Baseline Responses of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) to Insect Growth Regulators[1]

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ABSTRACT The cancellation of most organophosphate and carbamate insecticides, and the emerging resistance to currently available insecticides for managing the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), necessitate efficacy studies on other classes of insecticidal compounds. Baseline dose-response bioassays were conducted with three different types of insect growth regulators (IGRs), fenoxycarb, diflubenzuron, and 20-hydroxyecdysone (20E) on first and seventh instars, two day-old pupae, and one-week-old adult beetles. Insects were exposed to a range of concentrations through topical application, by residual contact with treated wood shavings, and through feeding on treated chicken feed diet. Insects were observed at 10-day intervals to estimate mortality, pupation, and abnormal growth for each treatment. The mean number of days to pupation increased and pupation was delayed in fenoxycarb-treated seventh instars in feeding, residual, and topical bioassays. Fenoxycarb-treated larvae continued to molt and gain weight, and died as deformed larvae, abnormal pupae, or intermediate larval-pupal and pupal-adult forms. In feeding bioassays, fenoxycarb was more toxic to seventh instars than to first instars or adults, whereas diflubenzuron was more toxic to first instars than it was to seventh instars or adults. The feeding bioassay was more suitable for the first and seventh instars, although the residual contact bioassay could also be used for seventh instars. Adults succumbed to lower concentrations of IGRs in a topical bioassay than in feeding or residual bioassays. All three bioassay methods produced usable dose-response curves and may be used for surveying temporal changes in the IGR susceptibility to lesser mealworm.

KEY WORDS Coleoptera, Tenebrionidae, *Alphitobius diaperinus*, feeding bioassay, susceptibility, weight gain, pupation

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is a serious, cosmopolitan pest present in poultry production facilities, where it consumes poultry feed and litter. This beetle causes decreased weight gains in broiler chicks that eat them (Despins & Axtell 1995). These beetles also transmit several disease agents such as avian influenza, Marek's disease, Coronavirus, the Newcastle disease virus (De Las Casas et al. 1973, 1976), *Salmonella typhimurium* (McAllister et al. 1994), *Campylobacter jejuni* (Strother

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et al. 2005), and infectious bursal disease (McAllister et al. 1995). Lesser mealworms also cause damage in poultry houses by tunneling through the insulation (Despins et al. 1987).

The cancellation of organophosphate and carbamate insecticides has reduced the number of residual insecticides for lesser mealworm control, and the remaining products are facing the potential of developing insecticide-resistant populations (Lambkin 2005). Fenitrothion was used for 20 years before the adoption of cyfluthrin, which is currently considered the industry standard in Australia. Unfortunately, resistance to both fenitrothion and cyfluthrin has been found in populations of lesser mealworms in broiler houses in Australia, resulting in failure of control measures (Lambkin 2005). Tetrachlorvinphos and cyfluthrin have been reported to have lost effectiveness against lesser mealworms in poultry houses in the United States (Hamm et al. 2006). Due to environmental and resistance concerns related to the use of conventional insecticides, research needs to be focused towards insect growth regulators (IGRs) that are more selective with reduced-risk and possess different modes of action (Dhadialla et al. 2005).

Ecdysone is a steroidal pro-hormone of the major insect molting hormone 20-hydroxyecdysone (20E), which is secreted from the prothoracic glands. Ecdysone, 20E, and juvenile hormone, regulate the physiological and behavioral processes in insects, and these compounds also play an important role in regulating the morphogenetic changes during metamorphosis (Willis 1974). Juvenile hormone analogs (JHAs) and ecdysone agonists that interfere with insect growth and development have a great potential in pest management (Ishaaya 1990). These ecdysone agonists cause premature cuticle synthesis around the head region where occlusion of the functional mouthparts resulted in feeding inhibition within 24 hours in lepidopterans regardless of the age or instar treated (Wing & Aller 1990). Treatment of two ecdysone agonists, methoxyfenozide (RH-2485) and halofenozide (RH-0345) at concentrations of 25, 50, and 100 mg/liter on multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), caused premature induction of larval molting, cessation of feeding, and incomplete pupation in the last instar (Carton et al. 2003).

Metamorphosis (larval-pupal and pupal-adult) is induced by 20E, but this needs to happen in the absence of juvenile hormone (Goodman & Granger 2005). JHAs are toxic during the embryonic, final larval, and reproductive stages of insects. Juvenile hormone treatment at any immature stage reduces adult emergence in yellow mealworm, *Tenebrio molitor* L., and confused flour beetle, *Tribolium confusum* Jacquelin du Val (Pallos et al. 1971). Fenoxycarb (a JHA) caused 93% reduction of several stored-product pests, including rice weevil, *Sitophilus oryzae* (L.), confused flour beetle, lesser grain borer, *Rhyzopertha dominica* (F.), and Indianmeal moth, *Plodia interpunctella* (Hübner), in stored wheat (Solomon 1985). Fenoxycarb significantly delayed the developmental times of larvae from the stage treated to adult emergence (Liu & Chen 2001).

The chitin synthesis inhibitors (CSI), such as diflubenzuron and flucycloxuron, prevent the molting cycle of insects, leading to abnormal endocuticular deposition and abortive molting (Dhadialla et al. 2005), ecdysial failure, and formation of globular bodies between the endocuticle and epidermis (Ren et al. 1988). CSIs retarded larval growth of the red flour beetle, *T. castaneum* (Herbst), and hence increased larval life. CSI-treated larvae were found to eat less, and consequently, they grew at a slower rate (Parween 1996). The khapra beetle, *Trogoderma*

granarium Everts, treated with diflubenzuron at the dose of 12.8 mg a.i./kg in their feed resulted in 94, 80, and 76% mortality in 3, 7, and 14 day-old larvae, respectively (Rajendran & Shivaramaiah 1983).

Lesser mealworm beetles in poultry houses are exposed to insecticides by three different ways. They are direct spray contact, tarsal residual contact with treated surfaces, and through feeding on treated litter and chicken feed. There have been no published reports of laboratory bioassays on lesser mealworm larvae, pupae, and adults exposed to insecticides in these three ways that mimic conditions in poultry facilities. Little data exists on the efficacy of IGRs against lesser mealworm.

The goal of the present study was to compare fenoxycarb, diflubenzuron, and 20E to determine the differences in their toxicity levels to lesser mealworm larvae, pupae, and adults. Topical, residual, and feeding bioassays were also compared to determine the most susceptible stage, percentage pupation, mean number of days to pupation, and adult survival among individuals treated with fenoxycarb, diflubenzuron, and 20E.

Materials and Methods

Laboratory colony of lesser mealworms. A large number of different stages of lesser mealworms were collected from the Applied Broiler Research Unit, in Savoy, AR, where pyrethroids, organophosphates, and spinosad had been used regularly over the previous four years for beetle control. Litter or manure was collected from these houses and mealworms were extracted by sieving samples through 2.8 and 2.0-mm screens. Lesser mealworms were collected and reared in the laboratory in covered plastic chambers maintained at 28°C with 60% RH, and a photoperiod of 16:8 (L:D). These beetles were fed commercial starter chicken feed that contained corn and soybean meal along with vitamin and mineral supplements (Chick Starter, Herider Farms, Fayetteville, AR), referred to hereafter as lesser mealworm diet. Rolled pieces of corrugated cardboard (30 cm^2) (Northwest Arkansas Paper Company, Springdale, AR) were added to serve as shelters and pupation sites for mature larvae. Beetles were provided water weekly by placing a 6-cm^2 piece of water-saturated cotton on the litter surface.

Lesser mealworm larvae undergo eight to eleven instars depending upon nutrition before pupatation. To get uniform age, seventh instar lesser mealworms for feeding, residual, and topical bioassays, approximately 10,000 sixth instars (7 mm in length according to Wilson & Miner 1969) were separated from field-collected beetles and placed in a plastic chamber (27.5 long by 17.5 wide by 17.5 cm deep, Penn Plax Large Animal Carrier, Garden City, NY) with wood shavings. Lesser mealworm diet and water were provided weekly to these larvae. After seven days, the chambers were checked regularly for cast skins to confirm that larvae had molted to the seventh instar (Wilson & Miner 1969). The newly molted seventh instars (light-colored exoskeleton) were removed for bioassays. A piece of rolled corrugated cardboard (30 cm^2) was placed in each plastic rearing chamber that contained approximately 10,000 seventh instars. Chambers were checked weekly to confirm molting, and uniform aged pupae were collected for bioassays. The unused pupae were allowed to eclose into adults that were used in later bioassays. The newly emerged adults were removed and held in groups of

100 per Petri dish (150 mm diameter). The adult exoskeletons were allowed to completely harden over a five-day period (Hopkins et al. 1992), during which time these adults were provided daily with 1 g of finely ground lesser mealworm diet and water in the form of moistened 2-cm^2 cotton ball. First instars of uniform age were obtained by placing about 1000 newly emerged adults in plastic chambers containing water, diet, and wood shavings. Eggs were laid within a week, and the first instars were sieved from these chambers after two weeks.

Three different types of IGRs were bioassayed. They were a juvenile hormone analog (JHA), fenoxycarb (98%); a chitin synthesis inhibitor (CSI), diflubenzuron (99%); and the molting hormone, 20-hydroxyecdysone (20E) (98%; Sigma-Aldrich Co., Milwaukee, WI).

Feeding bioassays. Stock solutions of each IGR compound were made by dissolving them in acetone. Aliquots of the stock solution were added to 100 g batches of lesser mealworm diet to achieve concentrations ranging from 0.01 to 100 ppm (μg of IGR/g of diet). Excess acetone was added to uniformly disperse the IGRs. Batches of diet treated with acetone alone served as the untreated controls. The treated diet mixtures were stirred and put in a fume hood for 24 h to evaporate the acetone completely. Batches of treated and untreated diet were divided into aliquots of 2 g in 30 ml plastic cups and then one beetle was placed in each cup. Individual 4-day-old first instars, newly eclosed seventh instars, and one-week-old adults were used in all experiments. One hundred replicates were completed for fenoxycarb, and 80 replicates each were completed for diflubenzuron and 20E. Individual insects were allowed to feed on treated feed for 10 d, and then they were transferred to normal lesser mealworm diet. Water-soaked cotton (2 cm^2) was placed on the surface of the diet and moistened twice weekly. Each beetle was observed at 10-day intervals for 60 d to estimate the percentage mortality and abnormal growth for each treatment.

Residual contact bioassays. To achieve concentrations ranging from 0.01 to 1000 ppm, IGR compounds were diluted in acetone, mixed uniformly with wood shavings (500 g) in a glass container (40 by 30 cm), and air dried for 12 h. Treated wood shavings (2 g) were transferred to 30-ml plastic cups. Wood shavings treated with acetone alone served as untreated controls. Individual, newly eclosed seventh instars and one-week-old adults were used in all tests, and there were 80 replicates per treatment. Each beetle was placed in a treated cup, and observations of percentage mortality and abnormal growth for each treatment were made at 10-day intervals for 60 d.

Topical bioassays. Newly eclosed seventh instars, two-day-old pupae and one-week-old adults were used in these tests. Before IGR application, beetles were removed from rearing boxes and counted into batches of 10 beetles. Treatment concentrations in nanograms per milligram (ng/mg) or ppm were calculated using the mean weight of seventh instars (15.55 ± 1.66 mg, N = 100) and adults (14.47 ± 1.52 mg, N = 100). The seventh instars or adult beetles were transferred to a small glass sheet (5 cm long by 4 cm wide) where 1 μl of an IGR concentration (ranging from 0.01 to 100 ng/mg) was individually applied to the thoracic dorsum with a Hamilton micro syringe (Hamilton Co., Reno, NV). Untreated control beetles were similarly treated with 1 μl of acetone. One beetle was placed in each 30-ml plastic cup with lesser mealworm diet. Sixty replicates per treatment were completed for pupae and adults, whereas 80 replicates were used for seventh instars.

All treatment cups were placed in the laboratory conditioned at 28°C with 60% RH, and a photoperiod of 16:8 (L:D). A lesser mealworm was considered morbid or dead if it was unable to right itself or walk, or there was no movement when they were prodded with a metal probe. Pupae were regarded as dead if they turned black with no abdominal gyrating movement, or showed signs of desiccation.

Weight gain. Larval weights were measured at 10-day intervals for seventh and first instars fed on fenoxycarb-treated feed and for seventh instars topically treated or exposed to fenoxycarb-treated wood shavings. The weights of all seventh instars were nearly identical at the beginning of the bioassays. However, weight gain was calculated as the difference between the final weight at the time of observation and the initial weight of each larva to eliminate the variations in the initial weights on analysis. The weight at 10 d was used as initial weight to determine weight gain in the analysis for the first instars since initial weight was extremely low.

Experimental plan and statistical analyses. Each experiment was setup in a randomized complete block (RCB) design with each cup representing a replicate. Each IGR concentration was replicated ten times in a block with one beetle per replicate. Ten blocks were used in feeding bioassays for fenoxycarb, whereas eight blocks were used in feeding bioassays for diflubenzuron and 20E, and all residual bioassays. Eight blocks were used in topical bioassays for seventh instars and six blocks were used for pupae and adults. Concentration-mortality responses were estimated with the PROC PROBIT option of SAS (SAS Institute 2004). Mortality of treated beetles at 10 days was corrected for natural mortality in the untreated control using Abbott's formula (Abbott 1925). Corrected data from bioassays were analyzed by probit analysis to estimate the IGR concentration that killed 50 or 90% of the lesser mealworms (LC_{50} and LC_{90}), regression coefficient (slope) and its standard error, Pearson's goodness-of-fit, Chi-square, and 95% fiducial limits for effective level of concentrations. Only pairs of LC_{50} values or pairs of LC_{90} values that did not have overlapping 95% fiducial limits were considered significantly different. Weight gains, percentage pupation, mean number of days to pupation, mortality in each stage, and adult survival were analyzed using ANOVA, and means were separated using Tukey test (SAS Institute 2004).

Results

Weight gain in feeding bioassays. The mean larval weight gains of seventh instars fed different concentrations of fenoxycarb-treated diet were significantly different after 10 d ($df = 5, 24$; $F = 50.42$; $P = 0.0001$), 20 d ($df = 4, 20$; $F = 271.96$; $P = 0.0001$), 30 d ($df = 4, 20$; $F = 814.30$; $P = 0.0001$), and 40 d ($df = 4, 20$; $F = 392.80$; $P = 0.0001$) post treatment. The mean larval weight gains were highest at 0.01 ppm after 10 d, at 0.1 ppm and 1 ppm after 20 d, and at 1 ppm and 10 ppm after 30 d post-treatment ($P < 0.05$) (Table 1). At 40 d post-treatment, larvae fed a dose of 10 ppm attained the maximum weight gain of 13.7 mg (Fig. 1). The weight gains of untreated check larvae were significantly lower than all the concentrations of fenoxycarb with the exception of the 100 ppm treatment, where the untreated acetone control and 100 ppm had similar low weight gains ($P < 0.05$). The majority of the untreated control larvae developed to pupae within 10 d, so the weight gains in this group were not reported thereafter

Table 1. Weight gain of first and seventh instar lesser mealworms after exposure to fenoxycarb-treated diet in feeding bioassays.

Larval stage	Concentration (ppm)	Initial wt (mg)	Weight gain (mg)				
			10 d	20 d	30 d	40 d	50 d
7^{th}	0.01	15.16 ± 0.17	3.61 ± 0.24b[a]	4.21 ± 0.31c	1.12 ± 0.18d	0.90 ± 0.18d	-
7^{th}	0.1	15.36 ± 0.66	4.80 ± 0.37a	8.80 ± 0.22a	4.90 ± 0.26b	2.72 ± 0.44c	-
7^{th}	1	15.26 ± 0.14	3.48 ± 0.28b	9.08 ± 0.19a	10.38 ± 0.10a	7.94 ± 0.21b	-
7^{th}	10	15.65 ± 0.15	1.62 ± 0.28c	7.53 ± 0.16b	9.98 ± 0.16a	13.66 ± 0.13a	-
7^{th}	100	15.54 ± 0.20	0.18 ± 0.15d	0.56 ± 0.15d	1.86 ± 0.11c	8.70 ± 0.19b	-
1^{st}	0.1	-	3.23 ± 0.21a	5.91 ± 0.37a	10.53 ± 0.49b	15.74 ± 0.34c	18.80 ± 0.32c
1^{st}	1	-	1.68 ± 0.12d	4.83 ± 0.15b	12.32 ± 0.39a	17.02 ± 0.39b	21.13 ± 0.25b
1^{st}	10	-	1.85 ± 0.13cd	4.76 ± 0.22b	11.90 ± 0.45a	18.18 ± 0.31a	25.14 ± 0.51a
1^{st}	100	-	1.84 ± 0.11cd	3.51 ± 0.18c	6.50 ± 0.27d	9.14 ± 0.60e	16.00 ± 0.57d
1^{st}	Control	-	2.17 ± 0.10c	4.62 ± 0.15b	7.65 ± 0.21d	14.70 ± 0.12d	-

[a]For each larval stage, means in the same column with a similar letter are not significantly different ($P > 0.05$, Tukey's HSD).

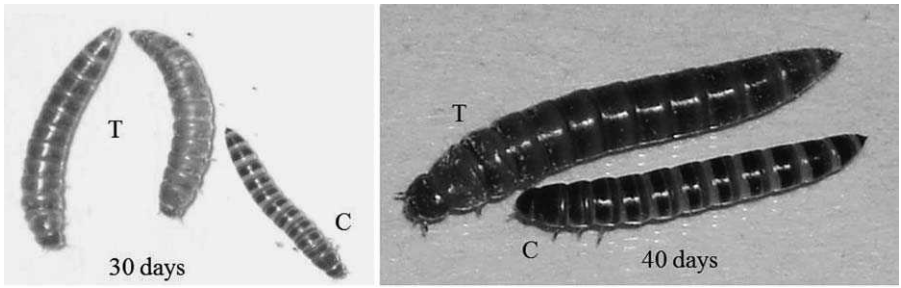

Fig. 1. Relative size of first instar lesser mealworms after 30 and 40 d exposure to fenoxycarb-treated diet at 10 ppm (T = Treatment) or acetone-treated control diet (C = Control) in feeding bioassays.

(Table 1). In the untreated control, the percentage pupation in the untreated control (86.5%) was significantly higher than for larvae fed on fenoxycarb treated diet (33.0%). The mean number of days to pupation for untreated control (13.7 d) was significantly lower than for larvae fed on fenoxycarb treated diet (26.3 d) (Table 2).

The mean larval weight gains of first instars fed different concentrations of fenoxycarb-treated diet were significantly different after 10 d (df = 5, 388; F = 13.64; $P = 0.0001$), 20 d (df = 5, 342; F = 6.41; $P = 0.0001$), 30 d (df = 5, 304; F = 32.22; $P = 0.0001$), 40 d (df = 5, 292; F = 45.63; $P = 0.0001$), and 50 d (df = 4, 179; F = 69.86; $P = 0.0001$) post-treatment. First instars exhibited the highest weight gains for 0.1 ppm fenoxycarb after 10 and 20 d, for 1 and 10 ppm after 30 d, and for 10 ppm after 40 and 50 d post-treatment ($P < 0.05$) (Table 1). The fenoxycarb-treated first instars exhibited a significantly lower rate of pupation (30.6%) than the untreated control (85.0%). The mean number of days to pupation for fenoxycarb-treated larvae (43.0 d) was not statistically different from the untreated control (41.0 d) ($P > 0.05$) (Table 2).

Weight gain in residual bioassays. The mean larval weight gains of seventh instars exposed to wood shavings treated with different concentrations of fenoxycarb were significantly different after 10 d (df = 6, 558; F = 16.63; $P = 0.0001$) and 20 d (df = 4, 45; F = 10.95; $P = 0.0001$), but they were not significantly different after 30 d (df = 1, 17; F = 0.21; $P = 0.65$) post-treatment. Seventh instars exposed to fenoxycarb-treated wood shavings exhibited significantly higher weight gains at concentrations of 10, 100, and 1000 ppm after 10 d, and at a concentration of 100 ppm after 20 d, compared to other concentrations (Table 3). Lower fenoxycarb concentrations of 0.1 and 1 ppm extended pupation of some seventh instars to 30 d. The larvae in the untreated control or in concentrations of 1000 ppm either pupated or died, respectively, after 20 d ($P < 0.05$) (Table 3). Untreated control larvae attained a significantly higher percentage pupation (93.3%) than seventh instars exposed to fenoxycarb (77.9%). The mean number of days to pupation for untreated control larvae (12.0 d) was significantly lower than the 28.4 d for larvae exposed to fenoxycarb ($P < 0.05$) (Table 4).

Table 2. Stage specific mortality, pupation rate, adult survival, and time to pupation for first and seventh instar lesser mealworms after exposure to diet treated with insect growth regulators (IGRs) in feeding bioassays.

IGR[a]	Larval stage	N	% Dead larvae	% Dead pupae	% Dead adults	% Alive adults	Total % pupated	No. of days to pupation
FXB	7th	10	67.0 ± 3.3a[b]	17.0 ± 2.0a	4.2 ± 1.2b	12.0 ± 1.4d	33.0 ± 3.3d	26.3 ± 0.45a
20E	7th	8	44.7 ± 3.8b	14.7 ± 1.0a	10.7 ± 0.9a	29.0 ± 3.2c	55.2 ± 3.8c	19.6 ± 0.88b
DFB	7th	8	33.0 ± 1.9c	13.0 ± 1.5a	14.0 ± 1.6a	40.0 ± 2.9b	67.0 ± 1.9b	15.7 ± 0.37c
Control	7th	8	13.4 ± 1.4d	4.2 ± 0.9b	3.4 ± 0.9b	80.0 ± 1.3a	86.5 ± 1.4a	13.7 ± 0.31d
FXB	1st	10	69.4 ± 3.0a	10.2 ± 1.2a	0.0b	20.4 ± 2.4b	30.6 ± 3.0b	43.0 ± 4.70a
20E	1st	8	70.0 ± 3.3a	4.8 ± 0.8b	1.2 ± 0.5a	24.0 ± 3.5b	30.0 ± 3.3b	46.0 ± 5.50a
DFB	1st	8	67.5 ± 2.6a	10.0 ± 1.8a	0.0b	22.5 ± 1.7b	32.5 ± 2.6b	45.0 ± 6.60a
Control	1st	8	15.0 ± 1.2b	5.0 ± 0.9b	0.0b	80.0 ± 5.7a	85.0 ± 1.2a	41.0 ± 3.80a

[a]FXB = fenoxycarb, DFB = diflubenzuron, 20E = 20-hydroxyecdysone, and control = untreated control treatment.

[b]For each larval stage, means in the same column with a similar letter are not significantly different ($P > 0.05$, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 3. Weight gain of seventh instar lesser mealworms exposed to fenoxycarb in residual (treated wood shavings) or topical bioassays.

		Weight gain (mg)				
Treatment method	Concentration (ppm)	Initial wt (mg)	10 d	20 d	30 d	40 d
Residual	0.01	16.69 ± 0.13	3.33 ± 0.24e[a]	5.88 ± 0.57c	-	-
Residual	0.1	16.08 ± 0.14	3.85 ± 0.25de	9.69 ± 0.40b	3.82 ± 0.45a	-
Residual	1	16.34 ± 0.15	4.44 ± 0.23c	9.46 ± 0.36b	3.06 ± 1.01a	-
Residual	10	16.71 ± 0.14	4.89 ± 0.16b	9.29 ± 0.46b	-	-
Residual	100	16.81 ± 0.09	5.91 ± 0.16a	10.90 ± 0.42a	-	-
Residual	1000	16.57 ± 0.12	5.65 ± 0.24ab	-	-	-
Residual	Control	16.68 ± 0.13	4.00 ± 0.20cd	-	-	-
Topical	0.01	15.46 ± 0.12	1.39 ± 0.41a	2.16 ± 0.32c	-	-
Topical	0.1	15.70 ± 0.11	−0.35 ± 0.13b	3.72 ± 0.22a	10.74 ± 0.93b	3.96 ± 0.47a
Topical	1	15.60 ± 0.10	−0.71 ± 0.27b	4.11 ± 0.45a	10.96 ± 0.88b	5.73 ± 0.24a
Topical	10	15.70 ± 0.10	−1.34 ± 0.14b	2.80 ± 0.64b	13.96 ± 0.55a	-
Topical	100	15.50 ± 0.11	−1.33 ± 0.23b	2.30 ± 0.22bc	10.15 ± 0.32b	-
Topical	Control	15.20 ± 0.25	1.83 ± 0.19a	-	-	-

[a]For each treatment method, means in the same column with a similar letter(s) are not significantly different ($P > 0.05$, Tukey's HSD).

Table 4. Stage specific mortality, percentage pupation, adult survival, and time to pupation for seventh instar lesser mealworms exposed to insect growth regulators (IGRs) in residual or topical bioassays.

Treatment method	IGR[a]	% Dead larvae	% Dead pupae	% Dead adults	% Alive adults	Total % pupated	No. of days to pupation
Residual	FXB	22.5 ± 1.6a[b]	34.3 ± 2.3a	10.2 ± 1.2a	34.3 ± 2.8d	77.9 ± 1.6b	28.4 ± 0.64a
Residual	20E	23.7 ± 2.1a	11.6 ± 0.9b	6.2 ± 0.7b	59.1 ± 2.0b	75.4 ± 2.1b	20.0 ± 0.43b
Residual	DFB	20.6 ± 2.3a	17.0 ± 1.9b	13.7 ± 1.0a	48.5 ± 2.3c	79.3 ± 2.3b	19.8 ± 0.55b
Residual	Control	5.4 ± 1.0b	8.3 ± 0.7c	5.0 ± 1.0b	80.4 ± 1.2a	93.3 ± 1.1a	12.0 ± 0.37c
Topical	FXB	52.0 ± 2.4a	25.2 ± 1.7a	0.3 ± 0.1b	22.5 ± 2.3c	48.7 ± 2.4c	22.8 ± 0.39a
Topical	20E	36.5 ± 3.0b	9.0 ± 0.8b	8.0 ± 0.6a	46.0 ± 2.7b	62.2 ± 3.1b	16.5 ± 0.31b
Topical	DFB	58.5 ± 3.7a	26.0 ± 2.0a	0.7 ± 0.4b	34.7 ± 5.5bc	42.2 ± 3.6c	16.7 ± 0.61b
Topical	Control	10.0 ± 1.3c	7.9 ± 1.5b	2.5 ± 0.9b	79.5 ± 1.2a	90.4 ± 1.4a	11.2 ± 0.27c

[a]FXB = fenoxycarb, DFB = diflubenzuron, 20E = 20-hydroxyecdysone, and control = untreated control treatment.

[b]For each treatment method, means in the same column with a similar letter are not significantly different ($P > 0.05$, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Weight gain in topical bioassays. The mean larval weight gains of seventh instars tropically treated with different concentrations of fenoxycarb were significantly different after 10 d (df = 5, 337; F = 8.85; $P = 0.0001$), 20 d (4, 120; F = 3.24; $P = 0.014$), and 30 d (df = 3, 62; F = 6.54; $P = 0.0006$), but they were not significantly different after 40 d (df = 1, 16; F = 3.72; $P = 0.07$) post-treatment. Seventh instars in the untreated control or those topically treated with 0.01 ppm fenoxycarb gained weight, whereas, weight was reduced by all other concentrations after 10 d ($P < 0.05$) (Table 3). The mean larval weight gains were significantly higher at the lower fenoxycarb concentrations of 0.1 and 1 ppm after 20 d and at the concentration of 10 ppm after 30 d ($P < 0.05$). The untreated control larvae pupated after 10 d, whereas pupation was extended to 40 d in larvae treated with lower concentrations of 0.1 ppm and 1 ppm fenoxycarb (Table 3). The percentage pupation in untreated control (90.4%) larvae was significantly higher than fenoxycarb treated larvae (48.7%). Larvae treated with fenoxycarb had a significantly longer time to pupation (22.8 d) compared to untreated control larvae (11.2 d) ($P < 0.05$) (Table 4).

Lethal concentration (LC_{50}) for feeding bioassays. The non-significant P values in the goodness-of-fit table for the Pearson's chi-square for each of the bioassays indicated an adequate fit for the model with the normal distribution. Comparisons of fiducial limits for LC_{50} values for seventh instars, showed that fenoxycarb (0.065 ppm) treated diet was significantly more toxic than 20E (0.26 ppm) or diflubenzuron (2.13 ppm) (Table 5). For first instars, the LC_{50} values of diflubenzuron (0.062 ppm), 20E (0.105 ppm), and fenoxycarb (0.144 ppm) were not significantly different. For adults, fenoxycarb (1426 ppm) was most potent, followed by diflubenzuron (11,660 ppm) and then 20E (982,937 ppm), however these values were not significantly different. Comparisons of LC_{50} values among life stages indicated that the fenoxycarb was significantly more toxic to seventh instars than first instars, whereas diflubenzuron was more toxic to first instars than seventh instars. The toxicities of 20E to seventh and first instar were not significantly different. Fenoxycarb, diflubenzuron, and 20E were more toxic to first and seventh instars than they were to adults (Table 5).

Lethal concentration (LC_{50}) for residual bioassays. For seventh instars, the LC_{50} values and fiducial limits indicated a significantly greater toxicity of wood shavings treated with fenoxycarb (0.20 ppm) than diflubenzuron (7.1 ppm) or 20E (112.4 ppm) (Table 6). For adults, the values for fenoxycarb (4350 ppm), 20E (12,201 ppm), and diflubenzuron (117,829 ppm) were not significantly different. The LC_{50} values for fenoxycarb, diflubenzuron, and 20E were much lower for seventh instars than for adults (Table 6).

Lethal concentration (LC_{50}) for topical bioassays. The lowest LC_{50} values within a life stage were recorded for topical applications compared to feeding or residual bioassays. For seventh instars, toxicity to diflubenzuron (0.003 ppm) and fenoxycarb (0.007 ppm) were significantly higher than for 20E (1.11 ppm) (Table 7). For pupae, the topical toxicities of fenoxycarb (0.07 ppm), diflubenzuron (0.08 ppm), and 20E (0.10 ppm) were not significantly different. Likewise for adults, LC_{50} values were not significantly different for fenoxycarb (18.0 ppm), diflubenzuron (15.2 ppm), and 20E (16.8 ppm). All three IGRs were more toxic to seventh instars and pupae than to adults. Fenoxycarb and diflubenzuron were more toxic to the seventh instars than pupae, whereas 20E was more toxic to pupae than to seventh instars (Table 7).

Table 5. Slope, lethal concentrations (ppm) (LC_{50} or LC_{90}), and 95% fiducial limits (FL) of mortality to field-collected adults, first, and seventh instar lesser mealworms exposed to diets treated with insect growth regulators (IGRs) in feeding bioassays.

IGR[a]	Life stage	Slope (±SE)	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2	*P*	df
FXB	7th	0.45 ± 0.05	0.065 (0.024–0.110)e[b]	42.06 (15.33–182.22)	42.5	0.7	48
DFB	7th	0.36 ± 0.05	2.130 (0.830–5.820)c	7307 (893.94–304,030)	33.8	0.6	38
20E	7th	0.50 ± 0.05	0.257 (0.110–0.510)d	90.59 (30.79–449.45)	22.1	0.9	38
FXB	1st	0.72 ± 0.08	0.144 (0.067–0.250)b	8.58 (4.50–21.07)	33.6	0.8	48
DFB	1st	0.33 ± 0.05	0.062 (0.012–0.180)b	385.05 (69.15–8,203)	18.0	0.9	38
20E	1st	0.54 ± 0.06	0.105 (0.045–0.200)b	23.47 (9.52–83.95)	40.4	0.3	38
FXB	Adult	0.40 ± 0.09	1426 (233–154,906)a	NA[c]	12.5	1.0	48
DFB	Adult	0.30 ± 0.10	11,660 (461–1.0×10^6)a	NA	12.5	0.9	38
20E	Adult	0.27 ± 0.13	982,937 (2,530–1.0×10^6)a	NA	20.6	1.0	38

[a]FXB = fenoxycarb, DFB = diflubenzuron, and 20E = 20-hydroxyecdysone.
[b]Within each life stage in the same column, means with a similar letter are not significantly different because 95% fiducial limits overlap.
[c]NA; due to low mortality in adults, calculated LC_{90} value exceeded saturation.

Table 6. Slope, lethal concentrations (LC_{50} or LC_{90}), and 95% fiducial limits (FL) of mortality to field-collected adults and seventh instar lesser mealworms exposed to wood shavings treated with insect growth regulators (IGRs) in residual bioassays.

IGR[a]	Life stage	Slope (±SE)	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2	*P*	df
FXB	7th	0.33 ± 0.04	0.20 (0.054–0.53)d[b]	1477 (316–16,202)	25.8	0.9	46
DFB	7th	0.25 ± 0.04	7.1 (2.1–25.6)c	NA[c]	10.2	1.0	46
20E	7th	0.23 ± 0.04	112.4 (26.5–1,107)b	NA	13.0	1.0	46
FXB	Adult	0.38 ± 0.07	4350 (902–109,869)a	NA	17.0	1.0	46
DFB	Adult	0.28 ± 0.07	117,829 (4,581–1.0×10^6)a	NA	11.4	1.0	44
20E	Adult	0.35 ± 0.08	12,201 (1,649–1.0×10^6)a	NA	9.8	1.0	46

[a]FXB = fenoxycarb, DFB = diflubenzuron, and 20E = 20-hydroxyecdysone.
[b]Means in the same column with a similar letter are not significantly different because 95% fiducial limits overlap.
[c]NA; due to low mortality in adults, calculated LC_{90} value exceeded saturation.

Table 7. Slope, lethal concentrations (LC_{50} or LC_{90}), and 95% fiducial limits (FL) of mortality to field-collected adults, pupae, and seventh instar lesser mealworms exposed to insect growth regulators (IGRs) in topical bioassays.

IGR[a]	Life stage	Slope (±SE)	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2	*P*	df
FXB	7^{th}	0.35 ± 0.05	0.007 (0.0006–0.020)d[b]	32.4 (8.2–345.8)	11.7	1.0	38
DFB	7^{th}	0.48 ± 0.07	0.003 (0.0004–0.010)d	1.5 (0.6–5.7)	18.4	0.9	38
20E	7^{th}	0.34 ± 0.05	1.11 (0.39–3.06)b	6996 (751–435,143)	11.3	1.0	38
FXB	Pupa	0.61 ± 0.08	0.072 (0.027–0.151)c	9.2 (3.6–37.2)	12.0	0.9	28
DFB	Pupa	0.47 ± 0.06	0.080 (0.023–0.198)c	43.0 (12.7–306.3)	10.4	0.9	28
20E	Pupa	0.43 ± 0.06	0.100 (0.026–0.262)c	100.4 (24.7–1,062)	8.9	0.9	28
FXB	Adult	0.41 ± 0.07	18.0 (6.3–85.0)a	NA[c]	9.0	0.9	28
DFB	Adult	0.40 ± 0.07	15.2 (5.3–69.8)a	NA	11.5	0.9	28
20E	Adult	0.36 ± 0.06	16.8 (5.2–102.7)a	NA	8.9	0.9	28

[a]FXB = fenoxycarb, DFB = diflubenzuron, and 20E = 20-hydroxyecdysone.
[b]Means in the same column with a similar letter are not significantly different because 95% fiducial limits overlap.
[c]NA; due to low mortality in adults, calculated LC_{90} value exceeded saturation.

Percentage pupation, mean number of days to pupation, adult survival, and stage-specific mortality. The percentage pupation, mean number of days to pupation, adult survival and stage-specific mortality were recorded for all lesser mealworms exposed to feeding, residual, and topical bioassays.

Feeding bioassays. The percentage of seventh instars that reached pupation were significantly different (df = 3, 152; F = 49.13; $P = 0.0001$) for the IGR treatments: untreated control (86.5%), diflubenzuron (67.0%), 20E (55.2%), and fenoxycarb (33.0%) (Table 2). The mean numbers of days to pupation were also statistically different (df = 3, 826; F = 97.81; $P = 0.0001$) for the IGR treatments: untreated control (13.7 d), diflubenzuron (15.7 d), 20E (19.6 d), and fenoxycarb (26.3 d). Significant differences were found in percentages of seventh instars that died as larvae (df = 3, 152; F = 49.13; $P = 0.0001$), pupae (df = 3, 152; F = 9.03; $P = 0.0001$), and adults (df = 3, 152; F = 15.40; $P = 0.0001$). The percentage of seventh instars that died as larvae was significantly higher for fenoxycarb (67.0%) than it was for 20E (44.7%), diflubenzuron (33.0%), or the untreated control (13.4%) ($P < 0.05$). Mortalities of adults in which larvae fed on diet treated with diflubenzuron (14.0%) or 20E (10.7%) were significantly higher than for larvae fed on diet with fenoxycarb (4.2%) or the untreated control (3.4%). Survival of emerged adults was significantly higher (df = 3, 152; F = 111.52; $P = 0.0001$) in the untreated control (80.0%) than in the diflubenzuron (40.0%), 20E (29.0%), or fenoxycarb (12.0%) treatments (Table 2).

There were significant differences in the percentages of first instars that died as larvae (df = 3, 152; F = 59.76; $P = 0.0001$), pupae (df = 3, 152; F = 4.43; $P = 0.005$), and adults (df = 3, 152; F = 5.38; $P = 0.001$), when fed IGR treated or untreated diet (Table 2). The percentage mortality of first instars treated with diflubenzuron (67.5%), 20E (70.0%), and fenoxycarb (69.4%) were significantly higher than in the untreated controls (15.0%) ($P < 0.05$). The IGR-fed first instars had significantly lower percentage pupation than the untreated control (df = 3, 152; F = 59.76; $P = 0.0001$). Adults had significantly greater survival (df = 3, 152; F = 36.11; $P = 0.0001$) in the untreated control (80.0%) than in treatments with diflubenzuron (22.5%), 20E (24.0%), or fenoxycarb (20.4%) ($P < 0.05$) (Table 2). However, the mean number of days to pupation was not significantly different (df = 3, 620; F = 1.32; $P = 0.10$) among treatments.

Residual bioassays. The percentage pupation for untreated seventh instars (93.3%) was significantly higher (df = 3, 164; F = 10.50; $P = 0.0001$) than for larvae exposed to wood shavings treated with diflubenzuron (79.3%), fenoxycarb (77.9%), or 20E (75.4%) (Table 4). Fenoxycarb significantly (df = 3, 164; F= 92.2; $P = 0.001$) increased the mean number of days to pupation (28.4 d) compared to that in diflubenzuron (19.8 d), 20E (20.0 d), or the untreated control (12.0 d) ($P < 0.05$). The percentage of seventh instars that died as larvae (df = 3, 164; F = 11.30; $P = 0.0001$), pupae (df = 3, 164; F = 40.40; $P = 0.0001$), and adults were significantly different (df = 3, 164; F = 14.0; $P = 0.0001$). Percentage larval mortalities were greater for residual treatments of fenoxycarb (22.5%), diflubenzuron (20.6%), and 20E (23.7%) than for larvae in the untreated control (5.4%) (Table 4). Pupal mortalities were significantly higher for larvae treated with fenoxycarb (34.3%) than for larvae treated with diflubenzuron (17.0%), 20E (11.6%), or the untreated control (8.3%) ($P < 0.05$). Adults from larvae treated with fenoxycarb (10.2%) and diflubenzuron (13.7%) suffered significantly higher

mortality than adults from the 20E (6.2%) treatment or the untreated control larvae (5.0%). The overall percentage survival of adults was significantly different among treatments (df = 3, 164; F = 49.72; P = 0.0001): untreated control (80.4%), 20E (59.1%), diflubenzuron (48.5%), and fenoxycarb (34.4%) ($P <$ 0.05) (Table 4).

Topical Bioassays. The percentage pupation for seventh instars in the untreated control (90.4%) was significantly greater (df = 3, 140; F = 39.09; P = 0.0001) than for larvae topically treated with 20E (62.2%), fenoxycarb (48.7%), or diflubenzuron (42.2%) (Table 4). However, the percentage pupation in larvae treated with fenoxycarb (48.7%) or diflubenzuron (42.2%) were significantly lower than larvae treated with 20E (62.2%), although they were not significantly different from each other. The mean number of days to pupation was significantly higher (df = 3, 827; F= 95.6; P = 0.001) for the topical fenoxycarb treatment (22.8 d) than it was for topical treatments of diflubenzuron (16.7 d) or 20E (16.5 d), which were not significantly different from each other but were significantly greater than the untreated control (11.2 d) ($P < 0.05$) (Table 4). There were significant differences in the percentage mortalities of seventh instar during the larval (df = 3, 140; F = 40.30; P = 0.0001), pupal (df = 3, 140; F = 34.20; P = 0.0001), and adult (df = 3, 140; F = 53.41; P = 0.0001) stages. Larval mortalities were higher for seventh instars treated topically with fenoxycarb (52.0%) or diflubenzuron (58.5%) than for those treated with 20E (36.5%), which was greater than the untreated control (10.0%). Significantly more larvae topically treated with fenoxycarb (25.2%) and diflubenzuron (26.0%) died in the pupal stage than larvae treated with 20E (9.0%) or in the untreated control (7.9%) ($P < 0.05$). The percentage adult survival was significantly greater (df = 3, 140; F = 35.06; P = 0.0001) for the untreated control (79.5%) than for adults resulting from larvae treated with 20E (46.0%), diflubenzuron (34.7%), or fenoxycarb (22.5%). More adults from 20E-treated larvae survived than did adults from larvae treated with fenoxycarb ($P < 0.05$) (Table 4).

Discussion

Lesser mealworm larvae in treatments with low IGR concentrations gained weight in the first few days, whereas in the treatments with higher IGR concentrations, larvae gained little weight initially, but later there were increased weight gains and prolonged development times. Similarly, Smet et al. (1989) found that for *T. confusum,* the larval period increased with increased dose of fenoxycarb and methoprene. In the present study, pupation was inhibited in fenoxycarb-treated seventh instar lesser mealworms, which continued to molt and gain weight. The same trend was reported for *T. castaneum* (Pallos et al. 1971), where JHAs targeted at the mature larval stage inhibited pupation, which caused continued molting and the production of 'giant' or 'supernumerary larvae'. It was reported that a JHA-treated diet prolonged the larval feeding period of the Indianmeal moth, *Plodia interpunctella* (Hübner) (Firstenberg & Silhacek 1976); and a similar JHAs treatment increased larval size by adding extra larval molts in the Khapra beetle, *Trogoderma granarium* Everts (Metwally & Sehnal 1973). Furthermore, when larvae of six stored products insects were fed, injected with, or put in contact with CSIs or JHAs, the percentage pupation was usually

reduced due to either larval death during metamorphosis or inhibition of pupation due to a prolonged juvenile period (Loschiavo 1976).

For feeding, residual, and topical bioassays, it took longer for fenoxycarb-treated seventh instar lesser mealworms to reach pupation than it did for larvae in the untreated control or in the diflubenzuron or 20E treatments. Many heavier larvae were deformed, which resulted in abnormal pupae or intermediate larval-pupal and pupal-adult forms. Whereas, untreated larvae pupated in 25 d, prolonged larval development periods of 120 and 150 d were reported (Loschiavo 1975, 1976), when mature instars of *Tribolium* species were treated with fenoxycarb and hydroprene, respectively. Last instars of *T. confusum* and *T. granarium* treated with juvenile hormone resulted in either abnormal pupae or intermediate larval-pupal and pupal-adult forms (El-Sayed 1987). In the present study, there was reduced pupation and higher mortality rates in lesser mealworm larval and pupal stages when seventh instars were treated topically with fenoxycarb or diflubenzuron. Seventh instars topically treated with 20E had higher mortality in the adult stage. Second and fourth instars of the 28-spotted potato ladybird beetle [*Henosepilachna vigintioctopunctata* (F.)] topically treated with diflubenzuron had ecdysal failure (Rao et al. 1992).

In our feeding bioassays, fenoxycarb was more toxic to seventh instars than to first instars or adults, whereas diflubenzuron was more toxic to first instars than to seventh instars or adults. Diflubenzuron was reported to be more effective in inhibiting the growth and development of first instars of *T. castaneum* than fourth instars (Ishaaya & Ascher 1977). In our feeding bioassays, 20E was more toxic to seventh instar lesser mealworms than diflubenzuron, whereas diflubenzuron was more toxic than 20E in residual bioassays. Lesser mealworm adults were less susceptible to all IGRs tested compared to other life stages in feeding, residual, and topical bioassays. Also, low concentrations of all IGRs were less toxic to adults initially in residual and feeding bioassays; whereas in topical bioassays, these lower concentrations were significantly more toxic to adults.

The slopes of the concentration-mortality lines give information about the phenotypic variation within the lesser mealworm population, including genetic and environmental variation. Steeper slopes with values greater than one represent reduced phenotypic variation within a population in response to IGRs, or in other words an increasingly homogeneous population. Slope values less than one represents an increasingly heterogeneous population (Plapp et al. 1979), where resistance may be possible. Probit analysis for all the bioassays yielded slope values less than one (Tables 5, 6, & 7), suggesting the increased variation within the lesser mealworm population is in response to the IGRs used.

The results of this study show that fenoxycarb, diflubenzuron, and 20E exhibit feeding (IGR-treated diet), residual, and topical toxicity to lesser mealworms. All three bioassay methods produced usable concentration-response curves (Tables 5, 6, & 7) that may be used for detecting temporal changes in the IGR susceptibility to lesser mealworm. Nevertheless, the bioassay using IGR-treated diet produced tighter fiducial limits, steeper slopes, and generally a better fit of the data for first and seventh instars of lesser mealworms. Residual contact also seems to be a promising method for controlling seventh instars. However, residual bioassay tests provide data that more closely simulates the exposure of lesser mealworm beetles to an insecticide on a substrate in field applications. This method can be used to detect susceptibility of older larvae to IGRs. But this method was not effective

against adults, and is not easy to use for first instars due to their small size. Adults responded to topical bioassays better than to feeding or residual contact bioassays. Therefore, these three different bioassays can be used to test different lesser mealworm stages and to determine temporal changes in susceptibility of local populations to various IGRs.

It is important to discuss some concerns about using JHA against lesser mealworm in poultry facilities. If JHA treatments cause lesser mealworm larvae to prolong feeding and/or increase size, these larvae could eat more chicken feed, fill the crops of chickens faster, carry and transmit disease for a longer period of time, further delay weight gain, and cause larger tunnels through the insulation causing more heat loss. However, the results from this study revealed that fenoxycarb is toxic to all stages of lesser mealworm which increases its potential for use in a resistance management program.

Results from the present study comprise initial efforts to establish baseline data on the susceptibility of lesser mealworms to IGRs that can be used as reference points for future concentration-response bioassays. This might evolve as an excellent option in management of lesser mealworm, where insecticides currently being used no longer provide adequate control. The use of selective pesticides should be a major consideration in developing an integrated control program for lesser mealworm. The combination of IGRs and other recommended insecticides should be tested to find if they have any synergistic effects greater than either alone on lesser mealworm. Also, adults that are less susceptible to IGRs may be controlled with an insecticide-IGR combination.

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