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Compatibility of the Parasitoid Wasp *Spalangia endius* (Hymenoptera: Pteromalidae) and Insecticides against *Musca domestica* (Diptera: Muscidae) as Evaluated by a New Index

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ABSTRACT Various insecticides for the control of the house fly *Musca domestica* L. were tested for compatibility with a biological control agent, the pupal parasitoid *Spalangia endius* Walker. Bioassays used the mode in which each organism was expected to be harmed by the insecticides, a surface contact bioassay for *S. endius* and a feeding bioassay for *M. domestica*. A Pesticide Compatibility Index (PCI) was created that allows comparison of LC_{50} values when the mode of exposure to a pesticide differs. First LC_{50} values were converted into units of prescribed dosages (LPR = LC_{50}-to-prescribed dosage ratio). This study used dosages from labels of granular baits. PCI is the ratio of $LPR_{\text{biological control agent}}$ to LPR_{pest}. For these 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, is consistent with conclusions from the LC_{50} values. Permethrin and nitenpyram were also tested, but their PCIs were not calculated. Permethrin is prescribed as a contact insecticide against flies rather than being consumed as a bait, and nitenpyram has not been formulated as a fly insecticide. Compared to the other insecticides in terms of LC_{50} values, permethrin was moderately toxic to *S. endius* but one of the most toxic for *M. domestica*; whereas nitenpyram was least toxic for both *S. endius* and the flies.

KEY WORDS biological control, efficacy index, IPM-compatibility index, lethal concentration, pesticide, selectivity index

Integrated Pest Management programs (IPM) often include biological controls as an environmentally friendly means of pest control (Tobin et al. 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-Ramora 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 2015; Agita® 1GB Scatterbait (thiamethoxam) (Novartis Animal Health, North Ryde NSW, Canada)). During male dispersal away from the natal site (Myint and Walter 1990) and as females move from one host location to another is another time when wasps may encounter sites with insecticide residue. 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 type 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 (Golden Malrin®, Wellmark, Schaumburg, IL), dinotefuran (Quikstrike® Fly Scatter Bait, Wellmark, Schaumburg, IL), thiamethoxam (Agita® 1 GB Scatterbait, Novartis, Greensboro, NC), and spinosad (Conserve® Fly Bait, Southern Agricultural Insecticides, Inc., Palmetto, FL). LPR values were not calculated for permethrin or for nitenpyram because neither is sold as a 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 ($LPR_{parasitoids}$, LPR_{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 $LPR_{parasitoid}$ by the LPR_{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 vindemmiae* 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 (Lofton et al. 2003, Stafford 2008).

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Table 1. *Spalangia endius* LC_{50}, prescribed dosage, and LC_{50}-to-prescribed dosage ratio (LPR)

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

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® (dinotefurna), 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)

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

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 = $LPR_{parasitoid}/LPR_{fly}$