Effect of Host Decoys on the Ability of the Parasitoids Muscidifurax raptor and Spalangia cameroni (Hymenoptera: Pteromalidae) to Parasitize House Fly (Diptera: Muscidae) Puparia

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Effect of host decoys on the ability of the parasitoids *Muscidifurax raptor* and *Spalangia cameroni* (Hymenoptera: Pteromalidae) to parasitize house fly (Diptera: Muscidae) puparia

Dana M. Johnson¹,*, Emily K. Rizzo², Caitlin Taylor¹, and Christopher J. Geden³

Abstract

The pteromalid pupal parasitoids *Muscidifurax raptor* Girault & Sanders and *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) are commonly released on livestock farms for management of house flies, *Musca domestica* L. (Diptera: Muscidae). To be effective, parasitoids must be able to locate live host puparia in complex environments that may include dead or formerly parasitized hosts and non-host physical objects. In this study, both species of parasitoids were examined for their ability to kill and parasitize live house fly puparia either alone or in mixtures with formerly parasitized (dead) hosts or similarly sized acrylic beads. *Muscidifurax raptor* killed significantly fewer hosts and produced fewer progeny when the parasitoids were provided with hosts that were mixed with formerly parasitized puparia. *Spalangia cameroni* was unaffected by the presence of formerly parasitized puparia for any of the measured variables. When beads were used as a decoy instead of formerly parasitized puparia, high bead-to-live-host ratios (90% decoys) resulted in significantly fewer numbers of hosts killed by *M. raptor* compared with the other treatments (50% and no decoys). Residual host mortality at the high bead-to-live-host ratio (90% decoys) was lower (31.2%) than in ratios of 50:50 and with no decoys (51.6 and 59.3%, respectively), so that progeny production by *M. raptor* was unaffected by the presence of beads. *Spalangia cameroni* killed over twice as many hosts and produced twice as many progeny in the absence of bead decoys than when beads made up 90% of the decoy–host mixture. The results support the scatter method for deploying parasitized puparia during releases, because the presence of formerly parasitized hosts did not interfere substantially with the ability of *S. cameroni* and *M. raptor* to locate and parasitize live pupae.

Key Words: *Musca domestica*; stable fly; *Stomoxys calcitrans*; biocontrol; host-finding

Resumen

Los parásitos pteromalídicos de las pupas, *Muscidifurax raptor* Girault & Sanders y *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) se suelen liberar en las fincas ganaderas para el manejo de las moscas domésticas, *Musca domestica* L. (Diptera: Muscidae). Para ser eficaz, los parasitoides deben ser capaces de localizar las pupas hospederas vivas en ambientes complejos que pueden incluir hospederos muertos o anteriormente parasitados y objetos físicos que no son hospederos. En este estudio, se examinó a ambas especies de parasitoides para determinar su capacidad para matar y parasitar las puparias de moscas vivas solas o en mezclas con hospederos anteriormente parasitados (muertos) o cuentas de acrílico de tamaño similar. *Muscidifurax raptor* mató significativamente menos hospederos y produjo menos progenie cuando los parasitoides fueron proporcionados con los hospederos que fueron mezclados con puparia parasitadas anteriormente. *Spalangia cameroni* no se vio afectada por la presencia de puparia anteriormente parasitadas para ninguna de las variables medidas. Cuando se utilizaron las cuentas de acrílico como señuelo sustituto en lugar de puparia anteriormente parasitada, altas proporciones de cuentas de acrílico –hospederos vivos (90% señuelos) resultó en un número significativamente menor de hospederos muertos por *M. raptor* en comparación con los otros tratamientos (50% y no señuelos ). La mortalidad residual del hospedero en la proporción alta de cuentas de acrílico -hospedero (90% señuelos) fue menor (31,2%) que en las proporciones de 50:50 y sin señuelos (51,6 y 59,3%, respectivamente), por lo que la producción de progenie por *M. raptor* no se vio afectada por la presencia de cuentas de acrílico. *Spalangia cameroni* mató más del doble del número de hospederos y produjo el doble del número de progenie en ausencia de señuelos de cuentas que cuando las cuentas constituían el 90% de la mezcla señuelo-hospedero. Los resultados apoyan el método de dispersión para el despliegue de puparia parasitada durante las liberaciones en que la presencia de hospederos anteriormente parasitados no interferiría sustancialmente con la capacidad de *S. cameroni* y *M. raptor* para localizar y parasitar pupas vivas.

Palabras Clave: *Musca domestica*; mosca del estable; *Stomoxys calcitrans*; control biológico; búsqueda de hospederos

House flies (*Musca domestica* L.; Diptera: Muscidae) are worldwide pests that are an agricultural nuisance and a major public health concern. These flies have the ability to mechanically vector a wide variety of pathogenic microorganisms to humans and livestock and may have a role in the dispersal of antibiotic-resistant bacteria (Graczyk et al. 2001; Zurek & Ghosh 2014). There is critical need for house fly management...
tools because of increasing resistance to conventional insecticides (Malik et al. 2007; Scott et al. 2013). Pteromalid pupal parasitoids provide one of the most common and readily available biological controls for fly management (Machtiger & Geden 2017). Commercial insectaries rear and sell a variety of species, including Muscidifurax raptor Girault & Sanders and Spalangia cameroni Perkins (Hymenoptera: Pteromalidae). Although releases of these species have proven effective as part of integrated pest management programs in a variety of production systems (Geden et al. 1992; Geden & Hogsette 2006; Birkemoe et al. 2009), questions remain about the numbers of parasitoids needed to provide satisfactory management and the best methods to deploy parasitized hosts in the field.

Parasitoids can be released by either scattering parasitized puparia in areas of known fly breeding (Rutz & Axtell 1981; Kaufman et al. 2002, 2012; Skovgård 2004) or by placing them in release stations that protect them from damage and accidental removal (Geden et al. 1992; Petersen et al. 1995; Crespo et al. 1998; Weinzierl & Jones 1998; Floate 2003; Skovgård & Nachman 2004; Geden & Hogsette 2006). Although release stations provide protection, scattering has the advantage of placing the parasitoids near the target fly puparia and mitigates concerns about the limited dispersal distances of some species (Tobin & Pitts 1999; Skovgård 2002; Machtiger et al. 2015). However, the scatter method results in an accumulation of formerly parasitized puparia in the habitat that must be searched through and avoided by parasitoids. Such accumulations may or may not impose increased handling time constraints on the parasitoids as they locate, inspect, and then reject unusable candidate hosts (Hubbard & Cook 1978; Waage 1979; Van Alphen & Galis 1983). The objective of the present study was to evaluate the effect of the presence of formerly parasitized hosts on M. raptor and S. cameroni parasitism of house fly puparia. We also examined whether the presence of an equivalent volume of inanimate objects roughly similar in size and shape to house fly puparia would affect the searching efficiency of both species.

**Materials and Methods**

**INSECTS USED IN BIOASSAY**

Spalangia cameroni and M. raptor females were from colonies maintained at the United States Department of Agriculture Agricultural Research Service (USDA-ARS) Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida. The original source material for both colonies was collected from a dairy farm in Gilchrist County, Florida. All tests with S. cameroni and the M. raptor tests involving previously parasitized puparia were conducted with colonies established in 2012. During the hiatus between tests with formerly parasitized puparia and bead decoys, the M. raptor colony developed Nosema disease and was no longer suitable for use in bioassays, so another colony was used that had been collected 1 yr earlier.

Parasitoids were provided with 2-d-old house fly puparia every 3 to 4 d at a host-to-parasitoid ratio of 5:1 in 32.5 × 32.5 × 32.5 cm cages (MegaView Science, Taiwan) and held at 25 °C and 80% RH under constant darkness. House flies were from a colony (“Orlando Normal”) originally collected in the 1950s near Orlando, Florida, and since then maintained at the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology. Fly larvae were reared on a 13:1:6.5 ratio of wheat bran, Calf-Manna (Manna Pro Products LLC, Chesterfield, Missouri), and water (by volume). Adults were reared under laboratory conditions of 27 °C, 45 to 70% RH, and a photoperiod of 16:8 h L:D. Adult flies were fed a diet consisting of granulated sucrose, powdered milk, dried egg, and sugar and maintained at 27 °C in wire mesh cages.

**HOST DECOYS**

Parasitoids were presented with either live fly puparia alone or in combination with “decoys” in the form of either fly puparia formerly parasitized by conspecifics or acrylic craft beads. Formerly parasitized puparia were obtained by examining spent puparia from parasitoid colonies and selecting those with exit holes indicating parasitoid emergence. The acrylic craft beads were included to provide an inanimate matrix comparable to an equal volume of puparia. The beads (item #6M145F, Gifts of Avalon, Gainesville, Florida) were obtained from a local craft shop and had outer dimensions of 2.9 × 4.4 mm, whereas fly puparia averaged 2.5 × 6.5 mm. The beads also had an open center for insertion of a string (Fig. 1). Although the beads had a somewhat smaller length-by-width aspect than the puparia (12.9 and 16.4 mm², respectively), the differences in shape meant that groups of beads occupied a somewhat larger volume than the puparia. The quantity of beads needed to achieve the desired equivalent volume as 1,000 live pupae (33 cm³) was 616 beads (Fig. 1).

**BIOASSAY**

For both types of decoys (formerly parasitized puparia and beads), the treatments consisted of 3 mixtures with live pupae: 1) 1,000 live puparia (1–2 d after pupation) with no decoys (100% live hosts); 2) 500 live puparia and either 500 parasitized puparia or 313
beads (50% live hosts); and 3) 100 live pupae and either 900 parasitized pupae or 554 beads (10% live hosts). These combinations were placed in 60 cm³ cups with muslin covers. Parasitoids were removed from colony containers, placed on a chill table to anesthetize them, and groups of 5 females were counted and placed into gelatin capsules (size 00, B&B Pharmaceuticals, Aurora, California). Parasitoids were released from a single capsule into the assay cups by opening the gelatin capsules and tapping the parasitoids onto 1 of the 3 puparia treatments. There were 5 replicates of pupae and decoys for each species, combination, and type of decoy (parasitized puparia or beads) tested, for a total of 10 observations. Parasitoids were removed after 24 h by placing puparia in a standard U.S. number 10 sieve (with 2 mm openings) and shaking gently until all 5 parasitoids came through the sieve. Live puparia were separated from formerly parasitized puparia by microscopic examination and removal of pupae with exit holes. Live puparia were separated from beads by sifting puparia through a U.S. standard no. 6 sieve (3.36 mm openings). Puparia were returned to the bioassay cups and held for fly emergence at 28 °C for 7 d. Dead adult house flies and empty puparia were discarded. Unclosed puparia were counted and then placed back into the 28 °C rearing chamber for parasitoid emergence. In tests involving M. raptor, progeny production was determined by counting the number of adult parasitoids present in the assay cups. Because S. cameroni will sometimes re-enter puparia through exit holes before dying, counting the number of adult parasitoids found in an assay cup can result in substantial underestimates of progeny production (Machtinger & Geden 2013). Therefore, for this species, we examined puparia for the presence of exit holes and used this as our measure of progeny production. Residual host mortality, or the percentage of killed hosts that produced neither a fly nor a parasitoid, was calculated for each bioassay cup (Taylor et al. 2016). The entire experiment was replicated on 2 occasions with different cohorts of flies and parasitoids. The bioassays with the 2 decoy types (parasitized puparia and beads) were conducted 2 yr apart, in 2014 and 2016, respectively.

Data on the number of hosts killed, progeny produced, and residual mortality were analyzed separately for each species and decoy type by 1-way ANOVA using the 3 live host–decoy combinations as the grouping variable. Preliminary analysis indicated no significant effect of replication for either species or decoy type, so results from the replicates were pooled (n = 10 sets of parasitoids and puparia for each species and host–decoy combination). Treatment means were compared using Tukey–Kramer honest significant difference (HSD) tests if the overall model F was significant at P ≤ 0.05. Analyses were conducted using PROC GLM with the MEANS/TUKEY statement using SAS® software version 9.4 (SAS Institute Inc. 2013).

Table 1. Mean (SE) numbers of hosts (live house fly puparia) killed and progeny produced by groups of 5 Muscidifurax raptor and Spalangia cameroni females over 24 h when hosts were either presented alone or in combinations with decoys in the form of dead fly puparia that had been parasitized by conspecifics (empty puparia that had produced adult parasitoids).

<table>
<thead>
<tr>
<th>Treatment (% decoys)</th>
<th>No. of hosts killed</th>
<th>No. of progeny</th>
<th>Residual mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% decoys</td>
<td>55.3 (4.6)a</td>
<td>43.1 (4.9)</td>
<td>22.2 (6.7)</td>
</tr>
<tr>
<td>50% decoys</td>
<td>39.6 (4.3)b</td>
<td>30.9 (3.9)</td>
<td>15.7 (13.6)</td>
</tr>
<tr>
<td>90% decoys</td>
<td>47.8 (3.1)b</td>
<td>32.1 (2.6)</td>
<td>32.8 (3.7)</td>
</tr>
<tr>
<td>ANOVA P</td>
<td>3.76*</td>
<td>2.93 ns</td>
<td>0.91 ns</td>
</tr>
</tbody>
</table>

Muscidifurax raptor

<table>
<thead>
<tr>
<th>Treatment (% decoys)</th>
<th>No. of hosts killed</th>
<th>No. of progeny</th>
<th>Residual mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% decoys</td>
<td>34.2 (3.9)</td>
<td>14.6 (1.2)</td>
<td>50.6 (9.2)</td>
</tr>
<tr>
<td>50% decoys</td>
<td>37.8 (3.3)</td>
<td>17.6 (2.4)</td>
<td>53.0 (6.0)</td>
</tr>
<tr>
<td>90% decoys</td>
<td>34.6 (3.1)</td>
<td>14.7 (2.2)</td>
<td>51.2 (12.1)</td>
</tr>
<tr>
<td>ANOVA P</td>
<td>0.33 ns</td>
<td>0.73 ns</td>
<td>0.02 ns</td>
</tr>
</tbody>
</table>

Spalangia cameroni

Means followed by the same letter within columns under the same species header did not differ at P = 0.05 (Tukey).

*Percentage of killed hosts that did not produce an adult parasitoid.

One-way ANOVA, df = 2,27; *, P ≤ 0.05; ns, P > 0.05.

Results

Muscidifurax raptor killed significantly more hosts (55.3) and produced more progeny (43.1) when decoys were absent than when the live pupae were mixed with formerly parasitized puparia (Table 1). The presence of formerly parasitized hosts had no significant effect on M. raptor residual mortality. Spalangia cameroni was unaffected by the presence of formerly parasitized puparia for any of the measured variables (Table 1). When beads were used as decoys rather than formerly parasitized puparia, the high decoy-to-live-host ratio (90% beads) resulted in significantly lower numbers of hosts killed by M. raptor (56.6) compared with the other treatments (79.2 and 97.2) (Table 2). Residual mortality at the high decoy-to-live-host ratio (90% beads) was lower (31.2%) than in the other treatments (51.6 and 59.3%), indicating that progeny production by M. raptor was unaffected by the presence of decoys. Spalangia cameroni killed over twice as many hosts (99.4) in the absence of bead decoys than at the high decoy-to-live-host ratio (37.9) (Table 2). Residual mortality was unaffected by the treatments (52.1–63.3%), as progeny production by S. cameroni was about twice as high (39.6) when decoys were absent than in the 90% decoys treatment (19.5).

Discussion

Muscidifurax raptor and S. cameroni are both cosmopolitan species with wide host ranges that are best known for attacking house flies and stable flies in a variety of animal production systems (Machtinger & Geden 2017). Although they occur sympatically, their differences in searching behavior result in a degree of niche partitioning, with Muscidifurax species searching near the surface of host-breeding substrates and S. cameroni searching at greater depths (King 1997; Geden 2002; Rueda & Axtell 1985; Pitzer et al. 2011). Recent studies have documented that Muscidifurax species rely on pupal odors to locate hosts, whereas Spalangia species are attracted by combinations of host larvae and breeding substrates (Machtinger et al. 2015; Machtinger & Geden 2015).

Little is known about how these parasitoid species locate and assess potential hosts at close range once they have discovered a host-
Both *M. raptor* and *S. cameroni* inflict higher host mortality and produce more progeny when hosts are widely distributed rather than clumped within a patch (Mann et al. 1990). Live puparia in natural settings must be discovered against a background of physical features of the habitat, as well as in a context of hosts that are unsuitable because they are currently parasitized or dead from various other causes, including past parasitism. Dead puparia that have already produced parasitoids can accumulate in substantial numbers in stable habitats, and live hosts may represent only a small proportion of those encountered by a parasitoid while searching. The time required to assess and reject numerous unsuitable hosts can reduce successful parasitism by increasing “handling time,” or the interval between parasitism events (Connor & Cargain 1994). One of our goals was to determine whether searching efficiency deteriorated when live hosts were presented along with formerly parasitized puparia. Our results indicate that *S. cameroni* is unaffected by the presence of such hosts, and that the effect on *M. raptor* was weak, even when live hosts made up only 10% of the potential hosts that had to be assessed. As a control for the spatial complexity that dead puparia provide, we also examined parasitism when live hosts were mixed with comparable volumes of inanimate decoys in the form of acrylic beads. Surprisingly, a stronger effect on search efficiency was observed with bead decoys than with dead puparia. The beads appear to have provided a degree of spatial complexity that the parasitoids found more difficult to navigate than the presence of formerly parasitized puparia. In this regard, the results with beads are perhaps analogous to other studies where *Spalangia* species and *Muscidifurax* species parasitoids were exposed to hosts alone or hosts within substrates (Geden 2002; Pitzer et al. 2011).

Comparison of Tables 1 and 2 indicate that performance of *M. raptor* in the absence of decoys differed between the tests involving formerly parasitized puparia and beads. Although progeny production was similar in the assays, parasitoids in the tests with formerly parasitized pupae attacked more hosts and had higher residual mortality rates than in the tests with beads. This is probably due to the use of different *M. raptor* strains in the 2 types of assays, which were conducted 2 yr apart. At the time of the bead tests, the *M. raptor* colony used in the earlier assays was compromised by *Nosema* disease. This required using a different colony for the bead tests, and house fly and stable fly parasitoid colonies can vary in their intrinsic residual mortality rates (Geden et al. 2006).

When parasitoids are released in augmentative fly management programs, the end-user must decide whether to scatter parasitized puparia in fly breeding areas or place them in discrete release stations. Both methods have advantages and liabilities. One potential disadvantage of the scatter method is that the accumulation of parasitized puparia in the environment could diminish the ability of foraging females to locate live puparia. To our knowledge, Birkemoe & Øyrehagen (2010) conducted the only study comparing the 2 methods and found no significant effect on house fly parasitism by *S. cameroni* on Danish pig farms. They suggested that any disadvantages of the scatter method were compensated for by the short distance that parasitoids needed to travel to find hosts (Birkemoe & Øyrehagen 2010). Our results support the practice of scattering parasitized puparia in that the presence of formerly parasitized hosts does not interfere substantially in the ability of *S. cameroni* and *M. raptor* to locate and parasitize live puparia.

## Acknowledgments

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## References Cited


