Resistance to cyfluthrin and tetrachlorvinphos in the lesser mealworm, *Alphitobius diaperinus*, collected from the eastern United States

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Abstract: The lesser mealworm, *Alphitobius diaperinus* (Panzer), is an important pest in poultry facilities. The toxicity of cyfluthrin and tetrachlorvinphos to five strains of the lesser mealworm was compared with the toxicity to a susceptible laboratory strain. Bioassays were carried out with both larvae and adults. For the susceptible strain, cyfluthrin and tetrachlorvinphos had similar toxicity to adults, but cyfluthrin was 5 times more toxic to larvae when compared with tetrachlorvinphos. High levels of resistance to tetrachlorvinphos in two beetle strains were detected in both larvae and adults, although these strains were heterogeneous and still contained susceptible individuals. Resistance to cyfluthrin ranged from 1.7- to 9.5-fold for adults and from 0.5- to 29-fold for larvae at the LC_{95}. Overall, the patterns of resistance did not mirror the insecticide use patterns reported at these facilities. The implications of these results to management of the lesser mealworms are discussed.
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Keywords: poultry; insecticide resistance; pyrethroid; organophosphate; *Alphitobius diaperinus*

1 INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus* (Panzer), is the primary structural pest of the poultry industry.[1] The lesser mealworm is a known reservoir of many avian pathogens and parasites, including *Salmonella typhimurium* Cast & Chalm, *Escherichia coli* (Mig) Cast & Chalm, tapeworms, avian leucosis virus, turkey coronavirus and turkey enterovirus.[2–7] Chicks feeding excessively on larvae have poor weight gain, and mortality may result.[8] High beetle populations consume significant feed.[9] Under dry conditions in the broiler house, beetles will bite the skin of birds resting at night. To prevent these bites, agitated birds will rest for only short periods of time, leading to reduced weight gain and feed conversion.[9] In addition, mature beetle larvae climb building walls and posts and chew into building support structures and insulation, seeking pupation sites.[10,11] The 1996 cost of lesser mealworm control in Georgia alone was over $1 000 000, with estimated damage costs of $8 476 000.[12]

Two insecticides registered for the control of lesser mealworm in poultry facilities in the USA are cyfluthrin and tetrachlorvinphos,[13] which are typically applied following manure removal in caged-layer systems. In spite of the widespread use of these compounds for control of lesser mealworm (and houseflies), minimal information about resistance to these materials in this species in the USA is available. Vaughan and Turner[10] examined several formulated insecticides for their toxicity to a field-collected (Virginia, USA) strain of lesser mealworm, and found that tetrachlorvinphos was highly effective.

Poultry facilities are typically treated with pesticides for lesser mealworm infestations following manure removal, when beetles in the pupal and adult stage are hiding in building structural and insulation harborages. Savage[9] states that, if litter is stockpiled near the poultry house, half the beetles will move back in when resources are no longer available or the temperature falls below optimum. He also describes how lesser mealworm adults can fly 1 mile in one night, although the methods used to determine this are not described. Therefore, it is reasonable to assume that, even with manure removal from the poultry house, many of the displaced beetles are capable of reinfesting poultry houses.

In the present study, the susceptibility of five strains of lesser mealworms from Georgia, Maine and New York was compared with that of a susceptible laboratory strain using a residual contact method of insecticide exposure. Bioassays were carried out with both larvae and adults against the two currently registered insecticides used for control, cyfluthrin and tetrachlorvinphos.

2 MATERIALS AND METHODS

2.1 Insects

An insecticide-susceptible strain (Denmark-S) of *A. diaperinus* was obtained from Saturnia (Bjerring-brovej 48 2610 Rødovre, Denmark). Lesser meal-worms were also collected in 2002 from four caged-layer poultry farms, three in New York (Cayuga County, Onondaga County and Wayne County) and one in Kennebec County, Maine. An additional strain was obtained from an infestation occurring in a com-mercial cricket colony in Waycross, Georgia.

Alphitobius diaperinus is likely affected by insecticides used for housefly (and possibly ectoparasite) control in poultry facilities, as well as insecticides used specifically for lesser mealworm control. Therefore, information was collected on all insecticide use at each facility. Insecticide use for fly control at the Cayuga County facility included premise applications of permethrin, tetrachlorvinphos and carbaryl, and feed mixtures containing cyromazine and methomyl fly bait. In this facility, walls and support beams were sprayed with either cyfluthrin or tetrachlorvinphos twice following each manure removal (approximately every 12–18 months). The birds were occasionally treated with carbaryl for northern fowl mites, *Ornithonyssus sylviarum* (Canestrini and Fanzago). Insecticide use (based on interviews with the facility managers) for fly control for at least the previous 5 years at the Wayne County and Onondaga County facilities was limited to pyrethrin space sprays and methomyl baits. Treatments for fly control at the Maine facility included nearly weekly applications of pyrethrins from 1998 to 2001, one application of dichlorvos, two applications of dimethoate and 10 weeks per year of cyromazine feed-through use, as well as methomyl and nithiazine baits. All facilities remove manure every 6–18 months. No pesticide use history is available for either the Denmark-S or Waycross County strains. However, because both of these strains were obtained from insect cultures, it is assumed that these strains received no pesticide exposure for at least the last 4 years. Insect colonies were maintained at 28 °C, 60–70% RH and provided a diet of 95 + 5 cracked corn + wheat bran (Agway, Ithaca, NY) *ad libitum*.

2.2 Insecticides and bioassays

Cyfluthrin (98% mix of isomers) and tetrachlorvinphos (99.5%) were from Chem Services (West Chester, PA) and were evaluated against six strains of beetles at two life stages. A residual contact bioas-say method was used for all the insecticides tested. Glass jars (30 mL volume, treated surface area 18 cm^2, total internal surface area 33 cm^2) were treated with 0.5 mL of insecticide in acetone, or acetone only for control treatments. Jars were put on an orbital shaker to ensure complete coverage of the bottom and about one-quarter of the wall (beetles could not climb up the jars). The acetone was allowed to evaporate for at least 2 h before the introduction of beetles. A minimum of three doses giving >0% and <100% mortality after insecticide treatment was used for each experiment. Each experiment was replicated at least 3 times. The specimens used in the assays were 2–7 day post-eclosion adult beetles or mature larvae that passed through a No. 14 US Standard sieve but were retained by a No. 16 sieve.[14] Some 10–20 insects were placed inside a glass jar that had been treated with either insecticide or acetone only. Mortality was assessed after 48 h. Typically, a total of 50–80 adults and 50–80 larvae were treated per concentration per strain. All bioassays were kept at 26.5 °C with a 16:8 h light:dark photoperiod. Adults and larvae were considered dead if they were unable to move out of a 5 cm circle within 15 min. Bioas-say data were pooled and analyzed on the basis of standard probit analysis[15] as adapted to personal com-puter use,[16] using Abbott's correction[17] for control mortality. Strains were considered 'resistant' if their LC_{50} value was significantly greater than that of the susceptible (Denmark-S) strain.

3 RESULTS AND DISCUSSION

Cyfluthrin and tetrachlorvinphos had similar toxicities to adults of the susceptible strain (Denmark-S). However, cyfluthrin was 4 times more toxic to susceptible larvae than tetrachlorvinphos (see Tables 1 to 4). Significant levels of cyfluthrin resistance (resistance ratios varied from 1.8- to 4.0-fold at the LC_{50} and from 2.5- to 9.5-fold at the LC_{95}) were detected in adults from four of the five strains (Table 1). The highest levels of resistance were

Table 1. Toxicity of cyfluthrin to lesser mealworm adults from six strains

Strain	n	LC_{50} (μg cm^{-2}) (95% CI)	LC_{95} (μg cm^{-2}) (95% CI)	RR_{50}[a]	RR_{95}[b]	Slope (SE)
Denmark-S	590	0.04 (0.03–0.05)	0.32 (0.23–0.48)	–	–	1.9 (0.2)
Kennebec	710	0.16 (0.26–0.44)	3.03 (1.69–6.78)	4.0*	9.5*	1.3 (0.1)
Waycross[c]	630	0.15 (0.12–0.19)	2.47 (1.54–4.71)	3.8*	7.7*	1.4 (0.1)
Cayuga	940	0.07 (0.06–0.09)	0.81 (0.55–1.36)	1.8*	2.5*	1.6 (0.1)
Onondaga	600	0.13 (0.11–0.17)	1.50 (1.02–2.51)	3.3*	4.7*	1.6 (0.1)
Wayne	760	0.07 (0.06–0.08)	0.53 (0.39–0.80)	1.8*	1.7	1.8 (0.1)

[a] Resistance ratio at LC_{50} (i.e. LC_{50} resistant strain/LC_{50} Denmark-S).
[b] Resistance ratio at LC_{95} (i.e. LC_{95} resistant strain/LC_{95} Denmark-S).
[c] Cricket colony collection, all others (except Denmark-S) are from caged-layer poultry facilities.
* Significantly different from 1.0 based on non-overlap of 95% CI.

Table 2. Toxicity of cyfluthrin to lesser mealworm larvae from six strains

Strain	n	LC$_{50}$ (μg cm^{-2}) (95% CI)	LC$_{95}$ (μg cm^{-2}) (95% CI)	RR$_{50}$[a]	RR$_{95}$[b]	Slope (SE)
Denmark-S	640	0.015 (0.013–0.017)	0.061 (0.048–0.085)	–	–	2.7 (0.2)
Kennebec	844	0.187 (0.16–0.22)	1.74 (1.29–2.51)	12*	29*	1.7 (0.1)
Waycross[c]	551	0.013 (0.009–0.017)	0.343 (0.197–0.731)	0.9	5.6*	1.2 (0.1)
Cayuga	627	0.008 (0.005–0.010)	0.122 (0.081–0.224)	0.5	2.0	1.4 (0.2)
Onondaga	384	0.013 (0.010–0.017)	0.095 (0.063–0.176)	0.9	1.6	1.9 (0.2)
Wayne	397	0.004 (0.006–0.012)	0.030 (0.014–0.060)	0.3*	0.5	2.1 (0.4)

[a] Resistance ratio at LC$_{50}$ (i.e. LC$_{50}$ resistant strain/LC$_{50}$ Denmark-S).
[b] Resistance ratio at LC$_{95}$ (i.e. LC$_{95}$ resistant strain/LC$_{95}$ Denmark-S).
[c] Cricket colony collection, all others (except Denmark-S) are from caged-layer poultry facilities.
* Significantly different from 1.0 based on non-overlap of 95% CI.

Table 3. Toxicity of tetrachlorvinphos to lesser mealworm adults from six strains

Strain	n	LC$_{50}$ (μg cm^{-2}) (95% CI)	LC$_{95}$ (μg cm^{-2}) (95% CI)	RR$_{50}$[a]	RR$_{95}$[b]	Slope (SE)
Denmark-S	440	0.08 (0.07–0.10)	0.23 (0.17–0.42)	–	–	3.6 (0.6)
Kennebec	690	1.26 (1.01–1.65)[d]	>1000	16*	>4000	1.5 (0.2)[d]
Waycross[c]	520	0.38 (0.31–0.49)[d]	>1000	4.8*	>4000	2.0 (0.2)[d]
Cayuga	640	0.13 (0.09–0.20)	0.73 (0.35–1.53)	1.6	3.2	2.2 (0.5)
Onondaga	580	0.18 (0.16–0.21)	0.77 (0.58–1.17)	2.3*	3.4*	2.6 (0.3)
Wayne	590	0.34 (0.28–0.41)	2.44 (1.69–4.18)	4.3*	11*	1.9 (0.2)

[a] Resistance ratio at LC$_{50}$ (i.e. LC$_{50}$ resistant strain/LC$_{50}$ Denmark-S).
[b] Resistance ratio at LC$_{95}$ (i.e. LC$_{95}$ resistant strain/LC$_{95}$ Denmark-S).
[c] Cricket colony collection, all others (except Denmark-S) are from caged-layer poultry facilities.
[d] Probit analysis was conducted using only the concentrations below the plateau of the line (see Fig. 1).
* Significantly different from 1.0 based on non-overlap of 95% CI.

Table 4. Toxicity of tetrachlorvinphos to lesser mealworm larvae from four strains

Strain	n	LC$_{50}$ (μg cm^{-2}) (95% CI)	LC$_{95}$ (μg cm^{-2}) (95% CI)	RR$_{50}$[a]	RR$_{95}$[b]	Slope (SE)
Denmark-S	740	0.07 (0.05–0.10)	0.25 (0.12–0.52)	–	–	2.9 (0.8)
Kennebec	620	~0.6[c]	>100[c]	8.6	>400	
Waycross	710	~0.4[c]	>100[c]	5.7	>400	
Cayuga	860	0.08 (0.06–0.11)	0.27 (0.17–0.44)	1.1	1.1	3.1 (0.5)
Onondaga	600	0.08 (0.07–0.09)	0.18 (0.15–0.22)	1.1	0.7	4.9 (0.6)
Wayne	510	0.09 (0.06–0.12)	0.27 (0.14–0.52)	1.3	1.1	3.4 (0.8)

[a] Resistance ratio at LC$_{50}$ (i.e. LC$_{50}$ resistant strain/LC$_{50}$ Denmark-S).
[b] Resistance ratio at LC$_{95}$ (i.e. LC$_{95}$ resistant strain/LC$_{95}$ Denmark-S).
[c] LC$_{50}$ and LC$_{95}$ values estimated from Fig. 2.

found with the Kennebec strain, which is somewhat surprising given the reported lack of cyfluthrin use at this facility. However, this population was from a facility that had been repeatedly treated with pyrethrins, which could result in cross-resistance to pyrethroids such as cyfluthrin.[18] Four of the five strains had less steep cyfluthrin log concentration–probit (lcp) lines relative to the susceptible strain, resulting in higher resistance ratios at the LC$_{95}$ and indicating that these strains are more heterogeneous than the susceptible strain. This suggests that resistance alleles are likely present in these populations. Adults from the Wayne strain were not significantly resistant to cyfluthrin at the LC$_{95}$.

Larvae from the Wayne strain were significantly more susceptible to cyfluthrin at the LC$_{50}$, but not at the LC$_{95}$ (Table 2), suggesting that there is some small amount of variation in cyfluthrin toxicity

between different susceptible strains. Larvae from the Kennebec strain proved most resistant, having resistance ratios of 12 and 29 at the LC$_{50}$ and LC$_{95}$, respectively. Larvae from the Waycross cricket colony strain were significantly resistant (5.6-fold), but only when LC$_{95}$ values were compared. Generally, the patterns of resistance to cyfluthrin in larvae were similar to those in adults, with the exception of the Onondaga and Cayuga strains in which resistance was detected only in adults.

Adults in two of the strains tested (Kennebec and Waycross) contained individuals that were highly resistant to tetrachlorvinphos, as indicated by the plateau in the lcp line for these strains (Fig. 1). Both strains contained individuals that survived a concentration that was approximately 1000-fold greater than the susceptible strain LC$_{99}$. Since the Waycross strain (from a cricket colony) had not been exposed to insecticides
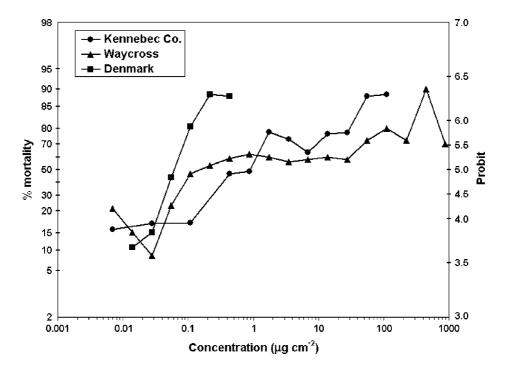
Figure 1. Log concentration–response lines for tetrachlorvinphos against lesser mealworm adults of a susceptible strain (Denmark-S) and two resistant strains (Kennebec and Waycross).
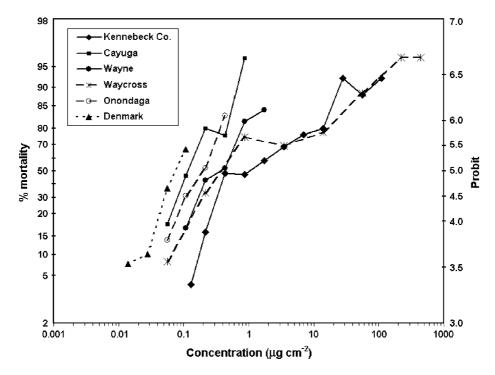

Figure 2. Log concentration–response lines for tetrachlorvinphos against lesser mealworm larvae of a susceptible strain (Denmark-S) and five field-collected strains including two resistant strains (Kennebec and Waycross).

for many years, and given that highly resistant individuals can still be found in this strain, the present data indicate that the gene(s) responsible for the resistance have a negligible fitness disadvantage in the absence of insecticide use. Adults from the Wayne and Onondaga strains from caged-layer poultry farms were also resistant to tetrachlorvinphos, but these strains did not have plateaus in their lcp lines. Adults from the Cayuga strain were susceptible to tetrachlorvinphos (Table 3),

even though this poultry farm reported using this insecticide for premise treatments.

The Kennebec and Waycross strains also contained individual larvae that were highly resistant to tetrachlorvinphos, as seen from the plateau in the lcp line for these strains (Fig. 2). Although similar to the results observed for adults of these strains, the plateau occurred at a slightly lower percentage mortality value. Larvae from the other three strains were

not significantly different from the susceptible strain in their response to tetrachlorvinphos (Table 4). The similar lcp lines obtained for adult and larval beetles in the Kennebec and Waycross strains suggest that a similar mechanism may be causing resistance in both life stages. Identification of this mechanism will require further study.

The present results indicate that resistance is very likely to be having a negative impact on the successful management of the lesser mealworm in caged-layer poultry facilities, especially in the case of tetrachlorvinphos at the Kennebec facility. A goal for any resistance monitoring study is to identify the level of resistance detected in the assay, and relate it to the level of control seen in the field. To achieve this goal it will be necessary to conduct additional studies with these strains to examine the mortality response of adults and larvae to formulated materials applied to plywood (as would occur in a commercial setting) or to monitor the effectiveness of these insecticides directly at the poultry facilities. Clearly, the gene(s) involved in resistance to tetrachlorvinphos (in adults and larvae) do not exert a strong fitness cost as they are maintained in the Waycross strain even after several years of being reared in the lab without insecticide exposure. Similarly, Lambkin[19] also reported retention of fenitrothion resistance for approximately 15 years in *A. diaperinus*. Since tetrachlorvinphos and fenitrothion are both organophosphates, it is possible that a similar mechanism of resistance exists for these two organophosphates in the USA and Australia. If so, this could explain why tetrachlorvinphos and fenitrothion resistance alleles are preserved in lesser mealworm populations in the USA. Identification of the gene(s) involved in the resistance will require further study.

ACKNOWLEDGEMENTS

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