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## **Behavior and Survival of the Filth Fly Parasitoids *Spalangia endius* and *Urolepis rufipes* (Hymenoptera: Pteromalidae) in Response to Three Granular House Fly Baits and Components**

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**ABSTRACT** Behaviors and mortality of two filth fly parasitoid wasps, *Spalangia endius* Walker and *Urolepis rufipes* Ashmead, were tested in response to granular fly baits containing one of three active ingredients (AI): Golden Malrin (methomyl), QuickBayt (imidacloprid), or Quikstrike (dinotefuran). Behavioral responses to each of two components of the baits, the AIs and the fly attractant pheromone (Z)-9-tricosene, were also examined independently. *S. endius* avoided contact with bait granules, regardless of bait type. However, when *S. endius* contacted bait residue, the imidacloprid bait appeared to be the least harmful of the baits for *S. endius*, at least in the short term. *S. endius* was attracted to imidacloprid by itself. However, *S. endius* avoided (z)-9-tricosene. In contrast to *S. endius*' attraction to imidacloprid, *S. endius* neither avoided nor was attracted to methomyl or dinotefuran. For *U. rufipes*, the methomyl bait appeared to be especially harmful. *U. rufipes* avoided bait granules with imidacloprid or dinotefuran but not with methomyl, died quickly in the presence of methomyl bait residue, and had a methomyl LC<sub>50</sub> that was lower than that for *S. endius*. The avoidance by *U. rufipes* of granules with imidacloprid or dinotefuran appears to be related to components other than the AIs or the (Z)-9-tricosene because *U. rufipes* did not avoid either individually. The behavioral avoidance of the parasitoids in the present study occurred despite no exposure recently, if ever, to these pesticides.

**KEY WORDS** bait, parasitoid, Hymenoptera, IPM, neonicotinoid

One of the most common filth flies in animal production facilities is the house fly, *Musca domestica* (Diptera: Muscidae) (Floate 2003, Geden 2012). An estimated 1.6 million USD is spent on house fly control in the United States per year (Malik et al. 2007). They are vectors of human pathogens (White et al. 2007) and of the bacterium *Corynebacterium pseudotuberculosis*, which can cause mastitis in milk-producing animals (Yeruham et al. 1996). Although management of manure and other waste is the most important aspect of controlling filth fly populations, additional controls are often used (Machtinger et al. 2012, Ferguson et al. 2014).

Pupal parasitoids of filth flies have the potential to significantly decrease filth fly populations when released en masse (Weinzierl and Jones 1998, Skovgård and Nachman 2004, McKay et al. 2007), but control is not always achieved (Andress and Campbell 1994), and chemical control remains widely used. However, challenges in using pesticides include: increased public awareness of pesticide residues in food and harm to beneficial insects, as well as the flies rapidly acquiring resistance to many modern formulations (Geden et al. 1992). Among pesticides, granular fly baits have the advantage of selectivity to pest species (Butler et al. 2007). To maximize the selectivity of baits, it is important to know how biological control agents respond to them. With this knowledge, it may be possible to reduce inadvertent exposure of biological controls, through either changes in bait formulations or changes in instructions on labels.

*Spalangia endius* Walker (Hymenoptera: Pteromalidae) is widely used and commercially available as a biological control of filth flies (van Lenteren 2012, Cranshaw and Broberg 2015). *Urolepis rufipes* Ashmead (Hymenoptera: Pteromalidae) is found in some of the same hosts and habitats as *S. endius* but is not currently

commercially available. *U. rufipes* has been suggested as a biological control agent that is especially suited to wet habitats in northern areas (Smith and Rutz 1985, Stenseng et al. 2003, Floate and Skovgård 2004). These and related parasitoids may encounter fly pesticides where baits are placed, such as on or near manure and other rotting organic material where filth flies breed (Stafford 2008), or around windows, where flies and parasitoids often congregate (Smith et al. 1989, Skovgård 2002). Only some granular fly bait labels explicitly discourage bait application directly to manure.

Granular fly baits containing methomyl, imidacloprid, or dinotefuran are widely available, and have been well-studied for their efficacy against house flies (Darbro and Mullens 2004, Butler et al. 2007, White et al. 2007), including their behavioral resistance or attraction to the baits (Murillo et al. 2014, Seraydar and Kaufman 2015). However, there are currently no studies on the behavioral response of filth fly parasitoids to granular fly baits.

The present study examined the behavioral and toxicological responses of *S. endius* and *U. rufipes* to three granular fly baits. The baits contained methomyl, imidacloprid or dinotefuran. Two important components of many granular baits, including those tested here, were also tested, the active ingredient (AI) and the house fly pheromone (Z)-9-tricosene (also called muscalure). The pheromone is used to increase the attraction of flies to bait (Chapman et al. 1998, 1999 and references therein, but see Butler et al. 2007). It is a cuticular hydrocarbon with low volatility that acts at short range (Hanley et al 2004). The pesticides tested are also of low volatility. Methomyl is an older pesticide that is more toxic to mammals than many of the more recent classes of pesticides, like neonicotinoids. Imidacloprid and dinotefuran are neonicotinoids. Both methomyl and neonicotinoids are known to adversely impact some beneficial insect species, including some hymenopterans (Kok et al. 1996, Prabhaker et al. 2011, Krupke and Long 2015). However, there are few studies of effects of these pesticides on parasitoids of filth flies (Burgess and King 2015, Owens et al. 2015, Whitehorn et al. 2015).

## Materials and Methods

**Laboratory Colonies.** The parasitoids used in this study were from laboratory-maintained colonies of *S. endius* and *U. rufipes*. The *S. endius* colony was established with parasitoids from a poultry farm in Zephyr Hills, FL, in 1996. Vouchers are at the Illinois Natural History Survey Center for Biodiversity, catalog numbers Insect Collection 6035 through 6054 for *S. endius* and 814731 through 814750 for *U. rufipes*. The *U. rufipes* colony was established with parasitoids from cattle feedlots in southern Alberta in 2008. As in any study with single strains, differences found between species may be colony specific rather than species specific. Parasitoid performance is known to vary among colonies, but mean longevity does not consistently increase or decrease with colony age (Machtinger et al. 2015). The pupae used to rear the parasitoids were the “NIU Strain” colony of *M. domestica* from Burgess and King (2015). None of the colonies had been exposed to pesticides since establishment.

All experiments were temporally blocked by treatment, but not by species; however, the species were tested with the same protocols at consistent temperature ( $22.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ ) within the same month; and RH during testing did not significantly differ between species ( $p > 0.05$ ).

**Pesticides, AIs and Fly Pheromone Sources.** The granular fly baits used in this study were methomyl bait (Golden Malrin, Wellmark, Schaumburg, IL), imidacloprid bait (QuickBait Fly Bait, Bayer, Shawnee Mission, KS), and dinotefuran bait (QuikStrike Fly Scatter Bait, Wellmark, Schaumburg, IL). The AIs that were tested were methomyl (99.5% purity), imidacloprid (99.5%), and dinotefuran (98.2%) (all from Chem Service, West Chester, PA). The AIs were dissolved in pesticide-grade acetone (Chem Service, West Chester, PA). The fly pheromone (Z)-9-tricosene is sold as a liquid (97%, Sigma-Aldrich, St. Louis, MO).

**Granule Behavior Experiments.** A 2 cm circle was drawn with marker on the outside, bottom, center of a Pyrex petri dish (9 cm diameter x 1.5 cm height). A single layer of one of the three baits was placed inside the dish to cover the entire area of the circle. A control dish with clean fine silica sand in place of bait was done simultaneously. One 0-4 d old female of either *S. endius* or *U. rufipes* was placed on the inside perimeter of each dish. Parasitoids were observed for 10 min. The number of times each parasitoid contacted the bait (or sand) or groomed herself was counted. A single grooming episode was defined as beginning when a female stopped walking and used her tarsi to stroke her antennae, wings, or other body parts, or rubbed her tarsi together. A grooming episode was considered complete when the female began walking again. For each species, four females were tested per treatment, including a control, on each of 6 d for a total of  $n = 24$  females for each

treatment, which was 96 females altogether. Each female was tested only once in this and subsequent experiments.

**Residue Survival Experiments.** The inside surfaces of a Pyrex petri dish (9 cm diameter x 1.5 cm height) were coated with the powdery residue of bait by gently rolling around 1 g of one of the three baits for approximately 30 s. After 30 s, any loose granules were poured out, and a glass microscope cover slip (22 mm x 22 mm) containing a small drop of honey mixed with water was placed in the center bottom of the dish. The control was a clean dish, also with a cover slip with honey solution in the center. Ten 0-3 d old female *S. endius* or ten 0-3 d old female *U. rufipes* were placed in the center of the cover slip in each dish. Number of dead and moribund parasitoids (defined in Burgess and King 2015) was assessed at three different time intervals, 10 min, 1 h, 2 h; but no parasitoids were removed. For each species, five females were tested per treatment, including control, which was 20 females altogether.

**AI LC<sub>50</sub>.** The LC<sub>50</sub> value was determined for each AI for *S. endius* and for *U. rufipes* at the same time and with the same contact assay protocol as in Burgess and King (2015). Test concentrations were created using a combination of serial and parallel dilutions from a 1 ml stock solution. The stock solution was made new for each replicate, by weighing the AI and dissolving it in 1 ml of acetone. Each test concentration, acetone was added to a calculated volume of the stock solution, bringing the volume to 1 ml. For each AI, twenty 0-3 d old females were tested in each 20 mL (42.8 cm<sup>2</sup> inner surface area) glass test vial, the insides of which had been coated with 0.5 ml of one of five concentrations of an AI; and mortality was assessed after 48 h. Thus there were 100 females per replicate, i.e., per set of five vials, one vial for each concentration). The concentrations produced mortalities > 0% and < 100%, and were determined by initial concentration fixing experiments. Honey and water solution (1:1) was provided on the cotton plug of each test vial. Each replicate was performed three to five times per AI per species. Results for *S. endius* are in Burgess and King (2015) but are reported again to facilitate comparisons (Table 1).

**Table 1. LC<sub>50</sub> values of three active ingredients (AI) found in three house fly granular baits, for *Spalangia endius* and for *Urolepis rufipes* under forced exposure to treated glass surfaces.**

Species	Treatment (AI)	<i>n</i>	Slope (SE)	LC <sub>50</sub> <sup>a</sup> (95% CI)	χ <sup>2</sup> ( <i>p</i> -value)	Recommended Application Rate <sup>a</sup>
<i>S. endius</i> <sup>b</sup>	Imidacloprid	300	0.5 (0.1)	17.9 (8.3 – 38.0)Ba	4.7 (0.19)	915.4
	Methomyl	400	4.8 (0.5)	14.7 (13.6 – 15.9)Ca	0.9 (0.63)	2684.0 <sup>c</sup>
	Dinotefuran	400	3.1 (0.3)	52.2 (46.4 – 58.4)Db	2.9 (0.41)	1220.6
<i>U. rufipes</i>	Imidacloprid	400	2.6 (0.2)	10.4 (9.0 – 11.8)Bb	4.2 (0.25)	915.4
	Methomyl	400	3.4 (0.3)	10.4 (9.3 – 11.6)Bb	3.7 (0.30)	2684.0
	Dinotefuran	500	3.1 (0.2)	0.8 (0.7 – 0.9)Aa	7.0 (0.07)	1220.6

Within each species, LC<sub>50</sub> values followed by the same lower case letter do not differ significantly based on overlap of their 95% CI. Between species within each AI, upper case letters do not differ significantly based on overlap of their 95% CI.

<sup>a</sup> In units of ng / cm<sup>2</sup>

<sup>b</sup> Data collected simultaneously with *U. rufipes*; reported previously in Burgess and King (2015), shown here for comparison.

<sup>c\*</sup> Concentration from more recent label than the 2441.25 ng/cm<sup>2</sup> reported in Burgess and King (2015).

**AI Behavior Experiments.** A choice test was done with *S. endius* and with *U. rufipes* to assess the level of aversion or attraction that the parasitoids have to the AIs. A polystyrene petri dish (10 cm x 1.5 cm) was used

as the testing arena. A quantity of AI equivalent to that found in a single granule of one of the three tested baits (11.07 µg imidacloprid, 192.27 µg methomyl, 20.73 µg dinotefuran) was dissolved in acetone and then pipetted onto a glass cover slip (22 mm x 22 mm). A clean cover slip was used as a control. One cover slip was placed far left and one far right, with each equidistant from a center line drawn on a piece of white 22 cm x 28 cm paper under the dish. Sides on which the treatment and control were placed were alternated to control for side bias. A 0-5 d old female was placed in the center of the dish, and the amount of time she spent on each half of the dish during 10 min of observation was recorded using two stopwatches. This was replicated 15 times for each of the three AIs for each of the species.

**Fly Pheromone Behavior Experiments.** A choice test was done both with *S. endius* and with *U. rufipes* to assess the level of aversion or attraction that the parasitoids have to (Z)-9-tricosene, the fly pheromone in many fly baits. The protocol was the same as in the AI experiment, except the treatment cover slip contained (Z)-9-tricosene. One set of females was tested with a large quantity (8.57 mg, 10.63 µL) and one set with a medium quantity (2.21 mg, 2.74 µL), each against a clean control. The large quantity corresponds to the approximate amount per 1000 granules of methomyl bait. The medium quantity corresponds to the approximate amount per 1000 granules of imidacloprid bait. (The approximate amount per 1000 granules of dinotefuran bait (1.66 mg, 1.34 µL) was not tested). 1000 granules of bait is an estimate for the amount of bait granules that will fit in a typical commercial bait station. Chapman et al. (1998, 1999) showed house fly attraction to 5g of 65% (Z)-9-tricosene, 15% (E)-9-tricosene; whereas Butler et al (2007) found no house fly attraction to 5 µL of 97% (Z)-9-tricosene (Aldrich Chemical, Milwaukee, WI).

**Statistical Analyses.** In the Granule Behavior experiments, the data on number of contacts by each female included many zeroes, so analyses were on the presence or absence of any contact by each female. The effect of treatment was tested for each species, using Pearson's chi-square tests of independence with Yates correction for continuity (Yates 1934). Number of grooming episodes was compared among treatments with generalized linear models for each species, using the glm procedure in R version 3.2.2 (R Core Team 2015). Model selection was by a step-down technique (Crawley 2013). A quasi-Poisson distribution was used to account for over dispersion in the data. Multiple comparison of treatments was with Tukey's test, using the R package 'multcomp' (Hothorn et al. 2008).

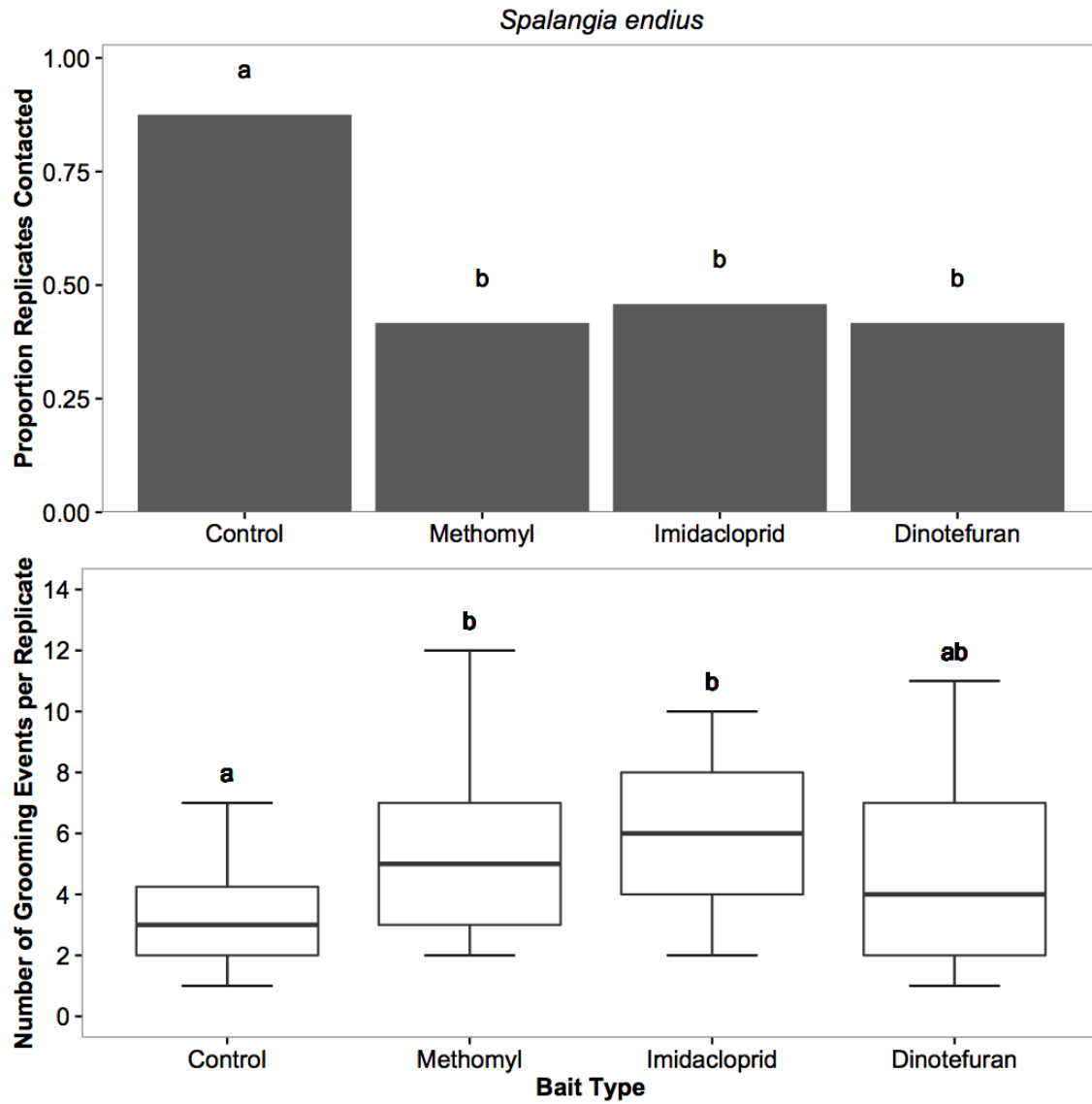
In the Residue Survival experiments, effect of treatment on number surviving versus not surviving was tested using contingency tests. For each test, if expected cell frequencies were five or smaller, Fisher's Exact Test with Monte Carlo simulation was used to generate a P value (based on 2000 replicates) (Freeman and Halton 1951); and if expected cell frequencies were greater than five, a chi square test of independence was used (Zar 2007).

AI LC<sub>50</sub> values and their 95% confidence intervals were calculated using probit analysis (SPSS 2012). Abbott's formula was used to correct for control mortality (Abbott 1925).

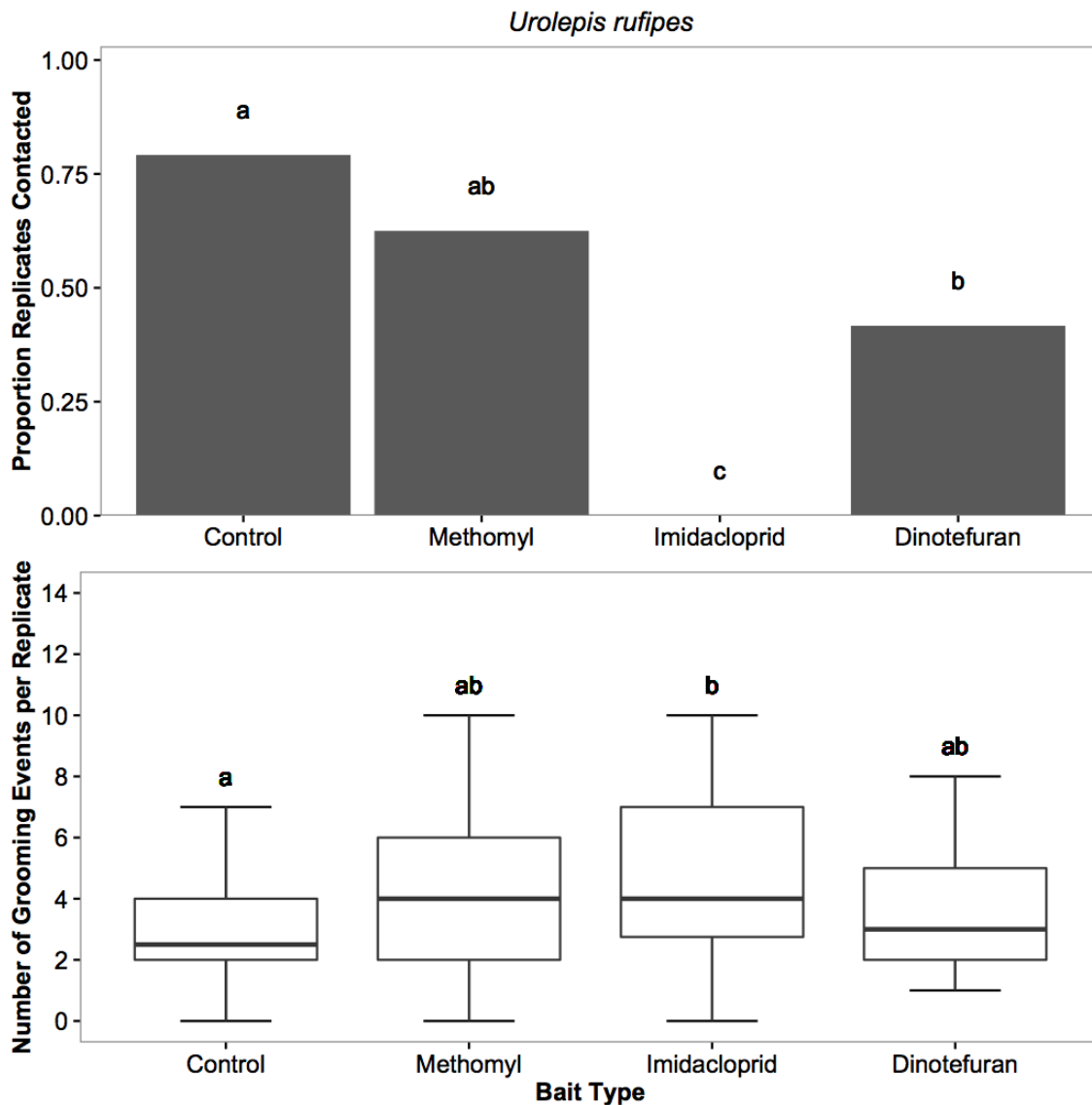
In the AI Behavior experiment and in the Fly Pheromone Behavior experiment the amount of time spent in each half of the arena was compared using a paired t-test. This is equivalent to asking whether the difference between the times spent on the two sides differs from 0.

## Results

**Granule Behavior Experiments.** In *S. endius*, the proportion of females that had any contact with the circle of bait or sand depended on treatment (Fig. 1;  $\chi^2 = 14.43$ , df = 3,  $p = 0.002$ ), but there was no significant difference in proportion that had contact among the baits ( $\chi^2 = 0.11$ , df = 2,  $p = 0.94$ ). Combining all observations of contact with baits, a significantly lower proportion of females contacted bait than the control ( $\chi^2 = 12.59$ , df = 1,  $p < 0.001$ ), about half as many females contacted bait, about 40% of females. There was a significant effect of treatment on number of times a female *S. endius* groomed (Fig. 1;  $F = 7.52$ , df = 3, 92,  $p < 0.001$ ). There was significantly more grooming with methomyl bait than with the control ( $z = 3.66$ ,  $p = 0.002$ ), and with imidacloprid bait than with the control ( $z = 4.31$ ,  $p < 0.001$ ); but no other pairwise comparisons were significant ( $p > 0.05$ ).



**Fig. 1. Proportion of female *S. endius* for which contact was observed during a 10 min exposure of single females to a sand pile control or to a methomyl, imidacloprid, or dinotefuran bait pile and number of grooming events during that 10 min. Bars in the interquartile ranges represent medians, and the whiskers represent the upper and lower range ( $n = 24$  females for each bait type). Letters that differ represent statistical differences at  $\alpha = 0.05$ .**



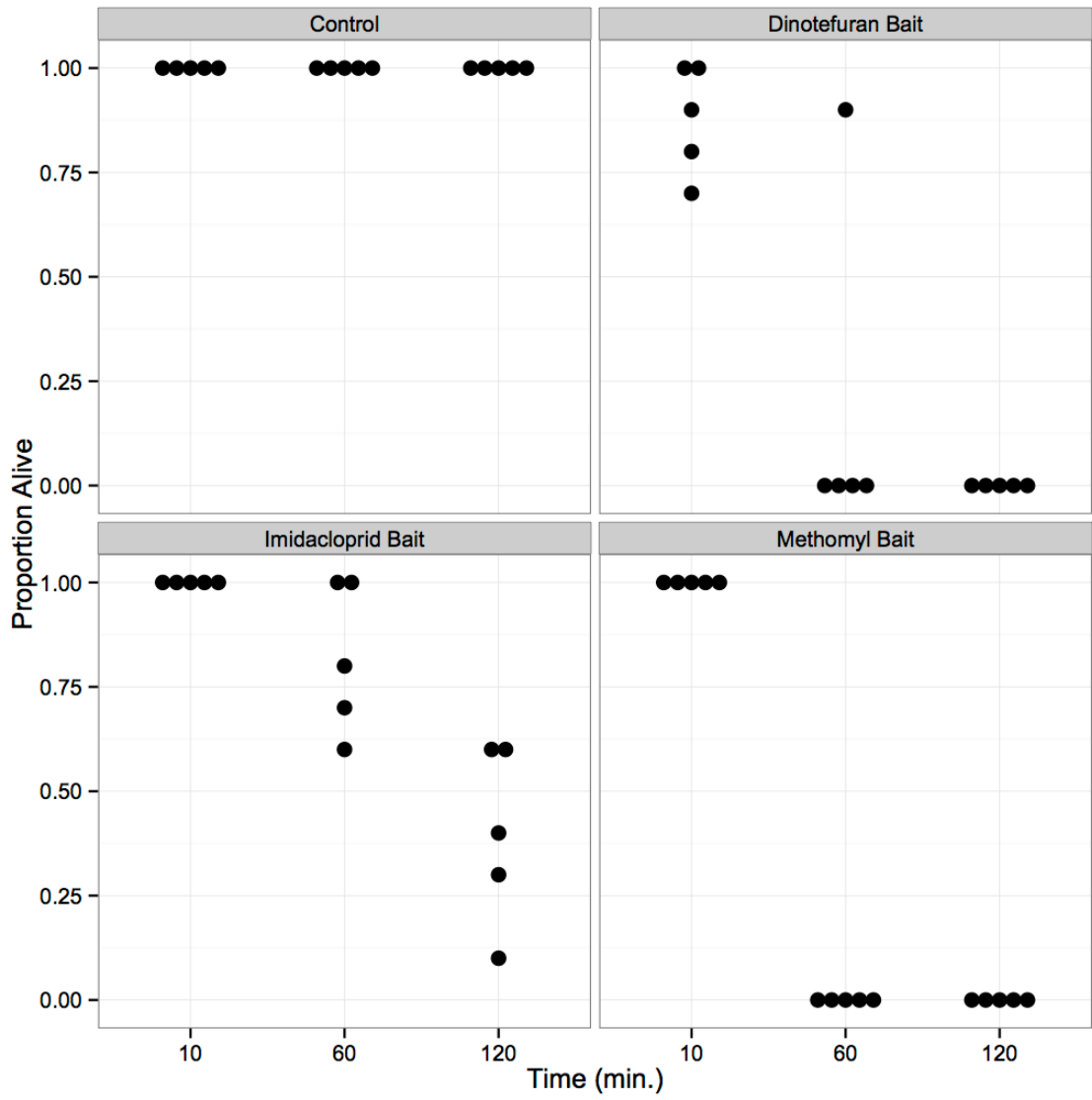
**Fig. 2. Proportion of female *U. rufipes* for which contact was observed during a 10 min exposure of single females to a sand pile control or to a methomyl, imidacloprid, or dinotefuran bait pile and number of grooming events during that 10 min. Bars in the interquartile ranges represent medians, and the whiskers represent the upper and lower range ( $n = 24$  females for each bait type). Letters that differ represent statistical differences at  $\alpha = 0.05$ .**

In *U. rufipes*, whether a female had any contact with bait granules or sand depended on treatment (Fig. 2;  $\chi^2 = 33.90$ ,  $df = 3$ ,  $p < 0.001$ ), and differed among the baits ( $\chi^2 = 21.45$ ,  $df = 2$ ,  $p < 0.001$ ). No females contacted imidacloprid bait, which was significantly less than contacted dinotefuran bait ( $\chi^2 = 10.23$ ,  $df = 1$ ,  $p = 0.001$ ) or methomyl bait ( $\chi^2 = 19.01$ ,  $df = 1$ ,  $p < 0.001$ ); but there was no significant difference between dinotefuran bait and methomyl bait ( $\chi^2 = 1.34$ ,  $df = 1$ ,  $p = 0.25$ ). Relative to the control, a significantly lower proportion of females contacted the imidacloprid bait ( $\chi^2 = 28.23$ ,  $df = 1$ ,  $p < 0.001$ ) and the dinotefuran bait ( $\chi^2 = 5.58$ ,  $df = 1$ ,  $p = 0.018$ ), but not methomyl bait ( $\chi^2 = 0.91$ ,  $df = 1$ ,  $p = 0.34$ ). In *U. rufipes*, there was a significant effect of treatment on number of grooms (Fig. 2;  $F = 2.90$ ,  $df = 3$ ,  $92$ ,  $p = 0.039$ ). There were significantly more grooms with imidacloprid bait than with the control ( $z = 2.77$ ,  $p = 0.029$ ), but no other pairwise comparisons were significant (all  $p > 0.05$ ).

**Residue Survival Experiments.** All controls in both species had 100% survival (Fig. 3, 4). There was little overall reduction of survival observed in *S. endius* based on treatment at 10 min. However, at 1 h, there was a notable reduction in survival, and survival was dependent on bait residue type ( $\chi^2 = 83.58$ ,  $df = 2$ ,  $p < 0.001$ ). Effect of bait residue starting with greatest mortality was methomyl bait > dinotefuran bait >

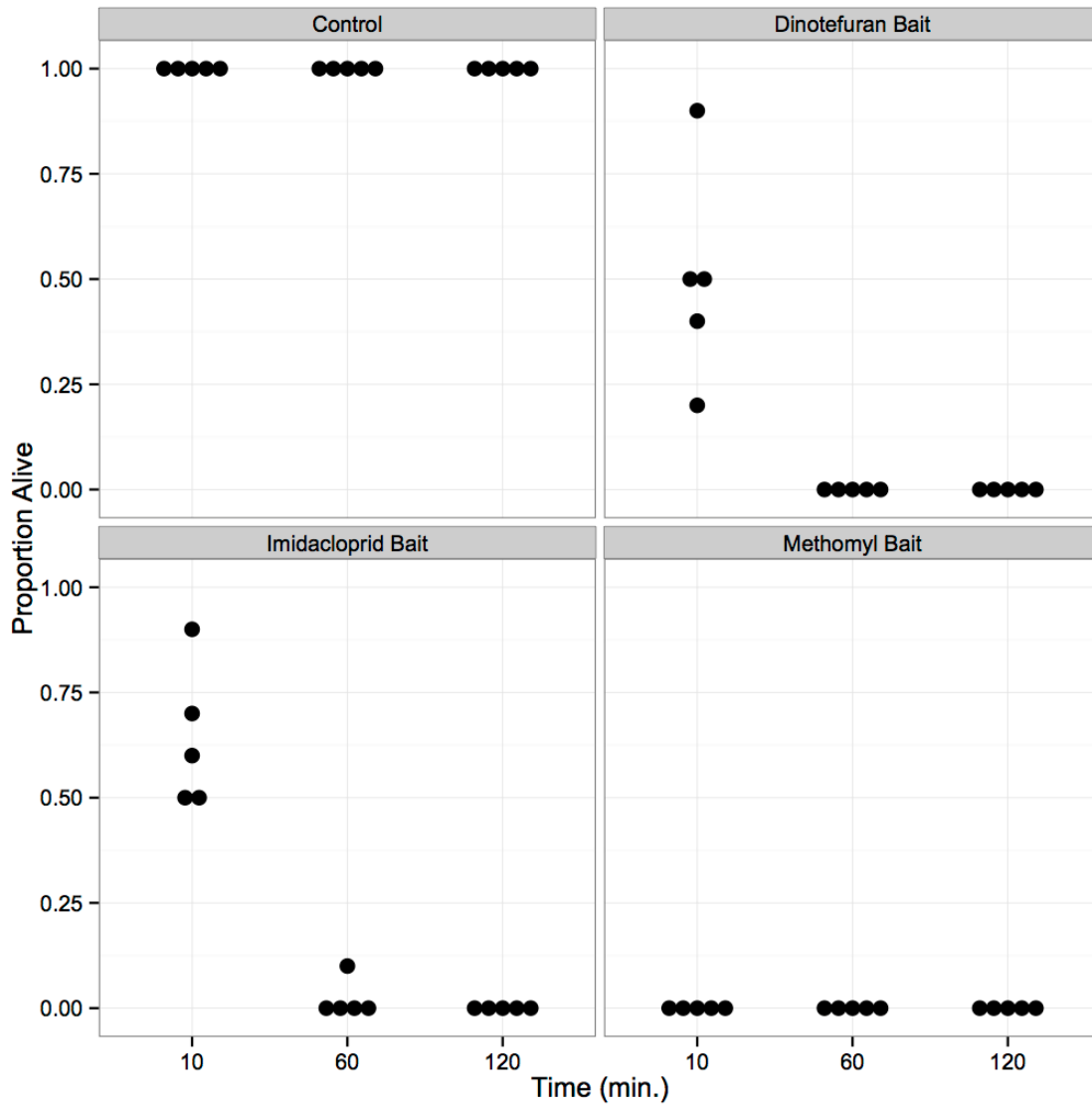
imidacloprid bait > control, with all pairwise comparisons significant (all  $p < 0.002$ ). By 2 h, all *S. endius* were dead in the dinotefuran and methomyl treatments.

By 10 min of contact with bait residues, the proportion of deaths in *U. rufipes* was dependent on bait type ( $\chi^2 = 48.05$ ,  $df = 2$ ,  $p < 0.001$ ). The proportion dead at 10 min did not differ between imidacloprid bait residue and dinotefuran bait residue, but all other pairwise comparisons were significant (all  $p < 0.001$ ). Thus effect of bait residue starting with greatest mortality was methomyl bait > dinotefuran bait = imidacloprid bait > control.



**Fig. 3. Proportion of *Spalangia endius* females surviving when exposed to bait residue.** Each black dot represents the proportion of ten female wasps surviving at each time interval in each of five replicates ( $n = 50$  wasps total for each bait type).





**Fig. 4. Proportion of *Urolepis rufipes* females surviving when exposed to bait residue.** Each black dot represents the proportion of ten female wasps surviving at each time interval in each of five replicates ( $n = 50$  wasps total for each bait type).

**AI LC<sub>50</sub>.** When exposed to just the AI, there was a difference in the order of toxicity between the two parasitoids, with dinotefuran being the most toxic of the three AIs for *U. rufipes*; but the least toxic for *S. endius* (Table 1). In *U. rufipes*, the order of toxicity was dinotefuran > imidacloprid = methomyl, with a 12.8-fold difference between the largest and smallest LC<sub>50</sub> values. In *S. endius* the order of toxicity was imidacloprid = methomyl > dinotefuran, with a 3.5-fold difference between the largest and smallest LC<sub>50</sub> values. *U. rufipes* was susceptible at lower concentrations of methomyl and dinotefuran than was *S. endius*, especially for dinotefuran.

**AI Behavior Experiments.** *S. endius* was attracted to imidacloprid but neither avoided nor was attracted to dinotefuran or methomyl (Table 2). *U. rufipes* neither avoided nor was attracted to any of the AIs.

**Table 2. Mean ± SE difference in times (s) spent in arena halves with and without AI by solitary female *Spalangia endius* and *Urolepis rufipes*.**

Species	Treatment	Difference (s)	$t^a$	df	$p$ -value
<i>S. endius</i>	Imidacloprid AI	168.27 ± 76.03	2.21	14	0.04
	Dinotefuran AI	-11.87 ± 81.76	-0.15	14	0.89
	Methomyl AI	40.27 ± 82.97	0.49	14	0.64
<i>U. rufipes</i>	Imidacloprid AI	-80.67 ± 107.79	-0.75	14	0.47

Dinotefuran AI	22.27 ± 111.05	0.20	14	0.84
Methomyl AI	76.80 ± 98.61	0.78	14	0.45

<sup>a</sup> paired t-test

**Fly Pheromone Behavior Experiments.** *S. endius* avoided the pheromone at both quantities, whereas *U. rufipes* neither avoided nor was attracted to the pheromone at either concentration (Table 3).

**Table 3. Mean ± SE difference times (s) spent in arena halves with a medium or large quantity of fly pheromone minus time spent in the arena half with the control by solitary female *Spalangia endius* and *Urolepis rufipes*.**

Species	Treatment	Difference (s)	<i>t</i> <sup>a</sup>	df	<i>p</i> -value
<i>S. endius</i>	Medium Quantity Pheromone	-328.13 ± 52.69	-6.23	14	< 0.001
	Large Quantity Pheromone	-282.80 ± 69.61	-4.06	14	0.001
<i>U. rufipes</i>	Medium Quantity Pheromone	-76.40 ± 86.35	-0.89	14	0.39
	Large Quantity Pheromone	-76.53 ± 64.79	-1.18	14	0.25

<sup>a</sup> paired t-test.

### Discussion

Results of the present study indicate that some baits may be of minimal harm to parasitoids because of the parasitoids' behavioral avoidance of the baits. This avoidance was seen in the present study even though the parasitoid strains had no recent exposure (if any) to these baits or to the active ingredients in them. Relative to the control, both *S. endius* and *U. rufipes* avoided contact with dinotefuran bait and imidacloprid bait. *S. endius* also avoided methomyl bait. *U. rufipes* was not significantly attracted or repelled by methomyl bait. *S. endius* may have avoided all three baits because the fly pheromone was repellent. In contrast, the fly pheromone had no apparent effect on *U. rufipes*' response to these baits; there was neither aversion nor attraction to fly pheromone alone. *U. rufipes* also did not have an aversion or attraction to any of the three AIs. The lack of aversions to both AI and fly pheromone suggest that *U. rufipes*' aversion to bait must be to another component of the bait or to an interaction among components. *S. endius* showed neither aversion nor attraction to any of the AI residues, except for imidacloprid, to which it had an attraction. Given that *S. endius* avoided all baits, this attraction may have been overcome by the strength of the aversion to the fly pheromone. As with *U. rufipes*, other bait components that were not tested may also have contributed to *S. endius*' avoidance of baits.

Some components of fly baits are proprietary, e.g., other chemical attractants (Butler et al. 2007). The imidacloprid bait contains "two fly attractants to lure flies" (BayerLivestock.com), although the ingredients list only imidacloprid 0.5% and Z-9 tricosene 0.1%. The present study suggests that imidacloprid AI is an attractant to some parasitoids. Whether aversion to imidacloprid will evolve in parasitoid populations exposed to imidacloprid, like it may have in house flies (Gerry and Zhang 2009), remains to be seen. The mechanism of attraction to imidacloprid in *S. endius* is unknown. When provided a choice, some honey bees and buff-tailed bumblebees preferentially eat sucrose if it contains the neonicotinoid imidacloprid or thiamethoxam, although recently emerged adult workers avoid sucrose solutions with low concentrations of imidacloprid (Kessler et al. 2015). None of the bees can taste the neonicotinoids with their mouthparts, based on recordings from gustatory neurons. The bees that prefer the sucrose solutions containing the pesticides are thought to do so as a learned response that results from the neonicotinoids binding to reward centers in the bees' brains. In contrast to *S. endius*, beetles and flies in a grassland in Scotland avoided traps containing imidacloprid (Easton and Goulson 2013).

Imidacloprid fly bait may also include Bitrex (denatonium benzoate), a bittering agent (Bayer HealthCare 2011). Denatonium benzoate has been used in many fly baits to reduce ingestion by pets and children (Payne and Tracy 1995). Denatonium benzoate is also a feeding deterrent to pest *Vespa* wasps (Sackmann et al. 2010), adult tobacco budworms, *Heliothis virescens* Fabricius (Ramaswamy et al. 1992), and blow fly spp. (Liscia et al. 2004). Its effect on house flies and their parasitoids, e.g., as a repellent or feeding deterrent, remains to be investigated.

Although both parasitoid species avoided at least some baits relative to the control, some *S. endius* and *U. rufipes* individuals contacted each bait, with the exception of no *U. rufipes* individuals contacting imidacloprid bait. Imidacloprid bait granules appear to irritate both parasitoid species, in that both groomed more in the bait's presence. Grooming is likely initiated when the insect's mechano- or chemoreceptors are stimulated by chemical or tactile stimuli (Reingold and Camhi 1978). Grooming included frequently contacting tarsi to mouthparts, potentially increasing the chances of pesticide ingestion.

That the parasitoids avoid some bait components that house flies find attractive is helpful in terms of developing baits. Unlike the parasitoids, house flies are attracted to (Z)-9-tricosene in many studies, although not all (reviewed in Butler et al. 2007).

*U. rufipes* was more sensitive to the three AIs tested here than *S. endius* was (Table 1). Likewise, in tests of earlier pesticides, *U. rufipes* was particularly susceptible to pesticides relative to other pupal parasitoids of filth flies, including *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) (Rutz and Scott 1990). Different metabolic pathways are required to breakdown the different chemical classes (Simon-Delso et al. 2015), but perhaps *S. endius* is better equipped than *U. rufipes* to metabolize all three pesticides. *U. rufipes*' greater sensitivity probably was not just a result of more contact (Table 2).

Of the AIs tested in the present study, imidacloprid appears to be the best choice for *U. rufipes*. Females avoided the granules the most, were less quickly killed by bait residue than with methomyl and had a higher LC<sub>50</sub> than with dinotefuran AI. Of the baits tested, imidacloprid bait appears to result in lower mortality for *S. endius* than the other baits. But that is in the short term (2 h, Fig. 3), and imidacloprid can cause delayed deaths (Suchail et al. 2001, Hu et al. 2010). Longer-term survival tests with the AIs suggest that dinotefuran may be the least bad choice for *S. endius*, even relative to the recommended dosage (0.04 relative ratio versus 0.02 for imidacloprid, 2 d, Table 1).

Results of the present study reinforce the importance of looking not only at physiological effects of pesticides, but also at behavioral effects. The pesticide AIs in current filth fly baits are enough to kill the parasitoid wasps *S. endius* and *U. rufipes* upon contact, with LC<sub>50</sub> values more than ten to a hundred times less than in the baits (Burgess and King 2015, present study). However, the behavior of the parasitoids is expected to reduce their exposure. In addition, much parasitoid habitat may have only runoff or residue from baits, meaning parasitoids there will encounter lower concentrations. Until how these pesticides disseminate through parasitoid habitat is known, we recommend that baits not be applied close to known parasitoid habitat. The bait labels currently recommend application outside livestock facilities, on walkways indoors, or in bait stations. However, manure and other rotting organic matter are stored outside of livestock buildings, and *S. endius* and other parasitoids are found there (personal observation; Smith et al. 1998). More data on where adult parasitoids travel would be helpful, e.g., studies like Skovgård (2002) for additional parasitoid species and at a variety of livestock-facility types.

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