) ranging from 0.0585 to 1.5 ppm and eight concentrations of pyriproxifen (Archer liquid, Zeneca, Wilmington, DE) ranging from 0.0316 to 4 ppm and six concentrations from 58 to 1,857 ppm. To achieve a hierarchy of concentrations, successive pyriproxifen concentrations were halved in water, whereas successive cyromazine concentrations were diluted one-third in water. Water was applied as the control. A replicate of each concentration consisted of 35 soldier fly larvae (5 d old) placed in a 454-ml Sweetheart plastic container (Sweetheart Cup Company, Chicago, IL) containing 180 g CSMA (Chemical Specialties Manufacturer's Association, Ralston Purina, St. Louis, MO) house fly larval media treated with 30 ml of the appropriate solution. Cups containing treated diet and soldier fly larvae were covered with a paper towel and placed in a rearing room (27°C, 60–70% RH, and a photoperiod of 14:10 [L:D] h).

The percentage of larvae to die and weights of those surviving to the prepupal stage were recorded 38 d after initiating the experiment. Prepupae were identified by change in pigment color from larval white to black (May 1961). Weights were determined with a Mettler AT261 DeltaRange balance. Percentage of the larvae failing to reach the adult stage for each treatment was recorded 68 d after initiation of the experiment. The experiment was replicated on three occasions from January through August 2000.

Responses of wild adult house flies and soldier flies to papers treated with λ-cyhalothrin or permethrin were determined. Wild adult house flies were exposed to 12 concentrations of λ-cyhalothrin (Demand CS, Zeneca, Wilmington, DE) ranging from 0.14 to 12.0 $\mu g/cm^2$, and eight concentrations of permethrin (Gardstar 40% EC, Y-Tex, Cody, WY) ranging from 6.25 to 800.0 $\mu g/cm^2$ per filter paper. Successive λ-cyhalothrin and permethrin concentrations used as treatments for the wild house flies were diluted with acetone by one-third and one-half respectively. We also exposed susceptible house flies from a Cornell colony to 12 λ-cyhalothrin concentrations ranging from 0.057 to 5.0 $\mu g/cm^2$, and 11 permethrin concentrations ranging from 1.18 to 11.0 $\mu g/cm^2$ with successive concentrations being diluted with acetone by one-third. Adult soldier flies from a wild colony were exposed to nine λ-cyhalothrin concentrations ranging from 0.061 to 1.5625 $\mu g/cm^2$ and 11 permethrin concentrations from 0.65 to 37.5 $\mu g/cm^2$. Successive λ-cyhalothrin concentrations used as treatments for the soldier flies were diluted with acetone by half, while the permethrin concentrations were diluted by one-third. Acetone was used as an untreated check in bioassays for both pyrethroids.

Methods used to examine the pyrethroids were adapted from Sheppard and Hinkle (1987). Pesticides in 1 ml of an acetone solution were applied to a 9 cm filter paper. Treated papers were allowed to air-dry for 24 h. Papers with the same concentration and pesticide were wrapped in aluminum foil and stored at room temperature until their use. Each pesticide concentration was represented three times in each bioassay. Adult black soldier flies <24 h in age were used in two bioassays and adults one to 12 d in age were used in a final bioassay. Results for experiments conducted with black soldier flies of different ages were compared and determined not to be significantly different ($P \leq 0.05$). House flies <6 d old were used in each experiment. Fifteen to 20 of the appropriate fly species were placed on a treated paper in each petri plate and mortality recorded 2 h later. Each experiment was replicated on three occasions in the laboratory under ambient conditions. Mortality was defined as the inability of a fly to walk or remain on its tarsi.

PROBIT analysis was used to determine dosage-mortality regression equations and to estimate LC_{50}s for larval and adult black soldier flies and house flies (Daum 1970, Russell et al. 1977). Percentages were arcsine transformed before being analyzed with Pro-

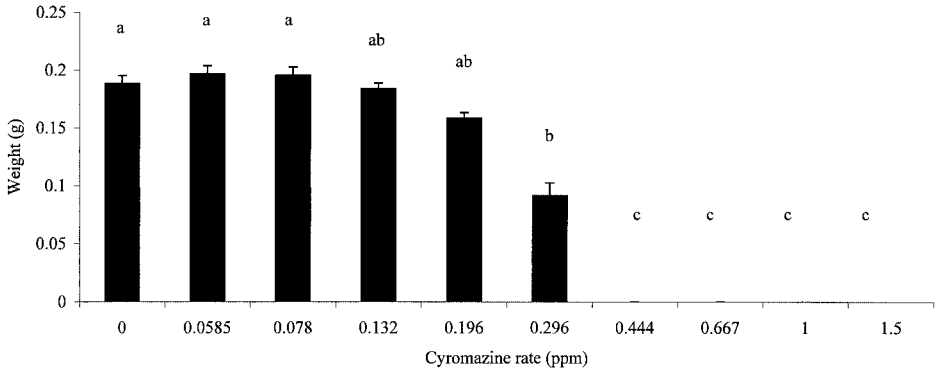

Fig. 1. Mean prepupal weight (g) ±SE for black soldier fly larvae feeding on diet treated with differing cyromazine concentrations (ppm). Measurements recorded 38 d after initiation of the experiment (treatments with different lower case letters were significantly different, $P < 0.05$, LSD).

cedure GLM (SAS Institute 1992). Least significant difference (LSD) test (SAS Institute 1992) was used to separate means per treatment following a significant F test ($P \leq 0.05$).

Results and Discussion

This study was the first to examine the effects of cyromazine and pyriproxifen on larval development of soldier flies. Cyromazine LC_{50}s for black soldier fly larvae dying before reaching the prepupal stage ranged from 0.25 to 0.28 ppm with dosage-mortality regression slopes between 8.14 and 12.04 (Table 1). The percentage of larvae that died before reaching the prepupal stage ranged from 8.6% in the 0.0585 ppm concentration to 100% in the 1.5 ppm concentration. Mean prepupal weight (Fig. 1) ranged from 188.4 mg in the control to 91.6 mg (51% < control) for those reared on diet containing 0.296 ppm cyromazine. LC_{50}s for larvae dying before reaching the adult stage ranged from 0.13 to 0.19 ppm with dosage-mortality regression slopes ranging from 3.94 to 7.69 (Table 1). The percentage of the larvae introduced into a treatment that did not emerge by the conclusion of the experiment ranged from 70.8% in the 0.078 ppm treatment to 100% in treatments ≥0.296 ppm.

The current cyromazine concentration recommended to suppress house flies in poultry facilities is five ppm in the feed with resultant manure having a concentration of 3.3 ppm. This concentration is greater than the LC_{50} range determined for the black soldier fly and would result in the reduction of soldier fly numbers and associated benefits. To use cyromazine to suppress house flies without reducing black soldier fly numbers, we suggest that the label spray concentration of ≈1,000 ppm be used as a spot treatment in areas with dense house fly larvae, while avoiding areas where soldier fly larvae are present.

Significant dosage-mortality regressions for larvae fed pyriproxifen-treated diet could not be determined because mortality at the highest concentration (1,857 ppm) was no more than 32%. Mean prepupal weight (Fig. 2) ranged from 169.3 mg in the control to 236.2 mg (40% > control) for larvae fed diet containing 58 ppm pyriproxifen. Prepupal weight decreased to 124.4 mg (27% < control) for larvae fed diet containing

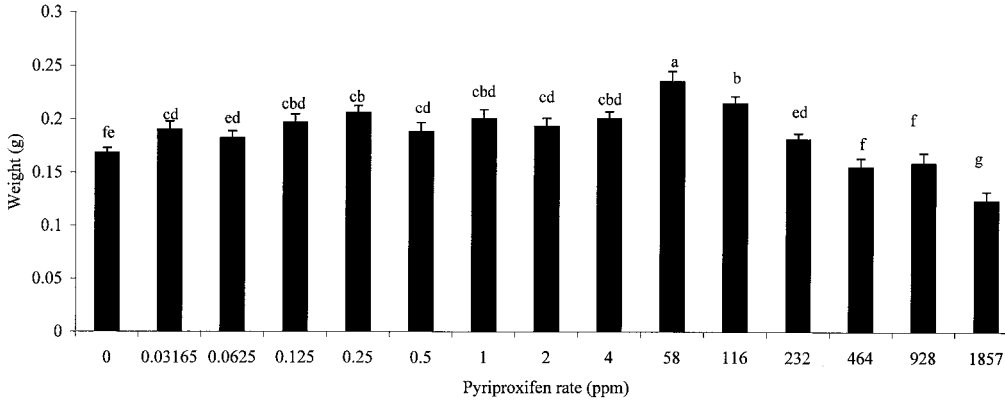

Fig. 2. Mean prepupal weight (g) ±SE for black soldier fly larvae feeding on diet treated with differing pyriproxifen concentrations (ppm). Measurements recorded 38 d after initiation of the experiment (treatments with different lower case letters were significantly different, $P \leq 0.5$, LSD).

Table 2. Resistance factors and susceptibility of wild *H. illucens* and *M. domestica* adults exposed to filter papers treated with either λ-cyhalothrin or permethrin in comparison to results for susceptible *M. domestica*

Hermetia illucens (L.)					*Musca domestica* L.							
Wild population					Wild population				Susceptible population			
LC_{50}s (95% CL) $\mu g/cm^2$	Slope (SE)	Resistance[a] ratio	n	χ^2	LC_{50}s (95% CL) $\mu g/cm^2$	Slope (SE)	Resistance ratio	n	χ^2	LC_{50}s (95% CL) $\mu g/cm^2$	Slope (SE)	n	χ^2
					λ-cyhalothrin								
0.34 (0.38–0.31)	5.29 (0.53)		27	9.48	2.12 (2.46–1.85)	2.89 (0.28)		36	12.44	0.18 (0.20–0.16)	5.04 (0.51)	36	9.48
0.31 (0.34–0.29)	5.76 (0.63)		27	7.81	3.34 (4.62–2.57)	2.58 (0.36)		36	14.06	0.22 (0.24–0.19)	4.84 (0.54)	36	9.48
0.32 (0.35–0.29)	5.34 (0.53)		27	9.48	2.26 (2.63–1.96)	2.89 (0.26)		36	15.50	0.21 (0.30–0.16)	4.65 (0.90)	36	9.48
Mean = 0.32		1.60			2.56		12.80			0.20			
					Permethrin								
8.43 (9.65–7.35)	4.01 (0.49)		33	9.48	184.23 (405.71–104.82)	4.88 (1.19)		24	7.81	4.17 (5.34–3.24)	8.12 (1.98)	33	11.07
6.75 (7.73–5.88)	3.71 (0.38)		33	14.06	94.24 (110.98–79.84)	2.88 (0.27)		24	11.07	4.00 (4.32–3.70)	7.95 (0.99)	33	7.81
6.46 (7.46–5.56)	3.36 (0.36)		33	12.44	108.24 (129.06–90.66)	2.68 (0.24)		24	12.44	3.96 (4.28–3.67)	8.18 (0.96)	33	9.48
Mean = 7.21		1.78			128.9		31.91			4.04			

Significance set at $P < 0.05$; [a] Resistance ratio, mean field black soldier fly or house fly population LC_{50}/mean Cornell house fly colony LC_{50}.

1,857 ppm. For larvae fed diet treated with ≥116 ppm, the percentage of larvae in the prepupal stage after 38 d was 16.7% lower than that recorded for the control, which might be due to pyriproxifen delaying pupation. El-Gazzar et al. (1986) recorded prolonged development for cat flea, *Ctenocephálides felis* (Bouché) (Siphonaptera: Pulicidae), larvae exposed to pyriproxifen. We determined that LC_{50}s for the percentage of larvae not reaching the adult stage ranged from 0.10 to 0.12 ppm with dosage-mortality regression slopes between 1.67 and 2.32 (Table 1).

We hypothesize that using pyriproxifen in poultry facilities colonized by the black soldier fly might prolong the larval stage, which could result in greater manure consumption per soldier fly larva. However, reduced adult emergence and possible sterility of those individuals that emerge, such as that recorded for the cat flea (Karhu and Anderson 2000, Meola et al. 2000), would reduce the black soldier fly population in time. This effect could also occur for other nontarget organisms residing in the manure, specifically other biological control agents, which could in turn result in a resurgence of the pest population.

Table two includes estimated LC_{50}s and slopes of regression lines (±SE) for the wild adult black soldier flies and house flies and the susceptible house flies exposed to each pyrethroid. LC_{50}s for the black soldier fly ranged from 0.314 to 0.320 $\mu g/cm^2$ for λ-cyhalothrin and 6.46–8.43 $\mu g/cm^2$ for permethrin. LC_{50}s recorded for wild house flies ranged from 2.12 to 3.34 $\mu g/cm^2$ for λ-cyhalothrin and 94.24–184.23 $\mu g/cm^2$ for permethrin. LC_{50}s for susceptible house flies ranged from 0.18 to 0.22 $\mu g/cm^2$ for λ-cyhalothrin and 3.96–4.17 $\mu g/cm^2$ for permethrin. These results demonstrate that the wild house flies were 12–31 fold more resistant to λ-cyhalothrin and permethrin respectively, while the black soldier flies responded to doses similar to those effective on susceptible house flies.

Permethrin and λ-cyhalothrin are used to treat sites in livestock and poultry facilities frequented by adult house flies, not black soldier flies. Black soldier fly adults tend to live in the outdoors, returning to livestock or poultry facilities only to oviposit. Our observations from a separate study indicate that male adults account for <3% of the adult soldier flies collected in poultry facilities, while the remaining 97% are individuals ovipositing in the manure (Tomberlin and Sheppard 2001), which is not directly treated with the pyrethroids. However, care should be taken to avoid treating potential black soldier fly oviposition sites with pesticides when colonization and associated benefits are desired.

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