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Sublethal effects of imidacloprid exposure on *Spalangia endius*, a pupal parasitoid of filth flies

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Abstract Parasitoids and neonicotinoids can both suppress economically harmful filth fly populations. However, sublethal effects of neonicotinoids have not previously been studied for commonly used species of filth fly parasitoids. Exposure to an LC_{50} of imidacloprid decreased the ability of surviving individuals of the parasitoid wasp *Spalangia endius* Walker (Hymenoptera: Pteromalidae) 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 exposed to the media in which hosts were buried. Our findings suggest that substrate may reduce pesticides on biological control agents that burrow, making them more effective.

Keywords Imidacloprid; Neonicotinoid insecticide; Nontarget; Sublethal; Pteromalidae; Muscidae

Introduction

Filth flies, such as house flies (*Musca domestica* L.) and stable flies (*Stomoxys calcitrans*) L. (Diptera: Muscidae), are a source of considerable economic loss in animal-rearing operations (Malik et al. 2007; Taylor et al. 2012). The flies may spread pathogens (Alam and Zurek 2004; Talley et al. 2009) and are a stress-inducing nuisance to livestock and humans. 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 (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.

Imidacloprid is one of the most widely used pesticides against house flies (Simon-Delso et al. 2015), and is frequently successful (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 (Pham-Delègue et al. 2002; Desneux et al. 2007). In parasitoids from multiple hymenopteran families, imidacloprid is known to affect host finding ability and parasitization rates (Tiphiidae: Rogers and Potter 2003; Eulophidae: Tran et al. 2004; Mymaridae: Liu et al. 2010; Aphelinidae: Sohrabi et al. 2014).

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 (Gibson and Floate 2004; Romero et al. 2010) and through augmentative releases (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. Adult females kill buried and unburied hosts by laying offspring and host

feeding. *Spalangia* spp. are known for burrowing, although they also parasitize hosts on the surface (Rueda and Axtell 1985; Geden 2002; Skovgård 2006).

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, b). 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 life 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 an 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 limited (Burgess and King 2015, 2016; Whitehorn et al. 2015).

Materials and methods

Laboratory colonies

The *S. endius* and the *M. domestica* were from laboratory colonies that had not been exposed to pesticides since colony establishment. The *M. domestica* were reared on a mixture of fly larva medium (Lab Diet, St. Louis, MO; http://www.labdiet.com, accessed 26 April 2015), pine shavings, fish meal, and water (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, a 20 ml scintillation vial was coated with an LC_{10} of 0.04 ng/cm^2 (low concentration) or LC_{50} of 17.92 ng/cm^2 (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.

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 had been 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 honey to water 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. 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 and the previous experiment were chosen so that a healthy female would be unlikely 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 one 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. 1, 2).

Media versus no media experiment

A female 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 (approx. 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. 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. That their movements are extensive was known from prior observation of *S. endius* that had been placed in this setup after having been dusted with paint that fluoresces under UV black light. 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 reverse osmosis filtered 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 set up and collection of five females for each treatment was replicated 20 times, for a total of 100 females. The validity of the test was assessed with positive and negative controls, also in tubes of reverse osmosis filtered 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 in the centrifuge tubes were homogenized 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) and 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 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 about 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 on OD values.

Statistical analyses

Analyses were performed with R version 3.1.2 (R Core Team 2015). In the buried and unburied experiments, effect of treatment on the number of emerged parasitoids and flies and parasitoid sex ratio were each analyzed using generalized linear models. Analyses of number of flies and parasitoids were best fitted with a quasi-Poisson distribution to account for over dispersion in the models, with a log link function. Analysis of sex ratio was with a binomial error and logit link function. Post hoc comparisons were with Tukey's honestly significant difference test. 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. 1; $F_{3, 12} = 17.22$, $p < 0.001$). 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 (Fig. 1; $F_{3, 12} = 7.40$, $p = 0.005$). 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 sex ratio among treatments ($\chi^2_3 = 5.31$, $p = 0.15$). 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 also no significant difference among treatments in the number of

days that tested parasitoids (i.e., parasitoids that survived the initial exposure) subsequently lived ($\chi^2_3 = 2.7$, p = 0.43).
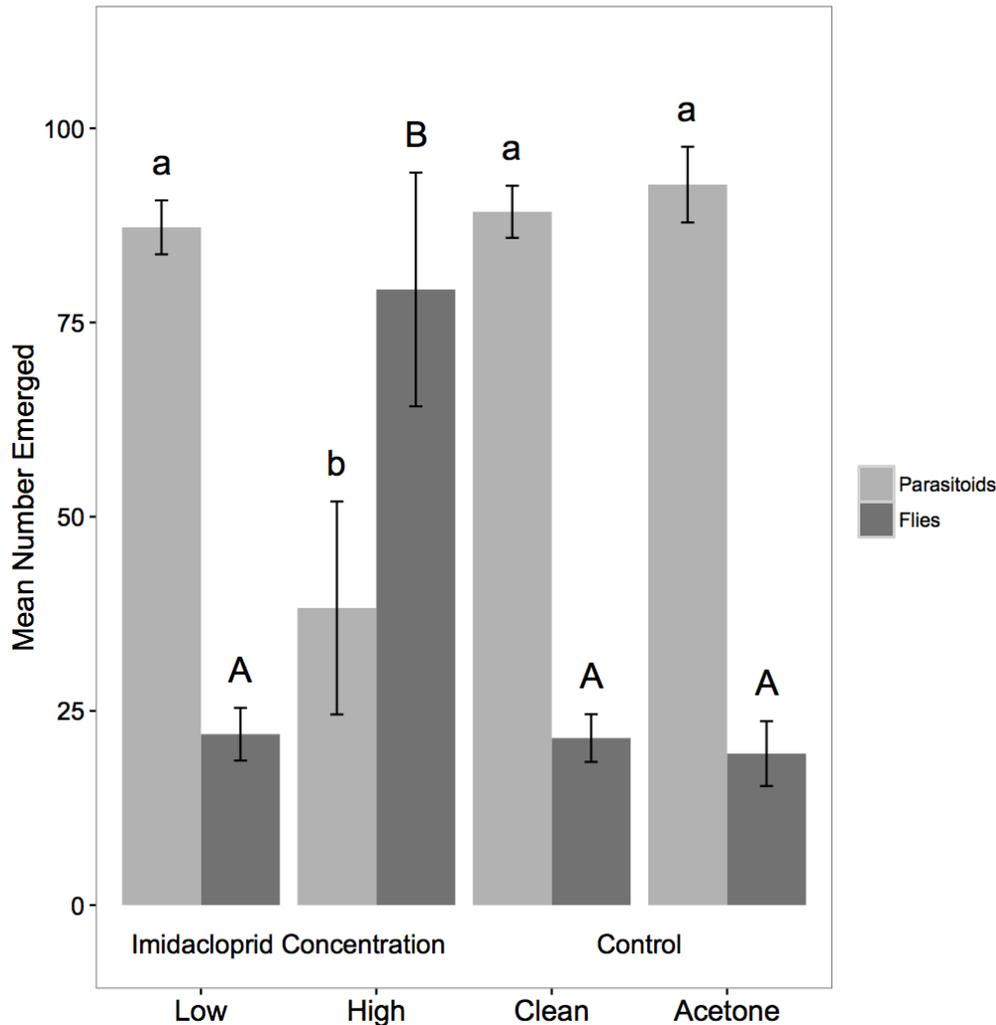

Fig. 1 Mean ± SE number of flies and parasitoid wasps emerged from the unburied hosts experiment in which a female parasitoid wasp had previously been exposed to a low concentration of imidacloprid, a high concentration, a clean vial control or an acetone control. The same lower case letter indicates no significant difference in number of parasitoids at $\alpha = 0.05$, and the same upper case letter indicates no significant difference in number of flies at $\alpha = 0.05$.

Buried hosts experiment

There was no significant treatment effect on the number of flies that emerged in this experiment (Fig. 2; $F_{3, 8} = 0.84$, p = 0.51). There was also no significant treatment effect on the number of parasitoids that emerged (Fig. 2; $F_{3, 8} = 1.52$, p = 0.28).
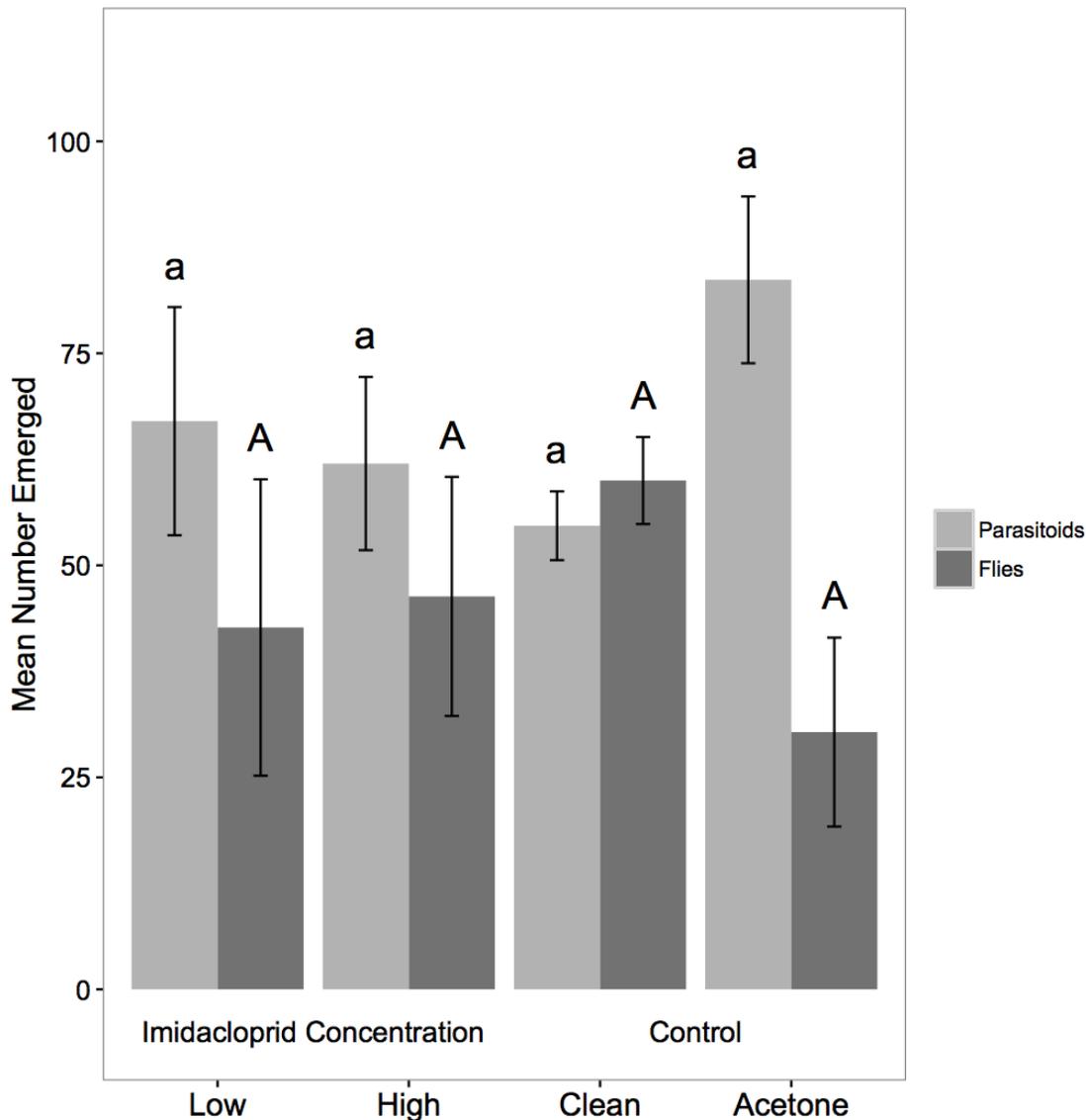
Fig. 2 Mean ± SE number of flies and parasitoid wasps that emerged from the buried hosts experiment in which a female parasitoid wasp had previously been exposed to a low concentration of imidacloprid, a high concentration, an acetone control or a clean vial control. The same lower case letter indicates no significant difference in number of parasitoids at $\alpha = 0.05$, and the same upper case letter indicates no significant difference in number of flies at $\alpha = 0.05$.

Media versus no media experiment

There was a significant effect of treatment on OD ($t_{38} = 3.76$, $p < 0.001$), indicating more imidacloprid in the no media treatment than in the media treatment.

Discussion

Exposure to a high concentration of imidacloprid caused some sublethal effects in *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 (Kurwadkar 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.

If a female *S. endius* managed to survive 48 h of exposure to the high concentration of imidacloprid, 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 its host searching behavior.

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 1985; 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 substrate, other than the containers in which the experiments were performed. Results of the buried hosts experiment and the media versus no media experiment 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. (1992) 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 residues.

The only other parasitoids of filth flies in which effects of imidacloprid have been examined are the confamilials *Urolepis rufipes* Ashmead and *Nasonia vitripennis* Walker. Both are in subfamily Pteromalinae, whereas *Spalangia* is in Spalanginae. The LC50 of imidacloprid is similar for *U. rufipes* and *S. endius* (Burgess and King 2016). *Spalangia endius* but not *U. rufipes*, is attracted to imidacloprid; although both species are repelled to a bait formulation that contains imidacloprid. In *N. vitripennis,* Whitehorn et al. (2015) were interested in exposure to imidacloprid through adults feeding on nectar from plants grown from pesticide-treated seeds. A realistic concentration of imidacloprid-contaminated sucrose solution decreased offspring production and increased the proportion of sons when multiple females were simultaneously parasitizing hosts.

The imidacloprid concentrations tested in the present study are within the range of what parasitoids will likely encounter in the field, which is for example 0 - 915.4 ng/cm^2. The 915.4 ng/cm^2 is the concentration of imidacloprid in the recommended application rate for Quickbayt Fly Bait (Bayer, Shawnee Mission, KS), which is greater than the LC_{100} for both *S. endius* and *U. rufipes* (Burgess and King 2016). Recommended reapplication is weekly as needed. The half-life of imidacloprid ranges from 40 d - 124 d in crop soil that has previously been fertilized with cow manure and in which imidacloprid has been applied as a seed dressing at 900 ng/cm^2 (Rouchaud et al. 1996). However, how field-relevant imidacloprid concentrations degrade and disseminate in substrates that more closely match those that parasitoids are likely to be in has not been studied.

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.

References

Alam MJ, Zurek L (2004) Association of *Escherichia coli* O157:H7 with houseflies on a cattle farm. Appl Environ Microbiol 70:7578-7580

Birkemoe T, Oyrehagen H (2010) Parasitism of the house fly parasitoid *Spalangia cameroni* on Norwegian pig farms: local effect of release method. BioControl 55:583-591.

Burgess ER, King BH (2015) Compatibility of the parasitoid wasp *Spalangia endius* (Hymenoptera: Pteromalidae) and insecticides against *Musca domestica* (Diptera: Muscidae) as evaluated by a new index. J Econ Entomol 108:986–992.

Burgess ER, King BH (2016) Behavior and survival of the filth fly parasitoids *Spalangia endius* and *Urolepis rufipes* (Hymenoptera: Pteromalidae) in response to three granular house fly baits and components. Environ Entomol.

Butler SM, Gerry AC, Mullens BA (2007) House fly (Diptera: Muscidae) activity near baits containing (Z)-9-tricosene and efficacy of commercial toxic fly baits on a southern California dairy. J Econ Entomol 100:1489–1495

Cox L, Koskinen WC, Celis R, Yen PY, Hermosin MC, Cornejo J (1998) Sorption of imidacloprid on soil clay mineral and organic components. Soil Sci Soc Am J 62:911–915

Cresswell JE, Robert F-XL, Florance H, Smirnoff N (2013) Clearance of ingested neonicotinoid pesticide (imidacloprid) in honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris*). Pest Manag Sci 70:332–337.

Desneux N, Decourtye A, Delpuech J-M (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106.

Ferguson H, Neal SO, Walsh D, Galvin K, Vásquez V, Yost M (2014) Fly control news to moo about: survey of pest management practices on Washington dairy farms April 2014. Washington State University Extension, Prosser, WA. http://wastatedairy.com/wp-content/uploads/2014/04/WSU-Extension-dairy-survey-results-Apr2014forprint.pdf Accessed 26 Sep 2016

Floate KD (2003) Field trials of *Trichomalopsis sarcophagae* (Hymenoptera: Pteromalidae) in cattle feedlots: a potential biocontrol agent of filth flies (Diptera: Muscidae). Can Entomol 135:599–608

Geden CJ (2002) Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: Pteromalidae, Chalcididae) of house flies (Diptera: Muscidae) in three types of substrates. Environ Entomol 31:411–417

Geden CJ, Rutz DA, Miller RW, Steinkraus DC (1992) Susceptibility of house flies (Diptera: Muscidae) and five pupal parasitoids (Hymenoptera: Pteromalidae) to abamectin and seven commercial insecticides. J Econ Entomol 85:435-440

Gerry AC, Zhang D (2009) Behavioral resistance of house flies, *Musca domestica* (Diptera: Muscidae) to imidacloprid. US Army Med Dep. J. 2009 Jul-Sep: 54–59.

Gibson GAP, Floate KD (2004) Filth fly parasitoids; on dairy farms in Ontario and Quebec, Canada. Can Entomol 136:407-417

Hinkle NC (2015) Animals: fly control in livestock facilities. In: Horton DL (ed) Georgia Pest Management Handbook, 36th Commercial Edition. Cooperative Extension Special Bulletin 28, University of Georgia. pp 63-65.

Kaufman PE, Nunez SC, Mann RS, Geden CJ, Scharf ME (2010) Nicotinoid and pyrethroid insecticide resistance in houseflies (Diptera: Muscidae) collected from Florida dairies. Pest Manag Sci 66: 290–294

King BH (2002) Breeding strategies in females of the parasitoid wasp *Spalangia endius*: effects of mating status and body size. J Insect Behav 15:181-193

King BH, Colyott KL, Chesney AR (2014) Livestock bedding effects on two species of parasitoid wasps of filth flies. J Insect Sci 14:185.

Kristensen M, Jespersen JB (2004) Susceptibility of spinosad in *Musca domestica* (Diptera: Muscidae) field populations. J Econ Entomol 97:1042–1048

Kurwadkar ST, Dewinne D, Wheat R, McGahan DG, Mitchell FL (2013) Time dependent sorption behavior of dinotefuran, imidacloprid and thiamethoxam. J Environ Sci Health Part B-Pestic Contam Agric Wastes 48:237-242.

Liu F, Bao SW, Song Y, Lu HY, Xu JX (2010) Effects of imidacloprid on the orientation behavior and parasitizing capacity of *Anagrus nilaparvatae*, an egg parasitoid of *Nilaparvata lugens*. BioControl 55:473-483.

Machtinger ET, Leppla NC, Sanders CS (2012) Pest management perceptions and practices for equine farms in north and central Florida. University of Florida, Institute of Food and Agricultural Sciences, Gainesville, FL. IFAS Publication ENY-2028 http://edis.ifas.ufl.edu/in983 Accessed 26 Sep 2016

Malik A, Singh N, Satya S (2007) House fly (*Musca domestica*): a review of control strategies for a challenging pest. J Environ Sci Heal B 42:453–469.

Mandeville JD, Mullens BA, Yu DS (1990) Impact of selected pesticides on field population dynamics of parasitic Hymenoptera (Pteromalidae) in caged-layer poultry manure in southern California, U.S.A. Med Vet Entomol 4:261–268.

McKay T, Steelman CD, Brazil SM, Szalanski AL (2007) Sustained mass release of pupal parasitoids (Hymenoptera: Pteromalidae) for control of *Hydrotaea aenescens* and *Musca domestica* (Diptera: Muscidae) in broiler-breeder poultry houses in Arkansas. J Agr Urban Entomol 24:67–85.

Mehlhorn H, Mencke N, Hansen O (1999) Effects of imidacloprid on adult and larval stages of the flea *Ctenocephalides felis* after in vivo and in vitro application: a light- and electron-microscopy study. Parasitol Res 85:625-637

Murillo AC, Gerry AC, Gallagher NT, Peterson NG, Mullens BA (2014) Laboratory and field assessment of cyantraniliprole relative to existing fly baits. Pest Manag Sci: 71:752–758.

Myint WW, Walter GH (1990) Behaviour of *Spalangia cameroni* males and sex ratio theory. Oikos 59:163-174

Nurita AT, Abu Hassan A (2010) Comparative performance of two commercial neonicotinoid baits against filth flies under field conditions. Trop Biomed 27:559–565

Pham-Delègue MH, Decourtye A, Kaiser L, Devillers J (2002) Behavioural methods to assess the effects of pesticides on honey bees. Apidologie 33:425-432

Pitzer JB, Kaufman PE, Geden CJ, Hogsette J (2011) The ability of selected pupal parasitoids (Hymenoptera: Pteromalidae) to locate stable fly hosts in a soiled equine bedding substrate. Environ Entomol 40: 88-93.

Pospischil R, Junkersdorf J, Horn K (2005) Control of houseflies *Musca domestica* (Diptera: Muscidae) with imidacloprid WG 10 in pig farms (Germany). Proceedings of the Fifth International Conference on Urban Pests. Perniagaan Ph'ng @ P&Y Design Network, Malaysia

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rogers ME, Potter DA (2003) Effects of spring imidacloprid application for white grub control on parasitism of Japanese beetle (Coleoptera: Scarabaeidae) by *Tiphia vernalis* (Hymenoptera: Tiphiidae). J Econ Entomol 96:1412–1419

Romero A, Hogsette JA, Coronado A (2010) Distribution and abundance of natural parasitoid (Hymenoptera: Pteromalidae) populations of house flies and stable flies (Diptera: Muscidae) at the University of Florida dairy research unit. Neotrop Entomol 39:424–429

Rouchaud J, Rouchaud J, Thirion A, Wauters A, VandeSteene F, Benoit F, Ceustermans N, Gillet J, Marchand S, Vanparys L (1996) Effects of fertilizer on insecticides adsorption and biodegradation in crop soils. Arch Environ Con Tox 31:98-106

Rueda LM, Axtell RC (1985) Effect of depth of house fly pupae in poultry manure in parasitism by six species of Pteromalidae (Hymenoptera). J Entomol Sci 20:444-449

Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke CH, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, Van Dyck H, Van Praagh J, Van der Sluijs JP, Whitehorn PR, Wiemers M (2015) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ Sci Pollut Res 22:5-34.

Skovgård H (2002) Dispersal of the filth fly parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) in a swine facility using fluorescent dust marking and sentinel pupal bags. Environ Entomol 31:425–431

Skovgård H (2006) Search efficiency of *Spalangia cameroni* and *Muscidifurax raptor* on *Musca domestica* pupae in dairy cattle farms in Denmark. BioControl 51:49-64.

Skovgård H, Nachman G (2004) Biological control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) by means of inundative releases of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Bull Entomol Res 94:555-567.

Smith L, Rutz DA, Scoles GA (1989) Influence of habitat and temperature on dispersal behaviour of two pteromalid parasitoids of houseflies during an inundative release at a dairy barn. Med Vet Entomol 3:169–178

Sohrabi F, Shishehbor P, Saber M, Mosaddegh MS (2014) Effects of buprofezin and imidacloprid on the functional response of *Eretmocerus mundus* Mercet. Plant Protection Science 50:145-150

Stafford KC, III (2008) Fly management handbook a guide to biology, dispersal, and management of the house fly and related flies for farmers, municipalities, and public health officials. Bulletin 1013. May 2008. The Connecticut Agricultural Experiment Station, New Haven

Talley JL, Wayadande AC, Wasala LP, Gerry AC, Fletcher J, DeSilva U, Gilliland SE (2009) Association of *Escherichia coli* O157:H7 with filth flies (Muscidae and Calliphoridae) captured in leafy greens fields and experimental transmission of *E. coli* O157:H7 to spinach leaves by house flies (Diptera: Muscidae). J Food Protect 72:1547-1552

Taylor DB, Moon RD, Mark DR (2012) Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. J Med Entomol 49:198-209.

Townsend L (2015a) Ent-28 Insecticide control on poultry - 2015. University of Kentucky College of Agriculture, Food and Environment, Lexington, KY http://pest.ca.uky.edu/EXT/Recs/ENT28-Poultry.pdf Cited 15 Sep 2015

Townsend L (2015b) Residual fly sprays. http://www.uky.edu/Ag/PAT/recs/livestk/recdairy/daires.htm Cited 15 Sep 2015

Tran DH, Takagi M, Takasu K (2004) Effects of selective insecticides on host searching and oviposition behavior of *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), a larval parasitoid of the American serpentine leafminer. Appl Entomol Zool 39:435-441.

Warkentin TE, Sheppard JI, Moore JC, Sigouin CS, Kelton JG (2008) Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. J Thromb Haemost 6:1304–1312.

White WH, McCoy CM, Meyer JA, Winkle JR, Plummer PR, Kemper CJ, Starkey R, Snyder DE (2007) Knockdown and mortality comparisons among spinosad-, imidacloprid-, and methomyl-containing baits against susceptible *Musca domestica* (Diptera: Muscidae) under laboratory conditions. J Econ Entomol 100:155–163

Whitehorn PR, Cook N, Blackburn CV, Gill SM, Green J, Shuker DM (2015) Sex allocation theory reveals a hidden cost of neonicotinoid exposure in a parasitoid wasp. Proc R Soc B 282:20150389

Zwicker JI, Uhl L, Huang WY, Shaz BH, Bauer KA (2004) Thrombosis and ELISA optical density values in hospitalized patients with heparin-induced thrombocytopenia. J Thromb Haemost 2:2133–2137.

Dr. E. R. Burgess This project is part of the Ph.D. work of Dr. Edwin R. Burgess IV. His research has focused on assessing the compatibility of filth fly pesticides with filth fly biological control parasitoids through toxicological and behavioral assays.

Aspen Kremer is a MS student interested in effects of various stressors, including pesticides, on insects.

Dr. Sherine Elsawa usually studies human tumor cell microenvironments.

Dr. B. H. King is a professor at Northern Illinois University and Edwin's advisor. She is a behavioral ecologist and has been doing research with parasitoid wasps of filth flies for more than 20 years.