

Attractiveness of an Aggregation Pheromone Lure and Chicken Droppings to Adults and Larvae of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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ABSTRACT The chemical cues by which lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) beetles find each other are still unknown. Laboratory two-choice pitfall bioassays were conducted to evaluate the attractiveness of synthetic aggregation pheromone lure to lesser mealworm adults and larvae. All components of this synthetic aggregation pheromone lure, including: (R)-(+)-limonene, (E)- β -ocimene, (S)-(+)-linalool, (R)-(+)-daucene, and 2-nonanone were also tested singly. Chicken dropping volatile compounds and fresh chicken droppings (CD) were evaluated singly or in combination with the pheromone lure. In Arkansas, trapping experiments were conducted in different poultry houses with low, moderate, and high lesser mealworm populations to evaluate the attraction of pheromone lure. Laboratory two-choice pitfall bioassay was found to be a useful and convenient tool for evaluating the attractants before testing them in the poultry house. Greater attraction of adults and larvae to a dose of 20–30 μ g pheromone lure was identified in laboratory two-choice pitfall bioassays. Adults and larvae were highly attractive to a combination of fresh CD and pheromone lure, whereas, a combination of chicken dropping volatile and pheromone lure was not significantly attractive. The low attraction of limonene and linalool in the laboratory two-choice pitfall bioassays suggest that either they are nonattractive or attractive only at a narrow range of concentrations. Higher numbers of lesser mealworm adults and larvae were found in traps treated with pheromone lure as compared with untreated controls in field experiments. Results indicate a potential for combining the pheromone lure with the attractive CD compounds to enhance trap efficacy.

KEY WORDS lesser mealworm, aggregation, poultry, pheromone, pitfall

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, and causes decreased weight gains in broiler chicks that eat these beetles (Despins and 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 et al. 2005), infectious bursal disease (McAllister et al. 1995), and also cause damage to poultry insulation by tunneling (Despins et al. 1987). Control of lesser mealworm adults and larvae is mainly through use of contact insecticides, which has resulted in insecticide-resistant strains (Lambkin 2005). Because of environmental and resistance concerns related to the use of conventional insecticides, research needs to be focused

toward other alternative tactics such as attractants, pheromones, kairomones, and other behavior-modifying chemicals.

Attraction of adults to pheromones is well documented in many tenebrionids including the red flour beetle, *Tribolium castaneum* Herbst (Rangaswamy and Sasikala 1991), the yellow mealworm, *Tenebrio molitor* L. (Tanaka et al. 1986), the broad-horned flour beetle, *Gnaticerus cornutus* F. (Tebayashi et al. 1998, Tashiro et al. 2004), and the desert tenebrionid beetle *Parasitizopus transgaripepinus* Koch (Geiselhardt et al. 2008). Volatile collections from lesser mealworm males and females revealed four male-specific compounds, including: (R)-(+)-limonene, (E)- β -ocimene, (S)-(+)-linalool, and (R)-(+)-daucene (Bartelt et al. 2009). A fifth compound, 2-nonanone was also identified from male, and in small amounts from female lesser mealworms. Synthetic lesser mealworm aggregation pheromone lure referred to hereafter as "pheromone lure" composed of these five compounds was attractive to both sexes in preliminary laboratory pitfall bioassays (Bartelt et al. 2009). However, the dose-dependent response to pheromone lure, and the attractiveness of individual pheromone lure compo-

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nents have never been tested to adults and larvae in laboratory conditions.

Adult and larval attraction to adult-produced pheromones have been described in codling moth, *Cydia pomonella* L. (Duthie et al. 2003), and *T. castaneum* (Mondal and Port 1984). Lesser mealworm adults and larvae are unevenly distributed in poultry houses in clumps or in aggregations (Falomo 1986). Adults and larvae placed in a petri dish with wood shavings form tight aggregations suggesting some sort of assembling scent present that leads to their aggregation behavior (Falomo 1986). These aggregations also suggest the existence of pheromonal communication among adults and larvae of lesser mealworm. The attractiveness of adult-produced pheromone lure to larvae of lesser mealworm needs to be explored in laboratory and field conditions.

Successful mass trapping using male-produced synthetic aggregation pheromones and plant co-attractants has been reported for bean weevil, *Acanthoscelides obtectus* (Say), pea leaf weevil, *Sitona lineatus* L. (Blight et al. 1984), smaller European elm bark beetle, *Scolytus multistriatus* (Marshall) (Dickens et al. 1990), the coconut rhinoceros beetle, *Oryctes rhinoceros* L. (Hallett et al. 1995), and the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Abraham et al. 2000). Adults and larvae of lesser mealworm were observed to be attracted to fresh chicken droppings (CD) in a poultry house. Strother and Steelman (2001) reported higher number of lesser mealworm adults and larvae along feed and water lines. This also suggests that the beetles may be attracted to the chicken droppings left behind from feeding birds in addition to their attraction for water and feed. The chicken dropping volatiles (CDV) may act as an attractant to adult and larval lesser mealworm beetles. Studying the interactions between pheromone lure and CDV has an immediate practical significance in designing more effective traps. These traps can be used to detect early infestations and to attract and kill lesser mealworm beetles in spot treatments rather than spraying the whole poultry house.

In this study, we first determined the attractiveness of pheromone lure, individual components of pheromone lure, CDV, and fresh CD to the lesser mealworm adults and larvae in laboratory pitfall bioassays. Field trials were then conducted to evaluate the attractiveness of pheromone lure to lesser mealworm adults and larvae.

Materials and Methods

Insect Rearing. Adult and larval lesser mealworms for all bioassays were collected from the Applied Broiler Research Unit, Savoy, AR. Beetles were extracted from litter or manure by sieving samples through 2.8 and 2.0 mm screens. They were transported to the laboratory and reared in covered plastic chambers (27.5 long \times 17.5 wide \times 17.5 cm deep, Penn Plax Large Animal Carrier, Garden City, NY) with wood shavings. The beetles were maintained at $28 \pm 1^\circ\text{C}$ under reverse L:D (14:10 h) photoperiod. Water

and chick starter feed were provided, and rolled cardboard was added to serve as shelter and pupation sites. To get the uniform age seventh instar lesser mealworms for bioassays, $\sim 5,000$ sixth instars (7 mm in length, according to Wilson and Miner 1969) were separated from field-collected beetles and placed in plastic chambers. Chick starter feed and water were provided weekly to these larvae. After 7 d, the chambers were checked regularly for cast skins to confirm that larvae had molted to the seventh instar (8 mm in length, Wilson and Miner 1969). The newly molted seventh instars (light colored exoskeleton) were removed for bioassays. For uniform aged adults, a piece of rolled corrugated cardboard (30 cm²) was placed in each plastic rearing chamber that contained $\sim 5,000$ seventh instars. Chambers were checked weekly to confirm molting, and pupae were collected and allowed to eclose into adults. The newly emerged adults were removed, and held in groups of 100 per petri dish (150 mm diameter). The adult exoskeleton was allowed to completely harden over a 5 d period (Hopkins et al. 1992), during which these adults were provided feed (1 g finely ground chicken feed) and water (daily moistened 2 cm² cotton ball).

Volatile Collection and Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. A 5 g fresh sample of CD was placed inside a glass headspace volatile collection chamber (5 cm diameter \times 0.5 m long). Volatiles were collected for 4 h from a chamber containing CD and the blank collection chamber (control). Three replications were used for CD and one for control. Carbon-filtered air flowing at the rate of 0.5 liter/min entered the glass chambers. Any CDV exiting the cylinders were adsorbed in a trap containing 30 mg SuperQ (Alltech Associates, Deerfield, IL). Each trap was eluted with 300 μl methylene chloride in a glass vial to obtain a CDV sample. A 90 μl aliquot from each CDV sample was then transferred to another vial and mixed with 10 μl ethyl caprate solution (100 ng ethyl caprate per 1 μl of methylene chloride, internal standard) to determine natural ratios of volatile components in each CDV sample. Samples were stored at -80°C .

A 1 μl aliquot of each CDV sample was analyzed on a Varian 450 GC coupled with Varian 320 MS at Statewide Mass Spectrometry Facility, University of Arkansas, Fayetteville, AR. The GC is equipped with a factory four Capillary column of 0.25 \times 0.25 mm ID \times 30 m length (Varian Inc., Palo Alto, CA). Injector temperature was set at 270°C in split mode (1:50) using helium as a carrier gas with a constant column flow of 1 ml/min. The oven was programmed to initially hold at 50°C for 3 min, and then ramp at $5^\circ\text{C}/\text{min}$ to 120°C and ramp at $10^\circ\text{C}/\text{min}$ to 270°C with a final hold time of 5 min. Each GC peak produced an ion spectrum after entering the MS. To identify each peak, its MS ion pattern was compared with known ion patterns of compounds in the National Institute of Standards and Technology (NIST) mass spectral database.

Four serial dilutions of ethyl caprate (99% pure) (Sigma-Aldrich Co., Milwaukee, WI) ranged from 1 ng to 1 μg were made in methylene chloride. A 1 μl

Table 1. Gas chromatograph/mass spectrometer analysis ($N = 3$) of fresh chicken dropping volatiles collected by SuperQ trap for 4 h

Compound	Retention time (min)	Mean amt (ng/ μ l) \pm SE
2-methyl-propanoic acid	3.72	21.0 \pm 3.6d ^a
2,3-butanediol	3.83	179.3 \pm 12.9a
Butanoic acid	4.74	69.4 \pm 7.5c
2-pentanone	5.75	34.9 \pm 4.5d
1-octen-3-ol	10.34	77.4 \pm 6.1c
2-chlorocyclohexanol	11.18	111.3 \pm 12.2b
Pentanoic acid	12.72	112.8 \pm 8.0b
Dodecanal	15.43	35.8 \pm 4.8d

^a Means in same column with different letters are significantly different ($P < 0.05$; Tukey Kramer HSD-test).

aliquot of each dilution was injected into the GC/MS following the procedure described above. The area was calculated for each chromatogram peak for each dilution and was plotted against four concentrations to generate a calibration curve. The area under each GC peak for each volatile peak from an injected CDV sample was related to the area for ethyl caprate peak in the sample. The area under the ethyl caprate peak in each sample was adjusted with a calibration curve to quantify the natural ratios of compounds in each sample. Eight compounds were identified from GC/MS analysis of CDV, including: 2-methyl-propanoic acid, 2, 3-butanediol, butanoic acid, 2-pentanone, 1-octen-3-ol, 2-chlorocyclohexanol, pentanoic acid, and dodecanal (Table 1).

Laboratory Two-Choice Pitfall Bioassay. The pheromone lure consisted of five compounds: (R)-(+)-limonene (97%), (E)- β -ocimene (98%), (S)-(+)-linalool (98%), (R)-(+)-daucene (98%), and 2-nonanone (99%) in a 9.6: 60.0: 9.6: 17.3: 3.5 ratios, respectively (Bartelt et al. 2009). The two commercial components, 2-nonanone and (R)-(+)-limonene were purchased from Aldrich, Milwaukee, WI. (E)- β -ocimene, (S)-(+)-linalool, and (R)-(+)-daucene were prepared in the USDA-ARS laboratory at Peoria, IL, by the procedures described in Bartelt et al. (2009). Pheromone lure doses ranging from 1 to 100 μ g were tested to determine responses of lesser mealworm adults and larvae. Because 20 μ g of pheromone lure was found to be more attractive in preliminary bioassays, this amount of pheromone lure, or individual components of pheromone lure was used in subsequent bioassays. Emission characteristics of the pheromone lure components placed on rubber septa were measured in the laboratory by the methods of Bartelt et al. (2009).

Based on the total amount of all compounds present per microliter in CDV sample, the amount of CDV sample needed to get 10 μ g of total compounds was calculated (Table 1). We decided to use 10 μ g based on preliminary bioassays results, where no significant differences were found among 10, 20, and 30 μ g CDV. This crude solution containing 10 μ g CDV compounds was then tested alone or in combination with the 20 μ g pheromone lure. Two grams of fresh CD was also tested singly or in combination with the 20 μ g pher-

omone lure. Red rubber septa (11 mm, Wheaton, Millville, NJ) were cleaned in advance by soaking them in methylene chloride overnight. Each of these four treatments: 1) pheromone lure, 2) individual pheromone lure components, 3) CDV, and 4) pheromone lure + CDV were applied separately to the rubber septa followed by 300 μ l methylene chloride, which was allowed to soak in and then the methylene chloride was allowed to evaporate for 2 h. Untreated control septa received 300 μ l methylene chloride.

Aluminum roasting pans (38 \times 25 \times 3.8 cm) were used as test arenas. Two 3.8 cm diameter circular holes were drilled directly opposite from each other on the floor of the pans, 15 cm apart and 2.5 cm from the pan lip. The pans rested on two open 120 ml glass specimen cups directly below the holes and these cups had either an odor stimulus or no odor (control). Two additional 120 ml specimen cups were placed at the other end of the pan to keep it level. The test arena tops were 43- \times 30-cm wide glass sheets. The glass sheets had two outlet holes 15 cm apart, parallel to holes on the pan floor but on the opposite end. Charcoal filtered air at 0.5 liter/min from the air delivery system (model no. OLFM-4C-ADS+V.1, ARS Inc., Gainesville, FL) was supplied separately through Teflon tubes (0.64 cm diameter, ARS Inc.) to the inlet ports of the 120 ml specimen cups containing an odor stimulus or no odor (control). The air then entered the test arenas and was exhausted from the outlet holes at a rate of 0.5 liter/min with a vacuum port of the air delivery system.

Lesser mealworm beetles are nocturnal and inhabit a darkened or subdued light environment (Asaniyan et al. 2007). Thus, all bioassays were conducted during the day, but in reverse L:D (14:10 h) conditions (perceived as night by the beetles). An 8 h period was shown to allow optimal insect dispersal in preliminary bioassay tests. Bioassays were conducted for 8 h in complete darkness at 28°C and 50 \pm 10% relative humidity (RH). One hundred and fifty test beetles were used in each replicate and four replicates were used for each treatment. Beetles were starved for 12 h before the bioassays. Beetles were placed under an inverted glass funnel (5 cm diameter at widest point) at the center of the arena for 8 h before release to allow for acclimation to the experimental conditions. These acclimated beetles were released at the down-wind end of the arena at 1 h after the dark cycle began, and their distribution recorded after 8 h. The treatment cup positions were reversed after each replicate to eliminate any positional cues. Two testing arenas 2.5 cm apart were used simultaneously. The beetles trapped in the odor or control cups and those made no choice were collected and counted. A new batch of beetles was tested in each replication. Each arena was washed with acetone and air-dried before any subsequent use.

Pitfall Trap for Field Test. The trap type used for the field study was a modified pitfall trap to exploit the thigmotactic behavior of lesser mealworm adults and larvae (Bartelt et al. 2009). The trap base was a 5 mm thick, 20 cm square piece of plywood with a 5 cm

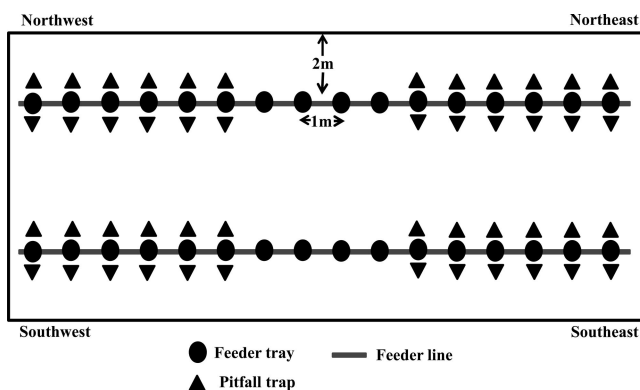


Fig. 1. Schematic diagram of pitfall trap locations inside a broiler production house.

circular hole in the center. A threaded metal canning ring, 7 cm in diameter, from a 250 ml canning jar, (Ball Inc., Muncie, IN) was attached, concentric to the hole, with three flat-head, machine screws, size 6–32 \times 1.25 cm, and hex nuts. Holes were predrilled, spaced equally around the canning ring. The trap top was a 10 cm², 5 mm thick piece of plywood. Three holes were drilled through the top and aligned with the machine screws protruding through the trap base. The ends of these screws served as “pins” to keep the trap top centered above the 5 cm hole, and the hex nuts provided a suitable separation between the trap top and base so that beetles could enter the trap. Trap assembly was completed by screwing the jar into the canning ring.

A pheromone lure consisting of the five synthetic components mentioned in the laboratory pitfall bioassays was applied to each rubber septum (100 μ g total, in 10 μ l methylene chloride), followed by 300 μ l methylene chloride. Untreated control septa received 300 μ l methylene chloride. The septa were loaded 2 h before use, stored in a tightly closed bottle and transported in an ice chest to the poultry house. The treated septa with control or pheromone lure were then dropped into the glass jar. A hole was made in the loose poultry house litter, and then the assembled trap was set in place, with the trap top slightly below the surface of the litter layer. Traps were cleaned with Alconox detergent, and dried at the end of the experiment.

Field Test to Determine Pheromone Lure Attractiveness to Adults and Larvae. Trapping experiments were done in three different poultry houses located in Savoy and Tontitown, AR, to evaluate the attraction of the pheromone lure to lesser mealworm adults and larvae. After bird removal, all the houses remained vacant for 2 wk before a new flock of broilers was introduced. There were always high numbers of beetles in the poultry houses for two reasons: 1) beetle-infested litter is normally removed from the poultry houses to surrounding pasture fields during the summer when the outside temperatures are suitable for larval development, adult emergence and adult flight, and 2) poultry houses are only treated with insecticides during the flock-free period (2 wk) when the

heat inside poultry house is turned off and majority of beetles are either hiding in building structures or hiding deep inside the litter layer. These beetles do not come in contact with insecticides. Savage (1992) speculated that if litter applied is 0.4 km from the poultry house, and the beetle dispersal is random, \approx 60,000 beetles for every one million applied to the field would return to the poultry house. Litter was not removed from the house before our study.

The first field test was conducted in a broiler production house with a moderate lesser mealworm infestation (1,000–2,000 lesser mealworm adults per trap), at the Applied Broiler Research Unit in Savoy, AR. These numbers were based on a 1 d trap catch before starting the experiment. The building was 99 \times 14 m wide with the long axis orientated east to west and was managed to produce six or 8 wk-old broiler chickens, but it did not contain birds at the start of this study. Two automatic feeder lines ran the length of the house, 2 m out from the wall. The floor of the house was covered with used pine wood-shaving litter \approx 8 cm deep. Plastic trays (56 \times 34 cm) containing chick feed were placed 2 d before chicks were introduced inside the house. They were used to supplement the automatic feeders so that newly hatched chicks had optimal feed access during the first 10 d of a production cycle. The trays were located in three parallel lines. One row of trays was located under feed spouts on either side of the permanent feeder pans. The remaining two rows were on either side of the feed line beneath each automatic feeder line and the tray spacing was 1 m. The beetle traps were placed parallel to the feeder lines or feeder pans and were spaced \approx 1 m apart. The experiment was a randomized complete block (RCB) design with four blocks: one and two on the north side of the house and three and four on the south side (Fig. 1). Each block consisted of two treatments randomly assigned within each block: six traps with pheromone-treated septa; and six traps with untreated (control) septa. Trapping was conducted over three consecutive days from 20 to 23 August 2008. The house temperature was 28°C when the traps were initially placed and baited in the afternoon. The beetles were collected from the traps after 24 h and trap baits were replaced. By then, propane heaters raised

the house temperature to 34°C (the rearing temperature of the young birds). After the second 24 h period, trapped beetles were again removed and baits replaced. At this time, 10,000 1-d-old chicks were released into the facility, and once again, beetles were collected from the traps after 24 h. Thus, the beetle response to the treated septa was monitored for 1 and 2 d before chick introduction and for 1 d afterward, and the time period of the study included a defined temperature change. Chicks were free to roam inside the poultry house. Chicken droppings had been observed all over on the floor in poultry house. However, more droppings were present near feeder lines.

The second field test was conducted from 15 to 19 September 2008 in a broiler production house with a low lesser mealworm infestation (<1,000 lesser mealworm adults per trap), in Tontitown, AR. The building had the same dimensions, feeder lines or pans and waterers set up as described in the field test. The experiment was a RCB design as described in field test. The house temperature was 28°C when the traps were initially placed and baited the afternoon of 15 September 2008. The beetles were collected from the traps after 24 and 48 h, and the trap baits were replaced each time. Then, the house temperature was raised to 34°C with propane heaters. At this time, the chicks were released free into the facility, and once again, beetles were collected from the traps after 24 and 48 h.

The third field test was conducted from 8 to 12 November 2008 in a broiler production house with a high lesser mealworm infestation (>2,000 lesser mealworm adults per trap), at the Applied Broiler Research Unit in Savoy, AR. The experimental set up, temperature change, introduction of chicks, collection schedule of beetles, and replacement of baits procedure was the same as in the second field test. Trapped beetles were transported back to the laboratory and counted.

Statistical Analyses. *Laboratory Two-Choice Pitfall Bioassay.* Attractiveness or repellency of treatments was expressed as a response index (RI) shown by Suzuki and Sugawara (1979). It was calculated as $RI = (T - C / Tot) * 100$, where T is the number responding to the treatment, C is the number responding to control, and Tot is the number of adult/larvae released per replicate into the test arena. Positive RIs indicate attraction to the treatment and negative RIs indicate repellency; and the values range from -100 (complete repellency) to 100 (complete attraction). The mean RIs among the treatments were compared using the nonparametric Kruskal-Wallis test followed by Tukey Kramer honestly significant difference (HSD) for multiple comparisons ($P = 0.05$) (JMP 2009).

Field Test. The experiment had a randomized complete block design, with six traps for each pheromone and control within each of four blocks. Data for adults and larvae captured in traps were $\log(X + 1)$ transformed to meet assumptions of normality and homogeneity of variance (Zar 1999). A multifactorial analysis of variance (ANOVA) was performed on transformed $\log(X + 1)$ trap catch data subjected to the SAS regression and general linear models proce-

dures (SAS Institute 2004). Interactions among treatments (pheromone treated or untreated controls), blocks (space) and days (time) were determined. One way ANOVA was also performed to compare treatments within each day.

Results

Laboratory Two-Choice Pitfall Bioassay. The treatments are abbreviated as follows: lesser mealworm pheromone lure (lesser mealworm lure); fresh chicken dropping volatiles collected on SuperQ adsorption powder trap (CDV); and freshly collected CD.

Adults. Significant differences in the mean RI values revealed the variability among fifteen different treatments tested for attractiveness to lesser mealworm adults ($\chi^2 = 58.30$; $df = 14$; $P < 0.0001$; Kruskal-Wallis Test). The mean percentage of adults trapped using the 30 μg lesser mealworm lure (59.0%) was significantly greater than the 1 μg lesser mealworm lure (18.7%), 10 μg lesser mealworm lure (34.5%), 50 μg lesser mealworm lure (27.0%), and 100 μg lesser mealworm lure (30.0%). In addition, 20 μg (R)-(+)-daucene (43.2%), and 20 μg 2-nonanone (42.0%) were more effective in capturing adults than 20 μg (E)- β -ocimene (22.2%), 20 μg (S)-(+)-linalool (5.5%), and 20 μg (R)-(+)-limonene (2.2%). The bait combination of 2 g CD + 20 μg lesser mealworm lure attracted significantly more adults than all other treatments. More adults responded to 2 g CD as compared with the other treatments except 2 g CD + 20 μg lesser mealworm lure or 30 μg lesser mealworm lure. The fresh 2 g CD was 3.3-fold more attractive than 10 μg CDV. Similarly, a combination of fresh 2 g CD + 20 μg lesser mealworm lure was three-fold more attractive than a combination of 10 μg CDV + 20 μg lesser mealworm lure (Fig. 2).

Larvae. Significant differences in the mean RI values were found among 15 different treatments exposed to lesser mealworm larvae ($\chi^2 = 52.56$; $df = 14$; $P = 0.0001$; Kruskal Wallis Test). A significantly higher mean percentage of larvae were trapped in traps baited with the 10 μg lesser mealworm lure (46.0%) than 1 μg lesser mealworm lure (8.5%), 50 μg lesser mealworm lure (12.0%), and 100 μg lesser mealworm lure (17.0%). Percent larval captures in traps baited with 20 μg 2-nonanone (33.0%) or 20 μg (R)-(+)-daucene (30.0%) were significantly higher than 20 μg (R)-(+)-limonene (0.2%) or (S)-(+)-linalool (1.3%). Larvae were most attracted to the 10 μg lesser mealworm lure, and the attractiveness of the lesser mealworm lure to larvae decreased with the increased dose. The lure using 2 g CD alone (78.0%) or combined with 20 μg lesser mealworm lure (76.0%) was statistically more attractive to larvae than all other treatments. The fresh 2 g CD was five-fold more attractive than 10 μg CDV. Similarly, the fresh 2 g CD + 20 μg lesser mealworm lure was four-fold more attractive than a combination of 10 μg CDV + 20 μg lesser mealworm lure (Fig. 3).

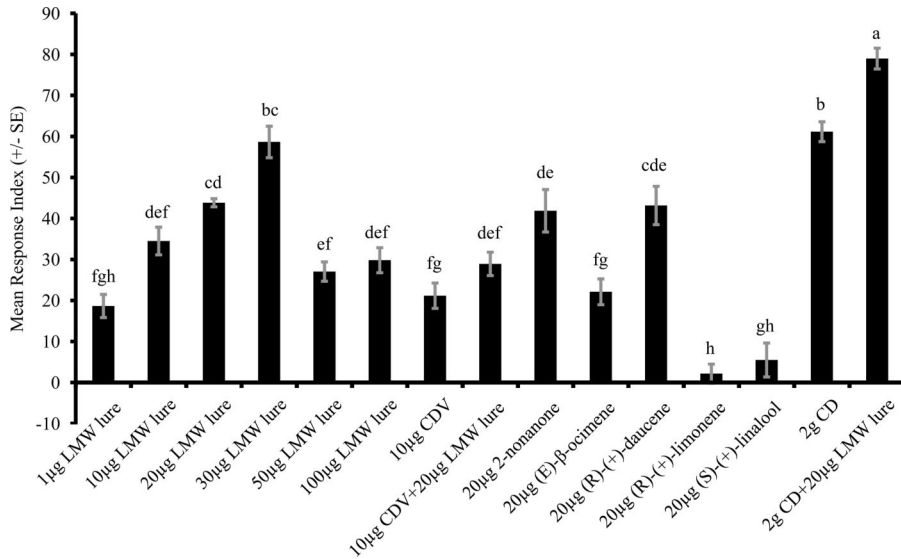


Fig. 2. Response of lesser mealworm adults to a range of pheromone lure (lesser mealworm lure) doses, 20 μg each component of lesser mealworm lure, 10 μg CDV, 2 g fresh CD, and a combination of 20 μg lesser mealworm lure with 10 μg CDV or 2 g CD in laboratory two-choice pitfall bioassays at air flow rate of 0.5 liter/min. Lesser mealworm lure contains: (R)-(+)-limonene, (E)- β -ocimene, (S)-(+)-linalool, (R)-(+)-daucene, and 2-nonanone in 9.6: 60.0: 9.6: 17.3: 3.5 ratios, respectively. CDV contains: 2-methyl-propanoic acid, 2,3-butanediol, butanoic acid, 2-pentanone, 1-octen-3-ol, 2-chlorocyclohexanol, pentanoic acid, and dodecanal in 3.3: 27.9: 10.8: 5.4: 12.1: 17.3: 17.6: 5.6 ratios, respectively. Response Index = $(T - C/\text{Tot}) * 100$, for which T is the number responding to the treatment, C is the number responding to the control, and Tot is the total number of adults released. Data are expressed as means \pm SE ($N = 4$). Bars with different letters are significantly different ($P < 0.05$; Tukey Kramer HSD-test).

Field Test to Determine Pheromone Lure Attractiveness in Moderate Lesser Mealworm Population.

Adults. The pheromone lure was significantly attractive in the moderate lesser mealworm population house (Table 2). The statistical analysis revealed the significant effect of pheromone on total trap catch ($F = 83.40$; $df = 1, 126$; $P = 0.0001$). Overall, the pheromone-baited traps caught 3.3-fold more adults than untreated controls. Captures decreased ≈ 10 -fold after the chicks were introduced (days 1 and 2 vs. day 3). Nevertheless, the factor difference between the pheromone and control was stable among the days (treatment-by-day interaction was not significant) ($F = 0.84$; $df = 2, 126$; $P = 0.43$). The significant interaction between blocks and days suggested that the population distribution changed over time ($F = 5.25$; $df = 6, 126$; $P = 0.0001$). Importantly, though, no interactions involving blocks and treatments were significant, indicating a consistent pheromone effect over space ($F = 1.25$; $df = 3, 126$; $P = 0.30$).

Larvae. The pheromone-baited traps caught two-fold more larvae than untreated controls. The introduction of chicks decreased the captures by nine-fold (days 1 and 2 vs. day 3) (Table 2). The pheromone-baited traps caught significantly more larvae than the controls over time (treatment-by-day interaction was not significant) ($F = 0.46$; $df = 2, 126$; $P = 0.62$). The distribution of the larval population changed somewhat over space and time as revealed from the significant interaction between blocks and days ($F = 6.30$;

$df = 6, 126$; $P = 0.0001$). Interactions involving blocks and treatments were significant, indicating an inconsistent pheromone effect over space ($F = 3.9$; $df = 3, 126$; $P = 0.011$).

Field Test to Determine Pheromone Lure Attractiveness in Low Lesser Mealworm Population. **Adults.** The trap catches of adults decreased six-fold with the introduction and presence of birds (days 1 and 2 vs. day 3 and 4). Significant differences in adult numbers were found in traps treated with the pheromone lure as compared with untreated controls for all 4 d (Table 3). The treatment-by-day interaction was not significant, suggesting the stable effect of pheromone over time ($F = 1.82$; $df = 3, 169$; $P = 0.14$). The pheromone lure-baited traps caught 3.3-fold more adults than their corresponding controls for the combined 4 d. The significant interaction between blocks and days suggested that the population distribution did change over space and time ($F = 7.46$; $df = 9, 169$; $P = 0.0001$). The inconsistent effect of pheromone over space in the poultry house was revealed by significant interactions between treatment and blocks ($F = 5.56$; $df = 3, 169$; $P = 0.001$).

Larvae. Significant differences in larval numbers were found in traps treated with pheromone lure as compared with untreated controls for all 4 d (Table 3). Overall, the baited traps caught three-fold more larvae than untreated controls. Trap catches only decreased two-fold with the introduction of chicks (days 1 and 2 vs. day 3 and 4). The treatment-by-day interaction was not significant suggesting the stable effect of pher-

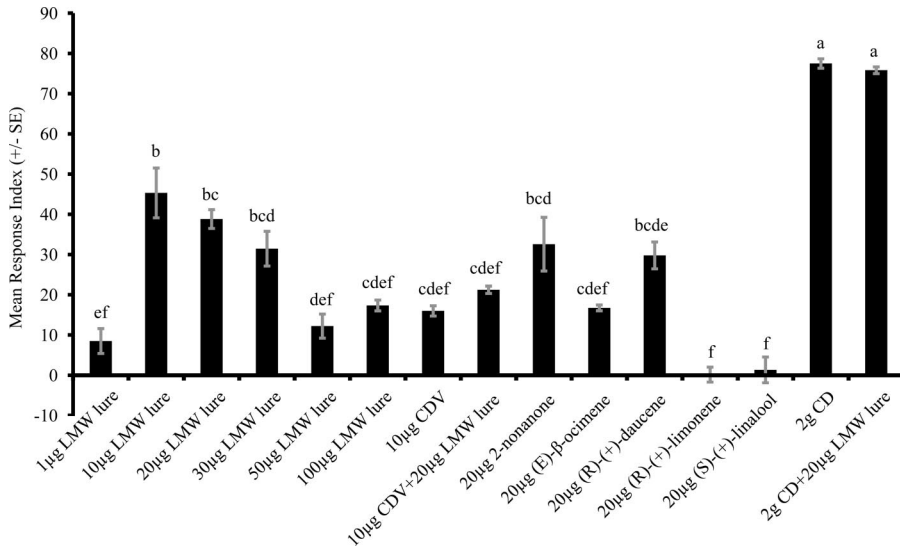


Fig. 3. Response of lesser mealworm larvae to a range of pheromone lure (lesser mealworm lure) doses, 20 µg each component of lesser mealworm lure, 10 µg CDV, 2 g fresh CD, and a combination of 20 µg lesser mealworm lure with 10 µg CDV or 2 g CD in laboratory two-choice pitfall bioassays at air flow rate of 0.5 liter/min. Lesser mealworm lure contains: (R)-(+)-limonene, (E)-β-ocimene, (S)-(+)-linalool, (R)-(+)-daucene, and 2-nonanone in 9.6: 60.0: 9.6: 17.3: 3.5 ratios, respectively. CDV contains: 2-methyl-propanoic acid, 2,3-butanediol, butanoic acid, 2-pentanone, 1-octen-3-ol, 2-chlorocyclohexanol, pentanoic acid, and dodecanal in 3.3: 27.9: 10.8: 5.4: 12.1: 17.3: 17.6: 5.6 ratios, respectively. Response index = (T - C/Tot)*100, for which T is the number responding to the treatment, C is the number responding to the control, and Tot is the total number of larvae released. Data are expressed as means ± SE (N = 4). Bars with different letters are significantly different (P < 0.05; Tukey Kramer HSD-test).

omone over time ($F = 1.80$; $df = 3, 169$; $P = 0.14$). The larval population distribution did not change significantly inside the poultry house over the space and time as evident from nonsignificant interactions between block and day ($F = 1.73$; $df = 9, 169$; $P = 0.08$). The treatment-by-block interaction was significant suggesting the variable effect of pheromone over space ($F = 7.30$; $df = 3, 169$; $P = 0.0001$).

Field Test to Determine Pheromone Lure Attractiveness in High Lesser Mealworm Population. Adults. No significant differences were found in day-by-treatment interaction suggesting the consistent ef-

fect of pheromone over time ($F = 2.20$; $df = 3, 169$; $P = 0.08$). The significant interaction among days and blocks revealed that population distribution inside the poultry house changed over space and time ($F = 3.57$; $df = 9, 169$; $P = 0.0004$). Interactions involving blocks and treatments were nonsignificant, indicating a consistent pheromone effect over space ($F = 2.09$; $df = 3, 169$; $P = 0.10$). A 30-fold reduction in adults trapped was observed after the introduction of chicks (days 1 and 2 vs. day 3 and 4). The pheromone-baited traps caught 2.6-fold more adults than untreated controls (Table 4).

Table 2. Attractiveness of pheromone lure to lesser mealworm adults and larvae at a broiler production facility with moderate lesser mealworm infestation, Savoy, AR, from 20 to 23 Aug. 2008

Treatment	Adults			Larvae		
	Untransformed (mean ± SE)	F-ratio	P value	Untransformed (mean ± SE)	F-ratio	P value
Day 1						
Pheromone	1555.5 ± 162.0a	6.9	0.0001	1465.8 ± 180.9a	11.1	0.001
Control	535.1 ± 72.1b			812.0 ± 95.1b		
Day 2						
Pheromone	2110.1 ± 170.9a	26.3	0.0001	1746.8 ± 254.6a	8.5	0.005
Control	568.1 ± 84.2b			751.4 ± 93.0b		
Day 3						
Pheromone	207.6 ± 56.4a	5.6	0.0002	256.6 ± 57.4a	7.3	0.009
Control	54.8 ± 17.7b			90.2 ± 25.7b		
Overall means (N = 72)						
Pheromone	1290.1 ± 123.6a	26.2	0.0001	1156.4 ± 129.5a	10.8	0.0001
Control	386.3 ± 46.2b			551.2 ± 59.0b		

Experiment had a randomized complete block design, with six traps for each pheromone and control within each of four blocks, replicated on 3 d. Analysis was on log (X + 1) transformed data. Within a day, or for overall means, values followed by the different letter are significantly different (P < 0.05; ANOVA).

Table 3. Attractiveness of pheromone lure to lesser mealworm adults and larvae at a broiler production facility with low lesser mealworm infestation, Tontitown, AR, from 15 to 19 Sept. 2008

Treatment	Adults			Larvae		
	Untransformed (mean ± SE)	F-ratio	P value	Untransformed (mean ± SE)	F-ratio	P value
Day 1						
Pheromone	485.4 ± 96.0a	28.9	0.0001	441.0 ± 78.0a	22.6	0.0001
Control	123.3 ± 22.5b			157.0 ± 26.3b		
Day 2						
Pheromone	439.9 ± 95.7a	16.5	0.0002	401.2 ± 79.0a	13.2	0.0007
Control	141.7 ± 30.3b			136.6 ± 16.9b		
Day 3						
Pheromone	69.2 ± 11.5a	13.6	0.0006	206.7 ± 38.1a	26.3	0.0001
Control	24.9 ± 3.1b			59.8 ± 8.5b		
day 4						
Pheromone	85.3 ± 11.1a	39.9	0.0001	207.6 ± 38.4a	19.9	0.0001
Control	21.3 ± 2.6b			70.8 ± 11.4b		
Overall means (N = 96)						
Pheromone	265.7 ± 39.0a	40.4	0.0001	314.1 ± 32.3a	65.1	0.0001
Control	77.6 ± 11.0b			106.0 ± 9.4b		

Experiment had a randomized complete block design, with six traps for each pheromone and control within each of four blocks, replicated on 4 d. Analysis was on log (X + 1) transformed data. Within a day, or for overall means, values followed by the different letter are significantly different (P < 0.05; ANOVA).

Larvae. Captures decreased ≈25-fold after the chicks were introduced (days 1 and 2 vs. day 3 and 4). Nonsignificant differences were found in day-by-treatment interaction suggesting the consistent effect of pheromone over time (F = 0.69; df = 3, 169; P = 0.55). The significant interaction between days and blocks revealed the population distribution change over space and time (F = 3.23; df = 9, 169; P = 0.001). The pheromone effect was consistent over space (treatment-by-block interaction was not significant) (F = 2.32; df = 3, 169; P = 0.06). Overall, the pheromone-baited traps caught 2.2-fold more larvae than did the untreated control (Table 4).

Discussion

The laboratory two-choice pitfall bioassay was found to be a useful and convenient tool for evaluating

the attractants for lesser mealworm adults and larvae before testing them in a poultry house. Similar studies were done to assess pheromone performance in laboratory pitfall bioassays for the rice weevil, *Sitophilus oryzae* (L.) (Phillips and Burkholder 1981), the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Mikolajczak et al. 1984), the maize weevil, *Sitophilus zeamais* Motschulsky and the red flour beetle, *Tribolium castaneum* Herbst (Phillips et al. 1993). In the current study, adults and larvae were strongly attracted to a dose of 20–30 µg synthetic lesser mealworm pheromone lure in laboratory pitfall bioassays. Lesser mealworm adults can perceive pheromones at lower doses, and were also more attracted than the larvae to higher doses of 50 and 100 µg. Our laboratory and field results confirm the larval attraction to adult pheromones. It is not known whether larvae of lesser mealworm produce compounds similar to adult emit-

Table 4. Attractiveness of pheromone lure to lesser mealworm adults and larvae at a broiler production facility with high lesser mealworm infestation, Savoy, AR, from 8 to 12 Nov. 2008

Treatment	Adults			Larvae		
	Untransformed (mean ± SE)	F-ratio	P value	Untransformed (mean ± SE)	F-ratio	P value
Day 1						
Pheromone	4799.3 ± 288.2a	88.1	0.0001	1522.6 ± 136.1a	39.0	0.0001
Control	1782.7 ± 113.0b			730.9 ± 54.3b		
Day 2						
Pheromone	3351.5 ± 321.9a	22.7	0.0001	1430.5 ± 172.2a	24.9	0.0001
Control	1398.9 ± 192.2b			632.0 ± 75.4b		
Day 3						
Pheromone	176.0 ± 38.3a	15.2	0.0003	81.9 ± 22.4a	8.0	0.007
Control	59.6 ± 12.5b			38.0 ± 4.3b		
Day 4						
Pheromone	202.6 ± 27.9a	30.4	0.0001	51.0 ± 13.2a	3.0	0.09
Control	58.0 ± 11.8b			29.7 ± 5.6b		
Overall means (N = 96)						
Pheromone	2132.4 ± 232.2a	17.4	0.0001	771.5 ± 90.0a	7.0	0.008
Control	824.0 ± 96.9b			357.7 ± 40.6b		

Experiment had a randomized complete block design, with six traps for each pheromone and control within each of four blocks, replicated on 4 d. Analysis was on log (X + 1) transformed data. Within a day, or for overall means, values followed by the different letter are significantly different (P < 0.05; ANOVA).

ted volatile profiles or not. Both adults and larvae feed on chicken feed and poultry manure (Pfeiffer and Axtell 1980). The larvae may have adapted and evolved the behavioral responses to male-produced aggregation pheromones for exploiting food resources and shelter occupied by adults.

The mean percentage of lesser mealworm adults caught in pitfall traps baited with 20 μg daucene (43.2%), or 20 μg nonanone (42.0%) were higher than the other three components of pheromone lure: 20 μg (E)- β -ocimene (22.2%), 20 μg (S)-(+)-linalool (5.5%), or 20 μg (R)-(+)-limonene (2.2%). 2-Nonanone has been reported to be attractive to caddisfly, *Molanna angustata* Curtis (Löfstedt et al. 2008), and olive bark beetle, *Phloeotribus scarabaeoides* Bernard (Szauman-Szumski et al. 1998). The very low attraction of limonene and linalool to lesser mealworm adults and larvae suggest that either they are nonattractive or attractive at a narrow range of concentrations in the laboratory two-choice pitfall bioassays. Limonene is a monoterpene that is found to be repellent against mealybugs and whiteflies (Hollingsworth 2005), and cattle tick, *Rhipicephalus microplus* (Canestrini) (Ferrarini et al. 2008). Linalool repelled 93% more mosquitoes than the unprotected control (Muller et al. 2009). The attractiveness of limonene and linalool needs to be assessed in dose-dependent response of lesser mealworm in laboratory pitfall bioassays. These compounds should be excluded if found nonattractive or repellent.

Chicken dropping volatiles (10 μg CDV) extracted in methylene chloride were 3.6-fold less attractive than the fresh chicken droppings (2 g CD), whereas, the 2 g CD + 20 μg pheromone lure was three-fold more attractive than a combination of 10 μg CDV + 20 μg pheromone lure. Pheromones are usually perceived at minute quantities and elicit strong behavioral and antennal responses. While multiple chemicals and correct blends can be critical for pheromone activity, kairomone activity in general is even more dependent on multiple compounds and correct ratios, and kairomones frequently require more material for activity (Witzgall et al. 2008, Trona et al. 2010). The concentration of CDV (kairomone) used was half that of the pheromone lure in the laboratory pitfall bioassays. Therefore, the low concentration of CDV might have accounted for the low attractiveness of CDV to adults and larvae as compared with fresh CD. The low concentration of CDV might not have released the correct natural ratios of different volatile components. Testing CDV at higher doses might have produced a more pronounced response. The fresh CD might have released the volatile profiles in ratios more similar to those released in the poultry house, and also the larvae and adults perceived the CD volatiles as they do in their natural habitat.

A combination of fresh CD and pheromone lure was observed to be very attractive to adults and larvae in laboratory two-choice pitfall bioassays. Adults were more attracted to the combination of pheromone lure and fresh CD than the larvae, whereas, larvae were more attracted to fresh CD alone than adults.

These results were in agreement with other pest insects that exhibit increased attraction to host volatiles. Human urine and chicken feces increased lure attraction to fruit flies (*Anastrepha* spp.) in a commercial mango orchard in Veracruz, Mexico (Pinero et al. 2003). Scarab dung beetles were most attracted to swine and opossum feces in laboratory bioassays (Fincher et al. 1970). Odors from fresh chicken feces in water elicited upwind flight of host-seeking female *Culex quinquefasciatus* Say mosquitoes in a two-choice olfactometer (Cooperband et al. 2008).

The CD left behind from previous flock might have some attractive volatiles that competed with the pheromone lure before introduction of chicks. The presence of chicks affected distribution of beetles, and their droppings strongly competed with the pheromone lure. Nevertheless, a consistent effect of the pheromone over time was observed in low, moderate, and high-infestation houses for adults and larvae. The pheromone effect was consistent over space in moderate and high lesser mealworm infestation houses for adults, and the high lesser mealworm infestation house for larvae. The low larval population or clumped aggregations in the house (Strother and Steelman 2001) might be the reason for the variable attractiveness of pheromone over space for adults in a low infestation house, and for larvae in low and moderate infestation houses.

Our results generally indicate that there is a potential for combining the synthetic lesser mealworm pheromone lure with the attractive CD compounds to enhance trap efficacy. The combinations that attract more beetles can be used to monitor the lesser mealworm populations and also combined with insecticide baits(s) to manage lesser mealworm populations in poultry houses. Bray et al. (2010) demonstrated the practical application of pheromones in experimental chicken sheds, where the addition of the synthetic pheromone (\pm)-9-methylgermacrene-B resulted in greater numbers of male and female sand fly, *Lutzomyia longipalpis* Lutz & Neiva being caught and killed with sprayed insecticide, compared with pheromone-free controls.

Further studies should be conducted to determine the dose-dependent attractiveness of identified CDV compounds (Table 1) alone or in combinations with or without pheromone lure in laboratory two-choice pitfall bioassays. The most attractive combination of attractive CD compounds and the synthetic pheromone should be then evaluated in poultry houses. Different release rates of pheromone lure and attractive CD compounds also need to be evaluated, and these release rates need to be optimized in poultry house conditions.

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