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C J. Geden
USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, Chris.geden@ars.usda.gov

D M. Johnson
USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology

P E. Kaufman
Entomology and Nematology Department, University of Florida

C K. Boohene
Polk County Mosquito Control

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Competition between the filth fly parasitoids *Muscidifurax raptor* and *M. raptorellus* (Hymenoptera: Pteromalidae)

C. J. Geden[1]✉, D. M. Johnson[1], P. E. Kaufman[2], and C. K. Boohene[3]

[1]*USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Dr., Gainesville, FL 32608, U.S.A.*
Chris.Geden@ars.usda.gov
[2]*Entomology and Nematology Department, University of Florida, 970 Natural Area Drive, Gainesville, FL 32611, U.S.A.*
[3]*Polk County Mosquito Control, 4177 Ben Durrance Rd, Bartow, FL 33830, U.S.A.*

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ABSTRACT: Competition bioassays were conducted with the filth fly pupal parasitoids *Muscidurax raptor* (Girault & Sanders) and *M. raptorellus* (Kogan & Legner) (Hymenoptera: Pteromalidae) using house fly *Musca domestica* L. (Diptera: Muscidae) hosts at different host densities. *Muscidifurax raptor* had a significant impact on *M. raptorellus* when hosts were limiting in sequential parasitism tests. Fewer than six *M. raptorellus* adult progeny emerged from groups of 50 fly pupae that were parasitized by *M. raptor* at the same time or when *M. raptor* parasitism preceded *M. raptorellus* by 48 h, respectively, compared with 42-55 *M. raptorellus* progeny produced when this species was tested alone. Production of *M. raptor* was significantly lower when parasitism by this species was preceded by *M. raptorellus* (25) than when *M. raptor* was tested alone (43). When the two species parasitized hosts at the same time in different proportions at low host:parasitoid densities (5:1), *M. raptorellus* produced 13 progeny per parent female when it was the sole species present and fewer than two when *M. raptor* was present. No negative impact of *M. raptorellus* on *M. raptor* was observed. Neither species had a substantial effect on the success of the other at higher host:parasitoid densities. ***Journal of Vector Ecology* 39 (2): 278-287. 2014.**

Keyword Index: *Muscidifurax raptor, Muscidifurax raptorellus, Musca domestica*, house fly, biocontrol, parasitoids.

INTRODUCTION

House flies (*Musca domestica* L.) and stable flies (*Stomoxys calcitrans* (L.)) (Diptera: Muscidae) are among the most important pests of animal agriculture globally. Economic losses due to these pests in the U.S. are estimated at $375 million and $2.2 billion for house flies and stable flies, respectively (Geden and Hogsette 2001, Taylor et al. 2012). House flies are mechanical vectors of animal and human disease organisms and have provoked legal disputes between livestock producers and neighbors (Malik et al. 2007). Stable fly biting pressure on livestock results in direct production losses in the form of reduced weight gain and milk production (reviewed in Taylor et al. 2012). Effective fly management requires integration of sanitation, biological control, and selective use of insecticides. Insecticidal control of house flies is problematic because of the rapidity with which insecticide resistance develops, even to new products that are thought to have novel modes of action (Malik et al. 2007, Kaufman et al. 2010).

Successful fly management using augmentative releases of pupal parasitoids was first demonstrated by Morgan et al. (1975). During the ensuing forty years, a vast literature on filth fly biological control has developed and many parasitoid products are commercially available. Despite the progress that has been made, the selection of species for effective augmentative releases is still an uncertain business. *Spalangia* spp. are valued for their ability to locate buried pupae (Geden 2002, Skovgård and Nachman 2004) and solitary *Muscidifurax* spp. (*M. raptor* and *M. zaraptor*) for their high attack rates and ease and economy of production (Rutz and Axtell 1981, Petersen et al. 1992). Since the 1990 discovery of the gregarious South American parasitoid *M. raptorellus* in the midwestern U.S., this species has received considerable attention (Petersen and Currey 1996, Kaufman et al. 2012) and become one of the prominent products carried by most commercial producers of filth fly parasitoids.

There has been a trend in the past 20 years away from single-species releases and towards species mixes for fly management in both the commercial and research community. In some cases, combinations of a *Spalangia* and *Muscidifurax* species have been used in the hope of exploiting between-genera niche differences to broaden the reach of the releases into a wider habitat range (Weinzierl and Jones 1998, Geden and Hogsette 2006). In others, combinations of solitary *Muscidifurax* species (*M. raptor* and *M. zaraptor* Kogan and Legner) and *M. raptorellus* have been used because of the cost-effectiveness that the latter offers (Kaufman et al. 2002, 2012). Competitive interactions between *Spalangia* spp. and solitary *Muscidifurax* are well understood, as are the life history strategies used by the parasitoids to avoid and resolve multiparasitism events (Wylie 1971, 1972, Ables and Shepard 1974, Propp and Morgan 1983, King 1997). In contrast, to our knowledge, nothing is known about such interactions within the genus *Muscidifurax*. Perhaps one reason for this has been the difficulty in identifying the three North American species using morphological characters (Kogan and Legner 1970). A red-eyed mutant strain of *M. raptor* became available to us when one of the authors (CKB) noted and selected for this trait in a Florida strain maintained at the USDA-ARS,

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Center for Medical, Agriculture and Veterinary Entomology (CMAVE). The availability of the mutant strain raised the possibility of examining competitive interactions between *M. raptor* and *M. raptorellus*. Assays were conducted to examine competition under conditions in which either the order or ratio of parasitism by each species varied.

MATERIALS AND METHODS

Colonies

House fly pupae were from a long-established insecticide-susceptible colony ("Orlando Normal") maintained at CMAVE. Larvae were reared in the diet described by Hogsette (1992) consisting of water added to a dry diet of 50% wheat bran, 30% alfalfa meal, and 20% corn meal. *Muscidifurax raptor* were from a red-eyed strain isolated from a colony established in 1992 from a poultry farm in Hernando County, FL. The *M. raptorellus* were from a colony established in Lincoln, NE, in approximately 1990. Parasitoids were maintained on two-day-old house fly pupae every three to four days at a host: parasitoid ratio of 5:1 in 32.5 x 32.5 x 32.5-cm cages (MegaView Science, Taiwan) and held at 25° C, 80% RH under constant darkness. All experiments were conducted using one-day-old fly pupae and three to four-day-old female parasitoids (presumed to have mated) that had been provided with hosts since wasp emergence. All experiments were conducted by holding pupae and parasitoids in an environmental chamber maintained at 25° C, 80% RH, and constant darkness.

Sequential parasitism

Two host densities were used, 50 and 300 pupae per group of parasitoids. For each replication, 21 sets of 50 and 300 fly pupae were placed in 60-ml plastic cups with muslin covers. Five female parasitoids were added to 15 of the 21 cups from each pupal group (50 or 300) on day 1 so that the cups contained either five *M. raptor* only (6 cups), five *M. raptorellus* only (6 cups) or ten total wasps consisting of five of each species (3 cups). Parasitoids were removed 24 h later (day 2). Following 24 h (day 3) without parasitoids, groups of five parasitoids were added to the unexposed pupae that had been set aside on day 1 (3 cups per host density per species) and to half of the cups previously exposed to the heterologous species. Parasitoids were removed 24 h later (day 4). This sequence resulted in the following treatments for both the 50- and 300-pupae host densities: 1) *M. raptor* only, on day 1; 2) *M. raptor* only, on day 3; 3) *M. raptorellus* only, on day 1; 4) *M. raptorellus* only, on day 3; 5) *M. raptor* on day 1 followed by *M. raptorellus* on day 3; 6) *M. raptorellus* on day 1 followed by *M. raptor* on day 3; 7) both species together, on day 1. House fly pupae with no parasitoids also were set up for each replication as a quality control check for stray parasitism..

Pupae were held until fly emergence was complete (7 d). Remaining uneclosed pupae were isolated in individual gelatin capsules (when numbers were low) or wells of 96-well tissue culture plates for parasitoid emergence. Plate wells were sealed to prevent emerging parasitoids from moving from one well to another by placing two layers of Parafilm and a rectangle of cardstock paper between the top and bottom of each plate. Tape was used to fasten the plate top to the bottom. Pupae that produced neither flies nor parasitoids were not dissected because the two species cannot be distinguished in the immature stages. The entire experiment was replicated on four separate occasions using different cohorts of flies and parasitoids.

Varying parasitoid ratios

In the second set of tests, groups of ten female parasitoids at varying species ratios were added simultaneously to pupae at varying host densities. For each replication, 15 sets each of 50, 200, and 400 fly pupae were placed in 60-ml plastic cups with muslin covers. Ten female parasitoids were added to the cups in the following ratios (*M. raptor:M. raptorellus)*: 10:0, 7:3, 5:5, 3:7, and 0:10. Unexposed pupae without parasitoid exposure were used as a quality control check for host viability and parasitoid contamination. Pupae with parasitoids were held at 25° C in an incubator until emerging adult flies were all dead. Uneclosed pupae were counted, dead parasitoids were removed, and isolated for parasitoid emergence as previously described. The experiment was replicated four times using different cohorts of flies and parasitoids.

Data analysis

For the sequential parasitism experiment, data on numbers of pupae successfully parasitized by each species (those that produced parasitoids) and the numbers of adult F1 parasitoid progeny produced were analyzed by two-way Analysis of Variance (ANOVA) using parasitoid exposure regimem (seven treatments) and host density (50 or 300) as the main effects plus their interaction. (Note: For *M. raptor,* these two response variables were the same). Because the interaction was significant in nearly all cases, the data were partitioned into the two host density groups, and one-way ANOVA was used to examine treatment effects and Tukey's multiple means separation was applied as appropriate (alpha=0.05). When analyzing treatment effects on one species, we excluded those treatments that did not include that species because there were only zero values for such treatments. Data analysis was completed using the GLM Procedure with the Means/Tukey Statement of the Statistical Analysis System version 9.2 (SAS Institute, Cary, NC). In the parasitoid ratio experiment, data were analyzed the same way, except that values for parasitized pupae and the number of adult F_1 parasitoid progeny produced were adjusted to a per-female basis to allow meaningful comparisons across treatments with varying numbers of parentals of each species.

RESULTS

Sequential parasitism

The interaction between parasitoid sequence and the number of pupae provided was significant for all variables except the number of pupae parasitized by both species, indicating that competition effects were modulated by host availability (Table 1). When data were partitioned by host density, it was evident that nearly 100% of hosts were killed

when only 50 pupae were provided to five *M. raptor* (Table 2). At these low host densities, the initial presence of *M. raptorellus* resulted in reduced parasitism by *M. raptor* by approximately 50%. The effect of *M. raptor* on the success of *M. raptorellus* was much stronger. Fewer than two pupae were successfully parasitized by *M. raptorellus* when both species were introduced at the same time or when *M. raptor* was the first species introduced compared with 18 to 22 parasitized pupae with *M. raptorellus* alone.

Similar results were observed with adult progeny of *M. raptorellus*; 42 to 55 progeny were produced when this species was alone compared with about six when *M. raptor* was introduced at the same time as *M. raptorellus* or when *M. raptor* was the first species introduced. *Muscidifurax raptorellus* was substantially more successful when it was allowed to oviposit before *M. raptor*, producing 11.4 parasitized pupae and 30.2 adult progeny (Table 2). Successful multiparastism occurred but was rare; only 0.9-1.5 pupae produced adult progeny of both species and there was no significant effect of parasitoid sequence on multiparasitism (Table 2).

When the same combinations were evaluated at a high host density (300 pupae per group of parasitoids), less than one-half of the pupae were killed (Table 2). In contrast to the low host-density treatments, no significant effect was observed for the presence of a second species on parasitism by either *M. raptor* or *M. raptorellus. Muscidifurax raptor* parasitized 85 to 109 pupae across all treatments. Parasitism by *M. raptorellus* was lower than *M. raptor*, with 18 to 28 pupae parasitized and 31 to 58 adult progeny produced. Successful multiparasitism occurred in a small number of pupae, accounting for <1% of the parasitized pupae (Table 2).

Varying parasitoid ratios

The interaction between parasitoid ratio and the number of pupae provided was significant for all variables except the number of pupae parasitized by both species, again indicating that competition effects were modulated by host availability (Table 1). Overall host mortality was substantially higher than in the previous assays and approached 100% except when host density was high (40:1 host:parasitoid ratio) and when few or no *M. raptor* were present (Table 3). Successful multiparasitism was higher as well, and accounted for 5-10% of parasitized pupae in some instances.

When expressed on a per-female basis, parasitism by *M. raptor* was highest when this species comprised 30% of the ratio and lowest when no *M. raptorellus* were present, and this effect was significant at all three host densities (Table 4). For example, when host availability was highest, *M. raptor* produced 70 progeny per conspecific female when it comprised 30% of the starting parasitoids compared with 31.5 when only *M. raptor* was present. It should be noted here that number of hosts available per female *M. raptor* was also 3.3 times higher in the 30% than in the 100% *M. raptor* treatments (three vs ten starting females per 400 pupae, respectively). This apparent intraspecific negative effect was most pronounced at the low host density of 50 pupae per group of ten wasps; only two *M. raptor* progeny were produced per female in the 100% *M. raptor* treatment as compared with 8.8 progeny when *M. raptorellus* made up 70% of the parasitoids.

In contrast, performance of *M. raptorellus* was significantly and strongly impacted by the presence of *M. raptor* at the low host density (Table 4). *Muscidifurax raptorellus* produced 13.4 progeny per female when it was the sole species and fewer than two in any of the treatments

Table 1. ANOVA F-values from experiments on interactions between *Muscidifurax raptor* and *M. raptorellus* when they were placed with house fly pupae (at varying quantities) in different time sequences or in different ratios at the same time.

ANOVA effect	Number of parasitized pupae:			Number of adult progeny produced	
	parasitized by *M. raptor*	parasitized by *M. raptorellus*	parasitized by both species	*M. raptor*	*M. raptorellus*
Varying the sequence of parasitism by species					
Parasitoid trt (Trt) (df= 6, 154)	120.24**[1]	7.14**	2.02ns	123.43**	28.28**
Host density (Den) (df=1, 154)	353.26**	24.38**	2.33ns	358.25**	10.91**
Trt X Den (df=6, 154)	25.43**	2.62**	0.53ns	25.25**	4.55**
Varying parasitoid species ratios					
Parasitoid trt (Trt) (df=4, 165)	120.38**	14.59**	4.10*	226.89**	10.55**
Host density (Den) (df=2, 165)	948.68**	25.27**	24.32*	1337.89**	58.54**
Trt X Den (df=8, 165)	23.42**	3.45**	1.09ns	42.15**	3.67**

[1] Results of two-way ANOVA using parasitoid treatment (parasitism sequence or ratios of *M. raptor* and *M. raptorellus*), host density, and their interaction. **, P≤0.01;* P≤0.05; ns, P>0.05.

Table 2. Parasitism of house fly pupae by *M. raptor* (MR) and *M. raptorellus* (MLS) either alone, together, or in sequence at two host densities[1].

Treatment	Mean (SE) number of pupae				Mean (SE) number of adult progeny	
	Uneclosed[2]	parasitized by *M. raptor*	parasitized by *M. raptorellus*	parasitized by both species	*M. raptor*	*M. raptorellus*
Tests with 50 pupae per group of parasitoids						
MR alone, time 0	48.4 (0.60)	42.6 (0.80)a[3]	0.0 (0.00)	0.0 (0.00)	43.2 (0.97)a	0.0 (0.00)
MR alone, 48 hr	49.2 (0.42)	42.0 (0.64)ab	0.0 (0.00)	0.0 (0.00)	42.7 (0.64)ab	0.0 (0.00)
MLS alone, time 0	25.5 (3.25)	0.0 (0.00)	21.8 (3.20)a	0.0 (0.00)	0.0 (0.00)	55.3 (9.79)a
MLS alone, 48 h	21.7 (2.40)	0.0 (0.00)	17.6 (1.95)ab	0.0 (0.00)	0.0 (0.00)	41.6 (6.22)ab
MR (time 0) then MLS (48 h)	50.0 (0.00)	35.5 (1.28)b	1.9 (0.48)c	1.5 (0.36)	35.5 (1.28)b	5.6 (1.08)c
MLS (time 0) then MR (48 h)	49.6 (0.33)	26.2 (2.72)c	11.4 (2.07)b	0.9 (0.29)	25.8 (2.87)c	30.2 (7.82)b
MR and MLS both at time 0	48.9 (0.40)	35.3 (2.66)b	1.9 (0.31)c	1.5 (0.34)	36.3 (2.09)b	5.8 (0.86)c
ANOVA F		14.48**[4]	22.4**		17.79**	12.43**
Tests with 300 pupae per group of parasitoids						
MR alone, time 0	133.2 (8.98)	109.1 (8.49)	0.0 (0.00)	0.0 (0.00)	109.1 (8.49)	0.0 (0.00)
MR alone, 48 hr	117.6 (5.01)	91.5 (4.88)	0.0 (0.00)	0.0 (0.00)	92.4 (5.30)	0.0 (0.00)
MLS alone, time 0	40.0 (3.37)	0.0 (0.00)	27.8 (3.27)	0.0 (0.00)	0.0 (0.00)	57.8 (8.37)
MLS alone, 48 hr	33.6 (3.05)	0.0 (0.00)	18.2 (2.09)	0.0 (0.00)	0.0 (0.00)	39.9 (5.98)
MR (time 0) then MLS (48 h)	149.0 (7.14)	92.9 (8.18)	18.9 (3.04)	1.2 (0.21)	94.2 (8.20)	31.3 (5.96)
MLS (time 0) then MR (48 h)	149.3 (5.58)	97.9 (4.92)	22.5 (3.59)	0.6 (0.29)	97.9 (3.92)	46.2 (8.03)
MR and MLS both, time 0	150.3 (8.08)	85.0 (6.46)	21.7 (3.06)	1.1 (0.23)	86.3 (6.70)	41.3 (4.84)
ANOVA F		1.75ns	2.16ns		1.59ns	2.38ns

[1] Groups of five female wasps were placed with and removed from live fly pupae for a single 24-h oviposition event at either time 0 (one-d-old pupae) or 48 h later.
[2] Pupae that did not produce flies; includes pupae killed by adult host-feeding, successful and unsuccessful parasitism, and unknown host mortality.
[3] Means within columns under the same subheading followed by the same letter are not significantly different (P>0.05, Tukey's).
[4] One-way ANOVA for treatment effects performed separately for each host density, **, P<0.01; ns, P>0.05 (df=4, 55 for all variables except number of pupae parasitized by both species [df=2, 33]).

Table 3. Total mortality and parasitism of house fly pupae by *M. raptor* (MR) and *M. raptorellus* (MLS) either alone or together in varying proportions (total, ten females) at three host densities (50, 200, 400)[1].

Species ratio treatment	Mean (SE) number of pupae				Mean (SE) number of adult progeny	
	Uneclosed[2]	parasitized by *M. raptor*	parasitized by *M. raptorellus*	parasitized by both species	*M. raptor*	*M. raptorellus*
	Tests with 50 pupae per group of parasitoids					
10 MR: 0 MLS	49.8 (0.25)	19.8 (2.72)	0.0 (0.00)	0.0 (0.00)	19.8 (2.72)	0.0 (0.00)
7 MR: 3 MLS	50.9 (0.66)	17.2 (1.32)	1.0 (0.30)	0.5 (0.15)	17.9 (1.36)	1.2 (0.37)
5 MR: 5 MLS	50.0 (0.00)	20.3 (1.10)	2.8 (0.49)	1.4 (0.29)	22.0 (1.22)	6.8 (1.54
3 MR: 7 MLS	50.0 (0.00)	22.6 (1.27)	3.8 (0.99)	3.1 (0.58)	26.4 (1.21)	12.7 (2.68)
0 MR: 10 MLS	49.0 (0.40)	0.0 (0.00)	39.3 (2.73)	0.0 (0.00)	0.0 (0.00)	134.0 (8.13)
	Tests with 200 pupae per group of parasitoids					
10 MR: 0MLS	197.9 (3.59)	169.6 (7.51)	0.0 (0.00)	0.0 (0.00)	169.6 (7.51)	0.0
7 MR: 3 MLS	196.0 (6.81)	134.2 (5.91)	18.6 (5.26)	10.8 (2.58)	155.2 (4.50)	46.5 (11.51)
5 MR: 5 MLS	193.8 (4.51)	142.9 (4.56)	18.9 (2.40)	11.6 (1.64)	162.5 (3.84)	47.2 (4.52)
3 MR: 7 MLS	167.1 (15.75)	113.0 (4.81)	47.1 (4.24)	13.4 (1.78)	130.9 (5.74)	105.6 (9.01)
0 MR: 10 MLS	140.1 (8.85)	0.0 (0.00)	128.0 (8.83)	0.0 (0.00)	0.0 (0.00)	187.1 (14.53)
	Tests with 400 pupae per group of parasitoids					
10 MR: 0MLS	387.7 (7.48)	314.8 (13.15)	0.0 (0.00)	0.0 (0.00)	314.8 (13.15)	0.0 (0.00)
7 MR: 3 MLS	390.6 (7.45)	301.4 (7.37)	21.2 (5.65)	9.0 (2.61)	321.5 (9.26)	44.5 (9.75)
5 MR: 5 MLS	374.9 (4.51)	283.3 (7.43)	41.3 (5.16)	13.9 (2.73)	303.1 (6.03)	98.6 (11.48)
3 MR: 7 MLS	310.0 (20.89)	211.1 (12.43)	101.1 (13.52)	20.5 (4.77)	234.4 (10.42)	227.8 (34.48)
0 MR: 10 MLS	209.2 (24.83)	0.0 (0.00)	176.7 (26.71)	0.0 (0.00)	0.0 (0.00)	268.8 (39.87)

[1] Parasitoids were placed with live 1-day-old fly pupae and left until adult fly emergence and death.
[2] Pupae that did not produce flies; includes pupae killed by adult host-feeding, successful and unsuccessful parasitism, and unknown host mortality.

Table 4. Per-capita parasitism of house fly pupae by *M. raptor* (MR) and *M. raptorellus* (MLS) either alone or together in varying proportions (total, ten females) at three host densities (50, 200, 400). Data adjusted to reflect parasitism per female of each species.

Treatment	Mean (SE) pupae per initial female			Mean (SE) progeny per initial female	
	parasitized by *M. raptor*	parasitized by *M. raptorellus*	parasitized by both species	*M. raptor*	*M. raptorellus*
		Tests with 50 pupae per group of parasitoids			
10 MR: 0MLS	1.98 (0.27)c	-	-	1.98 (0.27)c	-
7 MR: 3 MLS	2.46 (0.19)c	1.90 (0.93)	0.05 (0.02)b	2.56 (0.19)c	0.38 (0.12)b
5 MR: 5 MLS	4.05 (0.22)b	1.66 (0.52)	0.14 (0.03)b	4.40 (0.24)b	1.35 (0.31)b
3 MR: 7 MLS	7.53 (0.42)a	2.27 (0.79)	0.31 (0.06)a	8.79 (0.40)a	1.80 (0.39)b
0 MR: 10 MLS	-	3.93 (0.27)	-	-	13.40 (0.81)a
ANOVA **F**	74.47**	2.28ns	11.55**	114.38**	164.08**
		Tests with 200 pupae per group of parasitoids			
10 MR: 0MLS	17.00 (0.76)c	-	-	17.0 (0.76)d	-
7 MR: 3 MLS	19.17 (0.84)c	7.95 (2.35)ab	1.07 (0.26)	22.16 (0.64)c	15.52 (3.84)ab
5 MR: 5 MLS	28.58 (0.92)b	4.80 (0.71)b	1.17 (0.17)	32.50 (0.77)b	9.44 (0.91)b
3 MR: 7 MLS	37.67 (1.67)a	10.53 (2.10)ab	1.34 (0.18)	43.64 (1.91)a	15.09 (1.29)ab
0 MR: 10 MLS	-	`12.80 (0.88)a	-	-	18.71 (1.45)a
ANOVA **F**	76.85**	4.23*	0.45ns	120.43**	3.08*
		Tests with 400 pupae per group of parasitoids			
10 MR: 0MLS	31.48 (1.32)d	-	-	31.48 (1.32)d	-
7 MR: 3 MLS	43.05 (1.05)c	3.73 (0.97)b	0.91 (0.02)	45.92 (1.32)c	14.81 (3.25)b
5 MR: 5 MLS	56.66 (1.49)b	6.15 (1.52)b	1.39 (0.28)	60.63 (1.21)b	19.72 (2.30)ab
3 MR: 7 MLS	70.37 (4.15)a	8.91 (2.10)b	2.03 (0.48)	78.13 (3.47)a	32.54 (4.93)a
0 MR: 10 MLS	-	17.67 (2.67)a	-	-	26.88 (3.98)ab
ANOVA ***F***	51.01**	10.00**	2.55ns	98.78**	4.36**

[1] Parasitoids were placed with live 1-day-old fly pupae and left until adult fly emergence and death.
[2] Means within columns under the same subheading followed by the same letter are not significantly different (P>0.05, Tukey's).
[3] One-way ANOVA for treatment effects performed separately for each host density, **, P<0.01; ns, P>0.05 (df=3, 44 for all variables except number of pupae parasitized by both species [df=2, 33]).

where *M. raptor* also was present. When host availability was higher, the effect of *M. raptor* on *M. raptorellus* was less pronounced and inconsistent. For example, at the high host density of 400 pupae per group of ten parasitoids, the number of *M. raptorellus*-parasitized pupae per female was greatest (17.7) when only this species was present compared to fewer than ten in the treatments that included *M. raptor*. Numbers of *M. raptorellus* progeny showed only a weak trend of smaller numbers when high proportions of *M. raptor* were present (Table 4).

DISCUSSION

Solitary species of *Muscidifurax* have long been noted for their aggressive first stage larvae that move actively within the host puparium and attack supernumerary parasitoid immatures of their own and other species, which increases the odds for successful development of a single individual from each host (Wylie 1972). Moreover, these species show ovipositional restraint, preferring to oviposit in unparasitized hosts and those parasitized by non-conspecifics (Wylie 1971). This restraint breaks down when unparasitized hosts are scarce, leaving the immatures with the task of resolving competition.

The ability to avoid oviposition in parasitized hosts and aggressive larvae gives solitary *Muscidifurax* spp. a distinct advantage when competing with other species. An additional advantage that these species have is their more rapid development compared with other filth fly parasitoids such as *Spalangia* spp. (Mann et al. 1990b, Geden 1997). Wylie (1972) noted that *M. zaraptor* outcompeted *S. cameroni* Perkins under a variety of test conditions, and similar results were noted in competitions between *M. raptor* and *S. endius* Walker (Ables and Shepard 1974, Propp and Morgan 1983). Intergeneric conflict under field conditions may be mitigated by niche partitioning. *Muscidifurax* spp. concentrate their foraging efforts near the surface of fly larval habitats, whereas *Spalangia* spp. are more likely to attack pupae buried below the surface (Legner 1977, King 1997, Geden 2002). As a result, it has been argued that augmentative releases of parasitoids for fly management might be improved by releasing combinations of *Muscidifurax* and *Spalangia* spp. to take advantage of their complimentary host-searching strategies (Geden and Hogsette 2006), an approach that has been used for crop pests as well (Ehler 1978, 1992, Heinz and Nelson 1996).

Little is known about interactions among the species of *Muscidifurax.* The two native, solitary species *M. raptor* and *M. zaraptor* have been studied extensively and used with some success as augmentative biological control agents (Greene 1990, Mann et al. 1990a, b, Geden et al. 1992, Petersen et al. 1992, Lysyk 2000, 2001a, McKay et al. 2007). Both are also available as commercial products from several insectaries that provide parasitoids to livestock producers. The two species are biologically similar and occur sympatrically in many areas of central and western North America (Jones and Weinzierl 1997, Taylor et al. 1997, Floate et al. 1999). *Muscidifurax raptorellus* is an introduced species first collected in Chile in 1965 and subsequently released in California (Legner et al. 1990). Although it did not seem to become established at the time, this species was observed in Nebraska in 1990 (Petersen and Currey 1996). The Nebraska populations are believed to have originated from the original Chilean introduction (Antolin et al. 1996), and their movement eastward may have been hastened by releases of commercially-reared parasitoids.

Muscidifurax raptorellus in the U.S. is a gregarious species that typically produces two to five parasitoids per host pupa and is easily reared in large numbers (Petersen and Currey 1996). The ease and low cost required for its production make it an attractive candidate for augmentative releases and commercial production. Results of such releases have been mixed but encouraging. Inundative releases have resulted in high parasitism in cattle feedlots (Petersen and Cawthra 1995, Floate et al. 2000), dairy calf housing (Kaufman et al. 2012), and some poultry systems (Kaufman et al. 2001b, McKay et al. 2007). In addition to single-species releases, *M. raptorellus* sometimes has been paired with a second species in an effort to broaden niche coverage by the parasitoids (Meyer et al. 1990, Kaufman et al. 2001a, 2012, McKay et al. 2007, Geden and Hogsette 2006).

M. raptorellus shows considerable plasticity in the number of individuals produced per host pupa. Solitary emergence is not uncommon. Lysyk (2004) observed that 19% of house fly pupae parasitized by this species produced a single individual, and Geden and Hogsette (2006) observed a solitariness rate of 9% among *M. raptorellus*-parasitized pupae from a commercial insectary. Mean numbers of *M. raptorellus* progeny per host vary from 2.4-8.6 in the literature (Lysyk 2001b, Geden and Moon 2009). Lysyk (2004) and Petersen and Currey (1996) observed that the degree of gregariousness in this species is inversely density-dependent, with fewer parasitoids produced per pupa when hosts are abundant. Our results support those observations. Based on calculations using the data in Table 2, higher gregariousness was observed in the *M. raptorellus*-only treatments when hosts were scarce (3.4 parasitoids per pupa) than in the two higher host-availability groups (about 1.5 in both). From a fly management standpoint this could have a desirable outcome if the same number of parasitoids responds to rising fly populations by imposing a higher kill rate.

Parasitism by *M. raptorellus* typically increases during weeks when releases are made then declines rapidly once they stop (Floate et al. 2000, McKay et al. 2007, Kaufman et al. 2012). Such post-release declines, along with the rarity of this species in the absence of releases, suggest that natural populations of *M. raptorellus* may be competitively disadvantaged relative to native species such as *M. raptor*. Direct experimentation on competitive interactions among *Muscidifurax* spp. has been constrained by the fact that members of this genus are very difficult to identify using morphological characters (Kogan and Legner 1970, Doganlar 2007). The use of a red-eyed strain of *M. raptor* allowed the experiments presented here to be conducted with a degree of certainty of species identification that would otherwise be impossible without using molecular methods (Taylor et al. 1997, Geden et al. 1998, Taylor and Szalanski 1999). The mutant was discovered

and selected for by one of us (CKB) during routine colony maintenance and has equal or superior fitness to the wild-type strain from which it was isolated (unpublished data). In addition to interspecific competition studies, this strain may be useful for measuring the impact of released parasitoids or the distance that they travel after release.

Our experiments demonstrated that *M. raptor* has a substantially negative impact on *M. raptorellus* when hosts are in short supply. Impact is somewhat lessened when *M. raptorellus* is given a 48-h "head start" on *M. raptor*. This lead time may allow some of the larval *M. raptorellus* to reach a size that affords protection from attack by the time the aggressive *M. raptor* larvae hatch. Support for this can be found in Wylie (1972), who found that survival of the gregarious parasitoid *Nasonia vitripennis* Walker was high in competition tests with *M. zaraptor* only when parasitisim by the former species preceded that of its rival by one or two days. *Nasonia vitripennis* fared better overall with *M. zaraptor* than *M. raptorellus* did with *M. raptor* in our tests, at least at low host availability. *Nasonia vitripennis* produces many more parasitoids per host (up to 25) than *M. raptorellus,* and Wylie (1972) speculated that the sheer force of numbers sometimes gave the former an advantage in fending off attacks by *M. zaraptor* larvae, even under test conditions otherwise favorable to *M. zaraptor*.

Competition effects in our tests were highly influenced by host availability. In the sequential parasitism tests, in which parasitoid removal after 24 h had a dampening effect on overall parasitism, there were no significant competition effects when host supplies were abundant. Results were less clear in the varying-ratio bioassays, where overall higher parasitism resulted in negative intraspecific effects for *M. raptor* due to superparasitism. Even under these conditions, however, it was apparent that the negative effect of *M. raptor* on *M. raptorellus* was strongest when competition for host resources was highest, resulting in 87-97% reductions in production of *M. raptorellus* adults. At higher host densities these effects were muted in a manner similar to what was observed in the sequential parasitism bioassays. Under field conditions where fly pressure is high, it does not seem likely that the two species would interact in ways that would limit the effectiveness of either. Combinations of the two species do not appear to offer any advantage over single-species releases other than some cost savings due to the relative low cost of producing *M. raptorellus*, although this lower cost needs to be balanced against the lower per-capita kill rate of this species compared with solitary *Muscidifurax* spp. Another possible reason for the rarity of *M. raptorellus* in the field may be that the fitness of colonized *M. raptorellus* has degraded during the nearly 50 years that this species has been in culture in North America. A re-evaluation of this species using fresh material from its native South America (Marchiori et al. 2009) is overdue.

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