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## The Ability of Selected Pupal Parasitoids (Hymenoptera: Pteromalidae) to Locate Stable Fly Hosts in a Soiled Equine Bedding Substrate

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**ABSTRACT** The ability of *Spalangia cameroni* Perkins, *Spalangia endius* Walker, and *Muscidifurax raptorellus* Kogan and Legner to locate and attack stable fly hosts was evaluated under laboratory conditions. Postfeeding third-instar stable fly larvae were released and allowed to pupate in two arena types: large 4.8 liter chambers containing a field-collected, soiled equine bedding substrate; or 120-ml plastic cups containing wood chips. At the time of fly pupariation, parasitoids were released and permitted 72 h to locate and attack hosts. On average, parasitism rates of freely accessible stable fly pupae in cups were not significantly different between parasitoid species. However, parasitism rates in chambers containing either *Spalangia* spp. were  $\approx$ 50-fold more than *M. raptorellus*. Additional intraspecies analysis revealed that parasitism rates both by *S. cameroni* and *S. endius* were not significantly different when pupae were freely accessible or within bedding, whereas *M. raptorellus* attacked significantly more pupae in cups than in the larger chambers where hosts were distributed within bedding. These results suggest that *Spalangia* spp. are more suited to successfully locate and attack hosts in habitats created by equine husbandry in Florida. Therefore, commercially available parasitoid mixtures containing *Muscidifurax* spp. may be ineffective if used as a control measure at Florida equine facilities.

**KEY WORDS** *Spalangia cameroni*, *Spalangia endius*, *Muscidifurax raptorellus*, *Stomoxys calcitrans*, parasitism

Filth flies, in particular the stable fly, *Stomoxys calcitrans* (L.), continue to be a significant pest of confined and pastured livestock. Furthermore, the insecticides available for control of pests such as the stable fly result in modest control, and are becoming increasingly limited because of federal regulation such as the Food Quality Protection Act of 1996 (Kaufman et al. 2001b) and insecticide resistance expression in some populations (Cilek and Greene 1994, Marçon et al. 1997, Pitzer et al. 2010). In addition, pressure on livestock producers to control dispersing filth fly populations is mounting, as human population growth continues to decrease the gap between residential areas and nearby livestock operations. This can cause a greater quandary, as the flies' nuisance behavior and potential for disease transmission to urban areas may result in litigation (Tobin and Pitts 1999). In addition, stable fly feeding activity is known to drive tourists and other patrons from recreational areas, such as the Gulf coast beaches in Florida (Hogsette and Ruff 1985).

Many studies have been conducted to determine the effects of parasitoids, both released and naturally occurring, as an alternative method for filth fly control. However, the results of such studies have provided conflicting data. In New York state, sentinel pupae (laboratory reared and placed in field sites) parasitism rates were as high as 65% on dairies in New York state with a parasitoid release program, compared with 30% on control farms (Geden et al. 1992). Similarly, sentinel pupae mortality was as high as 37% on Nebraska cattle feedlots where parasitoid releases occurred, compared with 4% at control feedlots (Petersen et al. 1992). These results contrast those of a parasitoid study conducted on California dairies, where sentinel pupal parasitism increased from  $\approx$ 10% to 20% when wasps were released (Meyer et al. 1990). Concurrent evaluation of field-collected pupae indicated parasitism rates of only 4.4 and 12.5% for stable flies and house flies, respectively. In Nebraska, parasitoid releases at rates five-fold greater than the insectary-recommended amount were ineffective in reducing adult stable fly populations (Andress and Campbell 1994).

Several factors may account for the contrasting results of parasitoid release studies conducted in different geographical areas or livestock facilities. Habitat has been shown to play a major role in the host loca-

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tion success of many parasitoids. Filth fly breeding sites, such as tightly packed feed and manure, may cause wandering maggots to pupate closer to the surface or in easily accessible cracks, favoring attack by *Muscidifurax* spp. Differing conditions may require that parasitoids search at greater depths to locate pupae, thereby favoring attack by *Spalangia* spp. (Rueda and Axtell 1985, Meyer et al. 1991, Smith and Rutz 1991a). Furthermore, filth fly breeding substrates differing in abiotic factors such as moisture (Geden 1999) or light (Smith and Rutz 1991b) may influence the success of a parasitoid release program.

Studies concerning stable flies in Florida are numerous (King and Lenert 1936, Fye et al. 1980, Hogsette and Ruff 1985). However, most of this research has been directed toward nonanimal breeding sites and dispersal patterns, with limited research on livestock facilities (Greene et al. 1989). A large gap exists in research concerning equine facilities and filth flies, particularly in the area of resident pupal parasitoid populations. Moreover, in a previous study, we demonstrated that the unique filth fly breeding habitats created by equine husbandry practices influence the pteromalid pupal parasitoid species composition on these farms (Pitzer 2010).

Previously, nearly 100% of all parasitoids emerging from field-collected filth fly pupae were *Spalangia* spp. (Pitzer 2010). The primary goal of this study was to determine if the lack of other parasitoids such as *Muscidifurax* spp. is linked to the substrates commonly found at Florida equine facilities, especially regarding searching behavior and host location by parasitoids in those substrates. This study also was used to elucidate possible habitat-dependent interspecific differences between *Spalangia cameroni* Perkins, the most often collected parasitoid in our field studies, and a species less often collected, *Spalangia endius* Walker. To accomplish this, the abilities of *S. cameroni*, *S. endius*, and *Muscidifurax raptorellus* Kogan and Legner, to locate and attack hosts in an immature developmental substrate collected from a Florida equine facility were examined. Experiments were designed to evaluate the selected parasitoids for both inter- and intraspecies attack rates on stable fly host pupae that were either freely accessible or dispersed within a substrate.

### Materials and Methods

**Stable Flies.** A stable fly colony was established from wild individuals collected in February 2007 at the University of Florida Dairy Research Unit, in Hague, FL (UFD strain). The UFD colony flies were maintained at  $26 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and a photoperiod of 12:12 (L:D) h. Citrated bovine blood was provided daily on saturated cotton. Gatorade (The Gatorade Company, Chicago, IL) was provided ad libitum in a 500-ml cup fitted with dental wick as a sugar and electrolyte source. Eggs were collected one to two times weekly and added to a larval medium similar to that described by McPheron and Broce (1996). A modification of the described larval diet was made using maple wood chips in place of vermiculite.

Briefly, the diet was comprised of 2.8 liters water, 4.0 liters wheat bran, 1.2 liters Teklad maple sani-chips (Harlan Laboratories, Inc., Tampa, FL), and 0.4 liters fishmeal (Nelson and Sons Inc., Murray, UT). After a development period of  $\approx 14$  d, pupae were extracted from the rearing medium by flotation, dried, and placed in a 45 by 45 by 45-cm aluminum screened cage.

**Pupal Parasitoids and Test Substrate.** The pteromalid pupal parasitoids chosen for this experiment were *S. cameroni*, *S. endius*, and *M. raptorellus*. Both *S. cameroni* and *S. endius* were obtained on the day of their intended use from colonies maintained by author CJG at the USDA-ARS Center for Medical Agriculture, and Veterinary Entomology in Gainesville, FL. Colonies of *S. endius* and *S. cameroni* were originally established from specimens collected in 2006 from a dairy farm in Gilchrist County, FL. Parasitoids were maintained by providing them with 2-d-old house fly pupae twice per week at a host:parasitoid ratio of  $\approx 5:1$  in chambers maintained at  $25^\circ\text{C}$ , 60–80% RH under constant darkness. The *M. raptorellus* strain used in this study is maintained at the University of Florida Veterinary Entomology Laboratory in Gainesville, FL., under the conditions described above with the exception of a photoperiod provided at 12:12 (L:D) h. This colony was originally established from individuals collected from cattle feedlots in eastern Nebraska in 1990.

Soiled horse bedding was collected from an Ocala, FL., equine farm with recognized stable fly and house fly breeding populations. The bedding was composed of small particle (0.1–0.3-cm) wood shavings, and always contained varying amounts of discarded alfalfa hay, horse manure, and horse urine. To obtain uniform samples for our assays, soiled horse bedding (60 liters) was thoroughly mixed by shovel and divided among 3.75-liter plastic zipper bags. These bags were frozen at  $-20^\circ\text{C}$  for 1 wk before use to kill arthropods in the substrate.

**Parasitoid Release Arenas.** Cylindrical plastic bins (chambers), each having a 26-cm diameter, a 9-cm height, and a total volume of 4.8 liters, were used as chambers for the soiled horse bedding habitats for the experiment. The chambers had tight-fitting lids fitted with an 80-mesh, 20-cm-diameter screened area. Fifty postfeeding UFD stable fly larvae were released on the center surface of 16 chambers containing  $\approx 3.7$  liters (habitat depth, 7 cm) of soiled horse bedding, as well as into 16 120-ml plastic cups containing  $20\text{-cm}^3$  dry maple wood chips. Each replication contained a total of 16 chambers and 16 cups divided randomly into four treatment groups that included: *S. cameroni*-release, *S. endius*-release, *M. raptorellus*-release, and a no-parasitoid control. This arrangement resulted in treatments containing both four chambers and four cups per experimental setup. The cups served three purposes: 1) determination of the time of pupariation, and thus the appropriate time for parasitoid release; 2) a way to compare parasitoid efficiency between searching for dispersed pupae in chambers and freely accessible pupae in cups; and 3) a way to ensure that the pupae were suitable for parasitization.

When at least 90% of the larvae held in plastic cups had pupated (by visual inspection), five female parasitoids of the appropriate species were introduced into each chamber and cup of their respective treatment (Geden 2002). Parasitoids were allowed 72 h to search for and attack hosts (Kaufman et al. 2001a). Following the searching period, the pupae from each chamber and cup were recovered from the soiled horse bedding or wood chips by using a #6 brass sieve, and placed into new 120-ml plastic cups. All cups were held at 26°C, 70% RH, and a photoperiod of 12:12 (L:D) h for 3 to 5 d for adult fly eclosion. Thereafter, remaining uneclosed pupae were placed individually into #0 gelatin capsules and held at the same conditions for 40 d to allow parasitoid emergence (Mann et al. 1990, Lysyk 2001). Any pupae not producing an adult stable fly or parasitoid were dissected to determine the presence or absence of partially-developed parasitoids. This experiment was replicated three times, for a total of 12 chambers and 12 cups for each treatment group. For each replication, five 60-cm<sup>3</sup> samples of the soiled horse bedding were weighed and dried to assess the moisture content of the substrate.

**Statistical Analysis.** An analysis of variance (ANOVA) was conducted using the PROC GLM procedure of SAS 9.1 (SAS Institute 2004) to assess arena type (bedding versus no-bedding), species, and replication as main effects, as well as the potential arena and species interaction for both the percent whole pupae and percent parasitism (as defined below). Three evaluation parameters were subjected to further statistical analysis to determine if differences in searching behavior and parasitism existed between parasitoid species. These parameters included: 1) the percent whole pupae, or pupae that did not produce an adult stable fly, divided by the total pupae recovered from a particular container; 2) the percent parasitism, or pupae that produced an adult parasitoid divided by the total pupae recovered from a particular container; and 3) the percent parasitoid-induced mortality (PIM) (Petersen et al. 1991), or difference between percent whole pupae and percent parasitism (death because of host-feeding, unemerged parasitoid, or superparasitism).

For each ANOVA, species and replication were included as fixed effects in the model, with container type as a variable. An additional ANOVA was conducted for each parasitoid species to assess within-species differences in the efficiency of the aforementioned parameters because of arena type. Stable fly control mortality was assessed in cups and chambers designated as no-parasitoid treatments. Therefore, Abbott's correction was applied to the percent whole pupae to adjust for natural mortality factors (Abbott 1925). All data were transformed using an arcsine square root of the percent whole pupae, percent parasitism, and percent PIM. Multiple mean comparisons were conducted with the Ryan-Einot-Gabriel-Welsh multiple range test ( $\alpha = 0.05$ ). All data are presented as back-transformed means.

## Results

Pupal recovery rates were 100% in the cups and were between 92 and 100% in the chambers. The average moisture level of the soiled horse bedding was  $57.5 \pm 0.7\%$ . Preliminary tests (data not shown) confirmed that the cylindrical chambers inhibited stable fly maggot aggregation in the bedding as it was infrequent. Although the depth at which every pupa recovered was not recorded from the chamber portion of the experiment, these tests indicated that most pupation occurred at depths of 3–7 cm.

All ANOVA measures were found to be significantly different in overall percent whole pupae recovered, including the arena ( $F_{1,64} = 111.97$ ;  $P < 0.0001$ ), species ( $F_{2,64} = 55.91$ ;  $P < 0.0001$ ), and replication ( $F_{2,64} = 4.31$ ;  $P = 0.0175$ ) main effects, and the arena and species interaction ( $F_{2,64} = 15.12$ ;  $P < 0.0001$ ). Overall, percent parasitism was significant for arena ( $F_{1,64} = 33.21$ ;  $P < 0.0001$ ), species ( $F_{2,64} = 43.80$ ;  $P < 0.0001$ ), and their interaction ( $F_{2,64} = 30.95$ ;  $P < 0.0001$ ). Significant differences were detected in percent PIM for arena ( $F_{1,64} = 87.75$ ;  $P < 0.0001$ ) and species ( $F_{2,64} = 19.72$ ;  $P < 0.0001$ ). These findings prompted further analysis to determine the inter- and intraspecies differences in these response variables for both cups and chambers.

**Percent Whole Pupae.** Significant differences in percent whole pupae recovered were detected between parasitoid species in both the cups ( $F_{2,31} = 6.09$ ;  $P < 0.0059$ ) and chambers ( $F_{2,31} = 80.90$ ;  $P < 0.0001$ ) (Table 1). There was a significant difference ( $F_{2,31} = 5.28$ ;  $P = 0.0106$ ) in percent whole pupae between replications in the cups, but not between replications in the chambers. In both the cups and chambers, significantly fewer whole pupae were recovered for *M. raptorellus* (76 and 3%, respectively) than from either *Spalangia* spp., with no difference between *S. cameroni* (93 and 73%, respectively) and *S. endius* (96 and 69%, respectively) (Table 1).

The intraspecies analysis revealed that significantly more whole pupae were recovered from cups than from chambers for all species ( $F_{1,20} = 14.37$ ;  $P = 0.0011$ ) (Table 1). Significant differences between replications in whole pupae recovered were also detected for *M. raptorellus*, ( $F_{2,20} = 95.36$ ;  $P < 0.0001$ ) and *S. endius*, ( $F_{2,20} = 39.93$ ;  $P < 0.0001$ ).

**Percent Parasitism.** No significant difference in percent parasitism was detected between parasitoid species in cups, whereas in the chambers, percent parasitism was significantly greater ( $F_{2,31} = 57.16$ ;  $P < 0.0001$ ) for *S. cameroni* (56%) and *S. endius* (54%), than for *M. raptorellus* (1%) (Table 1). There were no significant differences detected in percent parasitism between replications for the cups or chambers.

No significant difference was detected in percent parasitism in the intraspecies analysis of *S. cameroni* or *S. endius*. However, *M. raptorellus* successfully parasitized significantly more ( $F_{1,20} = 160.45$ ;  $P < 0.0001$ ) pupae in cups (49%) than chambers (1%) (Table 1). A significant difference was detected between repli-



**Table 1.** Comparison of three parasitoid species following 72-h exposure to stable fly pupae that were freely accessible in cups containing 20 ml of dry maple wood chips or dispersed in chambers containing 3.7 liters (7-cm depth) of field-collected soiled horse bedding substrate

Evaluation parameter	Species	Mean (95% CI) <sup>a</sup>		ANOVA F	
		Cup	Chamber	Intraspecies <sup>c</sup>	Replication <sup>d</sup>
% Whole pupae (95% CI) <sup>b</sup>	<i>M. raptorellus</i>	76.4 (68.0–83.8)Ba	2.6 (1.4–4.1)Bb	95.36**	3.76*
	<i>S. cameroni</i>	92.8 (89.9–95.3)Aa	72.9 (67.8–77.7)Ab	14.37**	NS
	<i>S. endius</i>	96.0 (92.9–98.2)Aa	68.6 (63.2–73.8)Ab	39.93**	12.19**
ANOVA F <sup>e</sup>	Interspecies	6.09**	80.90**		
	Replication	5.28*	NS		
% Parasitized (95% CI) <sup>b</sup>	<i>M. raptorellus</i>	49.3 (44.4–54.3)Aa	0.7 (0.3–1.3)Bb	160.45**	NS
	<i>S. cameroni</i>	51.4 (48.0–54.9)Aa	56.4 (52.0–61.0)Aa	NS	4.56*
	<i>S. endius</i>	60.9 (56.8–65.0)Aa	53.7 (45.5–61.8)Aa	NS	NS
ANOVA F <sup>e</sup>	Interspecies	NS	57.16**		
	Replication	NS	NS		
% PIM (95% CI) <sup>b</sup>	<i>M. raptorellus</i>	19.7 (15.8–24.0)Ba	1.5 (0.8–2.5)Bb	28.84**	NS
	<i>S. cameroni</i>	38.8 (36.8–40.9)Aa	14.1 (11.1–17.4)Ab	32.09**	NS
	<i>S. endius</i>	30.9 (27.9–34.1)Aa	10.3 (7.9–12.8)Ab	28.41**	NS
ANOVA F <sup>e</sup>	Interspecies	10.18**	11.16**		
	Replication	5.35*	NS		

<sup>a</sup> Means in columns within an evaluation parameter followed by the same capital letter are not significantly different (Ryan–Einot–Gabriel–Welsh multiple range test [ $\alpha = 0.05$ ]).

<sup>b</sup> Means in rows within a species followed by the same lower case letter are not significantly different (Ryan–Einot–Gabriel–Welsh multiple range test [ $\alpha = 0.05$ ]).

<sup>c</sup> df = 1, 20.

<sup>d</sup> df = 2, 20.

<sup>e</sup> df = 2, 31.

\*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; NS,  $P > 0.05$ ; PIM = parasitoid-induced mortality; *M.* = *Muscidifurax*; *S.* = *Spalangia*.

cations for percent parasitism by *S. cameroni* ( $F_{2, 20} = 4.56$ ;  $P = 0.0234$ ), but not for *M. raptorellus* or *S. endius*.

**Percent PIM.** Significant differences in percent PIM were detected between parasitoid species in both the cups ( $F_{2, 31} = 10.18$ ;  $P = 0.0004$ ) and chambers ( $F_{2, 31} = 11.16$ ;  $P = 0.0002$ ). There was a significant difference ( $F_{2, 31} = 5.35$ ;  $P = 0.0101$ ) in percent PIM between replications for the cups. In both the cups and chambers, percent PIM was significantly lower in treatments containing *M. raptorellus* (20 and 2%, respectively) as compared with either *S. cameroni* (39 and 14%, respectively) or *S. endius* (31 and 10%, respectively), with no difference between the two *Spalangia* spp. (Table 1).

The intraspecies analysis revealed that percent PIM was significantly greater in cups than in chambers for all species ( $F_{1, 20} = 28.41$ ;  $P < 0.0001$ ) (Table 1). No significant differences in PIM were detected between replications for any species.

**Discussion**

Our laboratory findings support those of our field studies conducted in Ocala, FL., where nearly 100% of all pteromalids recovered from field-collected pupae were *Spalangia* spp. (Pitzer 2010). This is particularly evident in the results observed for percent parasitism between container types (Table 1). When parasitoids were forced to search for hosts dispersed in the soiled horse bedding, pupae in chambers containing either *Spalangia* spp. reproduced at a significantly higher level than in those containing *M. raptorellus* (Table 1). This was likely because of the depths at which most stable fly maggots ultimately pupated, and the ability of *Spalangia* spp. to search in the substrate. This fur-

ther corroborates our previous findings, where field-collected stable fly pupae were found at depths of 3 cm or greater, and nearly 100% of all parasitoids recovered were *Spalangia* spp. (Pitzer 2010).

Several studies demonstrating the effects of host dispersal and abiotic factors on pupal parasitoid activity may, in part, explain the findings of our laboratory experiments. A field study of poultry units demonstrated that most *M. raptor* were recovered from pupae collected at manure depths of 3 cm or less, whereas most *Spalangia* spp. were collected between depths of 5 and 10 cm (Rueda and Axtell 1985). This supports previous findings that *Muscidifurax* spp. foraged most often near the substrate surface (Legner 1967). Laboratory evaluations of both *M. raptor* and *S. cameroni*, demonstrated that host burial greatly reduced parasitism by the former species, whereas that of the latter was relatively unaffected (King 1997). Similarly, pupal parasitism by three *Muscidifurax* spp. greatly decreased if hosts were located at depths of  $\geq 1$  cm (Floate and Spooner 2002). In contrast, both *S. cameroni* and *S. endius* searched uniformly through a commonly used fly rearing medium, and regularly located hosts at 6-cm depths in this porous, relatively loose substrate (Geden 2002). Pupae were also attacked by *M. raptor* at 6-cm depths, but only half as often as *Spalangia* spp. The substrate used in our assays more closely approximates the fly rearing medium than the dense sandy soil or manure also evaluated in that study (Geden 2002).

The impact of both substrate moisture and light on *Muscidifurax* spp. and *Spalangia* spp. also has been evaluated. With regard to moisture, *Muscidifurax* spp. attacked more hosts in drier substrates (1 and 45% respectively), whereas *Spalangia* spp. performed best

in those with higher moisture content (40–80 and 45–65%, respectively) (Smith and Rutz 1991b, Geden 1999). However, both species have been shown to prefer dimly lit conditions (Smith and Rutz 1991b). Because light conditions in the current study were similar for all treatments, negating its effect, it is likely that the near 60% average moisture content in the current study is another contributing factor in the increased searching activity of *Spalangia* spp. over that of *M. raptorellus*.

Percent whole pupae and percent PIM were significantly greater for *S. cameroni* and *S. endius* than for *M. raptorellus* in both cups and chambers, although differences were greatest between species in chambers. Causes for increased PIM other than host-feeding, unsuccessful oviposition events or immature parasitoid mortality, may include differences in oviposition restraint. *Muscidifurax zaraptor* Kogan and Legner has been shown to discriminate against previously stung hosts more often than *S. cameroni* (Wylie 1971, 1972). This behavior was similarly described in detail as a possible cause of PIM for several *Muscidifurax* spp. and *Spalangia nigroaenea* Curtis (Petersen et al. 1991). Therefore, oviposition restraint behaviors between the two genera may be a contributing factor for our observed PIM differences.

Differences in PIM levels between the two genera were also greater in cups than in chambers. Host encounter frequency was probably greater in the much smaller cups, where pupae were essentially freely accessible, than in the chambers, which required greater searching effort. This may have further accentuated differences in oviposition restraint between the two genera. Our significant interaction effect between arena type and species also suggests that host attacks by the selected species differed because of the environmental conditions present, i.e., the arena size to which wasps were subjected.

Further evidence of increased host encounter frequency in cups compared with chambers is provided by our intra-species analysis of the aforementioned response variables. Successful parasitism was similar between container types for both *S. cameroni* and *S. endius*, suggesting that these species located similar numbers of hosts regardless of the searching effort required (Table 1). However, this analysis revealed that *M. raptorellus* was less efficient in locating hosts when required to search in chambers (Table 1). The intraspecific analysis indicated that percent whole pupae and percent PIM were significantly less in chambers than in cups for all species, further confirming the likelihood that host encounter frequency was greater in cups than in chambers.

In a few cases, significant differences were detected between replications for percent whole pupae and percent PIM. However, these differences only occurred between replications in the cup treatment. This discrepancy can be explained by our use of a newly established stable fly colony. Pupariation in this colony is far less synchronous than in a long-established one and takes place continually between days 10 and 14 postoviposition. Therefore, it was difficult to

collect only postfeeding third-instar stable flies from our rearing media by using larval aggregation in rearing containers to identify appropriately-aged individuals. A proportion of those larvae at any given time, had not yet ceased feeding, and this proportion may have varied somewhat between test replications. This resulted in higher and less uniform control mortality in cups than in the chambers, as the latter contained equine urine- and feces-soiled substrate for those larvae still requiring nutrition to continue feeding and pupate.

Several studies evaluating the behavior or ability of pteromalid parasitoids to locate hosts under laboratory conditions have been used as supporting evidence for the findings in the current study (Wylie 1971, 1972; Petersen et al. 1991; Geden 1999, 2002; Floate and Spooner 2002). However, most of these studies used small chambers ( $\approx 100$  ml), or artificially made substrate conditions to evaluate parasitoid activity. Furthermore, all of these studies used previously-pupariated fly pupae, placed in the chamber at predetermined locations or depths. Both *M. raptorellus* and *S. cameroni* have demonstrated short dispersal distances (2–3 m) from release sites in previous studies (Tobin and Pitts 1999, Skovgård 2002). Therefore, this study is, to our knowledge, the first evaluation of pteromalid parasitoid host location by using sizable chambers that more closely approximate field conditions and the recommendation made for developmental site specific releases (Tobin and Pitts 1999, Skovgård 2002). In addition, this is the first study in which third-instar filth flies were allowed to wander and pupate on their own using a substrate where filth fly breeding was known to have occurred previously.

Our results clearly demonstrate the ability of *S. cameroni* and *S. endius* to search more efficiently, i.e., deeper, for hosts than *M. raptorellus*, using a substrate where fly breeding had previously occurred at a livestock farm. This experiment also corroborates our previous findings that host depth, due to the fly breeding substrates generated by equine husbandry practices, is likely the cause for the predominant proportions of *Spalangia* spp. found in those field studies (Pitzer 2010). Further experiments are needed to determine if *Spalangia* spp.-only releases at equine facilities in Florida can improve filth fly control.

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