Assessing the compatibility of filth fly pesticides with filth fly biological control parasitoids through toxicological and behavioral assays

Edwin R. Burgess IV

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ABSTRACT

ASSESSING THE COMPATIBILITY OF FILTH FLY PESTICIDES WITH FILTH FLY BIOLOGICAL CONTROL PARASITOIDS THROUGH TOXICOLOGICAL AND BEHAVIORAL ASSAYS

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Northern Illinois University, 2016
Dr. Bethia H. King, Director

Filth flies are a significant pest at animal production facilities, leading to economic losses that include reductions in animal weight and milk production. Pesticides and biological control organisms are two important components of Integrated Pest Management (IPM) programs against filth flies. Biological control against filth flies frequently involves augmentative releases of pupal parasitoids. The compatibility of pesticides and the parasitoids Spalangia endius Walker and Urolepis rufipes Ashmead was examined using one of the most common filth flies, the house fly, Musca domestica L. The specific goals were to 1) assess the effects of the active ingredients of several house fly pesticides on house flies relative to S. endius using a new index, 2) examine the behavior and survival of S. endius and U. rufipes in response to three granular house fly baits and components, and 3) examine sublethal effects of exposure to a common neonicotinoid,
imidacloprid, on *S. endius*.

For the first goal, a Pesticide Compatibility Index (PCI) was created. This index allows comparison of LC$_{50}$ values between pest and biological control organism even when the mode of exposure to a pesticide differs, e.g., exposure by contact versus by feeding, and the index takes into account recommended pesticide dosages. Bioassays of survival were performed using five pesticides presented in the mode in which each organism was expected to be exposed to the pesticides, a surface contact bioassay for *S. endius* and a feeding bioassay for *M. domestica*. The PCI index was computed by first converting LC$_{50}$ values into units of prescribed dosages (LPR = LC$_{50}$-to-prescribed dosage ratio). Prescribed dosages from labels of granular baits were used. PCI was calculated as the ratio of LPR$_{\text{biological control agent}}$ to LPR$_{\text{pest}}$. Based on PCI values, order of compatibility with *S. endius* was spinosad > thiamethoxam > dinotefuran > methomyl > imidacloprid. That spinosad was better than imidacloprid or methomyl, both for parasitoid survival and for killing flies, was consistent with conclusions from the LC$_{50}$ values. Permethrin and nitenpyram were also tested, but their PCIs were not calculated, so they were compared to the other pesticides using LC$_{50}$ values. PCIs were not calculated because prescribed bait dosages were not available: permethrin is prescribed as a contact pesticide against flies rather than being consumed as a bait and nitenpyram has not been formulated as a fly pesticide. Permethrin was moderately toxic to *S. endius* but one of the most toxic of the pesticides for *M. domestica*; whereas nitenpyram was the least toxic of the pesticides for both *S. endius* and *M. domestica*.

For the second goal, behaviors and mortality of *S. endius* and *U. rufipes* 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$_{50}$ 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 resistance of the parasitoids occurred despite no exposure recently, if ever, to these pesticides.

For the third and final goal, I determined if imidacloprid, the most commonly used pesticide against filth flies, would impact the ability of *S. endius* to act as an effective biological control if they did not initially die from exposure. Exposure to an LC$_{50}$ of imidacloprid decreased the ability of surviving individuals of the parasitoid wasp *S. endius* to kill house fly pupae under some conditions. In an unburied hosts experiment, significantly more flies and fewer parasitoids emerged in the LC$_{50}$ imidacloprid treatment versus the LC$_{10}$ or controls; parasitoid sex ratio and longevity were not affected. However, in a buried hosts experiment, parasitoid and fly emergence were independent of treatment. ELISA (enzyme-linked immunosorbent assay) showed lower imidacloprid residues in or on parasitoids that had been exposed to the media in
which hosts were buried. These findings suggest that substrate may reduce pesticides on biological control agents that burrow, making these agents more effective. Pesticides formulated to target filth flies are strong enough to kill *S. endius* and *U. rufipes* many times over. It is thus very important to carefully consider locations in which these pesticides are placed so as to minimize the likelihood of inadvertent exposure by biological control organisms like *S. endius* and *U. rufipes*. Based on these results, no current pesticide used for control of filth flies is 100% safe to *S. endius* or *U. rufipes*. That granular baits containing imidacloprid, methomyl, or dinotefuran were not attractive to either parasitoid is encouraging. However, use of *U. rufipes* with methomyl bait is not recommended. Of the parasitoids and baits tested, *S. endius* in conjunction with dinotefuran bait (QuikStrike) appears to be the best combination, based on the best PCI value and *S. endius*’s burrowing habit removing pesticide. The behavioral response of filth fly parasitoids to spinosad bait and thiamethoxam bait would be worth investigating. My results reinforce the importance of looking not only at traditional physiological effects of pesticides but also at behavioral responses.
ASSESSING THE COMPATIBILITY OF FILTH FLY PESTICIDES WITH FILTH FLY BIOLOGICAL CONTROL PARASITOIDS THROUGH TOXICOLOGICAL AND BEHAVIORAL ASSAYS

BY

EDWIN R. BURGESS IV
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DEDICATION

I dedicate this work to my father, Edwin R. Burgess III, whom I miss every day.
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CHAPTER 1

AN INTRODUCTION TO FILTH FLIES AND THEIR CONTROL

The house fly (*Musca domestica* L.) and stable fly (*Stomoxys calcitrans* L.) (Diptera: Muscidae) are members of an important group of pests known as filth flies (Gibson and Floate 2004). Their economic importance comes largely from their impact on animal rearing facilities. Animal confinements are attractive to filth flies because of their production of manure and decaying organic material, where filth flies breed.

Biting filth flies are a major stress inducer for dairy and feedlot cattle, reducing milk production in dairy cattle and decreasing weight gain in feedlot cattle (Taylor et al. 2012). They are also a vector for many disease-causing organisms of both humans and livestock, especially *Trypanosoma* (D’Amico et al. 1996), which are protists, and *Habronema*, which are nematodes (Foil and Hogsette 1994). Equine infectious anemia, a retrovirus transmitted by many hematophagous arthropods, can also be transmitted by filth flies.

Non-biting filth flies, such as the house fly, can induce stress and be a disease vector for both humans and livestock, transmitting gastroenteric pathogens such as *Escherichia coli*, *Shigella* spp., and *Salmonella* spp. (Geden 2012). Increased incidence of food-borne diarrheal diseases have also been connected to seasonal filth fly abundance (Graczyk et al. 2005). House flies can be difficult to control because of the range of substrates they can utilize, the rate at which they reproduce (Larrain and Salas 2008), and their rapidly acquired resistance to numerous
insecticides (Bong and Zairi. 2010). Attempting to control filth fly populations in livestock confinements often requires using several modalities (Malik et al. 2007).

At the forefront of all filth fly control regiments is sanitation, i.e., the removal of potential breeding sites by removing manure and other decaying organic matter and keeping such matter too dry or too wet for fly larvae to develop (Geden 1999). However, completely relying on sanitation is not always practical or cost effective. An Integrated Pest Management (IPM) approach stresses minimizing negative economic and environmental effects by considering all possible means of control and at all stages of planning. Insecticides are one widely used tool in IPM.

Insecticides are applied in numerous ways for filth fly control. Insecticides may be applied directly on the animal using back scrubbers or may be scattered or sprayed throughout their confinement (Malik et al. 2007). Sprays may be applied directly onto surfaces, e.g., walls and manure, or in the form of a mist or fog. A bolus impregnated with a larvicide can be placed in the animal’s stomach or in feed and acts as a source of continual chemical control as the animal defecates manure containing the larvicide.

Another, and among the most popular of the modalities of insecticide delivery, is insecticide-based granular fly baits. Such baits are thought to cause less harm to beneficial insect species because they are designed to attract flies (Loftin et al. 2008). They include sugar and often fly pheromone. Most of them can be applied by sprinkling particles on the ground, by putting the bait in a bait trap or jug, or by mixing with water and painting on, e.g., walls for example. Three popular insecticides and their fly baits are dinotefuran (Quikstrike), imidacloprid (Quickbayt), and methomyl (Golden Malrin) (White et al. 2007). However, there are numerous
reports of isolated populations of filth flies around the world with acquired resistance to all three of these popular insecticides (Kaufman et al. 2010, Memmi 2010, Geden 2012), as well as to permethrin and other pyrethroids (Pitzer et al. 2010). Rapidly acquired resistance is heritable, and the genetic mechanisms have been studied. The mechanism of rapidly acquired resistance does not seem to involve the pest fly eliminating only molecules of the specific insecticide to which the pest population was exposed. Rather, exposure seems to produce pervasive cross-resistances because common insecticidal synergists are used in many new insecticide formulations (Pospischil et al. 1996). The rampant onset of insecticide resistance has brought about interest in biological control agents as an additional means to control filth flies (Geden 2012), and some of these agents are sold commercially.

Pupal parasitoid wasps of the family Pteromalidae are one such biological control agent used against filth flies, and they naturally inhabit animal confinements (Gibson and Floate 2004, Romero et al. 2010, Pitzer et al. 2011). Efficacy depends on temperature as well as which species is being released (Geden and Hogsette 2006, McKay et al. 2007, Birkemo et al. 2008, Peterson et al. 2009). The efficacy of using parasitoid wasps to control fly populations also depends on the type and depth of the substrate in which the flies are breeding (Rueda and Axtell 1985a, Geden 2002).

Two such pupal parasitoids are Spalangia endius and Urolepis rufipes. S. endius is a 2-3 mm long parasitoid wasp that utilizes various dipteran hosts in their pupal stage, including house flies and stable flies (Morgan et al. 1978). Females drill into the hosts not only to oviposit but also to feed on host fluids that ooze out from wounds on the host caused by her drilling. Doing so often kills the host. Adults will also eat honey or sugar water in the laboratory and presumably
nectar and other liquid nutrients in more natural conditions (King 2002, King and Kuban 2012). The different parasitoid species of filth flies vary in their tendency to burrow in search of hosts (Geden 2002). *Spalangia* spp. is particularly well known for burrowing, capable of parasitizing hosts deeper than 3 cm (Rueda and Axtell 1985b, Gibson and Floate 2004, McKay et al. 2007). Some genera of Pteromalids stay primarily on the surface.

*S. endius* is a cosmopolitan species, widely distributed across the world, with much of its distribution being a result of either intentional release as a biological control agent or as accompaniment to human dispersal of fly populations (Taylor et al. 2006). *S. endius* is often found in or near livestock facilities (Pitzer et al. 2011); and where present, *S. endius* tends to be one of the more abundant parasitoid species (McKay et al. 2007, Romero et al. 2010). *S. endius* is a popular candidate for mass release, either as an augmentation to existing populations of *S. endius* or to diversify the native populations of other parasitoid wasp genera already established (Morgan 1980, Morgan et al. 1981).

*Spalangia endius* parasitizes a relatively broad spectrum of fly species, not only filth flies but also the tephritid fruit flies (Tephritidae) *Bactrocera correcta* and *Bactrocera dorsalis*, which are both noted agricultural pests (Kitthawee et al. 2004). Thus *S. endius* may encounter insecticides not only in livestock facilities but also in crops.

*Urolepis rufipes* is found in some of the same hosts and habitats as *S. endius*, specifically in the pupal stage of house flies and stable flies in livestock facilities (Smith and Rutz 1985, Stenseng et al. 2003). *U. rufipes* has been less well studied, only recently being found in such habitats and hosts. Brine fly pupae, which are semi-aquatic, are believed to be the original host (Smith and Rutz 1985, Gibson 2000). *U. rufipes* can be found across much of the Nearctic
region, as well as in Denmark and Northern Germany. *U. rufipes* has not yet been tested for its effectiveness as a released biological control agent in the field, and some strains have exhibited notably short life span, low progeny emergence, high fly eclosion (Matthews and Peterson 1989) and very male-biased sex ratios at high densities (Powell et al. 2003). However, the development rate of *U. rufipes* is the fastest of all known pteromalid filth fly pupal parasitoids (Smith and Rutz 1985), and some strains have higher intrinsic rates of growth than most other fly pupal pteromalid parasitoids (Stenseng et al. 2003). Stenseng et al. (2003) and Floate and Skovgård (2004) suggest the potential of *U. rufipes* for commercialization particularly in northern climates.

In an attempt to limit evolved resistance of insecticides, recommendations for filth fly control are for an integrated approach, using sanitation, biological control agents, and insecticides (Rutz and Patterson 1990, Loftin et al. 2003). Selective applications of insecticides against adult filth flies are recommended (Scott et al. 1990). Selectivity is defined as being particularly harmful to a specific target species relative to non-targets, such as biological control agents. Pteromalids such as *S. endius* and *U. rufipes* are non-targets. To better assess which pesticides are selective, knowing behavior of the flies but also of their natural enemies is important. In addition to toxicities defining the degree of selectivity of a pesticide to a target species, the modality in which the pesticide is being applied could also play a large role in determining its selectivity.

One might expect pesticides that affect insects that act as hosts to also affect parasitoid wasps of those hosts because the latter are necessarily smaller. There have been many studies showing adverse effects of pesticides on beneficial insect species, including other hymenopterans (Prabhaker et al. 2011). However, effects of pesticides cannot be predicted strictly from size or
from effects on hosts. This may be partly because endoparasitoids are somewhat protected by the host. For example, *Gonatocerus ashmeadi* is a mymarid egg parasitoid of the glassy-winged sharpshooter (*Homalodisca vitripennis*) (Byrne and Toscano 2007). Imidacloprid, a neonicotinoid pesticide, produces an LC$_{50}$ at just 39ng/cm$^2$ leaf for emerging glassy-winged sharpshooter nymphs, whereas emerging *G. ashmeadi* adults are susceptible to imidacloprid at an LC$_{50}$ of 66ng imidacloprid/cm$^2$ leaf. However, whether the lower concentrations needed to kill their hosts have sublethal effects on the behavior of the parasitoid has not been examined.

Sublethal effects are defined as any physiological or behavioral alteration in a pesticide-treated organism that has not suffered death due to the pesticide (Schneider et al. 2012). Not just lethal but also sublethal effects may affect parasitoid wasp effectiveness.

The sublethal effects of pesticides, especially neonicotinoids, have come under intense inspection due in part to the rapidly declining populations of beneficial honey bees (*Apis mellifera*) (Iwasa et al. 2004, Schneider et al. 2012). Honey bees fed with doses of imidacloprid ranging from 0.15 ng up to 6 ng show a dramatic increase in foraging time away from the hive immediately after treatment, with only 25% of individuals returning to the hive after treatment with 6 ng imidacloprid. Visible physiological aberrations such as motionlessness and trembling are seen in some treated individuals. A dose of 6 ng was thought to be beyond what honeybees would normally encounter until the recent discovery of leaf excretions consistent with known sublethal concentrations of neonicotinoids.

It is well documented that pteromalids disperse from their release site (Smith et al. 1989, Skovgård 2002); because they can travel relatively long distances, they may be exposed to areas targeted for pest control. In an inundative parasitoid release at a dairy barn, high rates of
parasitization by the Pteromalid *Muscidifurax raptor* were found around doorways, and individuals were seen walking near windows some 30m away from their release point (Skovgård 2002). These are sites were adult flies also congregate.
CHAPTER 2

A NEW INDEX TO EVALUATE COMPATIBILITY BETWEEN COMMONLY USED INSECTICIDES AND BIOLOGICAL CONTROL AGENTS

Introduction

Integrated Pest Management programs (IPM) often include biological controls as an environmentally friendly means of pest control (Tobin and Pitts 1999) that limits pesticide resistance in the target (Kristensen and Jespersen 2004, Birkemoe et al. 2008, Kaufman et al. 2010). Understanding the compatibility between chemical control and biological control organisms is important in developing robust sustainable IPM (Scott et al. 1990, Stark et al. 1995, Prabhaker et al. 2011). Measures to assess compatibility when both control methods are used have included life history or population monitoring of the biological control organism (Villanueva-Jiminez and Hoy 1998, Hardman et al. 2003, Stark et al. 2007, Gonzalez-Zamora et al. 2013) as well as calculations of selectivity ratios. Selectivity ratios compare the acute toxicity of the pesticide to the biological control organism relative to the acute toxicity to the pest, and a value greater than one indicates favorable selectivity to the control agent, that is, the amount of pesticide needed to kill the pest will be less than what kills the control agent (Scott et al. 1988, Scott et al. 1990). Comparing biological control and pest LC$_{50}$ values from topical bioassays is one way that selectivity ratios have been generated (Stark et al. 1995).

Larvae of filth flies such as the house fly, *Musca domestica* L. (Diptera: Muscidae), feed on decaying organic matter, including manure, and pupate in it. The adult flies are of significant economic importance in animal production (Malik et al. 2007). An estimated 1.6 million dollars is spent annually in the United States on house fly insecticides in poultry establishments alone. Mass releases of pupal parasitoids, including *Spalangia endius* Walker (Hymenoptera: Pteromalidae), have suppressed house fly populations in some (Morgan et al. 1975, Weinzierl and Jones 1998, Skovgård and Nachman 2004, McKay et al. 2007), but not all, situations (Meyer et al. 1990, Andress and Campbell 1994). But pesticides are still widely used. Newer pesticides used in filth fly control include neonicotinoids (Memmi 2010) and spinosad (Deacutis et al. 2006). However, there is currently no information on how compatible these pesticides are with parasitoids of these flies.

One common way that pesticides are used against filth flies is through feeding modalities, including granular baits (White et al. 2007, Ferguson et al. 2014). Baits are scattered or painted on. *Spalangia endius* does not readily eat these baits but may still suffer mortality through contact (Burgess unpublished data). Contact may occur when parasitoids are dispersing from a mass release, which may occur every second to fourth week throughout the summer (Floate 2003). Soon after release, *S. endius* are found in some of the same locations as adult flies, such as near windows and doorways (Smith et al. 1989, Skovgård 2002). Some extension publications and bait labels explicitly recommend putting insecticide near windows or doorways (Campbell 2006, Townsend 2015c; Agita® 1GB Scatterbait [thiamethoxam], Novartis Animal Health, North Ryde NSW, Australia). Other times when wasps may encounter sites with insecticide residue are during male dispersal away from the natal site (Myint and Walter 1990) and as females move...
from one host location to another. How much insecticide gets into house fly larval breeding sites, where the parasitoids emerge and parasitize hosts, is unclear. However, baits may sometimes be scattered in manure pits (Stafford 2008), and explicit advice against application to manure is not typically on labels. Pesticides inadvertently may get into manure and other decaying organic matter during treatment of livestock against ectoparasites, as a result of spills, or as a result of miscommunication between individuals knowledgeable about pesticide use and others working where the pesticides have been applied, such as during cleanup activities.

The compatibility of various pesticides with the parasitoid wasp S. endius was assessed using traditional LC$_{50}$ values as well as two new measures (see Methods for full details). The LPR (LC$_{50}$-to-prescribed dosage ratio) converts the LC$_{50}$ into units of prescribed dosage. The PCI (Pesticide Compatibility Index) compares LPR of the biological control agent to LPR of the pest, in this study the house fly. In the present study, the prescribed dosages used to calculate LPR, and thus PCI, were based on scatterbait formulations of pesticides. Other formulations may have different LPRs and thus different PCIs.

An advantage of LPR and PCI is when the deaths of the pest and its control agent result from different types of encounters with pesticides. For example, the pest may contact and eat a pesticide by design, whereas the biological control may experience only contact (Stark et al. 2004, Wang et al. 2005). In addition, the pest and the biological control agent may have different types of encounters because they visit different locations, and pesticides may be applied in multiple modalities in the same facility, even simultaneously, e.g., baits and surface applications. When type of encounter differs, if one uses the same bioassay method for both pest and biological control agent, e.g. a feeding bioassay for both, then the bioassay is unrealistic for one
of them. By converting LC$_{50}$ to LPR, then even when the type of bioassay differs, values are in the same units and thus comparable.

The pesticides tested here include five that are commonly used in granular house fly baits: three neonicotinoids (imidacloprid, dinotefuran, thiamethoxam), methomyl (a carbamate), and spinosad. For comparisons of LC$_{50}$ values, permethrin and the neonicotinoid nitenpyram also were tested, although neither is used in a granular bait against house flies. The LC$_{50}$ for permethrin can be used as a baseline against which to compare other compounds because effects of permethrin on other pteromalids have been well documented (Scott and Rutz 1988, Scott et al. 1990, Geden et al. 1992b). Permethrin is still widely used on dairy premises (Ferguson et al. 2014).

Materials and Methods

Laboratory Colonies

The Spalangia endius and the Musca domestica used in this study were from laboratory colonies. The S. endius colony was established with parasitoids obtained from Zephyr Hills, Florida in 1996, and has never been exposed to pesticides since colony establishment. Vouchers are at the Illinois Natural History Survey Center for Biodiversity, catalog numbers "Insect Collection 6035 through 6054." The Musca domestica colony, “NIU Strain,” is of unknown origin but has been maintained by B. King for more than twenty years without exposure to pesticides.
Determination of LC_{50}

First, LC_{50} values were determined for both *S. endius* and *M. domestica*. The pesticides used were pure analytical standards, purchased through Chem Service (West Chester, PA) and are as follows: imidacloprid (99.5% purity), methomyl (99.5%), dinotefuran (98.2%), thiamethoxam (99.5%), nitenpyram (99.0%), spinosad (98.6%), and permethrin (99.5%). Pesticide grade acetone was the solvent used to create dilutions (Chem Service, West Chester, PA). Test concentrations were made using a combination of serial and parallel dilutions from a 1 mL stock solution. New 1 mL stock solutions were made for each replicate by weighing the analytical standard and dissolving it in 1 mL of acetone. Each test concentration was made to a volume of 1 mL by mixing a calculated volume of the stock solution with acetone.

Pesticide sensitivities (LC_{50}) in *S. endius* were assessed using a surface contact bioassay. A volume of 0.5 mL of each test concentration was pipetted into a 20 mL glass test vial (42.8 cm\(^2\) inner surface area). The solution was spread within the vial by placing the vial on a commercial hot dog roller with no heat and allowing the vial to rotate for at least 30 min until the acetone was completely evaporated (Miller et al. 2010). Parasitoids were not observed preferentially standing on any particular part of the vial. Twenty female *S. endius*, which were 0-5 days old, were added to each test vial. A cotton plug was used to secure the parasitoids inside the test vials. A drop of 1:1 water-honey mixture on the cotton plug provided a food and water source. Each replicate consisted of one vial each of at least five concentrations and a control, with at least three replicates per pesticide. Test vials were held in an environmental chamber at 28°C ± 0.2°C and 52-64% RH. Parasitoid mortality was assessed after 48 h. Mortality was counted as any clearly dead or moribund parasitoids. A parasitoid was considered moribund if it
displayed any combination of two or more of the following: inability to right itself when laying on its back; jerky walking; abnormally slow walking; motionless and unaffected by poking; appendages that appeared to be paralyzed.

Pesticide sensitivities of *M. domestica* were tested using a feeding bioassay. Treatments were created by pipetting 0.5 mL of test solution onto a 4 g sugar cube (Domino Foods, Inc., Yonkers, NY) placed in the center of a 300 mL glass jar. The jars sat in a fume hood for at least 2 h to allow the acetone to fully evaporate. Twenty 0 – 2-day-old female *M. domestica* were anesthetized with carbon dioxide and added to each jar. A fiberglass screen cover was secured on the jar, and a dental wick soaked with water was placed on top of the screen cover to provide a water source. At least four concentrations, plus a control, were used per replicate, with at least three replicates per pesticide. Test jars were held in an environmental chamber at 28°C ± 0.3°C and 58-83% RH. Mortality was assessed at 48 h and was scored in the same way as for the parasitoids.

Percentage mortality was calculated for each concentration, pooling across replicates. Probit analysis was used to determine LC$_{50}$ values (SPSS 2012). Abbott’s formula was used to correct for control mortality (Abbott 1925).

**Calculation of LPR and PCI**

Equations are in footnotes of Tables 1 and 2. Basically, from each LC$_{50}$, LPR was calculated by dividing the LC$_{50}$ by the prescribed dosage of a reference granular bait formulation. The reference formulations that were chosen were readily available from commonly used house fly granular baits: imidacloprid (Quickbayt® Fly Bait, Bayer, Shawnee Mission, KS), methomyl
Table 1. *Spalangia endius* LC$_{50}$, prescribed dosage, and LC$_{50}$-to-prescribed dosage ratio (LPR)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Slope (SE)</th>
<th>LC$_{50}$ (95% CI)</th>
<th>$X^2$ (p-value)</th>
<th>Prescribed Dosage$^{ab}$</th>
<th>LPR$_{parasitoid}$$^c$,$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>400</td>
<td>4.87 (0.45)</td>
<td>14.72 (13.60–15.86) a</td>
<td>0.92 (0.63)</td>
<td>2441.25</td>
<td>60.3</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>300</td>
<td>0.48 (0.06)</td>
<td>17.92 (8.29–37.97) ab</td>
<td>4.73 (0.19)</td>
<td>915.35</td>
<td>195.8</td>
</tr>
<tr>
<td>Permethrin</td>
<td>400</td>
<td>5.25 (0.48)</td>
<td>36.80 (34.51–39.16) b</td>
<td>0.92 (0.82)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>300</td>
<td>2.17 (0.22)</td>
<td>41.94 (34.88–50.16) bc</td>
<td>3.56 (0.31)</td>
<td>2000.00</td>
<td>209.7</td>
</tr>
<tr>
<td>Spinosad</td>
<td>400</td>
<td>4.83 (0.52)</td>
<td>51.82 (48.22–55.23) cd</td>
<td>6.69 (0.08)</td>
<td>1220.63</td>
<td>424.5</td>
</tr>
<tr>
<td>Dinotefuran</td>
<td>400</td>
<td>3.10 (0.26)</td>
<td>52.20 (46.36–58.39) cd</td>
<td>2.90 (0.41)</td>
<td>1220.63</td>
<td>427.6</td>
</tr>
<tr>
<td>Nitenpyram</td>
<td>300</td>
<td>5.06 (0.59)</td>
<td>54.67 (50.86–59.44) d</td>
<td>1.46 (0.69)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

LC$_{50}$ values followed by the same lower case letter do not differ significantly based on overlap of their 95% CI.

$^a$ In units of ng/cm$^2$.

$^b$ Prescribed dosages were calculated by converting the recommended mass per area on the label to ng/cm$^2$ and then multiplying that value by the proportion by weight of active ingredient. Pesticide formulation name (active ingredient), percent by weight, and mass per area were Golden Malrin® (methomyl), 1.10%, 2.44 g/m$^2$; Quickbayt® (imidacloprid), 0.50%, 1.83 g/m$^2$; Agita® (thiamethoxam), 1.00%, 2 g/m$^2$; Conserve® (spinosad), 0.50%, 2.44 g/m$^2$; Quikstrike® (dinotefuran), 2.44 g/m$^2$.

$^c$ LPR$_{parasitoid}$ = Parasitoid LC$_{50}$/Prescribed dosage

$^d$ LPR$_{parasitoid}$ values are $10^{-4}$
Table 2. *Musca domestica* LC$_{50}$, prescribed dosage, LC$_{50}$-to-prescribed dosage ratio (LPR), and Pesticide Compatibility Index (PCI)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Slope (SE)</th>
<th>LC$_{50}$ $^a$ (95% CI)</th>
<th>$\chi^2$ (p-value)</th>
<th>Prescribed Dosage $^b$</th>
<th>LPR$_{fly}$$^c$, $^d$, $^e$</th>
<th>PCI $^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>320</td>
<td>6.10 (0.79)</td>
<td>4.48 (4.17 – 4.74) $^c$</td>
<td>5.56 (0.06)</td>
<td>1.00%</td>
<td>4.5</td>
<td>13.4</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>300</td>
<td>2.02 (0.23)</td>
<td>31.42 (26.16 – 37.81) $^d$</td>
<td>2.07 (0.59)</td>
<td>0.50%</td>
<td>62.8</td>
<td>3.1</td>
</tr>
<tr>
<td>Permethrin</td>
<td>300</td>
<td>5.53 (0.63)</td>
<td>1.72 (1.60 – 1.83) $^a$</td>
<td>2.94 (0.40)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>300</td>
<td>4.03 (0.42)</td>
<td>3.23 (2.94 – 3.55) $^b$</td>
<td>2.82 (0.42)</td>
<td>1.00%</td>
<td>3.2</td>
<td>65.5</td>
</tr>
<tr>
<td>Spinosad</td>
<td>300</td>
<td>4.292 (0.571)</td>
<td>1.85 (1.66 – 2.02) $^a$</td>
<td>2.185 (0.54)</td>
<td>0.50%</td>
<td>3.7</td>
<td>114.7</td>
</tr>
<tr>
<td>Dinotefuran</td>
<td>320</td>
<td>2.82 (0.27)</td>
<td>5.00 (4.37 – 5.71) $^c$</td>
<td>5.83 (0.05)</td>
<td>0.50%</td>
<td>10.0</td>
<td>42.8</td>
</tr>
<tr>
<td>Nitenpyram</td>
<td>400</td>
<td>3.70 (0.48)</td>
<td>63.54 (56.84 – 69.26) $^e$</td>
<td>1.95 (0.58)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

LC$_{50}$ values followed by the same lower case letter do not differ significantly based on overlap of their 95% CI.

$^a$ In units of µg/g sugar

$^b$ In units of percentage by weight from labels listed in Table 1 footnote

$^c$LPR$_{fly}$ = Fly LC$_{50}$/Prescribed dosage

$^d$ LC$_{50}$ values were converted to percentage by weight (i.e., multiplied by 100) and then divided by prescribed dosage in order to obtain LPR$_{fly}$ values.

$^e$LPR$_{fly}$ values are 10$^{-4}$

$^f$PCI = Pesticide Compatibility Index = LPR$_{parasitoid}$/LPR$_{fly}$
bait; in fact, nitenpyram has not yet been developed as a filth fly pesticide in any form. The list of bait formulations and how prescribed dosages were calculated are in the footnotes in Tables 1 and 2.

LPR values were calculated for both parasitoids and flies ($LPR_{\text{parasitoids}}$, $LPR_{\text{fly}}$). An LPR value is the number of prescribed dosage equivalents in an $LC_{50}$. Comparison of LPR values among pesticides thus uses the units in which the pesticides are actually applied. For the flies, $LC_{50}$ values and label information both were per weight, specifically, micrograms of active ingredient per gram of sugar for $LC_{50}$ and weight of the active ingredient (AI) per weight of bait for prescribed dosage. For the parasitoids, $LC_{50}$ values were in ng/cm$^2$, so the prescribed dosages needed to be converted to mass of AI per area to match. AI per area was calculated by multiplying two values from the labels, the weight of prescribed bait per area and proportion AI by weight.

After calculating LPRs, PCI was calculated for each pesticide formulation by dividing the $LPR_{\text{parasitoid}}$ by the $LPR_{\text{fly}}$. A large PCI value is good in that it indicates a pesticide that requires more units of prescribed dosage to kill the biological control than to kill the pest.

Results

The order of pesticide toxicity differed between the parasitoids and the flies, except that nitenpyram was least toxic for both (Tables 1, 2). Based on $S. endius$ $LC_{50}$ values, there was about a four-fold difference from the most toxic to the least toxic pesticides. Based on overlap of 95% confidence intervals, there was no significant difference in toxicity between methomyl and
imidacloprid; among imidacloprid, permethrin and thiamethoxam; among thiamethoxam, spinosad, and dinotefuran; and among spinosad, dinotefuran, and nitenpyram.

Based on *M. domestica* LC_{50} values there was a 37-fold difference in toxicity between the most toxic and least toxic pesticides. Based on overlap of 95% confidence intervals, permethrin and spinosad were of equal toxicity. They were about two times as toxic as thiamethoxam, about three times as toxic as methomyl and dinotefuran, 18 times as toxic as imidacloprid, and 37 times as toxic as nitenpyram.

LPR values from most to least toxic for *S. endius*, were as follows: Golden Malrin® (methomyl) > Quickbayt® (imidacloprid) > Agita® (thiamethoxam) > Conserve® (spinosad) > Quikstrike® (dinotefuran). This order is similar to the order of LC_{50} values. There was a seven-fold difference in toxicity between the most toxic and least toxic reference formulae based on LPR.

LPR values starting with the reference formula that was the most toxic for *M. domestica* were as follows: Agita® (thiamethoxam) > Conserve® (spinosad) > Golden Malrin® (methomyl) > Quikstrike® (dinotefuran) > Quickbayt® (imidacloprid). This order is similar to the order of LC_{50} values, except that the order of thiamethoxam and spinosad were reversed. There was a 20-fold difference in LPR values between the most toxic and least toxic of the pesticides.

PCI values starting with the pesticide that appears to be the least toxic to *S. endius* relative to *M. domestica* were as follows: Conserve® (spinosad) > Agita® (thiamethoxam) > Quikstrike® (dinotefuran) > Golden Malrin® (methomyl) > Quickbayt® (imidacloprid) (Table 1). Thus the Conserve® appeared to be the most compatible for use with *S. endius*, and Quickbayt®
appeared to be the least compatible. The PCI value for Conserve® was 37 times that of Quickbayt®.

Discussion

Based on our LC$_{50}$ values, no generalization could be made about the effectiveness of a given pesticide based on it being a neonicotinoid. Although Scott and Rutz (1988) did not test neonicotinoids, they likewise found no generalizations about toxicity could be made based on class of pesticides when testing house flies and another parasitoid.

The LC$_{50}$ values were all less for the parasitoids than for the flies. Parasitoids are necessarily smaller than their hosts, but smaller size is not always associated with greater susceptibility. The parasitoid Muscidifurax raptor Girault and Saunders is 14.5 times less sensitive than M. domestica to fenvalerate (Scott et al. 1990). The fold change in LC$_{50}$ values among pesticides was much greater for the house flies than for the parasitoids, a pattern also seen by Scott and Rutz (1988) with different pesticides and a different parasitoid of house fly pupae, Urolepis rufipes Ashmead (Hymenoptera: Pteromalidae).

Although LPR and PCI values are specific to the reference formulation used in their calculation, based on both the LC$_{50}$ and PCI values, imidacloprid (Quickbayt®) and methomyl (Golden Malrin®) were more harmful for the wasps and less effective for the flies than spinosad was. Imidacloprid is widely used in house fly control (Kaufman et al. 2006, Kaufman et al. 2010) and persists in the environment for long periods of time (Federoff et al. 2008). Methomyl is acutely toxic to mammals (IPCS 1996).
Looking at our study together with previous research, spinosad and thiamethoxam may be better choices than imidacloprid or methomyl for killing house flies but allowing parasitoids to survive. Thiamethoxam is more toxic than imidacloprid and methomyl to house flies based on LC$_{50}$ values from fly feeding bioassays in the present study and in Kristensen and Jespersen (2008). Among granular baits, Agita® (thiamethoxam) is no less effective than QuickBayt® (imidacloprid) in fly knockdown in the field (Nurita and Abu Hassan 2010). Spinosad appears to be more effective against house flies than methomyl or imidacloprid based on their EC$_{50}$ values (White et al. 2007), their LC$_{50}$ values (the present study), and tests of attraction and mortality with baits in the field (Murillo et al. 2014). Fortunately, when house flies evolve resistance to spinosads, the resistance may make the flies more susceptible to neonicotinoids (Markussen and Kristensen 2012). Spinosad, like imidacloprid, exhibits low mammalian toxicity, but in contrast to imidacloprid, spinosad has relatively short environmental persistence (Liu and Li 2004, Zhao et al. 2007).

For S. endius, nitenpyram was the least toxic pesticide tested based on LC$_{50}$. (Its LPR and PCI were not determined.) However, for M. domestica, the LC$_{50}$ by weight of nitenpyram was approximately double that of imidacloprid, so, all else being equal, developing nitenpyram into a competitive granular house fly bait would necessitate its manufacture being half the cost per weight of imidacloprid baits. Nitenpyram may be appealing in terms of public concerns about environmental safety because it has low photostability and breaks down quickly in both water and in soil (Yamamoto and Casida 1999), although these traits make designing long lasting pesticide formulations challenging.
The advisability of using permethrin appears to be variable for both house flies and their parasitoids. Permethrin had one of the lowest LC$_{50}$ values in the fly feeding bioassays. Furthermore, there was no detectable change in rate of parasitization of sentinel *M. domestica* pupae by *Spalangia* spp. and *Muscidifurax* spp. in manure that had been contaminated with permethrin during treatment of mites on poultry (Mandeville et al. 1990), suggesting that permethrin may not be a large risk to parasitoids in fly breeding sites. Scott and Rutz (1988) ranked permethrin favorably for use in conjunction with some parasitoids of filth flies, including some *Spalangia* spp. In surface contact bioassays, permethrin was less toxic than the six other pesticides tested for *M. raptor* and *Urolepis rufipes*, was fourth most toxic to *Pachycrepoideus vindemmmiae* Rondani and second most toxic to *S. cameroni* Perkins (Rutz and Scott 1990). Further evidence of compatibility of at least some pesticides and parasitoids is provided by Geden et al. (1992a), who found that parasitoid releases combined with limited targeted use of pesticides provided better fly suppression than on control farms that relied more exclusively on pesticides. That parasitoids spend much of their life cycle within a puparium when in manure may provide some protection. Scott et al. (1991) tested pesticides on house fly pupae that had or had not been parasitized. They used seven pesticides, all different than the ones tested here except permethrin. Flies were generally more susceptible to all seven pesticides than were *S. cameroni* within their hosts; *M. raptor* was more susceptible than the flies to Pyrenone (pyrethrins + piperonyl butoxide) but not to the other pesticides.

In conclusion, studies to date suggest that pesticides are sometimes compatible with conservation of existing populations of parasitoids and their mass release, although some pesticides appear to be more compatible than others. The levels of pesticides in manure and other
decaying organic matter remain to be determined, at least for the pesticides tested here. Adequate communication between those applying a pesticide and those involved in cleanup will be important in avoiding inadvertent environmental contamination. Mass releases of parasitoids should be timed to minimize overlap with pesticide use, particularly if pesticides will be near windows and doors. The pesticides tested in the present study should be kept away from areas that parasitoids frequent. Education on where the parasitoids live and their importance is essential, including more consistent and explicit instructions against pesticides getting in manure, such as seen on the label for Vectothor Bait™ (imidacloprid) (Ensystex Australasia Pty Ltd, Auburn, NSW, Australia) and in some extension service publications (Loftin et al. 2003, Stafford 2008).
CHAPTER 3

BEHAVIORAL INTERACTIONS OF PTEROMALIDS AND GRANULAR FILTH FLY BAITS

Introduction

One of the most common filth flies in animal production facilities is the house fly, *Musca domestica* L. (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). Filth flies negatively affect livestock by causing stress, which can reduce animal weight and slow milk production (Gibson and Floate 2004). They are also 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. 1992b). 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 agent 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). 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 for *S. endius* are at the Illinois Natural History Survey Center for Biodiversity, catalog numbers “Insect Collection 6035 through 6054.” 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°C ± 0.5°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 (QuickBayt 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 2.0 cm circle was drawn with marker on the outside, bottom, and center of a Pyrex petri dish (9.0 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-day-old female of either *S. endius* or *U. rufipes* was placed in each dish. Parasitoids were observed for 10 min. The number of times each parasitoid contacted the bait (or sand) or groomed herself was counted. For each species, four replicates of each treatment, including a control, were done on each of 6 d for a total of $n = 24$ replicates of each treatment, which was 96 dishes altogether.

Residue Survival Experiments

The inside surfaces of a Pyrex petri dish (9.0 cm diameter x 1.5 cm height) were coated with the powdery residue of bait by gently rolling around 1.0 g of bait 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-day-old female *S. endius* or ten 0-3-day-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
For each species, five replicates of each treatment, including control, were done, which was 20 dishes altogether.

**AI LC$_{50}$**

The LC$_{50}$ 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). Twenty 0-3-day-old females were tested in each of five 20 mL (42.8 cm$^2$ inner surface area) glass test vials, the insides of which had been coated with 0.5 ml of a concentration of AI; and mortality was assessed after 48 h. Results for *S. endius* are in Burgess and King (2015) but are reported again to facilitate comparisons (Table 3).

**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.
Table 3. LC$_{50}$ values of three active ingredients (AI) found in three house fly granular baits, for *Spalangia endius* and for *Urolepis rufipes*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>n</th>
<th>Slope (SE)</th>
<th>LC$_{50}^b (95%$ CI)</th>
<th>$\chi^2$ (p-value)</th>
<th>Recommended Application Rate$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. endius</em>$^b$</td>
<td>Imidacloprid AI</td>
<td>300</td>
<td>0.48 (0.06)</td>
<td>17.92 (8.29 – 37.97)Ba</td>
<td>4.73 (0.19)</td>
<td>915.35</td>
</tr>
<tr>
<td></td>
<td>Methomyl AI</td>
<td>400</td>
<td>4.80 (0.45)</td>
<td>14.72 (13.60 – 15.86)Ca</td>
<td>0.92 (0.63)</td>
<td>2684.00$^c$</td>
</tr>
<tr>
<td></td>
<td>Dinotefuran AI</td>
<td>400</td>
<td>3.10 (0.26)</td>
<td>52.20 (46.36 – 58.39)Db</td>
<td>2.90 (0.41)</td>
<td>1220.63</td>
</tr>
<tr>
<td><em>U. rufipes</em></td>
<td>Imidacloprid AI</td>
<td>400</td>
<td>2.58 (0.23)</td>
<td>10.37 (9.01 – 11.78)Bb</td>
<td>4.15 (0.25)</td>
<td>915.35</td>
</tr>
<tr>
<td></td>
<td>Methomyl AI</td>
<td>400</td>
<td>3.37 (0.33)</td>
<td>10.44 (9.32 – 11.64)Bb</td>
<td>3.65 (0.30)</td>
<td>2684.00</td>
</tr>
<tr>
<td></td>
<td>Dinotefuran AI</td>
<td>500</td>
<td>3.06 (0.22)</td>
<td>0.82 (0.72 – 0.91)Aa</td>
<td>7.01 (0.071)</td>
<td>1220.63</td>
</tr>
</tbody>
</table>

Within each species, LC$_{50}$ 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.

$^a$ In units of ng / cm$^2$

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

$^c$ Concentration from more recent label than the 2441.25 ng/cm$^2$ reported in Burgess and King (2015).
0-5-day-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 imidaclorpid bait. (The approximate amount per 1000 granules of dinotefuran bait [1.66 mg, 1.34 µL] was not tested). 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 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 overdispersion in the models. 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$_{50}$ 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; $\chi^2 = 14.43, \text{df} = 3, p = 0.002$), but there was no significant difference in proportion that had contact among the baits ($\chi^2 = 0.11, \text{df} = 2, p = 0.94$).

Combining all observations of contact with baits, a significantly lower proportion of females contacted bait than the control ($\chi^2 = 12.59, \text{df} = 1, p < 0.001$). There was a significant effect of treatment on number of times a female *S. endius* groomed (Fig. 2; $F = 7.52, \text{df} = 3, 92, p < 0.001$). 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 ($p > 0.05$).

In *U. rufipes*, whether a female had any contact with bait granules or sand depended on treatment (Fig. 1; $\chi^2 = 33.90, \text{df} = 3, p < 0.001$) and differed among the baits ($\chi^2 = 21.45, \text{df} = 2, p < 0.001$). A greater proportion of females contacted imidacloprid bait than dinotefuran bait ($\chi^2 = 10.23, \text{df} = 1, p = 0.001$) or methomyl bait ($\chi^2 = 19.01, \text{df} = 1, p < 0.001$), but there was no significant difference between dinotefuran bait and methomyl bait ($\chi^2 = 1.34, \text{df} = 1, p = 0.25$). Relative to the control, a significantly lower proportion of females contacted the imidacloprid bait ($\chi^2 = 28.23, \text{df} = 1, p < 0.001$) and the dinotefuran bait ($\chi^2 = 5.58, \text{df} = 1, p = 0.018$), but not methomyl bait ($\chi^2 = 0.91, \text{df} = 1, p = 0.34$). In *U. rufipes*, there was a significant effect of treatment on number of grooms (Fig. 2; $F = 2.90, \text{df} = 3, 92, p = 0.039$). There were significantly more
Figure 1. Proportion of replicates where contact was observed for female *S. endius* and *U. rufipes* exposed to a methomyl, imidacloprid, or dinotefuran bait pile or to a sand pile control for 10 min. Letters that differ represent statistical differences at $\alpha = 0.05$ within each species.
**Figure 2.** Number of grooms for female *S. endius* and *U. rufipes* exposed to a methomyl, imidacloprid, or dinotefuran bait pile or to a sand pile control for 10 min. Each black dot found throughout the range of each boxplot represents the number of times a given female groomed in each 10 min replicate (*n* = 24 replicates for each bait type). Letters that differ represent statistical differences at $\alpha = 0.05$ within each species.
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 (Figs. 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.
Figure 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 for each bait type).
Figure 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 for each bait type).
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 3). In *U. rufipes*, the order of toxicity was dinotefuran > imidacloprid = methomyl, with a 12.8-fold difference between the largest and smallest LC$_{50}$ values. In *S. endius* the order of toxicity was imidacloprid = methomyl > dinotefuran, with a 3.5-fold difference between the largest and smallest LC$_{50}$ values. *U. rufipes* was susceptible at lower concentrations of methomyl and dinotefuran than was *S. endius*, especially for dinotefuran.

**AI Behavior Experiments**

*S. endius* neither avoided nor was attracted to dinotefuran or methomyl but was attracted to imidacloprid (Table 4). *U. rufipes* neither avoided nor was attracted to any of the AIs.

**Fly Pheromone Behavior Experiments**

*S. endius* avoided the pheromone at both quantities, whereas *U. rufipes* neither avoided nor was attracted to the pheromone (Table 5).
Table 4. Difference (s) in time spent in the half of the arena with an active ingredient (AI) minus time spent in the half of arena with the control in *Spalangia endius* and *Urolepis rufipes*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Mean difference ± (SEM)</th>
<th>$t^a$</th>
<th>df</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. endius</em></td>
<td>Imidacloprid AI</td>
<td>168.27 ± 76.03</td>
<td>2.21</td>
<td>14</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Dinotefuran AI</td>
<td>-11.87 ± 81.76</td>
<td>-0.15</td>
<td>14</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Methomyl AI</td>
<td>40.27 ± 82.97</td>
<td>0.49</td>
<td>14</td>
<td>0.64</td>
</tr>
<tr>
<td><em>U. rufipes</em></td>
<td>Imidacloprid AI</td>
<td>-80.67 ± 107.79</td>
<td>-0.75</td>
<td>14</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Dinotefuran AI</td>
<td>22.27 ± 111.05</td>
<td>0.20</td>
<td>14</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Methomyl AI</td>
<td>76.80 ± 98.61</td>
<td>0.78</td>
<td>14</td>
<td>0.45</td>
</tr>
</tbody>
</table>

$a$ paired $t$-test.
Table 5. Difference (s) in time spent in the half of the arena with a medium or large quantity of fly pheromone minus time spent in the half of arena with the control in *Spalangia endius* and *Urolepis rufipes*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Mean difference ± (SEM)</th>
<th>t&lt;sup&gt;a&lt;/sup&gt;</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. endius</em></td>
<td>Medium Quantity Pheromone</td>
<td>-328.13 ± 52.69</td>
<td>-6.23</td>
<td>14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Large Quantity Pheromone</td>
<td>-282.80 ± 69.61</td>
<td>-4.06</td>
<td>14</td>
<td>0.001</td>
</tr>
<tr>
<td><em>U. rufipes</em></td>
<td>Medium Quantity Pheromone</td>
<td>-76.40 ± 86.35</td>
<td>-0.89</td>
<td>14</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Large Quantity Pheromone</td>
<td>-76.53 ± 64.79</td>
<td>-1.18</td>
<td>14</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<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 behavioral resistance 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*’s 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 suggests that *U. rufipes*’s 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*’s 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. The bees that prefer the laced solutions may 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 *Vespula* 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 irritatted 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 3). 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 break down 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*’s greater sensitivity probably was not just a result of more contact (Table 4).

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$_{50}$ 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 best choice for *S. endius*, even relative to the recommended dosage (0.04 relative ratio versus 0.02 for imidacloprid, 2 d, Table 3).

The pesticide AIs in current filth fly baits are enough to kill the parasitoid wasps *S. endius* and *U. rufipes* upon contact, with LC$_{50}$ 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. Results of the present study reinforce the importance of looking not only at physiological effects of pesticides but also at behavioral effects.
CHAPTER 4
SUBLETHAL EFFECTS OF IMIDACLOPRID ON SPALANGIA ENDIUS

Introduction

Most dairy and equine facilities use insecticides for pest control (Machtinger et al. 2012, Ferguson et al. 2014). However, filth flies can rapidly develop resistance (Liu and Yue 2000, Kristensen and Jespersen 2004, Kaufman et al. 2010). Integrated Pest Management (IPM) programs seek to integrate biological control and chemical control, along with other measures, to keep pest populations in check (Villanueva-Jimenez and Hoy 1998).

Imidacloprid is one of the most widely used pesticides against house flies (Simon-Delso et al. 2015) and is frequently successful (Kaufman et al. 2006, Butler et al. 2007, White et al. 2007), although behavioral resistance can be a problem (Gerry and Zhang 2009, Murillo et al. 2014). Pesticide use can be a risk to beneficial insects, including natural enemies. Harm may be in the form of death or sublethal effects. Sublethal effects of pesticides can be behavioral or physiological and are effects recorded on individuals that survive an exposure to a pesticide, either an exposure to a lethal concentration or to a sublethal concentration (Pham-Delège et al. 2002, Desneux et al. 2007). A lethal concentration kills some individuals, whereas a sublethal concentration does not. Sublethal effects include effects on fecundity (Amarasekare and Shearer 2013), sex allocation (Whitehorn et al. 2015), and foraging behavior (Liu et al. 2010, Schneider et al. 2012), any of which can reduce the effectiveness of beneficial arthropods. Imidacloprid has

The solitary parasitoid *Spalangia endius* Walker (Hymenoptera: Pteromalidae) is one of several parasitoid wasp species that can provide some control of filth flies, both through naturally occurring populations (Jones and Weinzierl 1997, Gibson and Floate 2004, Romero et al. 2010) and through augmentative releases (Weinzierl and Jones 1998, Skovgård and Nachman 2004, McKay et al. 2007). Most parasitoids of filth flies parasitize the pupal stage, which is found in manure or other rotting organic material (Rueda and Axtell 1985b). Adult females kill buried and unburied hosts by laying offspring and host feeding. Against filth flies, imidacloprid is commonly sold as granular fly bait, which is scattered on the ground, placed in bait stations, or dissolved in water and sprayed or painted on surfaces on which adult flies commonly rest (Pospischil et al. 2005, Nurita and Abu Hassan 2010). Parasitoids of filth flies may inadvertently be exposed to imidacloprid as adults disperse from natal or mass release sites, males seeking out mates (Myint and Walter 1990) and females searching for hosts. To be effective, augmentative releases are typically made every two to four weeks throughout the summer (Floate 2003). After being released, *Spalangia* spp. adults are sometimes found near windows and doorways (Smith et al. 1989, Skovgård 2002). Some imidacloprid labels and extension service recommendations include doorways or windows as preferred locations for pesticide applications because flies tend to congregate at these sites (Hinkle 2015, Townsend 2015a, c). Application instructions on some granular bait labels do not explicitly discourage applying baits to manure and other filth fly
breeding sites (QuickBayt Fly Bait, Bayer Healthcare LLC 2014), which may explain why baits are also sometimes scattered in such sites (Stafford 2008) even though natural enemies such as *S. endius* spend much of their lives there.

The present study tested for sublethal effects of imidacloprid on the ability of surviving adult *S. endius* females to subsequently kill hosts and produce parasitoid offspring. These abilities were measured in an experiment with unburied hosts and in another experiment with hosts buried in used host media. Then how the media affects imidacloprid residues on females was examined. Published data on the compatibility of imidacloprid and other neonicotinoids with parasitoids of filth flies are very limited (Burgess and King 2015, Whitehorn et al. 2015).

**Materials and methods**

**Laboratory Colonies**

The *S. endius* and the *M. domestica* used in this study were from laboratory colonies that had not been exposed to pesticides since colony establishment and for which lethal concentrations of imidacloprid are known (Burgess and King 2015). The *M. domestica* were reared on a mixture of water, a commercial fly larva medium (Lab Diet, St. Louis, MO; http://www.labdiet.com, accessed 26 April 2015), pine shavings, and fish meal (following King et al. 2014). Once the larvae finished feeding, they crawled out of their media box into a larger clean box underneath and pupated, allowing easy collection of fly pupae. The parasitoids were reared in a 25°C incubator with a photoperiod of 12L:12D. Females came from petri dishes of
parasitized hosts from which males had already begun emerging. The females used in experiments were of relatively uniform size and were randomly assigned to treatments.

**Imidacloprid Exposure Treatments**

The ability of exposed females to parasitize hosts was examined in two experiments, one in which hosts were not buried and one in which hosts were buried in used fly rearing media. A third experiment addressed how much imidacloprid was present in or on treated parasitoids after being in a media treatment or a no media treatment.

In the first two experiments, prior to use, female *S. endius* were prepared in one of four treatments: two imidacloprid exposure treatments and two control treatments. For the imidacloprid exposure treatments, a 20mL scintillation vial was coated with one of two concentrations of imidacloprid, LC$_{10}$ (low concentration) or LC$_{50}$ (high concentration). LC$_{10}$ and LC$_{50}$ values were interpolated previously from probit analysis for this same strain of *S. endius* with the same method of exposure (Burgess and King 2015). These concentrations produced approximately the same mortality in the present experiments. The low and high concentrations were generated by dissolving the appropriate amount of imidacloprid (99.5% purity, Chem Service West Chester, PA) in pesticide grade acetone (Chem Service, West Chester, PA). The two control treatments were an acetone-treated vial and a clean vial. Twenty female *S. endius* (0-2 d old) were placed in each vial. The cotton plug of each vial had a drop of 1:1 water-honey mixture on it as a food and water source.

The vials containing the parasitoids were held in an environmental chamber at 28°C ± 0.2°C for 48 h. Then five parasitoids that were still alive as defined by the criteria in Burgess and
King (2015) were randomly selected from each vial and used for testing in experiments. On a given test day for a given imidacloprid treatment or control, all five parasitoids that were tested came from the same exposure vial; number of fly and parasitoid emergences were thus pooled by day prior to analyses. In the third experiment, the media versus no media experiment, prior to use, female *S. endius* (0-2 d old) were exposed to either the high concentration vial or a clean vial. Again, all five parasitoids that were tested from the same exposure vial were pooled for analysis.

The LC$_{10}$ and the LC$_{50}$ are less than in imidacloprid-based granular baits used for house fly control. What parasitoids encounter in the field will range from the level in pesticide applications, which is greater than LC$_{100}$, to zero. Concentration may decrease over time and space from the source as a result of degradation and dissemination, with rate of decrease dependent on environmental conditions (Akoijam and Singh 2014, Herner et al. 2014, Schaafsma et al. 2015). How quickly imidacloprid degrades and disseminates in substrates that parasitoids frequent remains to be studied.

**Unburied Hosts Experiment**

Each treated or control female was placed alone with 25 fly pupae (0-2 d old) for 24 h in a 20 ml glass vial (70 mm high by 20 mm diameter) plugged with cotton. A small drop of 1:1 water-to-honey mixture was placed on the side of the vial. After the 24 h, the female was removed from the vial and placed in a test tube (12 mm in diameter, 75 mm in height), and her longevity was assessed. A 1:1 water-to-honey mixture was administered ad libitum to the cotton plug of the test tube. Each female was checked every 24 h for mortality until she died.
Meanwhile, the parasitized fly pupae were left for 5 weeks; flies that emerged from the pupae were counted, and emerged parasitoids were counted and sexed. This experiment was replicated five times per treatment per day on four different days, with a total of 20 females for each treatment (80 females total).

**Buried Hosts Experiment**

Each female was placed alone in a 150 ml jar filled about two-thirds full (6 cm deep) with spent fly-rearing media, and the jar was covered securely with cloth. Twenty-five fly pupae (0-2 d old) had been placed 2 cm under the media’s surface. *Spalangia* spp. are known for burrowing, although they also will parasitize hosts on the surface (Rueda and Axtell 1985a, Geden 2002, Skovgård 2006). After 48 h with the female *S. endius*, the fly pupae were transferred to an empty 20 ml glass vial (70 mm high by 20 mm diameter), and parasitoids and flies were allowed to complete emergence for five weeks. The number of emerged parasitoids and the number of emerged flies were counted. This experiment was replicated five times per treatment per day on three different days, with a total of 15 females tested for each treatment (60 females total).

The number of hosts and exposure duration in this experiment and the previous experiment were chosen so that a healthy female would be unlikely to be able to parasitize all of her hosts (King 2002). Females were given twice as much time to parasitize hosts in this experiment as in the previous experiment in order to end up with females parasitizing roughly the same number of hosts (King 2002), which appears to have been the case based on number of flies and offspring produced in the controls of each experiment (Figs. 5, 6).
Figure 5. Mean ± SE number of flies and parasitoid wasps emerged from the unburied hosts experiment in which a female parasitoid wasp had previously been exposed to a low concentration of imidacloprid, a high concentration, a clean vial control or an acetone control. The same lower case letter indicates no significant difference in number of parasitoids, and the same upper case letter indicates no significant difference in number of flies.
Figure 6. Mean ± SE number of flies and parasitoid wasps that emerged from the buried hosts experiment in which a female parasitoid wasp had previously been exposed to a low concentration of imidacloprid, a high concentration, an acetone control or a clean vial control. The same lower case letter indicates no significant difference in number of parasitoids, and the same upper case letter indicates no significant difference in number of flies.
Media Versus No-Media Experiment

A female *S. endius* that had been exposed to a high concentration of imidacloprid was given hosts to parasitize either in the presence or in the absence of media. The media treatment was a polystyrene petri dish (85 mm diameter) with a barrier of used fly-rearing media (approximately 5 cm wide, 8 mm high) across the center. This was done in lieu of the experimental setup in the buried hosts experiment to facilitate recovery of the parasitoid with minimal disturbance of the wet media. Ten *M. domestica* pupae (0-2 d old) were placed on one side of the barrier. A female *S. endius* was placed on the other side of the media strip. Females walked on and through the media. The no media treatment was the same as the media treatment except for the absence of the media barrier.

Both treatments were done five at a time. Females were left 24 h in the dishes and then collected for imidacloprid residue analysis, with all five of a given treatment placed in a 1.5 mL microcentrifuge tube that contained 120 µL of autoclaved RO water. Hosts were discarded. Solvent volume and pooling of five wasps were determined from initial trials to find a concentration that was detectable by the ELISA kit (enzyme-linked immunosorbent assay). This setup and collection of five females for each treatment was replicated 20 times, for a total of 100 females. In addition, the validity of the test was assessed with positive and negative controls, also in microcentrifuge tubes of RO water. Each positive control consisted of five females taken directly from a high imidacloprid concentration exposure vial, and each negative control consisted of five females from a clean vial. The positive and negative controls were each replicated six times, for a total of 30 females in each.
The parasitoids that had been collected in the centrifuge tubes were homogenized in the tubes by sonication and then centrifuged at 10 x g for 5 min. The supernatant was analyzed for imidacloprid, using a competitive ELISA Kit (Envirologix, Portland, ME) following manufacturer recommendations. Briefly, samples, positive controls, negative controls, and analytical standards were added to wells in a pre-coated plate provided with the kit. Imidacloprid-enzyme conjugate was immediately added to each well. The analytical standards ranged from 0.2 – 6.0 ppb and were provided with the ELISA kit. The plate was sealed with tape to prevent evaporation and then incubated at room temperature for 1 h on an orbital shaker (200 rpm). Wells were washed with tap water four times, followed by addition of substrate to each well. After approximately 15 min incubation at room temperature on an orbital shaker, stop solution was added to each well. Absorbance (OD, optical density) was read immediately on an Epoch plate reader (BioTek, Venooski, VT). OD is inversely proportional to the concentration of imidacloprid present in a sample. The kit can detect quantities of imidacloprid less than 0.2 ppb, but conversion to ppb is not recommended below 0.2 ppb because converting from OD to ppb introduces extrapolation error beyond the range of the standards. The majority of OD values obtained in testing fell outside of the 0.2 ppb standard, so analysis was conducted on OD values.

Statistical Analyses

Analyses were performed with R version 3.1.2 (R Core Team 2015). In both the buried and unburied experiments, the effect of treatment on the number of emerged parasitoids and flies was analyzed using generalized linear models. Analyses of number of flies and parasitoids were best fitted with a quasi-Poisson distribution to account for overdispersion in the models. Sex
ratio from the unburied experiment was analyzed using a general linear model, and the response
variable (male-to-total ratio) was transformed using the Box-Cox transformation to correct for
violations of normality and homoscedasticity of the data (Box and Cox 1964). Longevity was
analyzed by Kaplan-Meier survival analysis. For the media versus no media experiment, OD was
analyzed (Zwicker et al. 2004, Warkentin et al. 2008), using a two-sample Student’s *t* test.

Results

**Unburied Hosts Experiment**

There was a significant treatment effect on fly emergence (Fig. 5; \( p < 0.001 \)). There was
no significant difference in fly emergence between the low concentration and the controls.
However, significantly more flies emerged from the high concentration treatment.

There was a significant treatment effect on parasitoid emergence (\( p = 0.004 \)). There was
no significant difference in the number of parasitoids that emerged for the low concentration
compared to the controls. Significantly fewer parasitoids emerged from the high concentration.
There was no significant difference in the sex ratio among treatments (Fig. 7; \( p = 0.17 \)). Across
treatments, 296 males and 933 females emerged, i.e., 76% female. The high concentration
treatment produced no parasitoids in 6 of 20 replicates.

There was no significant difference among treatments in the number of days that tested
parasitoids (i.e., parasitoids that survived the initial exposure) subsequently lived (Fig. 8; \( p =
0.43 \)).
Figure 7. Mean ± SE ratio of male-to-total parasitoid wasps emerged from the unburied hosts experiment in which a female parasitoid wasp had previously been exposed to a low concentration of imidacloprid, a high concentration, an acetone control or a clean vial control. The same lower case letter indicates no significant difference.
Figure 8. Survivorship curves of female parasitoid wasps in the unburied hosts experiment. Females had previously been exposed to a low concentration of imidacloprid, a high concentration, an acetone control or a clean vial control.
Buried Hosts Experiment

There was no significant treatment effect on the number of flies that emerged in this experiment (Fig. 6; \( p = 0.51 \)). There was also no significant treatment effect on the number of parasitoids that emerged (Fig. 6; \( p = 0.28 \)).

Media versus no media experiment

There was a significant effect of treatment on OD (Fig. 9; \( p < 0.001 \)), indicating more imidacloprid in the no media treatment than in the media treatment.

Discussion

Exposure to lethal concentrations of imidacloprid had some sublethal effects on *S. endius* under some conditions. Specifically, an effect on abilities to kill flies and to reproduce was seen in an experiment in which hosts were not buried, but not in an experiment in which hosts were buried. This may be because imidacloprid levels in and on female *S. endius* are decreased by their navigating through media in search of hosts. Liquid or solids in the used fly media may have removed imidacloprid in or on the parasitoids to levels that did not reduce fly killing and parasitoid reproduction. Imidacloprid is highly soluble in water (Kurwadker et al. 2013) and adsorbs to organic matter (Cox et al. 1998). In contrast, when imidacloprid remains on a female, it may be absorbed through non-sclerotized parts of the insect’s cuticle, as seen in fleas (Mehlhorn et al. 1999); or females may also consume it as they groom because grooming frequently involves pulling the antennae and legs through the mouth.
Figure 9. Optical density (OD) distributions of the media treatment, no media treatment, positive control, and negative control. Different lower case letters indicate a significant difference.
Imidacloprid is the most toxic neonicotinoid documented for *S. endius* females (Burgess and King 2015). However, if a female managed to survive 48 h of exposure to the high concentration (equivalent of LC$_{50}$), her subsequent longevity was unaffected (unburied hosts experiment) even though her parasitizing ability was reduced. Honey bees and bumble bees have been observed clearing ingested imidacloprid as quickly as 24 h and 48 h, respectively (Cresswell et al. 2013), which is another way that *S. endius* may reduce harm to itself from pesticides besides by removal of pesticides on its exoskeleton.

In their natural environment, the harm to *S. endius* is probably less than what the unburied host experiment suggests; *Spalangia* spp. frequently parasitize buried hosts (Rueda and Axtell 1985a, Geden 2002), and in the buried host experiment, females that had survived their initial 48 h of exposure to imidacloprid were able to parasitize hosts as well as unexposed females. In the presence of imidacloprid, and perhaps other pesticides, species of parasitoid wasps that do not burrow as extensively, such as *Muscidifurax* spp. (Geden 2002, Pitzer et al. 2011), may not do as well as *Spalangia* spp. When releasing parasitoids to control filth flies, *Spalangia* spp. may also be easier to keep away from pesticide treatment than *Muscidifurax* spp. because *Spalangia* spp. seem not to disperse as far (Birkemoe and Oyrehagen 2010).

Most studies of how pesticides affect parasitoids of filth flies have not included the presence of substrate other than the container in which the experiments were performed. Results of the buried hosts experiment and the media versus no-media experiments suggest that manure may lessen negative effects of pesticide on *S. endius*. In a field study by Mandeville et al. (1990) poultry manure was treated with cyromazine, dimethoate, or permethrin, and there was no detectible difference in rate of parasitization of house flies by *Muscidifurax* spp. and *Spalangia*
spp. compared to untreated controls. Manure in Mandeville et al. (1990) and straw in a study by Geden et al. (1992b) may have reduced harm to parasitoids of filth flies by blocking the pesticide from reaching the parasitoids, whereas here we suggest that substrate may also aid in removing pesticide.

The other parasitoid of filth flies for which effects of imidacloprid have been examined is a confamilial in a different subfamily, Pteromalinae (versus Spalanginae), *Nasonia vitripennis* Walker (Hymenoptera: Pteromalidae) (Whitehorn et al. 2015). Instead of examining effects of exposure through contact of treated substrates, Whitehorn et al. (2015) were interested in exposure through adult *N. vitripennis* feeding on nectar from plants grown from pesticide-treated seeds. Whitehorn et al. (2015) found that in *N. vitripennis*, a realistic concentration of imidacloprid-contaminated sucrose solution decreases offspring production and increases the proportion of sons when multiple females are simultaneously parasitizing hosts. Studies of the effects of imidacloprid on other biological control insects suggest that besides effects on offspring production, sex ratio, and longevity, other effects may include changes to the shape of functional response to their prey (He et al. 2012, Malaquias et al. 2014) and decreased response to host cues (Rogers and Potter 2003).

Results of the present study show that the impact of imidacloprid on the effectiveness of *S. endius* through sublethal effects will depend on what concentrations *S. endius* are exposed to and the substrates they encounter. Whether *S. endius* is also likely to encounter imidacloprid while searching for nectar or dispersing through crop areas is unclear because field observations of *Spalangia* species in such locations are lacking.
CHAPTER 5
SYNTHESIS

The insecticidal active ingredients in pesticides used for controlling filth flies in animal rearing facilities pose some risk to biological control agents, specifically the pupal parasitoids *Spalangia endius* and *Urolepis rufipes*. However, granular bait formulations containing the fly pheromone (Z)-9-tricosene in conjunction with either imidacloprid, dinotefuran, or methomyl, as well as other proprietary ingredients, appear to be adequately selective towards filth flies in that neither *S. endius* nor *U. rufipes* were attracted to these baits. *Spalangia endius* may be particularly advantageous to release; for their aversion to the three tested baits, for their burrowing habit, which can reduce pesticide residue on their bodies; and for their well-established ability to reduce fly populations when released en masse (Morgan 1980, Morgan et al. 1981).

Spinosad appears to be one of the best insecticidal active ingredients to use in conjunction with mass releases of *S. endius*. Spinosad is currently used in granular baits that target filth flies, though none of those baits were tested in the present studies. In the house flies tested, spinosad was tied with permethrin for most toxic. Permethrin is mainly used as a contact pesticide and may be more effective against flies than spinosad-based baits when applied to maximize contact (Faulde et al. 2003). However, permethrin is moderately toxic to *S. endius*.

Given that parasitoids such as *S. endius* and *U. rufipes* are not attracted to such baits, then the most likely modality of pesticide risk to parasitoids appears to be from contamination of the
environments in which the parasitoids search for hosts. This contamination could result from an inadvertent spill of granular bait, emulsified concentrate, runoff or dust during farm cleanup, wettable granules, etc. into parasitoid habitat, or even from an intentional misapplication of various forms of pesticides perhaps initiated by the sight of significant fly activity on manure and other rotting organic matter; places where parasitoids are also found.

The present studies highlight various potential risks of commonly used filth fly pesticides to the parasitoids *Spalangia endius* and *Urolepis rufipes* as assessed in the laboratory. How misapplied or spilled pesticides affect parasitoids under field conditions and at field-relevant pesticide quantities remains to be seen. An important initial step in determining this effect will be to measure how these pesticides disseminate through the parasitoid’s environment when applied at field-relevant quantities. From this information, improvements could be made to fly-pesticide clean-up protocol, which will help minimize the negative impacts on biological control agents such as parasitoids. Or perhaps these results will not show any harm to parasitoids under field conditions. Parasitoids that come into contact with pesticides and then burrow into moist, uncontaminated substrates may have pesticide residues reduced in or on them.

Here I have made several recommendations for utilizing pesticides and the filth fly pupal parasitoids *Spalangia endius* and *Urolepis rufipes* together. I would also recommend that pesticide labels be updated to include restrictions on pesticides being applied to manure and other rotting organic matter. With pesticides that are sufficiently selective to the target species and with strict adherence to updated pesticide labels, filth fly pesticides and filth fly pupal parasitoids can safely and effectively be combined to reduce filth fly populations in animal rearing facilities.
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