Development of Spalangia cameroni and Muscidifurax raptor (Hymenoptera: Pteromalidae) on Live and Freeze-Killed House Fly (Diptera: Muscidae) Pupae

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Source: Florida Entomologist, 92(3) : 492-496

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.092.0311

DEVELOPMENT OF *SPALANGIA CAMERONI* AND *MUSCIDIFURAX RAPTOR* (HYMENOPTERA: PTEROMALIDAE) ON LIVE AND FREEZE-KILLED HOUSE FLY (DIPTERA: MUSCIDAE) PUPAE

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ABSTRACT

The use of pteromalid parasitoids for muscoid fly control is becoming increasingly common. Two species that are often recommended for releases are *Spalangia cameroni* Perkins and *Muscidifurax raptor* Girault and Sanders. This study was conducted to determine if the reduced suitability of freeze-killed pupae for Pteromalidae, particularly *S. cameroni*, is due to freezing, freezing duration, or the type of freezer used. Processing pupae through freezing had a considerably greater negative effect on rearing of *S. cameroni* than on *M. raptor.* Although freezing pupae did reduce *M. raptor* progeny production, there was no effect of length of storage or type of freezer used. Freezing pupae for 5 months resulted in significantly fewer *S. cameroni* progeny than freezing pupae for 2 weeks. Although not significant, pupae held in a frostfree freezer produced more progeny than those in a non-frostfree freezer. Measurements of weights of parasitized pupae over time indicated that freezing did not result in accelerated desiccation of hosts. These results document that the use of prolonged freezing and type of freezer minimally impacts *M. raptor*, but that commercial insectaries and researchers should be cautious in the use of frozen pupae when rearing or surveying for *S. cameroni*.

Key Words: *Muscidifurax raptor*, *Spalangia cameroni*, Pteromalidae, house fly, *Musca domestica*, biological control

RESUMEN

El uso de parasitoides de la familia Pteromalidae para el control de moscas del tipo muscoidea es cada día más común. Dos especies que se recomiendan a menudo para liberar son *Spalangia cameroni* Perkins y *Muscidifurax raptor* Girault y Sanders. Este estudio fue realizado para determinar si la reducida susceptibilidad de las pupas (de las moscas) matadas por congelación para criar las especies de Pteromalidae, particularmente en *S. cameroni,* si es debido a la congelación, la duración de la congelación o a la clase de congelador que se usa. Al procesar las pupas por la congelación se presento un efecto negativo notablemente mayor sobre la crianza de *S. cameroni* en comparición con lo de *M. raptor.* Aunque la congelación de las pupas redujo la producción de las progenies de *M. raptor*, no hubo un efecto causado por la duración del almacenamiento o de la clase de congelador que se uso. La congelación de las pupas por 5 meses resulto en un numero signativamente menor de progenies de *S. cameroni* en comparición a las pupas congeladas por 2 semanas. Aunque no es significativo, las pupas mantenidas en el congelador de clase "frostfree" (que no crea escarcha) producieron mas progenies que las pupas mantenidas en congeladores que no son de clase "frostfree". Las medidas del peso de las pupas parasitizadas sobre tiempo indicaron que la congelación no resulto en la desecación acelerada de los hospederos. Estos resultados documentan que el uso de congelación prolongada y la clase de congelador tiene un impacto mínimo sobre *M. raptor*, pero los insectarios y los investigadores deben ser precavidos con el uso de pupas congeladas cuando se cría o monitorea la especie *S. cameroni*.

House flies are a primary pest with confined livestock, particularly dairy and poultry. With the continued development of insecticide resistance and the general movement toward more sustainable management systems, including IPM, increasing numbers of producers are using biological control. On livestock and poultry facilities, augmentation of naturally-occurring pteromalid parasitoids can be helpful in managing fly populations and has become more common (McKay & Galloway 1995; Kaufman et al. 2001; Geden & Hogsette 2006). Evaluation of the effectiveness of parasitoid releases continues to rely on the use of either live sentinel muscoid pupae or collection of wild pupae as hosts of the parasitoids. There are merits to both approaches, and Kaufman et al. (2001) summarized their advantages and disadvantages. A compromise system that would allow for longer field exposures of sentinel pupae is desirable because this would combine the operational advantages of the sentinel pupae approach (repeatability, rapid location, high recovery rate)

with the increased species diversity characterized by collections of wild pupae.

Manipulation of muscoid pupae to increase field longevity of sentinel hosts over live pupae has received considerable attention (McKay & Galloway 1995; Floate & Spooner 2001; Gibson & Floate 2004; Geden & Hogsette 2006). In addition to surveillance, the use of killed pupae has 2 advantages to the biocontrol community. First, use of killed and stored pupae would allow commercial insectaries to stockpile hosts in anticipation of increased demands for products (Floate 2002). Second, placement of masses of killed pupal hosts in livestock facilities has been demonstrated to enhance parasitism of natively occurring and augmented parasitoids by providing an opportunity for in situ amplification of local populations (Pickens & Miller 1978; Petersen 1986).

The impacts of killed pupae on pteromalid development and parasitization have been investigated by several authors (Morgan et al. 1986; Petersen & Mathews 1994; Geden & Kaufman 2007). Recently, we documented multiple methods of pupal preservation with heat shock being the most readily adoptable procedure (Geden & Kaufman 2007). In that study we also found that pupae that were killed by brief exposure to freezing followed by refrigerated storage conditions resulted in diminished suitability for parasitism by *Spalangia cameroni* Perkins.

Many researchers have reported that the use of freeze-killed pupae with *S. cameroni* results in significantly reduced productivity or utilization, suggesting that this species may be averse to killed hosts (Roth et al. 1991; Floate 2002; Geden & Kaufman 2007). However, in our previous publication, we reported that *S. cameroni* production remained at 73-78% of that with live pupae when freeze-killed pupae were stored for up to 8 weeks at 4°C. Additionally, with many of the early publications on freeze-killed pupae, pupae were held in frost-free freezers. These freezers remain free of frost by systematically raising the temperature in the freezer to liberate frozen water particles into a gaseous state, wherein the water is removed when the compressor removes the water vapor, resulting in lost moisture from the material in the freezer. We see this effect as freezer burn, and its effect on the quality of stored fly pupae is unknown.

The primary objective of this study was to determine whether the type of freezing conditions (frost-free versus non-frost-free) affects the suitability of freeze-killed pupae for parasitism by pteromalids, particularly *S. cameroni*. A second objective was to determine whether the length of time that pupae are held in either freezer type impacts host suitability for the parasitoids.

Materials and Methods

House flies were obtained from an established colony maintained at the University of Florida. Flies were held under laboratory conditions that included 27°C, 75% RH and a photoperiod of 16:8 (L:D) h. Larvae were reared on a diet of 2:3:15:8 ratio of calf protein supplement, wood chips, bran and tap water, respectively, and adults were maintained on water, nonfat dry milk, and sucrose. Following pupariation, extracted fly pupae were weighed to obtain individual pupal weights. The daily collection was then divided in half and placed into double-layered zippered plastic freezer bags. Air was expelled from the bags by hand to the greatest extent that was possible without crushing pupae. One double-bag each was placed into either a vertical frost-free or non-frost-free freezer (both set at -20°C) and held frozen until experiment initiation. Pupae were allowed to thaw on the benchtop for 4 h prior to use. Live pupae were collected from rearing bins the day of parasitoid introduction. Freezer treatments consisted of 2 freezer types (frost-free and non-frost-free) and 2 freezing durations (2 weeks or 5 months), resulting in 5 treatments. *Muscidifurax raptor* Girault and Sanders and *S. cameroni* were Florida strains and reared as previously described (Geden 2002).

Evaluation methodology was similar to that described by Geden & Kaufman (2007). Briefly, at experiment initiation, pupae were counted into groups of 100 with 5 sets of pupae per each pupal-treatment and parasitoid species. Parasitoid exposures consisted of 5 female *M. raptor* or 10 female *S. cameroni* and all containers were held at 27°C. Parasitoids were removed from pupae following 24 h exposure and pupae were held in a 75% RH, 27°C rearing chamber for fly and parasitoid emergence. Following house fly emergence, *S. cameroni*-exposed pupae were placed in gelatin capsules to aid in enumeration. The experiment was conducted on 3 separate occasions with different batches of pupae and parasitoids.

An additional test was conducted to measure water loss in live, freeze-killed, and irradiated pupae parasitized by the 2 parasitoid species. The pupae were all obtained from a single cohort of 10,000 fly pupae (2 d old) that was divided into 3 equal portions. One portion (live) was held at 8°C for 3 d before the test. The second portion (freeze-killed) was placed in a frost-free freezer as described in the previous section for 3 d. The third portion (irradiated) was irradiated as before and held for 3 d in a refrigerator set at 4°C. On the day of the test, pupae of each treatment were exposed to oviposition by *M. raptor* and *S. cameroni* at host:parasitoid ratios of 10:1 and 5:1, respectively, for 24 h. After the parasitoids were removed, the pupae were transferred to screen-topped plastic holding containers and placed in a growth cham-

ber maintained at 25°C, 70% RH, and constant light. Aliquots of 50 pupae were removed from the chamber and weighed individually for 2 (*M. raptor*) or 3 weeks (*S. cameroni*) after oviposition. Each pupa was dissected immediately after being weighed, and weight data were analyzed only for those pupae that contained live immature parasitoids. Numbers of observations ranged from 20 (live, day 7, *M. raptor*) to 49 (irradiated, day 21, *S. cameroni*). Initial live weights of the pupae on the day of collection averaged 16.55 + 0.35 mg per pupa.

Data on progeny production were analyzed separately for each species with pupal treatment and replication as main effects in the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS Institute 1995). Treatment effects were further evaluated by 3 single degree of freedom orthogonal contrasts with the Contrast statement in the GLM Procedure: (1) live versus frozen pupae; (2) pupae kept frozen for 2 weeks versus 5 months; and (3) pupae stored in a frost-free versus non-frost-freezer. Data were normalized by square-root transformation prior to ANOVA. Treatment effects on weights of parasitized pupae were evaluated separately for each species and time interval by one-way ANOVA in the GLM Procedure of SAS.

Results and Discussion

Processing pupae through freezing had a considerably greater negative effect on *S. cameroni* than on *M. raptor* (Table 1). The overall treatment effect for the ANOVA was significant for *M. raptor* ($F_{4,66} = 2.62; P < 0.05$). Live pupae produced nearly 33 *M. raptor* progeny, while previously frozen progeny production ranged from 21 to 27 ($F_{1,66} = 6.67; P < 0.05$). With *M. raptor*, no differences were observed between length of freezer exposure or type of freezer (frost-free or non-frost-free).

Our results are similar to those reported by Floate & Spooner (2002) who froze pupae for 1-4 wks at -20°C without significant reductions in *M. raptor* parasitism. In their study, overall parasitism was higher than in our study; however, parasitoids in their study were allowed to attack the killed pupae for 4 d. Additionally, the type of freezer used was not mentioned. In our study, we held pupae in the freezer in airtight, double-layered zipper-style bags, while Floate & Spooner (2002) held pupae in non-airtight containers. Geden & Kaufman (2007) also reported that *M. raptor* progeny production was not impacted by several pupal killing treatments that included freezing and subsequent refrigeration.

The overall treatment effect with *S. cameroni* was highly significant ($F_{4,66} = 34.13; P < 0.01$), demonstrating again that *S. cameroni* survival is highest when live puparia are used (Table 1). Live house fly pupae exposed to *S. cameroni* produced 42 progeny, which was significantly greater ($F_{1,66} = 91.32; P < 0.01$) than any of the frozen treatments, where a range of from 8 to 27 progeny were recovered. With *S. cameroni*, the length of time that pupae were in the freezer was significant ($F_{1,66} = 35.84; P < 0.01$) as was the type of freezer ($F_{1,66} = 4.71; P < 0.05$).

Several researchers have studied the suitability of killed pupae for use with *Spalangia* spp. with varied results. Rueda & Axtell (1987) determined that freezing for short periods of time did not reduce *S. cameroni* production in comparison to live pupae. Morgan et al. (1986) reported that

Table 1. Progeny production of *Muscidifurax raptor* and *Spalangia cameroni* on live house fly pupae and pupae killed by placing in frostfree and non-frostfree -20°C freezers for either 2 weeks or 5 months.

Treatment	Mean (SE) no. adult parasitoid progeny produced:	
	M. raptor	*S. cameroni*
Live	32.7 (3.3)	41.9 (3.1)
2-wk, Frostfree	27.3 (3.9)	26.9 (4.5)
2-wk, Non-frostfree	23.3 (4.8)	15.9 (3.0)
5-mo., Frostfree	25.3 (3.6)	7.9 (1.1)
5-mo., Non-frostfree	21.1 (4.7)	8.1 (1.2)
ANOVA *F*		
Treatment overall	2.62*	34.13**
Live vs frozen	6.67*	91.32**
2 wk vs 5 mo storage	0.57ns	35.84**
Frostfree vs Non	2.87ns	4.71*

$n = 3$ replicates of 5 sets of 100 pupae per treatment exposed to either 5 (*M. raptor*) or 10 (*S. cameroni*) female parasitoids for 24 h.
**, $P < 0.01$.
*, $P < 0.05$; ns, $P \geq 0.05$; $df = 4,66$ (overall treatment effect) or 1,66 (orthogonal contrasts).

gamma-irradiation did not impact the parasitoidism of *Spalangia endius* Walker. However, these results seem to be in the minority, particularly with respect to the use of frozen pupae. Considerably more research suggests that killed fly pupae, particularly frozen fly pupae are less suitable for *Spalangia* than are live pupae (Roth et al. 1991; Floate 2002; Geden & Kaufman 2007). Floate (2002) determined that refrigeration at 15°C reduced suitability as hosts for *S. cameroni*. Geden & Kaufman (2007) found that *S. cameroni* production from pupae killed by heat shock or gamma radiation was not different on the day the pupae were killed, but freezing (-80°C for 10 min) reduced progeny production by 16%. Furthermore, they report that production from freeze-killed and refrigerated was reduced to 73-78% during the first 8 weeks of storage and was 28% of live pupal production by 4 months of storage.

Petersen & Matthews (1984) using *Muscidifurax zaraptor* Kogan and Legner theorized that reduced suitability of previously frozen house fly pupae as hosts (over 96 h thawed) was due to desiccation rather than putrefaction of the host. Our theory held that the suitability of pupae held in the freezers for extended time periods would decrease more quickly with frost-free freezers as pupae moisture levels dropped. Our bioassay results show that the effects of freezing were more profound for *S. cameroni* than for *M. raptor*. Our data do not support the desiccation theory and suggest that the length of freezing had a more profound impact on pupal suitability than did the type of freezer. Data on weights of parasitized pupae over time provide further evidence that differential desiccation rates do not account for the unsuitability of freeze-killed hosts for *S. cameroni* (Table 2). Although parasitized pupae in all treatments lost substantial amounts of weight over time, there was no evidence for accelerated weight loss as a result of freezing.

After eliminating desiccation as a hypothesis, the reason for the poor performance by *S. cameroni* on frozen pupae remains uncertain. Because we found in our previous study that this species readily attacked and developed on pupae that were killed by other means (heat and irradiation), the poor suitability of freeze-killed pupae is not simply due to the fact that they are not alive (Geden & Kaufman 2007). While dissecting puparia for this experiment, we found that the freeze-killed hosts were rapidly putrefying compared with live hosts. From these observations as well as from examination of the condition of hosts killed by heat shock and irradiation, we suggest that freezing results in damage to the puparium that makes the killed host within more vulnerable to colonization by decomposing and saprophytic microorganisms. We suggest that hosts killed by freezing simply deteriorate and decay faster than hosts that were initially live or that were killed by irradiation and minimal heat. If this is the case, the reason for the relatively high survival of *M. raptor* on freeze-killed hosts may be that its shorter development time allows this species to complete larval development before host quality deteriorates below a critical threshold.

The application of the results of this study confirms that the use of pupae that are killed by and stored under freezer conditions will substantially impact progeny production with *S. cameroni* regardless of freezer type. Although such pupae could be useful for emergency provisioning of hosts in a rearing program, their use for routine production and in surveillance programs is not recommended.

Acknowledgments

We thank L. Wood, A. Campbell, M. Geden, J. Matta, C. Scipioni, and J. Pecora for assistance with this project. Support was provided in part by the University of Florida Agricultural Experiment Station Federal Formula Funds, project FLA-ENY-04598 (Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture).

Table 2. Weights of live, freeze-killed, or irradiation-killed house fly pupae after parasitization by *Muscidifurax raptor* and *Spalangia cameroni*.

Species	Days since oviposition	Mean (SE) weight (mg) of parasitized pupae			ANOVA *F*
		Live	Irradiated	Frozen	
M. raptor	7	13.37 (0.26)	13.43 (0.47)	13.35 (0.45)	0.01 ns
M. raptor	14	11.39 (0.34)	10.82 (0.22)	10.72 (0.31)	1.44 ns
S. cameroni	7	13.48 (0.31)	13.04 (0.32)	13.63 (0.34)	0.85 ns
S. cameroni	14	10.37 (0.26)	10.08 (0.30)	11.00 (0.28)	1.97 ns
S. cameroni	21	9.50 (0.17)	9.11 (0.25)	9.04 (0.23)	1.25 ns

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