Susceptibility of lesser mealworm (Coleoptera: Tenebrionidae) adults and larvae exposed to two commercial insecticides on unpainted plywood panels

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Abstract

BACKGROUND: The susceptibilities of adult and larval lesser mealworms, *Alphitobius diaperinus* (Panzer), to two commercially formulated insecticides, cyfluthrin and tetrachlorvinphos, were examined through exposure on treated plywood panels. Lesser mealworms were collected from four caged-layer poultry farms, three in New York and one in Maine. An additional strain was obtained from an infestation occurring in a cricket colony.

RESULTS: In all poultry farm derived strains, a portion of the population (1.8–16.2%) survived cyfluthrin exposure. The Maine and cricket colony strains were tolerant of tetrachlorvinphos exposure as both larvae and adults, with 55–74% mortality, whereas nearly 100% mortality was observed with New York strains. The cricket colony adult beetles were highly susceptible to cyfluthrin, with 100% mortality following exposure, but larvae were considerably less susceptible (87.7%). Pesticide use histories for the poultry farms and their impact on the results are discussed.

CONCLUSION: The results document that tetrachlorvinphos, an active ingredient with a long use history, may be losing its effectiveness against lesser mealworms in some poultry operations; however, it is still effective in many others.

Keywords: Insecta; resistance; insecticides; *Alphitobius diaperinus*; organophosphate; pyrethroid; poultry

1 INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus* (Panzer), is the predominant beetle pest of poultry production in the United States. This beetle can be a pest in several ways, e.g. causing extensive damage to poultry housing and dispersing from agricultural fields when infested manure is spread during warmer months. The importance of this beetle in pathogen transmission is well documented.[1–4]

Because beetle populations peak when poultry manure accumulations are large, pesticide application is difficult and often reserved for the time when manure is removed from housing. Thus, pesticide resistance has been slow to develop and therefore has not been tracked until recently.[5–7] Other major livestock pests, such as the housefly, *Musca domestica* L., have been followed quite closely for many years.[8–11] Significant resistance to several pyrethroids has been reported from field populations of lesser mealworms in eastern Australia.[5,7] However, in a study performed in the eastern United States, overall resistance was not widespread and was quite high in only one of the four field strains examined.[6] In the same study, a population from Maine showed considerable resistance to tetrachlorvinphos, and this resistance was observed at a tenfold greater level in adults, suggesting a stage-influenced mechanism. Cyfluthrin resistance was also quite high in this Maine population; however, larvae had higher resistance ratios than adults.

Lesser mealworm larvae and adults generally leave poultry manure by climbing up the unpainted wooden support posts found in most poultry facilities. During this time, beetles are exposed to pesticides that have been applied to the support posts. Therefore, examination of lesser mealworm susceptibility to commercially available insecticides on unpainted plywood in controlled laboratory settings would simulate conditions in animal facilities. Similar studies conducted previously with houseflies and pteromalid parasitoids demonstrated that the surface to which the formulated insecticide was applied had considerable

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influence on efficacy.[11–13] For example, several pteromalid species were found to be more tolerant to dimethoate (Cygon) when exposed to treated wooden panels rather than to treated glass jars.[13]

The objective of the present study was to determine the level of insecticide resistance in adult and larval lesser mealworm populations from selected poultry facilities in the eastern United States. The study was conducted to simulate conditions in poultry facilities using field-collected populations of lesser mealworm adults and larvae placed on insecticide-treated, unpainted plywood panels. The authors are not aware of any studies documenting susceptibility of lesser mealworms exposed under similar field-simulation conditions.

2 MATERIALS AND METHODS

2.1 Insects, farms and chemicals

Insect colony sources and pesticide history of field colonies have been described previously.[6] Briefly, the insecticide-susceptible strain of lesser mealworms was obtained from a colony in Denmark (Saturnia, Bjerringbrovej 48 2610, Rødovre, Denmark). Lesser mealworms were field collected in 2002 from three caged-layer poultry farms in New York (Cayuga, Onondaga and Wayne counties) and from a fourth in Kennebec county, Maine. An additional strain was obtained from an infested cricket colony in Waycross, GA. Insecticide use at the Cayuga Co. facility included premise applications of permethrin, tetrachlorvinphos and carbaryl, and feed mixtures containing cyromazine and methomyl fly bait. Insecticide use at the Wayne Co. and Onondaga Co. facilities for at least the previous 5 years was limited to pyrethrin space sprays and methomyl fly baits. Kennebec facility treatments included nearly weekly applications of pyrethrin space sprays from 1998 to 2001, and variably timed premise applications of dichlorvos and dimethoate, cyromazine feed-through use and methomyl and nithiazine fly baits. No pesticide use history is available for either the Denmark or Waycross Co. strains. However, because each strain was obtained from insect cultures, it is assumed that these strains received minimal pesticide exposure. Lesser mealworm colonies were maintained at 28 °C, 60–70% RH and provided with a diet of cracked corn and wheat bran *ad libitum*.

2.2 Bioassays and analysis

Lesser mealworm adults and larvae were assayed using commercially available, formulated materials as residual contact applications, as described previously.[11] Two formulated insecticides were evaluated: cyfluthrin 200 g kg^{-1} WP (Tempo; Bayer, Kansas City, MO, USA) and tetrachlorvinphos 500 g kg^{-1} WP (Rabon; Fermenta, Kansas City, MO, USA). The plywood sheets (1.2 × 2.4 m) were exposed to natural summer weather conditions for 10 days and then cut into 900 cm^2 panels. Formulated insecticides were prepared according to label directions (= 1 gal per 750 ft^2) and applied to the plywood panels at a uniform rate of 5 mL per 929 cm^2 with calibrated trigger-pump hand sprayers. Equal volumes (5 mL) of water were applied to untreated control panels in the same manner. All panels were allowed to dry for 1 h before use. Panels were used only once.

Lesser mealworms were exposed in groups of either 20 larvae or 20 adults per strain, and each strain was represented on each of three replicate panels per treatment. Insects were confined to panels with plastic petri dish bottoms (90 mm inner diameter, 1 cm thick) (Fig. 1) secured to the plywood panels with rubber bands stretched across the petri dishes and fastened to push pins. After petri dishes were secured, panels were placed horizontally on a table for a 2 h holding period at 25 °C. Throughout the holding period, lesser mealworms were observed walking on the surface of the panels.

After 2 h, lesser mealworms were removed from panels by tapping the insects into the petri dish bottoms, transferred to holding dishes, provided with water on cotton wicks and observed for mortality after 48 h. Adults and larvae were considered dead if they were ataxic. For each life stage, the assays were repeated 3 times (60 insects per replication, strain and chemical), with three panels per insecticide at each replication (total of nine treated panels).

Percentage mortality was calculated and corrected for control mortality.[14] Data from each chemical and beetle life stage were analyzed using a two-way analysis of variance.[15] The statistical model contained the fixed effects of study replication and beetle strain. Data within each chemical and beetle life stage were tested for strain differences using a Tukey's mean separation.

3 RESULTS AND DISCUSSION

The Waycross strain originated from lesser mealworms that were infesting a cricket colony. Therefore, it was assumed that this strain would be quite susceptible to cyfluthrin owing to the recent introduction of the chemical and would be, at most, moderately

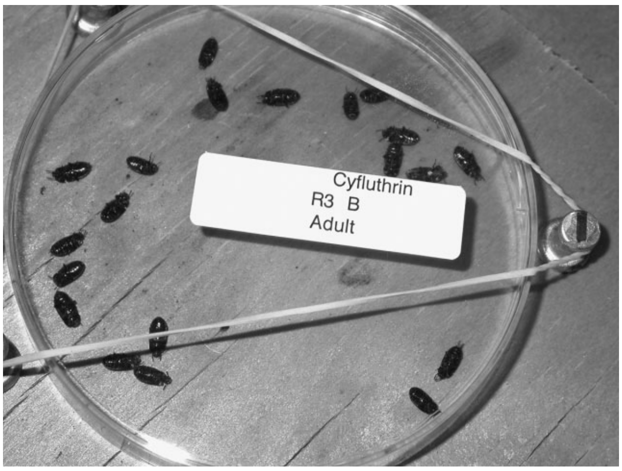

Figure 1. Adult lesser mealworms confined to treated plywood panel (i.e. simulated field bioassay).

susceptible to tetrachlorvinphos. Results confirmed that adult beetles of the Waycross strain were highly susceptible to cyfluthrin, with 100% mortality following exposure (Table 1). Surprisingly, mortality of the larvae was considerably lower (87.7%), but this was not significantly different from the other strains examined (Table 2). In all poultry farm derived strains, a portion of the lesser mealworm population survived for 48 h following the 2 h cyfluthrin exposure. At the time the field strains were collected, cyfluthrin had been registered for use on poultry farms for less than 8 years, and prior pesticide surveys suggest that minimal amounts were applied during that time.[16] Therefore, it would appear that cross-resistance with other pyrethroids that had been used against both lesser mealworm and houseflies is likely the cause for the reduced efficacy.

Both the Kennebec and Waycross strain adults and larvae were significantly less susceptible to tetrachlorvinphos than all the New York strains and the Denmark strain (Tables 1 and 2). Nearly 100% of all adults and larvae from the three New York populations were killed following exposure to tetrachlorvinphos. This is surprising given that this product has been available for use in poultry systems for many years.

In a previous study, these same lesser mealworm strains had been evaluated for resistance using technical-based tetrachlorvinphos and cyfluthrin exposures on glass,[6] where the Waycross and Kennebec strain adult lesser mealworms expressed 7.7- and 9.5-fold greater cyfluthrin resistance, respectively, than the Denmark strain beetles. This is contrary to the present results, which showed 100 and 91.8% mortality of the adult Waycross and Denmark (insecticide susceptible) beetles, respectively, when exposed to formulated cyfluthrin on plywood. In fact, using resistance ratios (RRs), Hamm *et al.*[6] reported that all strains were significantly less susceptible than the Denmark strain at the RR_{50} level and all but one strain at the RR_{95} level.

Previous studies documented that larvae from the Kennebec and Waycross strains were significantly less susceptible to cyfluthrin than the Denmark strain.[6] However, in the present study there were no differences between any of the strains (Table 2). Additionally, the Kennebec strain showed the highest resistance levels in the Hamm *et al.*[6] study, but in the present study the greatest survival was with the Waycross strain. Although these results seem counterintuitive, this illustrates the importance of multiple evaluation techniques and suggests that the exposure of larvae to cyfluthrin-treated glass does not necessarily simulate results expected in the field. It also suggests that product formulation affects efficacy.

In Australia the lesser mealworm was found to be highly resistant to the organophosphate fenitrothion in areas where the material had been used for up to 20 years, but resistance was weak or non-existent in areas with five or fewer years of use.[5] In the present study there was little resistance to cyfluthrin and tetrachlorvinphos in the New York strains where cyfluthrin had been available to producers for a short time and tetrachlorvinphos for much longer. That lesser mealworms were killed following tetrachlorvinphos exposure is likely due to its limited use by New York poultry producers.[16] Although tetrachlorvinphos has been registered for use in poultry since 1966, pesticide use frequencies were not well

Table 1. Percentage mortality of adult lesser mealworms from three New York state poultry farms, a Maine poultry farm (Kennebec), a Georgia cricket colony strain (Waycross) and an insecticide-susceptible strain (Denmark) exposed to the labeled rate of cyfluthrin and tetrachlorvinphos on unpainted plywood panels

Insecticide[a]	Strain	Mortality (%) (± SE)[b,c]
Cyfluthrin	Denmark	91.8 (±4.13)AB
	Kennebec	95.0 (±3.33)AB
	Waycross	100 (±0.00)A
	NY-R	96.6 (±1.19)AB
	NY-S	87.1 (±2.42)AB
	NY-W	83.8 (±6.05)B
Tetrachlorvinphos	Denmark	100 (±0.00)A
	Kennebec	65.2 (±6.87)B
	Waycross	73.9 (±5.26)B
	NY-R	100 (±0.00)A
	NY-S	100 (±0.00)A
	NY-W	99.4 (±0.55)A

[a] Cyfluthrin applied as Tempo 20WP and tetrachlorvinphos applied as Rabon 50WP.
[b] $N = 9$.
[c] Within a chemical, means followed by the same letter are not significantly different ($\alpha = 0.05$; df = 5, 53; Tukey's multiple range test). Cyfluthrin ($F = 3.36$, $P < 0.011$). Tetrachlorvinphos ($F = 19.55$, $P < 0.001$).

Table 2. Percentage mortality of lesser mealworm larvae from three New York state poultry farms, a Maine poultry farm (Kennebec), a Georgia cricket colony strain (Waycross) and an insecticide-susceptible strain (Denmark) exposed to the labeled rate of cyfluthrin and tetrachlorvinphos on unpainted plywood panels

Insecticide[a]	Strain	Mortality (%) (± SE)[b,c]
Cyfluthrin	Denmark	99.4 (±0.55)
	Kennebec	97.7 (±1.47)
	Waycross	87.7 (±11.03)
	NY-R	Not tested
	NY-S	98.2 (±1.29)
	NY-W	97.2 (±1.51)
Tetrachlorvinphos	Denmark	100 (±0.00)A
	Kennebec	72.0 (±5.74)B
	Waycross	55.7 (±10.52)B
	NY-R	100 (±0.00)A
	NY-S	100 (±0.00)A
	NY-W	100 (±0.00)A

[a] Cyfluthrin applied as Tempo 20WP and tetrachlorvinphos applied as Rabon 50WP.
[b] $N = 9$.
[c] Within a chemical, means followed by the same letter are not significantly different ($\alpha = 0.05$; cyfluthrin df = 4, 44; tetrachlorvinphos df = 5, 53; Tukey's multiple range test). Tetrachlorvinphos ($F = 15.34$, $P < 0.001$).

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documented in the earlier decades. The use of this material was probably much greater prior to the advent of pyrethroid materials. However, the much higher tetrachlorvinphos resistance in the Maine (Kennebec) strain suggests that the producer-reported higher use of organophosphate materials has selected for a more resistant population.

Cyfluthrin resistance was associated with its prior use against lesser mealworms.[7] With as few as 20 cyfluthrin applications in 4 years, 22-fold resistance was observed in Australia. This suggests that many researchers in this field may underestimate the rapidity with which lesser mealworms can attain resistance. Additionally, the use of this and other products targeting houseflies and other pests must be considered as contributing to inadvertent resistance development. That greater resistance is not occurring may well be related to the exceptional protection from insecticides that lesser mealworms acquire from the manure they inhabit.

The present results agree with those of Hamm *et al.*[6] with tetrachlorvinphos. Both studies showed very high levels of resistance in the Waycross and Kennebec strains. The present data suggest that control of lesser mealworm adults and larvae is still possible with tetrachlorvinphos, but that results may depend on local insecticide use histories.

The variability between technical-based assays, particularly on glass, as compared with studies with formulated materials on wood, either in laboratory conditions or in field situations, often leads to confusing results. However, both types of assay are valuable and point out the importance of restraint in data interpretation with any evaluation method. Comparative studies provide a valuable tool for bridging these inconsistencies. Although an arthropod population may be developing resistance levels that are quite high, the response in field situations may be masked by unique conditions including pesticide formulation and substrate interactions, such as a refugium provided by manure or interaction of formulations and treatment surface (plywood in this case).

The present results document that tetrachlorvinphos, an active ingredient with a long use history, may be losing its effectiveness against lesser mealworms in some poultry operations; however, it is still effective in many others.

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