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## **Dissemination of Imidacloprid through Dairy Cattle Manure and its Effect on the Biological Control Agent, *Spalangia endius* (Hymenoptera: Pteromalidae), and a Filth Fly Host, *Musca domestica* (Diptera: Muscidae)**

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### **Abstract**

Filth flies, including house flies, *Musca domestica* L. (Diptera: Muscidae), develop in animal manure. Adult house flies often are controlled with pesticides such as imidacloprid. How imidacloprid disseminates and persists after it contaminates manure was measured at a dairy farm. A week after application of imidacloprid via fly bait to cattle manure, a mean of approximately 4 ppm of imidacloprid, and as high as 15 ppm, was quantifiable up to 12 cm from the application site, but not farther. Laboratory experiments addressed the impact of 15 ppm of imidacloprid in manure on egg-to-adult development of house flies and on the biological control ability of a house fly pupal parasitoid, *Spalangia endius* Walker (Hymenoptera: Pteromalidae). In uncontaminated manure, 93% of eggs developed to adults, versus 7% in contaminated manure. In the parasitoid experiment, fly pupae were placed in contaminated or uncontaminated manure with or without *S. endius*. In the absence of *S. endius*, nearly 100% of flies emerged, with or without imidacloprid. In the presence of *S. endius*, only 11% of flies emerged from uncontaminated manure, versus 36% from contaminated manure; and parasitoids emerged from 82% of hosts in uncontaminated manure versus 53% in contaminated manure. These results suggest that realistic concentrations of imidacloprid in filth fly breeding habitat may interfere with house flies developing to the pupal stage, but also with parasitoids locating and utilizing house flies. However, after 1 wk, the effects on parasitoids will be low 12 cm beyond where bait was applied.

**Keywords:** house fly, fly bait, contamination, HPLC, natural enemies, pesticide

Livestock manure provides copious developmental habitat for pestiferous filth flies, including house flies, *Musca domestica* (Diptera: Muscidae). House flies are vectors of human and animal pathogens (Pugh et al. 2014; Nayduch and Burrus 2017). In addition to sanitation and physical control, adult house flies often are controlled with space sprays (Chapman et al. 1993) and baits (Butler et al. 2007). Larvicides can be included in livestock feed, which is subsequently deposited into manure (Ode and Matthyse 1964, Schmidt 1983); House flies also may be managed by natural enemies, naturally occurring ones and released ones, e.g., parasitoid wasps such as various species of *Spalangia* and *Muscidifurax* (Hymenoptera: Pteromalidae) (Morgan et al. 1975, Weinzierl and Jones 1998, Skovgård and Nachman 2004, McKay et al. 2007).

House fly baits typically include an aggregating pheromone, an active ingredient (AI), and sucrose (Yu 2015). The baits are sometimes applied on or near manure in livestock facilities (Stafford 2008). Bait labels and video commercials recommend their use both inside and outside livestock buildings (e.g., Bayer Healthcare 2006). Accidental spills of baits may occur where there is a high level of animal activity and foot traffic. What risks are imposed on natural enemies of filth flies by accidental or intentional pesticide exposure in their breeding habitat is not known.

Imidacloprid is one common AI in granular baits used in fly management programs (White et al. 2007). Imidacloprid is a neonicotinoid that is among the best-selling insecticides in the world (Jeschke et al. 2011) and one of the most widely used against filth flies (Simon-Delso et al. 2015). Pupal parasitoids of house flies and other filth flies especially are sensitive to imidacloprid, at least compared to other AIs used in filth fly control (Burgess and King 2015). Imidacloprid exposure can also reduce the effectiveness of pupal parasitoids subsequently finding and processing hosts in uncontaminated filth fly breeding habitat (Burgess and King 2017).

Persistence and dissemination of imidacloprid that is relevant to arthropod natural enemies of pest species and other non-target organisms primarily has been examined in crop situations, such as the crops themselves, and the soil they grow in, but not in manure alone (Blacquiere et al. 2012; Bonmatin et al. 2015). How pesticides disseminate and persist in manure also is important, as this substrate often is used as fertilizer in commercial agriculture and because of the potential for environmental contamination through runoff.

The proportion of organic matter in soil has a large effect on sorption of imidacloprid (Liu et al. 2006), which in part may explain the reduced dissemination and degradation of imidacloprid when the substrate includes organic matter (Rouchaud 1996). Reduced dissemination and degradation subsequently may affect invertebrate and microbial communities (Dittbrenner et al. 2011). The fate of imidacloprid in soil can also be affected by interactions of pH, moisture content, and microbial communities (Ping et al 2010; Lu et al. 2016).

The present study examined the dissemination and resulting concentrations of imidacloprid in dairy cattle manure one week after a label-recommended quantity of granular fly bait was applied to fresh dairy cattle manure under field conditions. Further, a method for extraction and quantification of imidacloprid in manure was developed. The greatest quantity of imidacloprid observed was then used as a basis to test its potential impact on house fly development, as well as on the ability of a pupal parasitoid, *Spalangia endius* Walker, to locate and utilize house fly pupae. These data may be a useful resource for future risk assessments of imidacloprid on dairy farms.

## Materials and Methods

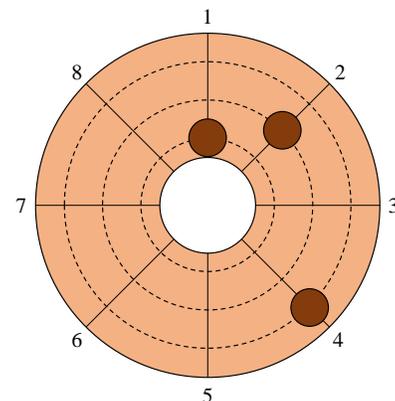
### Field Experiment

**Manure Pool Setup and Sampling.** A field experiment was conducted in a centrally-located, three-sided shed (4.0 m wide, 5.8 m long, 4.6 m high) on a dairy farm in northern DeKalb County, Illinois (450 cows, 650 hectares, a double-8 parallel parlor, sand-bedded free stalls and freedom stalls). The cattle diet was primarily a mixture of gluten and silage. The experiment was conducted during August - September 2016, with 22 – 35 °C air temperature. The shed was warm with minimal wind, which matched conditions recommended on the bait label (Bayer Healthcare 2006). Pesticides were not used for years prior to sampling.

At the start of each week, three clean plastic wading pools were filled with moist, 0-2 d old dairy manure (approx. 0.37 m<sup>3</sup> manure volume, 3.66 m<sup>2</sup> manure surface area) (Fig. 1). Eight equal-sized, pie-shaped sectors were measured around the circumference of the pool starting from the center. The center of near, middle and far samples were 6, 18, and 30 cm, respectively, from the outer edge of a central bait application. Each sector sample was chosen randomly. Each sample (12 cm diameter, 10 cm deep) was removed with two gardening trowels and put in a freezer bag, which was immediately placed on ice. Between each sample, both trowels were rinsed with water twice and dried.

After initial samples were collected to assess pH and moisture, 40 g of QuickBayt granular fly bait (Bayer Healthcare, Shawnee Mission, KS) was spread uniformly on the surface of the manure in the center of each pool. Because the shed floor area was 23.2 m<sup>2</sup> area, this Quickbayt application of 40 g corresponds to 1.72 g/m<sup>2</sup>, which is similar to the 1.83 g/m<sup>2</sup> recommended on the bait label. Given that QuickBayt is 0.50% imidacloprid by weight, approximately 200 mg of imidacloprid was applied to each pool.

**Fig. 1.** A dairy cattle manure pool (108 cm diameter) with bait in the center (30 cm diameter). Near, middle and far samples (12 cm diameter each) were taken from random sectors. On day 1 a pool was setup; specifically, 1) manure was collected; 2) two control samples were taken from the manure to confirm no imidacloprid; 3) the pool was filled; 4) to assess moisture and pH, an initial three samples were taken from the pool, one sample from each of the three distances; and 5) bait was applied. On day 8, to assess moisture and pH, three samples were taken, one from each of the three distances; and to detect and quantify imidacloprid, an additional three samples were taken, one from each of the three distances.



The bait label recommends reapplication weekly. Thus, one week after bait application, samples were taken again from each pool to assess moisture content and pH, as well as imidacloprid. All manure pools were disposed of, and three new pools were set up in their place. This experiment was replicated once per week for four total weeks (n = 12 pools total). All manure samples were stored at -80°C until analysis.

**Manure pH and Percent Moisture.** To determine moisture and pH, 10 g of each thawed manure sample was dried at 60 °C for 48 h. Then samples were reweighed. Moisture content was the percent loss in mass from drying, relative to starting mass. For pH, each dried manure sample was ground to a fine powder using a mortar and pestle, and remaining large particles were filtered out with a 1 mm mesh. A 1 g subsample of the filtered manure was stirred into 10 mL of Nanopure H<sub>2</sub>O, and the pH was measured after settling, using an electronic pH meter (Mettler Toledo SevenCompact, Columbus, OH).

**Imidacloprid Extraction from Manure.** To prepare for imidacloprid extraction, each thawed bag of manure was homogenized thoroughly by hand in its bag, and a 10 g subsample was measured into a 50 mL centrifuge tube. Each tube then had 10 mL HPLC grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) and 5 mL Milli-Q water (Millipore Milli-Q-Plus Purepak 2 water purification system EMD Millipore, Bellerica, MA) pipetted into it. All manure control samples were shown to lack detectable imidacloprid as expected given imidacloprid not being used on the farm. Positive controls were created by spiking centrifuge tubes of control manure with 2 mL of 5.30 mM imidacloprid stock solution made by dissolving 33.0 mg imidacloprid (99.5%; Chem Service, West Chester, PA) in 25 mL mobile phase (20:80, acetonitrile:water). Each sample was then vortexed for 1 min before extraction.

Extractions were performed by adding a QuEChERS Q-Sep Q100 unbuffered extraction salt packet (4 g MgSO<sub>4</sub>, 1 g NaCl; Restek, Bellefonte, PA) to each 50 mL centrifuge tube. This was followed by 1 min of hand shaking, and 1 min of vortexing. All samples were then centrifuged at 5000 rpm for 5 min at room temp. An aliquot of 1 mL from each centrifuge tube was transferred to 2 mL Q-Sep QuEChERS dSPE tubes (150 mg MgSO<sub>4</sub>, 25 mg PSA, 25 mg C<sub>18</sub>; Restek, Bellefonte, PA) and hand shaken for 30 s. These tubes were centrifuged for 5 min at 3000 rpm at room temp. From each tube, 0.5 mL was transferred to a glass vial for

condensing on a rotary evaporator. Each sample was then dissolved in 0.5 mL mobile phase, vortexed, and moved to a Millipore Ultrafree centrifugal filter unit (0.22  $\mu\text{m}$  GV Durapore; Merck-Millipore, Carrigtwohill, Cork, Ireland) and centrifuged for 3 min at 5000 rpm at room temp. The filtered solution was then added to a HPLC vial with 0.5 mL mobile phase and shaken before analysis.

**Table 1.** Line equations, coefficient of determination values, and percent recovery calculated from analytical grade imidacloprid calibration standards for each of 4 (weeks).

Week	Line Equation	$R^2$	Percent Recovery <sup>a</sup>
1	$y = 13055x + 43.887$	0.9999	69.95
2	$y = 13009x + 47.407$	0.9998	76.39
3	$y = 13171x + 30.056$	0.9999	66.05
4	$y = 12921x + 68.554$	0.9998	70.62

<sup>a</sup> Calculated as the average parts per million (ppm) of all samples within a week divided by the ppm recovered from the positive control for that week.

Imidacloprid content was assessed on an Agilent 1100 (Santa Clara, CA) utilizing a biphenyl Kinetex LC Column (100 x 4.6 mm, 5 micron particle size with 100 Å pore size) from Phenomenex using ChemStation. A stock solution was made by dissolving 13.1 mg imidacloprid in 25 mL mobile phase (20:80, acetonitrile-water). Six standards for calibration were prepared, either from aliquots of the stock solution or from aliquots of serial dilutions, resulting in a calibration range of 1.0  $\mu\text{M}$  – 1.0 mM. Analyses were performed isocratically (20:80, acetonitrile:water) at a flow-rate of 1.00 mL/min for a duration of 13 min. Injections of 10  $\mu\text{L}$  occurred via autoinjector. Injections of mobile phase as a blank were applied between each sample. Samples and controls were analyzed with standards for each of the 4 wk of sampling. At the end of the 4 wk, all samples and controls were reanalyzed, with standards repeated between every eleven samples (Table 1). A diode array detector was adjusted to monitor absorption at 270 nm. The limit of detection (LOD) for these parameters was 0.56  $\mu\text{M}$  (0.14 ppm), and the limit of quantitation (LOQ) was 1.72  $\mu\text{M}$  (0.44 ppm). The equation used to determine the LOD was 3.3SD/slope of calibration curve, and the equation used for the LOQ was 10SD/slope of calibration curve (Shrivastava and Gupta 2011). The slope of the calibration curve was calculated by combining all lines from each week into one unified slope.

## Laboratory Experiments

**House flies and *Spalangia endius*.** The “NIU” house fly strain was used for maintenance of *S. endius* and in the laboratory experiments. The strain is more than 20 yr old and has not been exposed to pesticides since establishment. The adult flies were fed diluted evaporated-milk, granulated sugar and water ad libitum. To facilitate egg collection for the house fly experiment (below), a 30 ml plastic cup was half filled with wet fly media that was covered with a piece of black cotton cloth in such a way as to create spaces for oviposition in wrinkles and along the edges. Details of the fly media and rearing are in King et al. (2014).

The colony of *S. endius* was established in fall 2016 from parasitized pupae collected from the dairy farm used in the field study. Vouchers for this strain of *S. endius* are at the Illinois Natural History Survey Center for Biodiversity, catalog numbers 833640 - 833651. The parasitoids were maintained in a 25°C incubator with a 12:12 L:D photoperiod.

**Manure Preparation.** Manure was collected in April 2018 at the same dairy farm as previously mentioned and stored in a freezer at -80.0°C prior to use. The manure was of similar consistency and moisture content as the manure used in 2016. Bags of thawed manure were either pre-treated with solutions of analytical grade imidacloprid (99.5% purity; Chem Service, West Chester, PA) in reverse osmosis water and acetone, or water and acetone-only, immediately before experimentation.

Imidacloprid treatment solution was determined for each treatment bag of manure by first weighing each bag and then calculating the appropriate mass of imidacloprid needed to bring the total imidacloprid-to-manure concentration to 15 ppm (mg AI/kg manure). The imidacloprid was initially dissolved in 0.8 g acetone (1 mL), and then that solution was added to 5 g of water (5 mL). This additional 5.8 g was accounted for in the overall mass of the manure in each bag to keep the mixtures consistently at 15 ppm. Once the treatment solution or control solution was added to each bag, they were then resealed and homogenized manually by hand for 5 min.

**House Fly Experiment.** This experiment compared the ability of house flies to develop from egg to adult in imidacloprid-treated versus untreated manure. Each replicate consisted of two clean 237 mL glass jars (8.8 cm height x 7.5 cm dia.), one filled with imidacloprid-treated manure and one with untreated manure, to a depth of 6 cm. For each replicate, 60 eggs were collected from colony cages; and in each jar, 30 eggs were placed on a piece of moistened paper towel, which was set egg-side down on the surface of the manure. The tops of the jars were covered with mesh (1 mm opening fiber glass screen), which was secured with a rubber band, and held at 25°C and 12:12 L:D photoperiod until adult flies finished emerging and died. Unclosed pupae, empty puparia, and adult flies were collected by floatation and counted. This was replicated five times.

**Parasitoid Experiment.** This experiment compared the ability of *S. endius* to manage house flies and produce offspring in imidacloprid-treated manure versus untreated manure. For this experiment, manure was placed in each jar to a depth of 4 cm; 25 house fly pupae (0-2 d old) were added on the center of the surface of the manure, with no pupae touching the sides of the jar, then additional manure was placed over the pupae to a depth of 2 cm (6 cm total depth). A replicate is defined as one of each treatment, and there were four treatments: imidacloprid-treated manure with a parasitoid (IP), untreated (control) manure with a parasitoid (CP), imidacloprid-treated manure with no parasitoid (INP), and control manure and no parasitoid (CNP). This experiment was replicated 20 times (80 vials in total) with between one and five replicates done per day over multiple days and parasitoid generations. The two treatments without a parasitoid were used to control for fly emergence that fails even in the absence of parasitoids. For the treatments with a parasitoid, a single 0-2 d old female was tapped out of a test tube and onto the surface of the manure. The females were from polystyrene dishes (10 mm deep, 100 mm diameter) of parasitized hosts from which males had already begun emerging; thus, females likely had mated.

All jars were held for 48 h at 25°C and 12:12 L:D photoperiod. Then the pupae were removed from the manure and placed in 20 mL glass vials (70 mm high, 20 mm diameter) and held at 25°C. After 5 weeks, the total number of male and female parasitoid offspring, puparia with parasitoid emergence holes, unclosed pupae, and empty puparia, were counted.

**Statistical Analyses.** All statistical analyses were with R version 3.3.0 (R Core Team 2016). Percent moisture and pH among weeks were each analyzed via linear mixed models, using the ‘nlme’ package (Pinheiro et al. 2016). Sample time (pre- versus post-treated) was analyzed as a repeated measure, and pool was nested within week. Week and pool were treated as random effects, sample time and distance as fixed effects.

For the laboratory house fly experiment, data were pooled across replicates and analyzed with a 2 x 2 chi-square test of independence (number of eggs that developed into adults versus not; treated versus untreated manure). For the parasitoid experiment, the effect of treatment on number of adult flies that emerged, and number of hosts that were successfully parasitized as evidence by a parasitoid emergence hole, were analyzed as a function of total pupae in each jar, with a generalized linear model. As a result, fly emergence and parasitization were each best-represented by a quasi-binomial distribution and a log link function. A general linear model was used to analyze sex ratio of the resulting parasitization. Pairwise comparisons were by Tukey’s HSD test using the ‘multcomp’ R package (Hothorn et al. 2008) whenever applicable among all analyses.

## Results

## Field Experiment

**Manure pH and Percent Moisture.** Percent moisture was significantly higher for the pre-treated manure than for the post-treated manure (Table 2;  $F = 27.50$ ,  $df = 1, 55$ ,  $P < 0.001$ ) and differed with distance ( $F = 3.34$ ,  $df = 2, 55$ ,  $P = 0.042$ ). Far samples were slightly more moist than middle samples ( $Z = 2.704$ ,  $P = 0.019$ ). There were no other significant differences between distance and moisture. The pre-treated manure samples' pH was slightly higher than for the post-treated samples ( $F = 10.98$ ,  $df = 1, 55$ ,  $P = 0.002$ ), and pH did not differ by distance ( $F = 0.53$ ,  $df = 2, 55$ ,  $P = 0.590$ ).

**Table 2.** Mean percent moisture and mean pH by sample distance in cattle manure pre- and post-treatment with imidacloprid granular bait.

Distance	Pre-treatment % moisture (mean $\pm$ SE)	Post-treatment % moisture (mean $\pm$ SE)	Pre-treatment pH (mean $\pm$ SE)	Post-treatment pH (mean $\pm$ SE)
Near	75.96 $\pm$ 0.93	71.78 $\pm$ 1.80	10.70 $\pm$ 0.05	10.51 $\pm$ 0.05
Middle	75.50 $\pm$ 1.26	68.70 $\pm$ 1.66	10.66 $\pm$ 0.05	10.48 $\pm$ 0.08
Far	77.03 $\pm$ 0.57	73.13 $\pm$ 0.91	10.60 $\pm$ 0.06	10.51 $\pm$ 0.05

Manure was collected from a dairy farm in northern DeKalb County, Illinois. Time between pre- and post-treatment was 1 wk.

**Imidacloprid Extraction from Manure Samples.** Post-treatment, imidacloprid was only quantifiable with HPLC in near samples, where it was detected in 6 of 12 samples (Table 3). Of the pools with quantifiable imidacloprid, the mean concentration was  $7.73 \pm 2.89$  ppm. The range of mean concentration of imidacloprid across all 12 samples, including those with no quantifiable imidacloprid, was  $3.87 \pm 1.81$  to  $3.94 \pm 1.79$  ppm. The lower mean was calculated as 0 ppm when no imidacloprid was detected (ND) in samples, and the upper mean was calculated using the LOD (0.14 ppm) for the ND samples. No value fell between the LOD and the LOQ. Percent recovery of imidacloprid from samples ranged from 66.05 to 76.39% by week. This likely represents the bioavailability of imidacloprid, with the remainder adsorbed to the manure matrix.

**Table 3.** Quantity of imidacloprid (ppm =  $\mu\text{g}$  imidacloprid per g of manure) found via HPLC for each of 4 weeks at 6 cm from the application site (near sample).

	Week 1	Week 2	Week 3	Week 4	Across all 12 samples
<b>Pool 1<sup>b</sup></b>	1.44	0.73	ND <sup>a</sup>	ND <sup>a</sup>	
<b>Pool 2<sup>b</sup></b>	1.72	ND <sup>a</sup>	14.47	13.05	
<b>Pool 3<sup>b</sup></b>	ND <sup>a</sup>	ND <sup>a</sup>	14.97	ND <sup>a</sup>	
<b>Where detected: mean (SE)<sup>c</sup></b>	1.58 (0.14)	0.73	14.72 (0.25)	13.05	7.73 (2.89)
<b>Across all pools<sup>d</sup></b>	1.05 (0.53) – 1.10 (0.49)	0.24 (0.24) – 0.34 (0.20)	9.81 (4.91) – 9.86 (4.86)	4.35 (4.35) – 4.44 (4.30)	3.87 (1.81) – 3.94 (1.79)

<sup>a</sup> ND = None Detected

<sup>b</sup> Dairy cattle manure setup was in plastic wading pools (approx. 60 cm diameter, approx. 10 cm deep). Manure volume was approx.  $0.37 \text{ m}^3$ , and manure surface area was approx.  $3.66 \text{ m}^2$ .

<sup>c</sup> Mean (SE) where detected: ND values were excluded.

<sup>d</sup> Across all pools is presented as a range of means because ND may be 0 ppm to as high as the LOD (0.14 ppm); the minimum mean (SE) was calculated by assigning a value of 0 ppm for any ND, and the maximum

mean (SE) was calculated by assigning a value of 0.14 ppm for any ND. All 18 cm and 30 cm dairy cattle manure samples had no detectable imidacloprid, thus their ranges could fall between 0 ppm and 0.14 ppm.

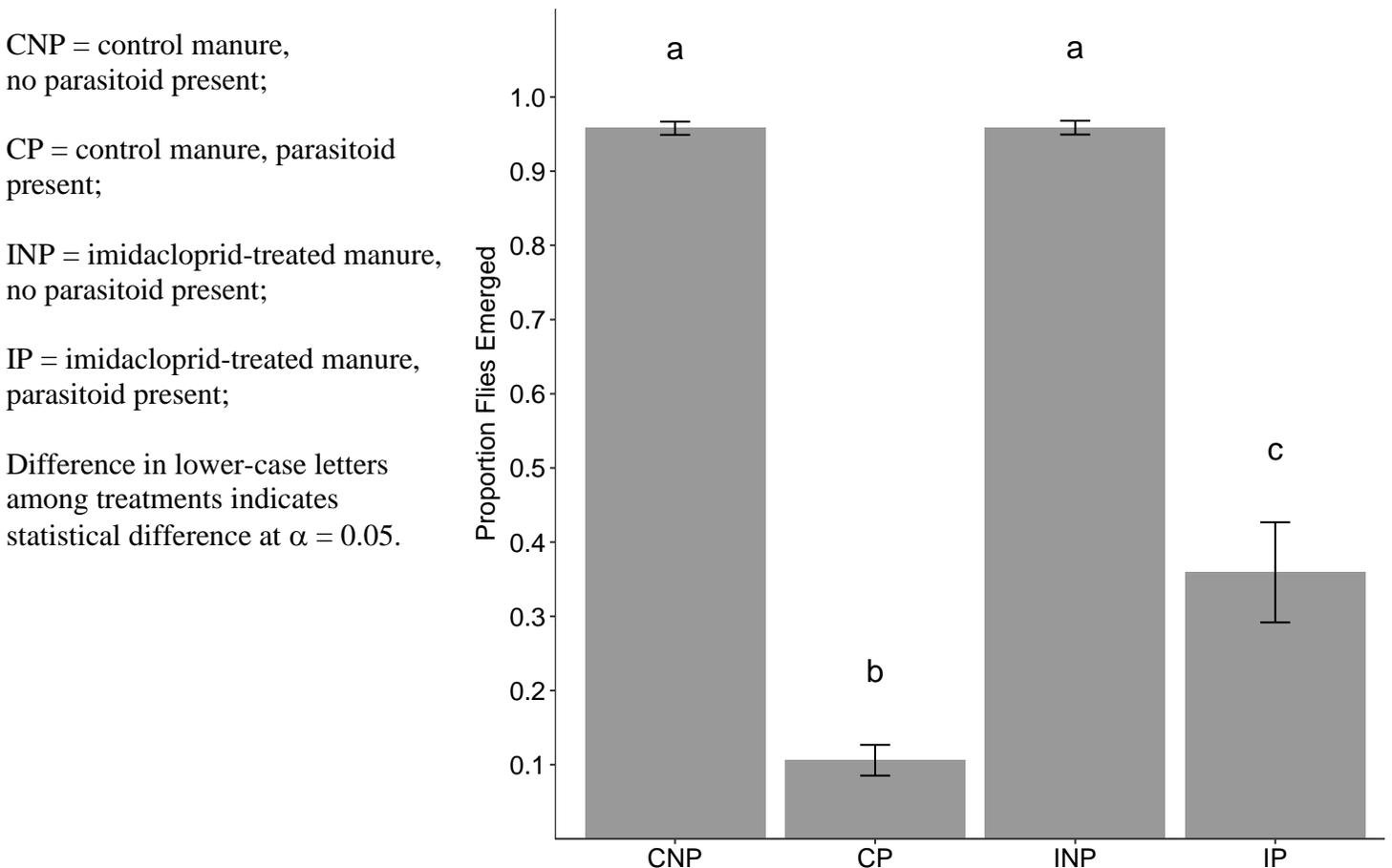
## Laboratory Experiments

**House Fly Experiment.** The proportion of house fly eggs that developed into adults was dependent on treatment ( $\chi^2 = 218.46$ ,  $df = 1$ ,  $P < 0.001$ ). In the control, 93.3% of fly eggs developed to adults, whereas in the imidacloprid treatment, 7.3% became adults. The low adult emergence resulted primarily from aspect(s) of development prior to adult eclosion, not from adults failing to eclose from pupae. Among all replicates of both the treatment and control, only one uneclosed pupae was found (in an imidacloprid treatment) (0 unenclosed pupae of 140 pupae in the control versus 1 unenclosed pupae of 12 pupae in the treatment).

**Parasitoid Experiment.** Treatment significantly affected the proportion of flies that emerged from the placed pupae (Fig 2;  $F = 83.89$ ,  $df = 3, 76$ ,  $P < 0.001$ ). Control parasitoid (CP) had significantly less fly emergence ( $10.6\% \pm 2.1\%$  emerged) compared to imidacloprid parasitoid (IP) ( $35.9\% \pm 6.7\%$ ), control no parasitoid (CNP) ( $95.8\% \pm 0.9\%$ ), and imidacloprid no parasitoid (INP) ( $95.9\% \pm 0.9\%$ ) (all  $P < 0.001$ ). IP also had significantly lower fly emergence compared to CNP and INP (both  $P < 0.001$ ).

There also was a significant effect of treatment on the proportion of dud pupae, where neither a fly nor a parasitoid emerged ( $F = 7.98$ ,  $df = 3, 76$ ,  $P < 0.001$ ). The proportion of dud pupae was significantly greater in IP ( $11.1\% \pm 1.7\%$ ) compared to both the CNP ( $4.2\% \pm 0.9$ ), and the INP ( $4.1\% \pm 0.9\%$ ) (both  $P < 0.001$ ). The proportion of dud pupae in CP was  $7.4\% \pm 1.1\%$ . The proportion of pupae with a parasitoid emergence hole was significantly lower in IP ( $52.9\% \pm 6.3\%$ ) compared to the CP ( $82.0\% \pm 2.1\%$ ; Fig 3;  $F = 19.77$ ,  $df = 1, 38$ ,  $P = 0.001$ ). There was no significant difference in the proportion of parasitoids that were male between IP and CP ( $0.16 \pm 0.03$  versus  $0.15 \pm 0.02$ ;  $F = 0.12$ ,  $df = 1, 36$ ,  $P = 0.73$ ).

**Fig 2.** Proportion of house fly emergence from pupae buried for 48 h under 2 cm of manure:

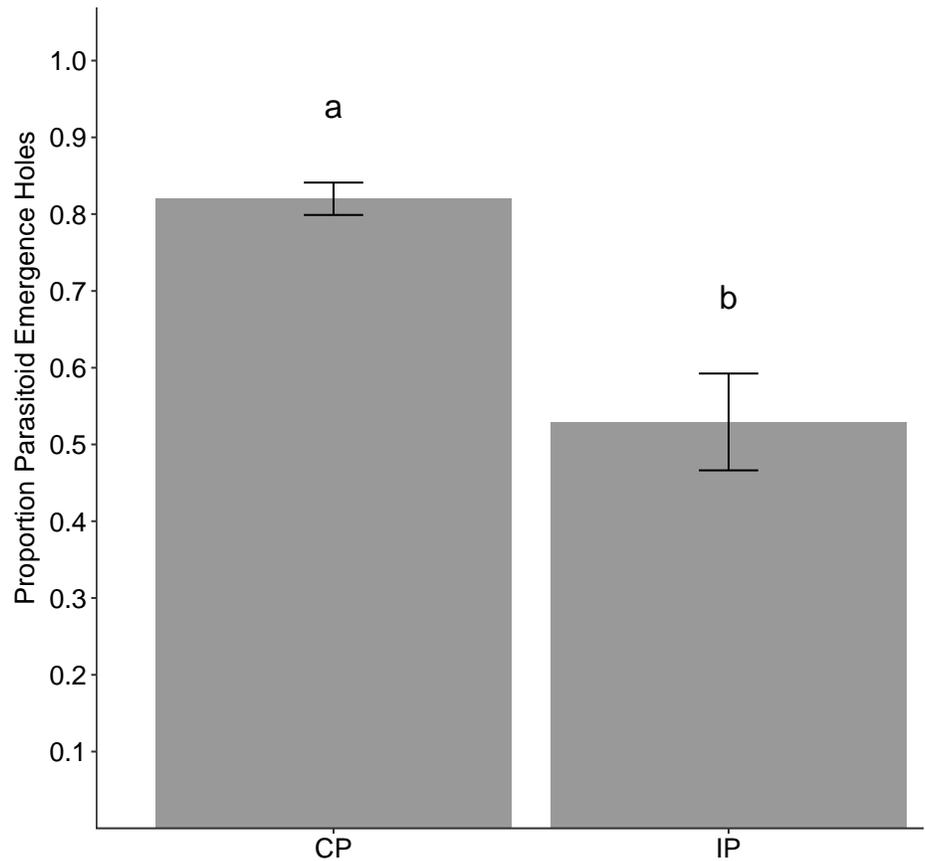


**Fig. 3.** Proportion of parasitoid emergence holes from pupae buried for 48 h under 2 cm

CP = control manure, parasitoid present;

IP = imidacloprid-treated manure, parasitoid present;

Difference in lower-case letters between treatments indicates statistical difference at  $\alpha = 0.05$ .



## Discussion

A week after bait application, the mean imidacloprid concentration was 4 ppm, with a maximum of 15 ppm in samples that spanned 0 - 12 cm from the application site. Imidacloprid was not detectable in samples past 12 cm up to the maximum distance of 36 cm from the application site under our extraction and HPLC protocols. In the present study, bait granules appeared to have completely dissolved into the manure after one week, despite being sheltered from rain, as recommended on the bait label. Because Quickbayt granules are water-soluble (Bayer Healthcare 2006), solvation with fluids present in the manure was a likely driver of imidacloprid dissemination. The manure generally decreased in percent moisture over the week. Dissemination may be farther when manure moisture content is retained or increased. Dissemination and degradation also may be affected by pH. A pH as alkaline as what was found in the manure is rare among soils (Slessarev et al. 2016). This is one reason that data regarding imidacloprid dissemination and degradation in soil is not an adequate proxy for what occurs in manure. Effects of pH on imidacloprid degradation are complex and can include effects on adsorption (Ping et al. 2010) and on microbial activity (Lu et al. 2016).

Dry granular imidacloprid bait is 0.50% AI by weight, i.e. 5000 ppm (Burgess and King 2015), but this value will decrease rapidly in wet, or even humid, conditions because the granular bait is hydrophilic (Parker et al. 2015). Imidacloprid in water in the presence of light has a half-life of a few hours, whereas the half-life of imidacloprid in field studies is 27 - 229 d (Wagner 2016). Imidacloprid-based granular baits that sit in a dish outside with partial sun exposure, become less effective against adult house flies after 2 wk, from more than 90% mortality initially to less than 70% mortality after 1 wk and less than 40% after 2 wk (Parker et al. 2015). However, dissemination of imidacloprid into manure may protect the imidacloprid from light.

The maximum amount of imidacloprid that was quantified in any of our samples, 15 ppm, was still sufficient to kill most house flies, by interfering with their development from the egg to pupal stage in treated manure. Flies that reached the pupal stage prior to imidacloprid exposure appear to be protected from harm at 15 ppm in manure, with almost 100% adult emergence. The strain tested in the present study was from a laboratory-reared colony, so care should be taken when translating these results to field strains.

Imidacloprid at 15 ppm in manure not only killed developing house flies directly, but also interfered with the ability of *S. endius* to control house flies, with increased numbers of flies and fewer parasitoid offspring produced. These results from the present study are consistent with studies in which adult house flies and adults of their pupal parasitoids are exposed to imidacloprid in sugar and on glass surfaces (Burgess and King 2015, 2016). There is often overlap in larval stages of multiple generations of house flies in colonized cattle manure (Broce and Haas 1999). How larval stages later in their development affect physical and chemical parameters of cattle manure and how that may in turn affect imidacloprid dissemination and persistence remains to be studied. The parasitoids are more sensitive than house flies, i.e. have lower LC<sub>50</sub> values (Burgess and King 2015, 2016). Nevertheless, in the present study, at levels that reduced successful house fly development, parasitoid females managed to parasitize approximately half of all hosts.

Manure contaminated with imidacloprid reduced the effectiveness of *S. endius* in the present study. Female *S. endius* burrow in search of hosts (Rueda and Axtell 1985, Geden 2002) The effectiveness of *S. endius* that have been exposed to imidacloprid is greater in the presence than in the absence of manure-like fly media (Burgess and King 2017). The presence of media reduces the amount of imidacloprid on females.

The results of the present study lead us to recommend that 1 wk after application of imidacloprid based bait is too soon to release parasitoids for fly control in the same location, but that the low dissemination of imidacloprid reduces the chance that imidacloprid will harm parasitoids more than 12 cm away. Future studies might examine whether the levels of imidacloprid seen in the present study are toxic to other organisms living in cattle manure, what imidacloprid metabolites are present in manure, and the effect of imidacloprid metabolites in manure on filth flies and their parasitoids. Some imidacloprid metabolites are toxic, at least to the honey bee, *Apis mellifera* (Suchail et al. 2004).

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