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Host location by *Spalangia cameroni* (Hymenoptera: Pteromalidae) in equine associated substrates

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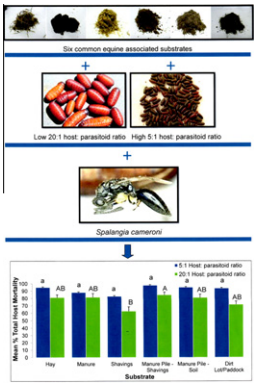
HIGHLIGHTS

- Substrate influenced host seeking of *Spalangia cameroni* at low host densities.
- With fewer hosts, *Spalangia cameroni* had greater flexibility for host seeking in substrates.
- Species of host did not have any effect on host seeking of *Spalangia cameroni*.
- Increased host:parasitoid ratio did not increase parasitoid progeny production.
- *Spalangia cameroni* appears to be a suitable candidate for release.

GRAPHICAL ABSTRACT



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ABSTRACT

House flies, (*Musca domestica* L.) and stable flies (*Stomoxys calcitrans* L.) are common pests on equine facilities. Biological control of these flies with pupal parasitoids has become increasingly popular with horse owners but has not been evaluated on equine facilities. Little is known of the substrate preferences of filth fly parasitoids on equine facilities, but the success of release programs may be affected by microhabitat preferences. *Spalangia cameroni* Perkins was evaluated for location preferences for parasitization of house fly and stable fly puparia in six substrates commonly found on equine farms in Florida. Substrates were evaluated at 20:1 and 5:1 H:P ratios and during the experiment parasitoids had access to all substrates simultaneously. No differences were observed between filth fly host species in any of the measured parameters: total host mortality, parasitoid progeny production and residual host mortality. Significant effects of H:P ratio on host mortality and residual mortality were found but not on progeny production. While there were significantly more hosts killed in the aged shavings than the fresh shavings at the 20:1 ratio, no differences were observed at the 5:1 ratio. Additionally, no differences were found in progeny production across substrates at the 20:1 ratio, but higher reproductive success was observed in several substrates at the 5:1 ratio. These results demonstrate that *S. cameroni* had substrate preferences but that these preferences were absent with reduced host density. This parasitoid species appears to be effective at parasitizing hosts in the common equine substrates of Florida.

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1. Introduction

Biological control of house flies, (*Musca domestica* L.), and stable flies, (*Stomoxys calcitrans* (L.), using pupal parasitoids (Hymenoptera: Pteromalidae) has been used in a variety of confined livestock facilities for many years. Pupal parasitoids used in augmentative control programs for filth flies is an alternative to chemical management and use on equine farms has the potential to reduce environmental contamination by reducing the need for insecticide applications and slow insecticide resistance development. Horse owners in particular have shown an increasing interest in the use of biological control agents equine (USDA, 2006; Machtinger, 2011) and commercial parasitoid products often are marketed specifically to this stakeholder group (Leppla and Johnson, 2010).

Though Pteromalid pupal parasitoids frequently are used for fly control, the results of augmentative parasitoid control programs on confined livestock and poultry operations have been inconsistent and evaluations on equine facilities are lacking. Several studies have documented parasitism and increased control of filth flies following releases (Morgan and Patterson, 1977; Rutz and Axtell, 1979; Morgan, 1980; Morgan and Patterson, 1990; Weinzierl and Jones, 1998; Skovgard, 2004; Geden and Hogsette, 2006), whereas others saw no effects on fly control (Petersen and Meyer, 1983; Andress and Campbell, 1994).

Substrate preferences of the released parasitoid species may influence parasitism rates and contribute to the variable effects of control seen in previous studies. Parasitism levels can vary in response to moisture (Legner, 1977; Smith and Rutz, 1991b; Geden, 1999) and host accessibility (Legner, 1977; Rueda and Axtell, 1985; Geden, 2002; Pitzer et al., 2011b). Surprisingly, little is known about microhabitat preferences and the effects of release rates on habitat preference for parasitism by *Spalangia cameroni*, the most common pupal parasitoid species of filth flies in Florida equine facilities (Pitzer et al., 2011a).

The probability of the success of a biological control program using pupal parasitoids on equine farms could be increased y matching parasitoid species to their preferred regional or local microhabitats. Knowledge of effects of parasitoid release rate on substrate preferences and the types of habitats likely to draw specific parasitoids could guide horse owners in the management of their facility.

The purpose of the present study was to examine habitat preferences of *S. cameroni* in the laboratory among six substrates that are commonly found at equine facilities in Florida with two hosts, house flies and stable flies, and at two different host:parasitoid (H:P) ratios. The goal was to determine if substrates used on equine facilities in Florida were suitable for parasitism by *S. cameroni* and if substrate preferences different with increased parasitoid application rates.

2. Materials and methods

2.1. Test substrates

Six substrates commonly found on equine facilities in Florida were evaluated for substrate preference by *S. cameroni*. These substrates had previously been observed to serve as pupariation sites for house fly and stable fly hosts on various equine facilities (Machtinger, 2011) (Table 1).

Substrates were collected during 1 week in the fall of 2010 at equine farms in Alachua County, FL. Neither area-wide pesticides nor insect growth regulators were used at the facilities. Accumulations on the surface to a maximum depth of 10-cm were recovered. Samples were collected with a trowel, put in plastic freezer bags (Ziplock®, SC Johnson. Racine, WI), and immediately placed in a plastic cooler to protect from direct sunlight. A subsample of each substrate was weighed and dried in a drying oven at 45 °C to determine the field moisture level. The remaining substrate was frozen at −18 °C for ⩾1 week to kill any existing arthropods. To ensure homogeneity, individual substrates collected on different days were mixed thoroughly while frozen by breaking them with an ice pick.

Plastic cups (266 ml capacity, Plastic Container City, Brooklyn, New York) were filled with 200 cm^3 of each of the six substrates. The substrates used are detailed in Table 1. Briefly, the substrates were: soiled hay of variable age from around a coastal Bermudagrass round bale (Hay), fresh manure collected from a mare fed perennial peanut hay and concentrated feed (Man), freshly soiled pine shavings with small amounts of waste perennial peanut hay from a stall kept horse (Shav), aged soiled pine shavings from a waste manure pile containing small amounts of waste perennial peanut hay (Mps), soiled sand bedding from an aged waste manure pile containing small amounts of waste hay Bermuda grass hay (Mpd), and soil mixed with variable aged manure from a dirt paddock (DL). Standardized weights of each substrate were used for each experiment. These weights varied because of differing substrate densities. Substrate densities were obtained by dividing the standardized weights used by the volume of each substrate.

Prior to inoculation with fly larvae, substrates were adjusted to a moisture level of 45% by drying at 45 °C (Geden, 1999) or adding deionized water with a misting spray bottle then lightly mixing with a probe. Moisture adjustments were made based on field collected moisture levels of each substrate as determined by the University

Table 1
Analysis and descriptions of six equine facility fly breeding substrates evaluated for pteromalid searching preferences.

Substrate	Abbreviation	Field collected moisture content (%)[a]	Density (g/ml)	Standardized weight	Narrative description
Hay	Hay	68.6	1.11	50 g	Hay + urine + manure (variable age) from around a coastal Bermudagrass round bale.
Manure	Man	78.2	1.66	100 g	Fresh manure (<12 h old) collected from a mare fed perennial peanut hay and concentrated feed.
Shavings	Shav	35.2	0.83	50 g	Fresh pine shavings (0.1–0.3-cm long) +urine + fresh manure (<12 h old) +small amounts of waste perennial peanut hay from a stall kept horse.
Manure pile – shavings	Mps	54.9	0.89	50 g	Pine shavings (size as above) +urine + manure from a manure pile (>72 h old) containing small amounts of waste perennial peanut hay.
Manure pile – soil	Mpd	59.7	2.24	100 g	Soil + urine + manure (>72 h old) from an aged manure pile containing small amounts of waste hay from a mare fed coastal Bermudagrass hay and concentrated feed.
Dirt lot	DL	22.6	2.50	100 g	Soil + urine + manure (variable age) from a dirt paddock.

[a] As determined by the University of Florida Analytic Services Laboratory.

of Florida Analytical Services Laboratory (Gainesville, FL). The rate of daily substrate moisture loss was determined in a preliminary drying experiment. Three samples of each substrate at the standardized weight were air dried in the room where the experiment occurred. After 24 h, the substrates were weighed and the rate of moisture loss recorded. During the experiments, substrates were rehydrated daily at the predetermined rate to maintain 45% moisture by lightly misting with deionized water in a spray bottle.

2.2. House flies, stable flies and parasitoids

Third instar house flies or stable flies were obtained from the USDA-ARS, Center for Medical, Agriculture and Veterinary Entomology (CMAVE) insecticide-susceptible colony. Larvae were reared in a diet described by Hogsette (1992). This diet consisted of 3800 L water and 5000 L of F.R.M fly diet mix (50% wheat bran, 30% alfalfa meal and 20% corn meal) manufactured in Marion County, Florida.

Active *S. cameroni* females were obtained from an established colony maintained by at the USDA-ARS, CMAVE in Gainesville, FL. This colony was originally obtained by author CJG from a dairy in Gilchrist County, FL in November, 2010. Weekly maintenance consisted of providing parasitoids with 2-day-old house fly puparia at a H:P ratio of 5:1 in 32.5 × 32.5 × 32.5-cm cages (MegaView Science, Taiwan) and held at 25 °C, 80% RH under constant darkness. For each experiment, parasitoids were anesthetized by chilling on a cooling table to count and identify females.

2.3. Bioassay

House fly and stable fly hosts were tested individually. One day prior to expected pupariation, 100 3rd instars of the respective fly species were counted and placed on the surface of the experimental cups containing one of the six substrates for each trial and host species. Six cups were used per trial to represent each substrate for a total of 600 hosts per release arena. The same numbers of larvae were placed in control cups containing 50 g of moistened vermiculite that served a visual confirmation of pupariation and controls for fly mortality and parasitism. Cups were covered with cotton muslin fabric secured with plastic lid rims and larvae from both control and experimental cups were held at 27 °C, 80% RH and provided constant light, allowing them to pupariate naturally in each cup.

Release arenas (60 × 60 × 60-cm) composed of 2 side panels of polyester netting (72 × 26 mesh) for ventilation and 2 sides with transparent plastic sheeting (MegaView Science, Taiwan) were used for this study. When 95% of the fly larvae in the control cups were visually observed to have pupated, approximately 2 days after the introduction of larvae, the fabric covers were removed and 1 representative cup of each of the six substrates were placed randomly in a circle array around a central release point in each of the trial arenas. Parasitoids were released in the center of the array and given 72 h to parasitize hosts (Pitzer et al., 2011b). Two H:P ratios were tested for each host, a 5:1 and 20:1 ratio that consisted of 120 and 30 individual female parasitoids, respectively, with 600 total fly puparia in each release arena.

After 3 days, the cups were removed from the arenas. Puparia from each experimental cup were isolated by water flotation and air dried (Lee and Toyama, 1990). Puparia from control cups were sifted from vermiculite in a #12 sieve. Puparia were maintained in covered 60-cm^3 plastic cups at 25 °C and approximately 80% RH with constant light for adult fly eclosion. Adult flies were counted after 7 days. Flies and empty puparia were removed and the remaining puparia were held for 4 weeks for parasitoid emergence. When it was determined that parasitoid emergence in experimental cups had ceased for 1 week (ca. 6 weeks after test set up), puparia from the release arenas and control cups were examined for the presence of emergence holes to determine the number producing adult parasitoids. Emergence holes were used as the metric for parasitoid progeny production because adults of this species occasionally re-enter host puparia if there are no hosts available. Remaining puparia from control cups were counted for residual mortality, or those that did not produce a parasitoid or fly.

Three replicates were conducted on each test date for each species and H:P ratio and the entire experiment was replicated on 3 dates using different cohorts of parasitoids and flies.

2.4. Statistical analysis

Data were analyzed with a full factorial analysis of variance (ANOVA) using JMP v. 9 (SAS Institute Inc., Cary, NC 2010). Separate analyses were conducted for 3 response variables; (1) host mortality, defined as the number of puparia not producing an adult fly/total number of recovered puparia, (2) progeny production which is characterized as the number of puparia producing a parasitoid/total number of recovered puparia, and (3) residual host mortality, or unexplained host mortality, which equaled the number of puparia not producing a fly or parasitoid/number of recovered puparia.

Substrate, host species, and H:P ratio as well as their interaction were evaluated as independent factors. Response variables were analyzed by H:P ratio separately to further evaluate the significant interaction term in the full factorial ANOVA. No significance of fly species, species x H:P ratio or species × substrate × H:P ratio was observed for any of the measured parameters, therefore data were pooled for further analysis.

Percentage data were normalized with an arcsine square root transformation; results in tables and text are reported as original units. Means were calculated for H:P ratio and were separated with Tukey's HSD test for comparison ($\alpha = 0.05$).

3. Results

Natural substrate moisture content as collected from the field ranged from 22.6% in the DL to 78.2% in the Man (Table 1). The density of each substrate was variable, measuring between 0.83 g/ml and 2.5 g/ml. Density did not appear to affect any of the measured parameters. Substrates with the highest host mortality and lowest host mortality were very similar in density (0.89–0.83 g/ml, respectively).Other substrates with no significant difference in host mortality ranged in density from 1.11 to 2.50 g/ml.

Overall, host mortality was influenced by substrate, H:P ratio and there was a species x substrate in the full factorial ANOVA (Table 2). Progeny production of *S. cameroni* was influenced by substrate and significant species × substrate and substrate × H:P ratio interactions. Substrate had a significant influence on residual host mortality as well as H:P ratio and the interaction of substrate × H:P ratio.

The H:P ratios were analyzed separately to further refine interaction effects found in the full factorial analysis (Table 3). Cumulatively, at the 20:1 H:P ratio, parasitoids induced significantly greater most mortality in the Mps (84.6%) than the Shav (62.6%). There were no observed differences in progeny production at the by substrate at the 20:1 ratio but differences in residual mortality were observed between the Man (27.2% residual mortality) and three other substrates, the Shav, Mps and DL (16.2–19.0% residual mortality).

At the 5:1 ratio, there was no significant difference in host mortality or residual mortality in any of the substrates. Host mortality was high, ranging from 82.7% to 97.7% (Shave and Mps, respectively). However, differences were observed in progeny production at the 5:1 ratio that was not seen in the 20:1 ratio. A higher

Table 2
Full factorial ANOVAs for effects of the host species, H:P ratio and host development substrate on the rates of host attack, expressed as host mortality and residual mortality, and *Spalangia cameroni* Perkins progeny production in experimental arenas containing equine-generated substrates.

	Percent total host mortality		Percent parasitoid progeny production		Percent residual host mortality	
Variable	*F*	*P*	*F*	*P*	*F*	*P*
Fly species[1]	0.47	0.3113NS	0.88	0.3485NS	0.01	0.9356NS
Substrate[2]	5.9	<0.0001	4.26	0.0010	2.27	0.0486
H:P ratio[3]	4.99	0.0266	0.77	0.3824NS	4.17	0.0424
Species × substrate	3.99	0.0018	3.65	0.0035	1.25	0.2863NS
Species × H:P ratio	0.11	0.7387NS	0.00	0.9882NS	0.63	0.4274NS
Substrate × H:P ratio	2.19	0.0570NS	2.36	0.0410	2.65	0.0238
Species × substrate × H:P ratio	1.85	0.1042NS	2.09	0.0683NS	1.18	0.3185NS

$P \leqslant 0.05$.
[1] Fly species were house fly, *Musca domestica* L. and stable fly, *Stomoxys calcitrans* (L).
[2] Fly Puparia in substrates of hay; manure; shavings; manure pile – shavings; manure pile – soil; and dirt lot.
[3] H:P = host:parasitoid ratios which included ratios of 5:1 and 20:1.

Table 3
Influence of six equine husbandry test substrates at two house fly and stable fly host:parasitoid ratios on host puparia killed, progeny production and residual mortality by *Spalangia cameroni* Perkins.

Ratio	Substrate	Total host mortality($\bar{x}$ % ±SEM)	Parasitoid progeny production($\bar{x}$ % ±SEM)	Residual host mortality ($\bar{x}$ % ±SEM)
5:1	Hay	94.9 + 1.2^{a}	66.9 + 2.9^{a}	28.2 ± 2.1^{a}
	Manure	88.0 ± 5.6^{a}	45.1 ± 6.1^{b}	42.9 ± 5.5^{a}
	Shavings	82.7 ± 5.3^{a}	52.1 ± 6.7^{ab}	33.4 ± 5.0^{a}
	Manure pile – shavings	97.7 ± 0.7^{a}	67.4 ± 3.4^{a}	30.4 ± 3.0^{a}
	Manure pile – soil	94.9 ± 2.0^{a}	55.8 ± 3.1^{ab}	39.1 ± 3.3^{a}
	Dirt lot	93.6 ± 2.6^{a}	61.4 ± 3.5^{ab}	32.2 ± 2.8^{a}
	ANOVA F(df = 5,120)	2.3377	4.4511	1.148
20:1	Hay	81.2 ± 3.9^{ab}	58.8 ± 4.9^{a}	22.4 ± 1.8^{ab}
	Manure	81.5 ± 5.2^{ab}	54.5 ± 5.7^{a}	27.2 ± 1.9^{a}
	Shavings	62.6 ± 5.9^{b}	44.0 ± 6.6^{a}	18.6 ± 2.7^{b}
	Manure pile – shavings	84.6 ± 3.9^{a}	63.5 ± 5.2^{a}	21.1 ± 2.1^{ab}
	Manure pile – soil	80.8 ± 4.7^{ab}	64.6 ± 4.2^{a}	16.2 ± 1.2^{b}
	Dirt lot	71.3 ± 4.7^{ab}	52.4 ± 5.3^{a}	19.0 ± 2.2^{b}
	ANOVA F(df = 5, 102)	2.7917	2.0712	3.4870

Within each host:parasitoid ratio, means in a column followed by the same letter are not significantly different (Tukey's HSD test, $\alpha = 0.05$).

percentage of puparia produced parasitoid progeny in the Mps and Hay substrates (66.9 and 67.4% progeny, respectively) than the Man (45.1% progeny) when there were a greater number of parasitoids present per host. There was no significant difference between the substrates on residual host mortality.

4. Discussion

Although previous studies have examined the role of microhabitat on filth fly parasitoid searching behavior, none have included substrates that are found on equine facilities (Petersen and Meyer, 1983; Rueda and Axtell, 1985; Smith and Rutz, 1991a,b; Geden, 1999, 2002). Our results demonstrate that *S. cameroni* is effective at locating hosts across the range of substrates typically encountered on Florida equine facilities and reinforce the importance of microhabitat associations on *S. cameroni*, especially when hosts are in abundant supply. These results further suggest that greater numbers of parasitoids per host (crowding) diminish habitat discrimination by S. *cameroni,* and that this results in higher overall host mortality in those substrates.

Little is known about the qualities of different substrates that affect parasitoid searching preferences or ability. In some cases, differential host location may be a result of density or textural factors that limit the ability of parasitoids to forage successfully. In a field survey, Smith and Rutz (1991a) determined that *S. cameroni* preferred loose substrates. Geden (2002) found that searching by *S. cameroni* in the laboratory was hindered substantially in dense sandy loam soil. In the present study, substrate density did not appear to be a factor in the success of host attack, although none of the tested substrates were as dense as the soil in the latter study (Geden, 2002). Indeed, the density and texture was similar between the most and least preferred substrate at the 20:1 ratio (0.89 and 0.83 g/ml, respectively) (Table 1).

The lack of density textural influence on substrate preference suggests that cues other than physical and textural properties play a role in host location. Our results indicated that substrates containing relatively fresh manure were less preferred by *S. cameroni*. Considering this, chemical cues may have a greater influence on habitat selection that the physical characteristics of the substrate. Carbon dioxide (CO_2) emissions increase as horse manure decomposes (Jeanbourquin and Guerin, 2007). It is possible that increased CO_2 emissions from aged manure serve as a semiochemical cue for attracting *S. cameroni* to the aged equine substrates where stable fly hosts are more likely to be found (Skoda et al., 1993). The results also suggest that parasitoid releases may be more effective if areas with aging manure are targeted, especially waste manure piles with a pine shavings base.

A greater percent of host mortality and residual mortality was observed on all substrates when more female parasitoids were present, but the percent of parasitoid progeny production was not different between H: P ratios. This is in contrast to the findings of Legner (1967) who saw increased progeny production with increased numbers of female *S. cameroni*. In the current study, the lack of increased progeny production when more females were present may be due to increased parasitoid host feeding (Geden, 1999) or superparasitim discrimination (Legner, 1967; Wylie, 1971a, 1972). Propp and Morgan (1983) determined that *Spalangia endius* Walker could discriminate and avoid ovipositing in previously parasitized pupae, but only after drilling into the puparia. This behavior may be shared with *S. cameroni*. Under population

pressure, the assessment and subsequent host-drilling to avoid superparasitism could be potentially fatal to the developing fly or wasp immature, increasing overall host mortality and decreasing successful progeny production. In this way, increasing the numbers of parasitoids released in augmentative programs may have a proportionally greater impact on fly mortality than on progeny production.

The results of these studies demonstrate that this species shows considerable plasticity in its searching behavior, and that host availability can mediate innate substrate preferences. Overall, substrate effects on *S. cameroni* were only modest at the "high" H:P ratio of 20:1 and were absent when hosts were in short supply. In a related study, Geden (1999) found that *S. cameroni* had a strong preference for dry poultry manure at high (50:1) H:P densities but that moisture preferences were much weaker at a ratio of 2.5:1. It is possible that the "high" H:P ratio used in the present study (20:1) was not high enough to reveal the full extent of innate preferences for the substrates tested. However, in augmentative release programs, the demonstrated behavioral plasticity would be beneficial and expected to result in broad distribution of host mortality among accessible substrates because high parasitoid abundance should result in spillover away from the preferred substrates. In equine facilities with a range of suitable substrates for fly development, high numbers of released parasitoids may ensure parasitism in all substrates.

5. Conclusions

In summary, this is the first study to our knowledge to evaluate the searching behavior and preferences of *S. cameroni* in substrates found on horse farms. House flies and stable flies were equally vulnerable to parasitism and none of the substrates tested fell outside the range of moisture and density properties that have previously been found to limit searching behavior by this species. Innate preferences among these substrates were small and were overwhelmed when parasitoid abundance was high. It does not appear as though equine-generated substrates would negatively influence the success of filth fly parasitism by *S. cameroni.* Therefore, these results suggest that *S. cameroni* is a suitable candidate for augmentative releases for fly management programs on Florida horse farms. Further research is needed to determine appropriate release rates for such facilities, but the results indicate that sustained releases would be required to keep fly mortality high enough to provide satisfactory control.

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