

This is an electronic version of an article published in Environmental Entomology:

King, B.H. 1997. Effects of age and burial of house fly pupae (Diptera: Muscidae) on parasitism by *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae). Environmental Entomology 26:410-415.

RUNNING HEAD: KING: HOST AGE AND BURYING EFFECTS ON PARASITOIDS

Effects of age and burial of house fly pupae (Diptera: Muscidae) on parasitism by *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae)

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KEY WORDS: pteromalids, parasitoid wasps, species interactions, biological control, house flies, host age

ABSTRACT. The parasitoid wasps *Spalangia cameroni* Perkins and *Muscidifurax raptor* Girault and Sanders commonly co-occur in nature and are sometimes released together in efforts to control pest fly populations. Laboratory experiments were conducted to determine how the effectiveness of these wasps in killing house flies (*Musca domestica* L.) and producing wasp progeny is affected by: the wasp specie(s) used, host burial, and host age. For effectiveness in killing flies, there was a significant three-way interaction. *S. cameroni* alone was consistently more effective than *M. raptor* alone or than the two species combined, regardless of host age and burial. However, *S. cameroni*'s greater effectiveness was most pronounced for buried hosts, and among unburied hosts for young hosts. *S. cameroni* produced offspring regardless of host burial and host age. Host burial significantly decreased *S. cameroni*'s offspring production only when just *S. cameroni* was present and hosts were young. Host burial significantly reduced *M. raptor*'s offspring production in all situations. *M. raptor* produced fewer offspring from young hosts than from old hosts under all conditions, producing no offspring from young buried hosts. Combining *S. cameroni* and *M. raptor* did not increase their effectiveness at killing hosts. Being with the other species versus a conspecific had no significant effect on *M. raptor*'s offspring production and increased *S. cameroni*'s offspring production only from young buried hosts.

Spalangia cameroni Perkins and *Muscidifurax raptor* Girault and Sanders (Hymenoptera: Pteromalidae) are widely distributed, frequently co-occurring species which parasitize the pupal stage of certain fly species found in manure or decaying organic matter and associated with humans (e.g., Butler et al. 1981; Mullens et al. 1986; Meyer et al. 1991). Both *S. cameroni* and *M. raptor* are solitary species, meaning that usually only one offspring completes development on each host. They kill hosts not only by laying offspring on them but also by host feeding (drilling into a host and feeding on fluids exuding from the host). A major and natural host is the house fly, *Musca domestica* L. (Diptera: Muscidae), one of the most important pests in livestock and poultry production in

the United States and worldwide (Greenberg 1973; Busvine 1980; Patterson & Rutz 1986).

Control of muscoid flies has relied heavily on insecticides (Rutz & Scott 1990). However, use of natural enemies such as parasitoid wasps to control flies may become increasingly important because flies are becoming more resistant to insecticides (Georghiou 1967, 1969; Farnham et al. 1984; Levot & Hughes 1989; Scott et al. 1989; Patterson & Morgan 1986), insecticides can be costly, availability of new insecticides is limited (Meyer 1990), and there are concerns with health risks and environmental risks associated with insecticide use (Rutz & Scott 1990).

Species of *Spalangia* and *Muscidifurax* are considered the most effective of the parasitoids of pest muscoids and are sometimes released alone or together to control pest flies (Legner 1981). Understanding more about interactions between *Spalangia* and *Muscidifurax* species and of these species with their environment will assist in deciding how to improve the effectiveness of biological control. Interactions are of interest with wasps released for biological control because densities will be higher and thus the potential for interaction greater.

Here I use laboratory experiments with *S. cameroni* and *M. raptor* to examine whether single species or pairs of species are more effective at killing flies and producing additional parasitoids and how this is affected by two environmental factors - host burial and host age. In the past, effects of environmental factors have usually been investigated for single species, i.e., in the absence of interspecific competition (e.g., Ables & Shepard 1976; Legner 1977; Siafacus 1980; Mann et al. 1990; Pawson & Petersen 1990; Smith & Rutz 1991; but see Ables & Shepard 1974). However, wasps may respond differently in the presence of another wasp species. Such information is relevant in deciding whether to release one or multiple species.

Materials and Methods

The colony of *S. cameroni* was established in 1985 with wasps collected in northern Indiana (King 1990, 1991) and the colony of *M. raptor* was established in 1990 with wasps collected in northern Illinois (King & Seidl 1993; Seidl & King 1993). The wasps had been in colony for 9 years and 4 years, respectively, at the time of the experiments. Wasp colonies were maintained at 24-28°C, 24L on house flies (King 1988).

The experiment was a full-factorial design with three factors: parasitoid combination, host age, and host burial. There were three parasitoid combinations: MM = *M. raptor* with *M. raptor*, MS = *M. raptor* with *S. cameroni*, and SS = *S. cameroni* with *S. cameroni*. Females were given either Y = young hosts or O = old hosts. When initially presented to the wasps, young hosts were 0-24 h old from the initiation of pupal tanning; old hosts were 3 days older. The hosts were approximately the size of naturally occurring house fly pupae, about 2.7 mm wide (King 1990). Hosts were either U = unburied, placed in a vial without any media or B = buried, placed on the bottom of a vial and covered with 2 cm of used house fly larval medium from which fly pupae had recently pupated and been removed. Both *S. cameroni* and *M. raptor* can reach and parasitize hosts at 2 cm (Legner 1977; personal observation). The twelve combinations of treatments were designated MMYU, MMOU, MMYB, MMOB, MSYU, MSOU, MSYB, MSOB, SSYU, SSOU, SSYB, and SSOB. Host mortality in the absence of the parasitoids was estimated for each of the four combinations of host age and burial by leaving 50 hosts unexposed to wasps (n = 9 to 13 per treatment); the number of fly pupae from which no adult flies emerged was recorded; and the mean was subtracted from host mortality in the presence of parasitoids to give number of hosts killed by parasitoids.

Each experimental female had been isolated in a test tube prior to her emergence so that she was of known age and had no contact with other females prior to use in experiments. Each

female was observed to mate with a virgin male when both female and male were a day old or less. Females were presented with hosts in 20 ml glass shell vials (70 mm high by 20 mm diameter) with cotton plugs. A streak of honey was provided on the side of each vial as food for the females. Each pair of females received 50 hosts for 24 h (Ables & Shepard 1974). Vials were placed in 900 ml jars (17 cm high by 9 cm diameter) containing a saturated sodium chloride solution to produce about 70-75% relative humidity (Winston & Bates 1960), and the jars were placed in an environmental chamber at 23-28°C, on a 12 h light: 12 h dark photoperiod.

After being exposed to females for 24 h, hosts were removed from the substrate. Emerging flies were counted, and the remaining hosts were individually isolated in test tubes with cotton plugs for further development of wasps. This was necessary to ensure that emerging *M. raptor*, which take about a week less to develop than *S. cameroni*, would not hyperparasitize any of the hosts from which wasps had not yet emerged. (Under natural conditions, *M. raptor* would presumably disperse.) After wasps finished emerging, all hosts were dissected and number of adult females and males of each species counted. Dissection of hosts was necessary because *S. cameroni* adults will crawl back into hosts (personal observation).

Ideally one would look at lifetime reproductive success rather than offspring production over one day, but it was not practical to do so and still maintain adequate sample sizes. Lifetime reproductive success is highly correlated with reproductive success over a period of a day for *S. cameroni* (King & King 1994; King & Lee 1994) and *M. raptor* (King & Seidl unpublished).

Statistical analyses were done using SPSS-PC version 4.0 (Norusis 1988). I used alpha of 0.05 for comparisons of means.

I analyzed the number of flies killed per parasitoid, using a three factor ANVOA of parasitoid species combination (just *M. raptor*, just *S. cameroni*, or both), host age (young or old), and host burial (buried or not buried). For each parasitoid species, I examined offspring production per female using a three factor ANVOA of companion (conspecific or heterospecific), host age (young or old), and host burial (buried or not buried). ANOVAS with significant interaction terms were followed up with one-way comparisons, ANOVA or Kruskal-Wallis, depending on normality. Significant one-way comparisons were followed up with multiple comparisons, Student-Newman-Keuls or pairwise t-tests or Mann-Whitney U tests, depending on normality.

In analyses of variance (ANOVAS), host age was treated as a random effect, the other main effects as fixed effects (Sokal & Rohlf 1981).

Results

Depending on treatment, parasitoids killed 0-65% of hosts (Table 1). For number of hosts killed per parasitoid, there was a significant three-way interaction between parasitoid combination, host burial, and host age (Table 2). For all four combinations of host burial and host age, the pattern was that the greatest number were killed when there was just *S. cameroni*, the least when there was just *M. raptor*, and an intermediate number when there was one parasitoid of each species (Table 1). However, the difference between both species present versus just *M. raptor* present was not significant for old unburied hosts. The number of hosts killed by the two species combined was not significantly different from the average of the two single species treatments for each of the four host age, host burial combinations (t-tests, $P = 0.05$ for each combination).

More hosts were killed per parasitoid when hosts were not buried than when they were buried in all treatments except for old hosts parasitized by just *S. cameroni*. More hosts were killed when hosts were old versus young in all treatments except for unburied hosts parasitized by just *S. cameroni*.

For number of offspring, for both *M. raptor* and *S. cameroni*,

there was no significant three way interaction between companion species, host age, and host burial (Tables 3-6).

Depending on treatment, *M. raptor* produced a mean of 0.00 - 7.76 offspring per female. The only significant two-way interaction was between host age and host burial. No *M. raptor* offspring were produced from young buried hosts. More offspring were produced from old hosts than from young hosts and from hosts that were not buried than from buried hosts under all conditions. There was no significant effect of *M. raptor*'s companion being *S. cameroni* versus a conspecific. (The nonsignificant pattern was more offspring with a heterospecific versus a conspecific in 3 of 3 comparisons (Table 4).)

Depending on treatment, *S. cameroni* produced a mean of 7.50 - 14.41 offspring per female. There were significant two-way interactions of host age with parasitoid companion and host age with host burial. *S. cameroni* females produced more offspring when with a *M. raptor* female than when with a conspecific when parasitizing young buried hosts but not from other host situations. Host age had no significant effect on *S. cameroni*'s offspring production except that more *S. cameroni* offspring were produced from old hosts than from young hosts when the hosts were buried and just *S. cameroni* was present. Burying had no significant effect except that more *S. cameroni* offspring were produced from unburied than from buried young hosts when just *S. cameroni* was present.

Discussion

Burying of hosts and host age affected the relative effectiveness of the three different wasp combinations in degree, not in direction. *S. cameroni* alone was consistently more effective than *M. raptor* alone or the two species combined, regardless of host age and host burial. Thus, under the conditions of the present study, *S. cameroni*'s advantages over *M. raptor* must have outweighed *M. raptor*'s advantages over *S. cameroni*.

In contrast to this study, some previous studies have concluded that *Muscidifurax* is competitively superior to *Spalangia* (Wylie 1972b; Markwick 1974; Legner 1977). In interactions among adult females, *M. raptor* is more aggressive toward *S. cameroni* than vice versa (King & Lee 1994). *Muscidifurax* females show less tendency to avoid pupae parasitized by *Spalangia* than vice versa (*Spalangia endius* Walker and *M. raptor* (Propp & Morgan 1983); *S. cameroni* and *Muscidifurax* zaraptor Kogan and Legner (Wylie 1971, 1972a)). When *M. zaraptor* and *S. cameroni* or *S. endius* parasitize the same host, *M. zaraptor* is more likely to survive (Wylie 1972b; Markwick 1974).

Laboratory experiments with *M. raptor* and *S. endius* suggest that which species will produce more offspring varies with temperature, at least for single species (Ables & Shepard 1976).

In the present study, *S. cameroni*'s consistently superior performance over *M. raptor*, both at killing hosts and at offspring production was likely due, at least in part, to the presence of a microsporidium infection in *M. raptor* (Becnel & Geden 1994). The colony was not tested; however, the microsporidium has been found in almost all colonies of *M. raptor* that have been surveyed and usually at very high rates (Zchori-Fein et al. 1992). The microsporidium severely reduces *M. raptor*'s fecundity (Geden et al. 1995), and fecundity values observed in my colony were similar to those of infected wasps (Zchori-Fein et al. 1992). The microsporidium also occurs in natural populations of *M. raptor* and in parasitoids from insectaries but has not been found in *S. cameroni* (Zchori-Fein et al. 1992; Geden et al. 1995). Although it is possible to reduce or even eliminate the infection (Geden et al. 1995), whether this will be done in commercial insectaries remains to be seen.

S. cameroni's greater effectiveness than *M. raptor* was most pronounced for buried hosts. This is consistent with the observation that *Spalangia* parasitizes hosts at greater depths than does *Muscidifurax* (*S. cameroni*, *S. endius*, *S. nigra* Latrielle, *S. nigroaenea* Curtis versus *M. zaraptor*, *M. uniraptor* Kogan and

Legner, *M. raptor* (Legner 1977; Rueda & Axtell 1985)). Both *S. cameroni* and *M. raptor* can burrow 2 cm to reach hosts as required in the present study (Tables 4 and 6). However, more unburied than buried hosts were killed by the parasitoids, except when the hosts were old and just *S. cameroni* was present, in which case burying had no effect.

Among unburied hosts, *S. cameroni*'s greater effectiveness than *M. raptor* at killing hosts was most pronounced for young hosts. *S. cameroni* produced offspring regardless of host age. *M. raptor* produced fewer offspring from young hosts than from old hosts under all conditions, producing no offspring from young hosts when they were buried. Effects of host age have not previously been examined for *M. raptor*. *M. raptor* produces the most offspring from hosts of ages intermediate to those used in the present study (Markwick 1974). The effect of host age on offspring production in *S. cameroni* appears to be context dependent. In the present study, offspring production of *S. cameroni* increased or was unaffected by host age, whereas when *S. cameroni* females are solitary and at lower humidity, offspring production decreases with host age (King, unpublished).

Spalangia and *Muscidifurax* species are sometimes released together for biological control. Multiple species might be more effective than single species -- if the different species each parasitize hosts that the other(s) would not through differences in factors such as when and where they are active and which stages of the host they use (e.g., Ehler 1978). However, under the conditions tested here, there was little evidence of this. Offspring production per *S. cameroni* female was greater in the presence of *M. raptor* than in the presence of a conspecific only from young buried hosts, from which *M. raptor* did not produce offspring. Whether there is an advantage to multiple species under other conditions remains to be tested and would be most expected in heterogeneous environments, e.g., with heterogeneous temperature, host age, and/or depth at which hosts are buried.

The results presented here suggest what the outcome of interactions will be with one generation of offspring production. Such short term effects are relevant to situations with repeated releases. Over a longer period, without repeated releases, it is possible that *M. raptor* might outcompete *S. cameroni* to extinction. This might occur because of *M. raptor*'s shorter development time and because *M. raptor* superparasitizes and hyperparasitizes house flies containing *S. cameroni* pupae (personal observation).

Acknowledgments

I thank M. Van Buren and J. Cline for assistance in the laboratory. I thank R. King and an anonymous reviewer for comments on this manuscript. This research was supported by Northern Illinois University Graduate School.

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Table 1. Mean \pm s.e. number of hosts killed per female (MM = 2 *M. raptor* females, MS = 1 *M. raptor* female + 1 *S. cameroni* female, SS = 2 *S. cameroni* females)

	Parasitoid MM	Combination MS	SS	ANOVA among parasitoid combinations
Unburied young hosts	4.5 \pm 0.7 a 1 - 10	10.8 \pm 0.9 b 3.5 - 15	14.1 \pm 0.6 c 10 - 17.5	F = 44.11 P < 0.0001
Unburied old hosts	11.9 \pm 0.6 a 8.3 - 15.3	13.6 \pm 0.8 a 6.8 - 18.3	15.8 \pm 0.9 b 7.8 - 19.8	F = 6.56 P = 0.003
Young versus old hosts	t = 8.21 P < 0.001	t = 2.43 P = 0.02	t = 1.62 P = 0.12	
Buried young hosts	-1.0 \pm 0.5 a -3 - 5.5	5.4 \pm 0.8 b -3 - 10.5	8.6 \pm 1.0 c 2.5 - 15	X ² = 27.95 P < 0.0001
Buried old hosts	2.9 \pm 1.0 a -.5 - 14	9.7 \pm 0.8 b 2 - 15	16.3 \pm 0.7 c 11 - 20	X ² = 34.75 P < 0.0001
Young versus old hosts	U = 24.5 P = < 0.0001	t = 3.71 P = 0.001	t = 6.44 P < 0.001	
Unburied versus buried young hosts	U = 11.5 P < 0.001	t = 4.44 P < 0.001	t = 4.78 P < 0.001	
Unburied versus buried old hosts	U = 14.0 P < 0.001	t = 3.41 P = 0.002	t = 0.42 P = 0.68	

n = 16 for all treatments, except 15 for 2 *S. cameroni* with unburied young hosts and 17 for both species with unburied old hosts. Within rows, different letters indicate significant differences. Negative values indicate fly deaths exceeded those in control.

Table 2. ANOVA of the number of hosts killed per parasitoid by species combination (*M. raptor* only, *S. cameroni* only, or both parasitoid species), host age (young or old), and host burial (buried or not buried)

	Mean Square	F	df	P
Species	1334.54	80.39	2	< 0.03
Host age	1037.75	105.60	1	< 0.001
Host burial	1109.60	51.37	1	> 0.05
Species X host age	16.60	1.69	2	0.19
Species X host burial	89.77	1.03	2	> 0.50
Host age X host burial	21.64	2.20	1	0.14
3-way interaction	87.34	8.89	2	< 0.001
Residual	9.83		180	

Table 3. ANOVA of the number of *M. raptor* offspring by companion (conspecific versus heterospecific), host age (young or old), and host burial (buried or not buried)

	Mean Square	F	df	P
Companion	24.45	4.69	1	> 0.25
Host age	286.32	74.67	1	< 0.001
Host burial	415.39	3.45	1	> 0.25
Companion x host age	5.21	1.36	1	0.25
Companion x host burial	20.26	5.99	1	> 0.10
Host age x host burial	120.50	31.42	1	< 0.001
3-way interaction	3.38	0.88	1	0.35
Residual	3.84		121	

Table 4. Mean \pm s.e. and range of the number of offspring produced per *M. raptor* female when the female was with a conspecific or heterospecific (*S. cameroni*) female

	Conspecific	Heterospecific
Unburied young hosts	1.19 \pm 0.33 0 - 4	2.13 \pm 0.46 0 - 6
Unburied old hosts	5.37 \pm 0.59 1 - 10	7.76 \pm 0.69 3 - 12
Unburied young versus old hosts	t = 6.24 P < 0.001	t = 6.74 P < 0.001
Buried young hosts	0.00 \pm 0.00 0 - 0	0.00 \pm 0.00 0 - 0
Buried old hosts	0.97 \pm 0.58 0 - 8	1.13 \pm 0.63 0 - 9
Buried young versus old hosts	U = 96.0 P = 0.04	U = 96.0 P = 0.04
Unburied versus buried young hosts	U = 48.0 P < 0.001	U = 24.0 P < 0.001
Unburied versus buried old hosts	U = 23.0 P < 0.001	U = 14.5 P < 0.001

Sample sizes as in Table 1

Table 5. ANOVA of the number of *S. cameroni* offspring by companion (conspecific versus heterospecific), host age (young or old), and host burial (buried, not buried)

	Mean Square	F	df	P
Companion	21.09	0.18	1	> 0.50
Host age	104.99	5.07	1	0.03
Host burial	33.23	0.17	1	0.75
Companion x host age	111.92	5.41	1	0.02
Companion x host burial	4.88	0.28	1	> 0.50
Host age x host burial	197.35	9.53	1	0.003
3-way interaction	17.43	0.84	1	0.36
Residual	20.71		120	

Table 6. Mean \pm s.e. and range of the number of offspring produced per *S. cameroni* female when the female was with a conspecific or heterospecific (*M. raptor*) female

	Conspecific	Heterospecific	
Unburied young hosts	12.13 \pm 0.96 4.5 - 16.5	13.69 \pm 1.29 0 - 21	t = 0.96 P = 0.35
Unburied old hosts	12.59 \pm 0.89 6 - 18.5	11.88 \pm 1.28 0 - 18	t = 0.45 P = 0.66
Unburied young versus old hosts	t = 0.35 P = 0.73	t = 0.99 P = 0.33	
Buried young hosts	7.50 \pm 0.93 2 - 13	11.31 \pm 1.37 0 - 20	t = 2.31 P = 0.03
Buried old hosts	14.41 \pm 0.72 8 - 18	13.00 \pm 1.40 0 - 20	t = 0.89 P = 0.38
Buried young versus old hosts	t = 5.87 P < 0.001	t = 0.86 P = 0.40	
Unburied versus buried young hosts	t = 3.47 P = 0.002	t = 1.26 P = 0.22	
Unburied versus buried old hosts	t = 1.58 P = 0.13	t = 0.59 P = 0.56	

Sample sizes as in Table 1