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Decaying Organic Matter Does Not Remove Sublethal Effects of Imidacloprid on Mating in *Spalangia endius* (Hymenoptera: Pteromalidae), a Parasitoid of Filth Flies

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Abstract

Both the parasitoid wasp *Spalangia endius* Walker and the insecticide imidacloprid are used to control house flies. A recent study found that negative sublethal effects of imidacloprid on killing flies and on offspring production by this parasitoid wasp are eliminated when females have the opportunity to crawl through decaying matter. An ELISA (enzyme-linked immunosorbent assay) showed that the presence of decaying matter reduces the amount of pesticide on their bodies. The present study examined whether this was also true for sublethal effects on mating. *S. endius* were exposed to a realistic concentration of imidacloprid that induces very low mortality. Then individual parasitoids were allowed to burrow through decaying organic matter or not, followed by mating tests in the absence of decaying matter. Even after 24 h with the decaying matter, copulation for both males and females that had previously been exposed to imidacloprid was delayed compared to no-pesticide controls. Furthermore, for pesticide-exposed males, subsequently burrowing through media made copulation even more delayed than if they were not exposed to media. For pesticide-exposed females, subsequently burrowing through media neither increased or decreased the negative effect of the pesticide exposure. Together with other studies, these results reinforce that use of *S. endius* and use of imidacloprid are incompatible, even at much lower than recommended concentration, unless application is sufficiently separated in place and time.

Key words: biological control – parasitoids and predators, pesticide residues, mating strategies, Hymenoptera, Pteromalidae

House flies, *Musca domestica* L. (Diptera: Muscidae) can cause substantial economic loss to animal rearing operations (Taylor et al. 2012, Baldacchino et al. 2013). In warm temperatures, large accumulations of manure can lead to large populations of adult flies, which may become a significant nuisance and health hazard to livestock and humans on the farm and to neighbors (Clean Neighborhoods 2005, Pugh et al. 2014, Khamesipour et al. 2018, Onwugamba et al. 2018)

House fly populations are primarily controlled by sanitation (Stafford 2008; Calvo et al. 2009; Higginbotham et al. 2010), but chemical control is also common (USDA 2007). Because of concern about resistance and health and environmental risks associated with insecticides, natural enemies may also be released. *Spalangia endius* Walker (Hymenoptera: Pteromalidae) is a parasitoid wasp that is sold commercially to augment natural populations in order to control filth flies (van Lenteren 2012, Cranshaw and Broberg 2015).

Mass releases of pupal parasitoids, including *S. endius*, are effective in some situations (Morgan et al. 1975, Weinzierl and Jones 1998, Skovgård and Nachman 2004, McKay et al. 2007; but see Meyer et al. 1990, Andress and Campbell 1994).

Imidacloprid is a neonicotinoid that is widely used against house flies in livestock facilities (Simon-Delso et al. 2015). Imidacloprid is applied on or near rotting organic material, sometimes along proximate hard surfaces (White et al. 2007, Stafford 2008, Pospischil et al. 2005); but *S. endius* and other parasitoids of filth flies occur naturally in these areas (Weinzierl and Jones 1998, Olbrich and King 2003, Romero et al. 2010). Thus, these parasitoids may receive inadvertent exposure to pesticides while searching for hosts and mates, which can reduce their effectiveness at controlling pest flies (Smith et al. 1989, Skovgård 2002).

In a Florida strain of *S. endius*, survival and subsequent ability to produce progeny and reduce fly numbers was reduced by contact with imidacloprid on a glass surface at concentrations well above recommended application rates (Burgess and King 2015). However, if females then had to move through manure-like media to locate hosts, parasitization ability was no longer affected (Burgess et al. 2017). An ELISA (enzyme-linked immunosorbent assay) demonstrated that the media reduced the amount of imidacloprid in-on the parasitoids. The media was damp and may have reduced the amount physically or by dilution.

In a previous study of the Illinois strain of *S. endius* that was used in the present study, media was absent, and imidacloprid caused sublethal reductions in mating (Kremer and King 2019). Specifically, exposed males were less successful at mating than unexposed males; and males did not avoid exposed females, but exposed females almost always failed to open their genital to complete copulation. The present study examined whether the presence of media mitigates deleterious sublethal effects on mating as it did with parasitization rate.

Materials and Methods

General Methods

The *S. endius* colony was started with parasitized fly pupae collected in fall 2016 from manure at a dairy farm in northern DeKalb County, Illinois. Neonicotinoids had never been applied to this farm. Vouchers from this colony are catalog numbers 833640 - 833651 in the Insect Collection at the Illinois Natural History Survey Center for Biodiversity. The parasitoids were reared on pupae of the Northern Illinois University strain of *M. domestica* at ~24^{o}C with a 12:12 light dark cycle. See Kremer and King 2019 for *M. domestica* rearing procedures.

Parasitoids used in experiments were 0-to 2- d old. To obtain virgins, males were removed from dishes of parasitized hosts before females emerged, whereas females were obtained by isolating parasitized hosts in test tubes. Experiments were no choice, i.e., had one parasitoid of each sex. One wasp is referred to as unexposed and was taken directly from the original test tube in which it had been isolated. The other parasitoid was from one of the three treatments described below.

Parasitoid Exposure to Imidacloprid

For the two pesticide treatments, parasitoids were exposed by contact to 0.01792 μg/cm^2 of imidacloprid for 48 h under a 12:12 light dark photoperiod at 28^{o}C ± 0.2^{o}C, following Kremer and King 2019. Such exposure kills ~10% of females and no males. Parasitoids might encounter this concentration on a farm because 0.91535 μg/cm^2 of imidacloprid is the recommended application rate for imidacloprid when it is in house fly bait (Burgess and King 2015), and the pesticide disseminates and degrades over time (Rouchaud et al. 1996, Burgess et al. 2018).

Male Experiment

The male experiment had three treatments, pesticide males (PNM♂ = pesticide, no media; n = 28), pesticide males that had then been allowed to burrow through media (PM♂ = pesticide, media; n = 28), and control males that had been allowed to burrow through media (CM♂ = control, media; n = 28) (Fig. 1). After pesticide exposure or lack of exposure (controls), then for 24 h, males were put in a polystyrene petri dish (85 mm diameter) with or without a strip of moistened used fly media (approximately 5 cm wide, 8 mm high) across the center of the dish and contacting both sides and the top and bottom of the dish (Burgess et al. 2017). The used fly media consisted of a decaying mixture of fly larva medium (ground oat hulls, ground barley, wheat bran, dehydrated alfalfa meal) (Lab Diet, St. Louis, MO, USA), large flake pine shavings, fish meal, and water. Ten *M. domestica* pupae were added to one side of each dish, to encourage the male to contact the media. Preliminary experiments with dry florescent paint on *S. endius* demonstrated that males and females walk on and through media. After the 24 h in a dish, the male was removed and immediately placed in a test tube with a virgin unexposed female. Duration to first contact, mount, and copulation (complete aedeagus entry) were recorded for up to 5 min. If a pesticide male manages to copulate, then his mate's sex ratio and ability to control flies is not affected by his exposure (Kremer and King 2019). Within a replicate, the control parasitoid and the parasitoid that was exposed to pesticide were matched for approximate size and age to the nearest day. No wasp was used more than once, and new containers and media were used for each trial. Trials were completed under full light between 22 and 24^{o}C.

Female Experiment

Methods were as described in the male experiment but replacing males with females and vice versa (n = 24 PNM♀, n = 35 PM♀, n = 34 CM♀. Thus, for each female, mating interactions with a virgin unexposed male was recorded.

Statistical Analyses

Alpha was set at 0.05 for each response variable (contact, mount and copulation) within each experiment, except as noted below, because patterns can differ among response variables (King et al. 2005). Duration until first contact, duration from contact to mount, and duration from mount to copulation were compared using log-rank tests on Kaplan-Meier survival curves (Goel et al. 2010) generated with the 'survival' package (Therneau 2015) in R version 3.1.2 (R Core Team 2015). Survival analysis accounts for the possibility that nonresponders might have responded if the duration of observation had been longer.

Results

Male Experiment

Whether media reduced effects of pesticide was examined by looking at PM♂ versus PNM♂. There was no significant effect on duration to first contact of a female (Fig 2; χ^2 = 1.80, df = 1, p = 0.19). However, PM♂ was delayed in duration to mount (χ^2 = 5.40, df = 1, p = 0.02) and duration to copulation (χ^2 = 4.70, df = 1, p = 0.03).

Pesticide still had an effect even after exposure to media for some aspects of mating as determined by comparing PM♂ versus CM♂. There was no significant difference in duration to first contact of a female (Fig 2; χ^2 = 1.90, df = 1, p = 0.16). However, PM♂ was delayed in duration to copulation relative to CM♂ (χ^2 = 5.10, df = 1, p = 0.02), although PM♂ was not significantly delayed in duration to mount (χ^2 = 3.80, df = 1, p = 0.052).

Female Experiment

Unlike for males, media had no significant effect on pesticide-exposed females, i.e., PM♀ versus PNM♀. There was no significant difference in duration to first contact (Fig 3; χ^2 = 0.30, df = 1, p = 0.58), duration to first mount (χ^2 = 0.80, df = 1, p = 0.39), and duration to copulation (χ^2 = 0.40, df = 2, p = 0.51).

Females exposed to media after having been exposed to pesticide or not (i.e., PM♀ versus CM♀), exhibited a similar pattern to that of males. There was no significant effect on duration to first contact (Fig 3; χ^2 = 3.2, df = 1, p = 0.08) and duration to mount (χ^2 = 3.40, df = 1, p = 0.07). However, duration to copulation was delayed for PM♀ females (χ^2 = 5.30, df = 1, p = 0.02).

Discussion

Exposure of male or female *S. endius* to imidacloprid interferes with subsequent copulation. This was true in choice experiments in which mating was examined immediately after exposure (Kremer and King 2019). This was also true in no choice experiments when mating of an exposed individual was examined after it had 24 h with media (the present study: PM versus CM). Burrowing through media after exposure (PM versus PNM) did not mitigate the negative effect of exposure for either sex; in fact, mounting and copulation by exposed males was delayed. That pesticide males, but not pesticide females, were negatively affected by subsequent exposure to media may be related to females burrowing in search of hosts, whereas males tend to stay on the surface seeking out mates (King 2002). As a result, males may be less effective than females at removing from their bodies media to which pesticide has absorbed or attached.

These results contrast with results of the previously described study of parasitization rates of *S. endius*, where hosts being in media eliminated the negative effect of imidacloprid on parasitization (Burgess et al 2017). The mass per area of imidacloprid that wasps were exposed to in Burgess et al. (2017) and in the present study were the same, but the lethality of that exposure is less for the present study. Explanations for the effect of media differing in these two studies include: the Illinois strain being less sensitive to imidacloprid, removal by media being sufficient to mitigate imidacloprid's effects on parasitization but not on mating, and burrowing through a larger quantity of media (Burgess et al 2017) removing more imidacloprid than walking on and maybe through a smaller quantity (the present study).

Previous studies of imidacloprid's effect on *S. endius* document negative effects beginning when exposure to imidacloprid ends. The present study shows that pesticide effects can persist to even 24 h from the time of exposure.

The present study examined effects of moving through clean (pesticide-free) manure-like media after exposure to imidacloprid, and there were no effects of the media on mating for females and negative effects for males. In contrast, clean female *S. endius* are affected by moving through manure that has been contaminated by imidacloprid relative to moving through uncontaminated manure (Burgess et al. 2018).

Unfortunately, parasitoids may stay in areas where they encounter pesticides for extended periods, because female *S. endius* preferentially contact imidacloprid (Burgess and King 2016), and parasitoids, especially males, do not move far from their emergence site in their natural environment (King 2006). Future studies should examine effects of additional substrates in which flies develop and parasitoids are found, such as silage, grain, and animal bedding (Greene et al. 1989, Olbrich and King 2003, Romero et al. 2010).

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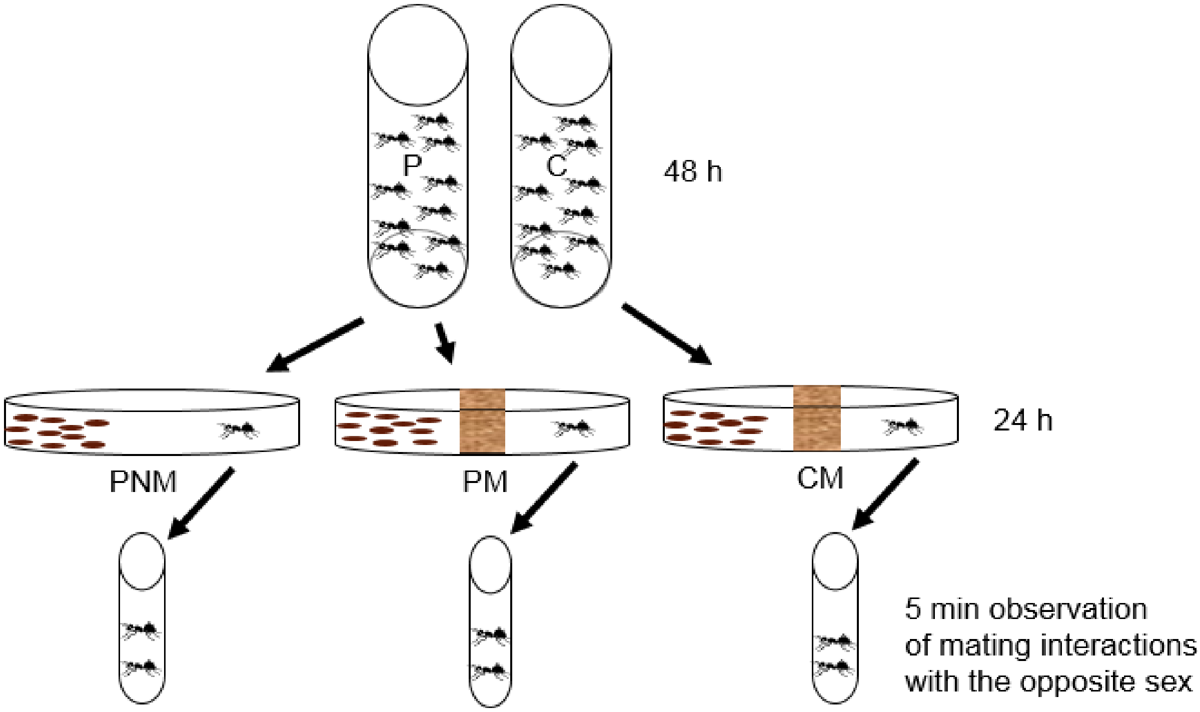

Fig 1. Experimental protocol: wasps exposed to pesticide (P) or control (C) were used to create three treatments, pesticide-exposed wasps given hosts but no media (PNM), pesticide-exposed wasps given hosts and media (PM), and control wasps given hosts and media (CM), and then their mating behavior with an unexposed wasp of the opposite sex was observed.

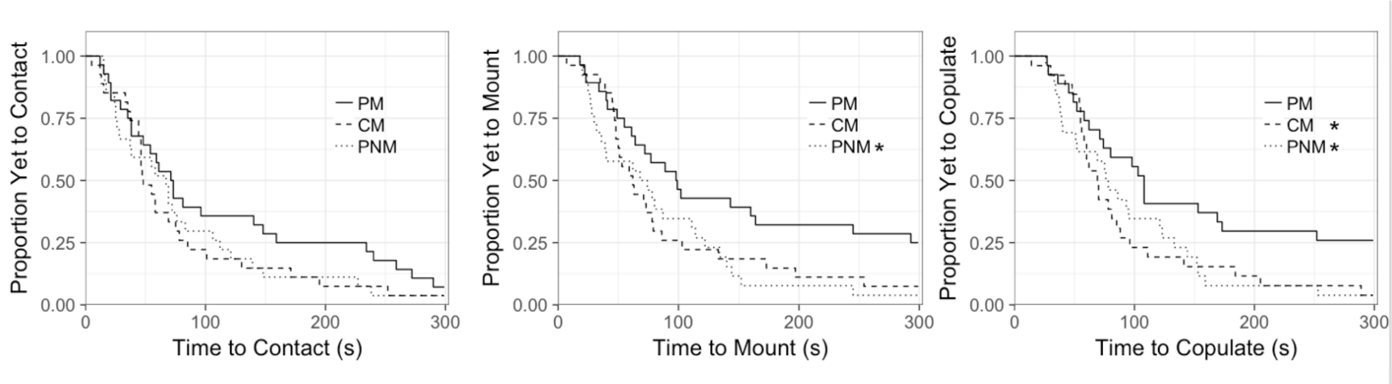

Fig. 2. Proportion yet to contact, yet to mount, and yet to copulate for males that were exposed to pesticide and allowed to burrow through media (PM♂), exposed to pesticide and not allowed to burrow through media (PNM♂), or exposed to an acetone control and allowed to burrow through media (CM♂). * = P < 0.05 when compared to PM♂

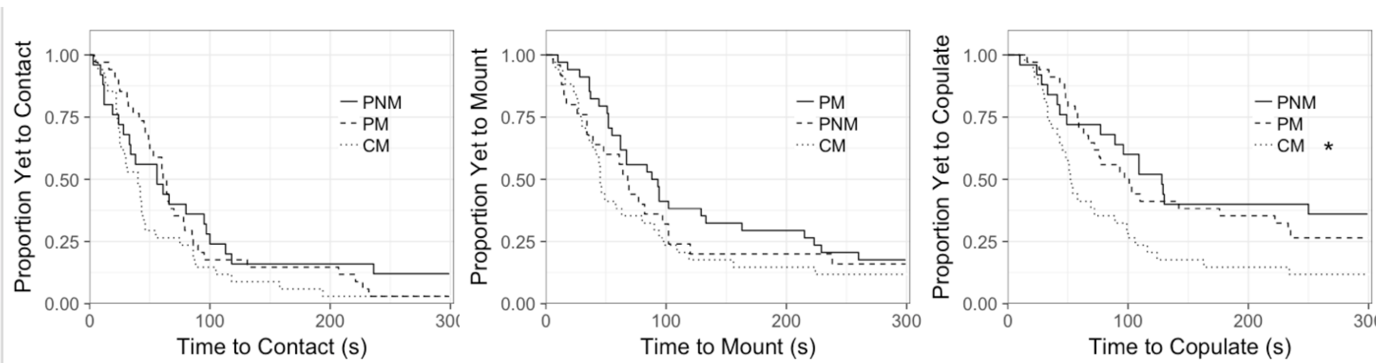

Fig. 3. Proportion yet to contact, yet to mount, and yet to copulate for females that were exposed to pesticide and allowed to burrow through media (PM♀), exposed to pesticide and not allowed to burrow through media (PNM♀), or exposed to an acetone control and allowed to burrow through media (CM♀) that had not yet been contacted by a male at different times. * = P < 0.05 when compared to PM♀