

Development of *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) on Live House Fly (Diptera: Muscidae) Pupae and Pupae Killed by Heat Shock, Irradiation, and Cold

C. J. GEDEN¹ AND P. E. KAUFMAN

USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Dr., Gainesville, FL 32608; and Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611-0620

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ABSTRACT The objective of this study was to evaluate the suitability of killed house fly (*Musca domestica* L.) pupae for production of two economically important pupal parasitoids. Two-day-old fly pupae were subjected to heat shock treatments of varying temperatures and durations in an oven at $\geq 70\%$ RH; exposure to temperatures of 55°C or higher for 15 min or longer resulted in 100% mortality. Exposure to 50°C resulted in 40 and 91% mortality at 15 and 60 min, respectively. All (100%) pupae placed in a -80°C freezer were killed after 10-min exposure; exposure times of <5 min resulted in <21% mortality. Progeny production of *Spalangia cameroni* Perkins and *Muscidifurax raptor* Girault and Sanders (Hymenoptera: Pteromalidae) from pupae killed by heat shock or 50 kR of gamma radiation was not significantly different from production on live hosts on the day when pupae were killed. Freeze-killed pupae produced 16% fewer *S. cameroni* than live pupae and an equivalent amount of *M. raptor* progeny on the day when pupae were killed. When killed pupae were stored in freezer bags at 4°C for 4 mo, heat-killed, irradiated, and freeze-killed pupae remained as effective for production of *M. raptor* as live pupae. Production of *S. cameroni* on heat-killed and irradiated pupae was equal to parasitoid production on live pupae for up to 2 mo of storage, after which production on killed pupae declined to 63% of that observed with live pupae. Production of *S. cameroni* on freeze-killed pupae was 73-78% of production using live pupae during weeks 2-8 of storage and declined to 41 and 28% after 3 and 4 mo, respectively. Killing pupae by heat shock provides a simple and low-cost method for stockpiling high-quality hosts for mass-rearing both of these filth fly biological control agents.

KEY WORDS *Muscidifurax raptor*, *Spalangia cameroni*, Pteromalidae, house fly, biological control

Inundative releases of pupal parasitoids in the genera *Muscidifurax* and *Spalangia* have been shown to increase parasitism in populations of house flies on livestock and poultry farms, sometimes to an extent sufficient to suppress flies to acceptable levels (Morgan and Patterson 1990, Geden et al. 1992, Petersen and Cawthra 1995, Crespo et al. 1998, Kaufman et al. 2001, Skovgard and Nachman 2004, Geden and Hogsette 2006). Mass rearing these species requires the simultaneous production of large numbers of both parasitoids and live hosts, usually house fly pupae. Although the wasps and the flies are fairly easy to produce (Morgan 1981), commercial insectaries that produce fly parasitoids face a dilemma during the winter and early spring, when customer demand for their products is low. Production during the off-season can be curtailed as a cost-saving measure, but scaling up again to meet anticipated demand can take several gener-

ations. The ability to stockpile high-quality hosts during this time would provide a way of scaling up parasitoid production more rapidly as the fly season approaches and would give producers a way to respond to fluctuations in demand for parasitoids (Klunker and Fabritius 1992).

Morgan et al. (1986) found that house fly pupae that were killed by exposure to 50 kR of gamma irradiation were suitable for production of *Spalangia endius* Walker for at least 5 wk after storage under refrigeration. Roth et al. (1991) reported that horn fly pupae subjected to similar levels of irradiation were suitable for production of *S. cameroni* initially, but that host quality declined after 2-wk storage at 5°C. Although gamma irradiation provides high-quality hosts for these and several other species of fly parasitoids (C.J.G., unpublished data), this approach requires access to an irradiator and is therefore not practical for most researchers or commercial insectaries.

The use of freeze-killed hosts for rearing fly parasitoids has been evaluated in several studies. *Muscidi-*

¹ Corresponding author: USDA-ARS, CMAVE, 1600 SW 23rd Dr., Gainesville, FL 32608 (e-mail: cgeden@gainesville.usda.ufl.edu).

furax spp. develop readily on freeze killed house fly pupae, and such pupae have been used for in situ augmentation of released parasitoids (Klunker 1982, Petersen and Matthews 1984, Petersen 1986, Petersen et al. 1992, Petersen and Currey 1996, Floate 2002). In contrast, freeze-killed hosts have generally been found to be unsuitable for development of *Spalangia* spp. (Morgan et al. 1986, Roth et al. 1991, Klunker and Fabritius 1992, Floate 2002). Most of these studies were conducted by placing pupae in freezers at conventional household freezer temperatures (approximately -20°C) until shortly before testing. It is not known whether pupae that are exposed briefly to lethal temperatures and stored under refrigeration are suitable for these parasitoids.

Another simple and economical way of killing fly pupae for subsequent rearing of parasitoids would be the use of heat-killed pupae; however, this approach has never been tested. The objectives of this study were to compare the relative suitability of live hosts, hosts killed by irradiation, and hosts killed by brief exposure to lethal levels of heat and cold.

Materials and Methods

Determination of Lethal Doses of Heat and Cold.

House flies and Florida strains of *S. cameroni* Perkins, and *Muscidifurax raptor* Girault and Sanders were from established colonies and raised using methods described previously (Geden 2002). Initial tests were conducted to determine minimal regimes of heat and cold needed to cause 100% mortality of fly pupae. For tests with heat, groups of 100 pupae in 30-ml cups were placed in an oven calibrated at 45, 47.5, 50, 55, 60, and 70°C for varying amounts of time ranging from 15 to 60 min (Table 1). Relative humidity in the oven was maintained at $>70\%$ by placing a large pan of water and sponges in the oven 30 min before introducing pupae. Pupae (five to seven replicates of 100 for each temperature and exposure time) were removed from the oven and transferred to a rearing room maintained at 27°C , 70% RH, and constant light (standard rearing conditions) for fly emergence. Pupae held continuously at 27°C were used as controls.

Two types of cold treatment were evaluated. In the first, groups of pupae (five replicates of 100 per treatment) were placed in an ultra-low freezer set at -80°C for 0 (controls), 1, 3, 5, 7, or 10 min and transferred to standard rearing conditions as before. In the second, an attempt was made to kill pupae by submersion in an ice water bath (2°C) for 0 (controls), 15, 30, 60, 90, 120, or 180 min. For water bath tests, pupae were placed in a mesh bag and anchored by a push-pin to the bottom of a foam cooler filled with ice water. Pupae were removed from the water bath, dried on paper towels, and transferred to standard rearing conditions for fly emergence.

Effect of Host-Killing Treatment on Parasitoid Production. Three host-killing treatments were selected for evaluation with parasitoids: (1) exposure to 50-kR gamma radiation from a sealed-source cesium irradiator as described by Morgan et al. (1986); (2) 15-min

Table 1. Mortality of 2-d-old house fly pupae after exposure to heat shock

| Temperature ($^{\circ}\text{C}$) | Mean (SE) mortality after exposure to temperature for time (min) | | | |
|------------------------------------|--|-------------|-------------|-------------|
| | 15 | 30 | 45 | 60 |
| 45 | Not tested | 14.7 (8.7) | 9.3 (5.8) | 20.3 (18.4) |
| 47.5 | Not tested | 19.0 (11.1) | 40.3 (13.9) | 62.3 (15.9) |
| 50 | 40.0 (13.3) | 69.4 (13.0) | 79.7 (13.6) | 90.8 (8.4) |
| 55 | 100.0 (0.0) | 100.0 (0.0) | 100.0 (0.0) | 100.0 (0.0) |
| 60 | 100.0 (0.0) | 100.0 (0.0) | 100.0 (0.0) | 100.0 (0.0) |
| 70 | 100.0 (0.0) | 100.0 (0.0) | 100.0 (0.0) | 100.0 (0.0) |

Control mortality (pupae held continuously at 27°C) = $6.7 \pm 1.2\%$.

exposure to 55°C (based on above tests); and (3) exposure to -80°C for 10 min (based on above tests). For each assay, a single batch of 2-d-old fly pupae was first counted out into 60 cups of 100 pupae in 30-ml plastic cups. The cups were grouped into four sets of 15 cups, and each set was subjected to either no treatment (27°C controls) or one of the three host-killing treatments. After a resting period of 30 min, five cups of the four sets of pupae were either left alone (no-parasitoid controls) or were exposed to either five female *M. raptor* or 10 female *S. cameroni* and held at 27°C . Parasitoids were from stock colony cages and were of mixed and undetermined age. Parasitoids were removed from the pupae after 24 h, and the pupae were transferred to a rearing chamber at 25°C for fly and parasitoid emergence (≈ 3 and 4 wk for *M. raptor* and *S. cameroni*, respectively). The experiment was conducted on four separate occasions with different batches of pupae and parasitoids. Data were analyzed using the General Linear Models procedure of SAS (SAS Institute 1995) by examining (separately for each species) progeny production and proportion females of progeny as a function of pupal treatment, replication and treatment \times replication interactions. Tukey's studentized range test was used to separate treatment means in those cases where the treatment analysis of variance (ANOVA) *F* was significant ($P \leq 0.05$). The no-parasitoid controls were not included in the data analysis but were used as a quality control measure to ensure that stray parasitoids were not attacking pupae during experimental setups.

Long-term storage effects were evaluated by first subjecting pupae to irradiation, heat shock, or freezing treatments as before, except that pupae were treated in masses of 7,000 pupae rather than small cups of 100. Treated pupae were placed in zippered freezer bags and placed in a refrigerator at 4°C after squeezing as much air from the bags as was practical without crushing the pupae. Pupae were removed from the bags, counted in batches of 100, and assayed with and without parasitoids at 2, 4, 6, and 8 wk and 3 and 4 mo after treatment using the methods described previously. The entire experiment was conducted twice (five sets of pupae per species, treatment, and storage time per replicate) using different production batches of pupae for each replication. Live pupae, with and without parasitoids, were included for comparison. Separate ANOVAs for each storage time were performed as

Table 2. Mortality of 2-d-old house fly pupae after exposure to freezing or submersion in an ice water bath

| Exposure time | Mean (SE) pupal mortality |
|--|---------------------------|
| Exposure to -80°C in ultra-low freezer | |
| 0 min (controls) | 1.0 (0.3) |
| 1 min | 1.0 (0.4) |
| 3 min | 20.4 (2.1) |
| 5 min | 96.8 (1.0) |
| 7 min | 99.2 (0.3) |
| 10 min | 100.0 (0.0) |
| Submersion in ice water (2°C) bath | |
| 0 min (controls) | 1.0 (0.5) |
| 15 min | 0.8 (0.3) |
| 30 min | 1.2 (0.3) |
| 60 min | 1.4 (0.4) |
| 90 min | 1.2 (0.5) |
| 2 h | 1.8 (0.7) |
| 3 h | 5.0 (0.9) |

before, examining treatment, replication, and treatment \times replication effects on progeny production and proportion females for each species.

Results

Determination of Lethal Doses of Heat and Cold.

Exposure of pupae to 45°C resulted in $<21\%$ mortality at all exposure times (Table 1). At 47.5°C , a maximum mortality of 62.3% was observed after 60-min exposures. Exposure to 50°C resulted in a maximum of 90.8% mortality after 60 min. Exposure to 55°C killed 100% of the pupae at all exposures, including the minimum dose of 15 min (Table 1). Exposure of pupae to -80°C for ≤ 3 min resulted in $<21\%$ mortality. Exposure times of ≥ 5 min killed $>96\%$ of the pupae, but 10 min was required to cause 100% mortality. Submersion of pupae in an ice water bath for up to 3 h never resulted in more than trivial mortality (maximum, 5%; Table 2).

Effect of Host-Killing Treatment on Parasitoid Production. In tests with *M. raptor* presented with live or freshly killed pupae, progeny production from live pupae (41.3 progeny per group of five female parasitoids) was not significantly different from any of the killed pupae treatments (Table 3). The only significant treatment effect was higher production from heat-killed (49.0) compared with freeze-killed (40.5) pupae. Sex ratios of progeny were the same across all treatments (62–64% females). In tests with *S. cameroni*, progeny production from live pupae (64.4 progeny per group of 10 female parasitoids) was not significantly different from pupae killed by irradiation or heat. However, progeny production from freeze-killed pupae was 17% lower (53.9) than from live pupae and significantly lower than any of the other pupal treatments (Table 3). Progeny production from irradiated pupae (69.9) was significantly higher than from the other killed-pupae treatments (53.9 and 61.0 for freeze and heat killed, respectively) but not from live pupae. As with *M. raptor*, sex ratios of *S. cameroni* did not differ significantly among treatments (63–70% females).

Progeny production by *M. raptor* on heat-killed and irradiated pupae and stored for up to 4 mo under refrigeration did not differ significantly from production using fresh live hosts (Table 4). Freeze-killed pupae were as suitable as live hosts for the first 6 wk after storage. Progeny production from freeze-killed hosts was significantly lower after 8 wk and 2 mo ($\approx 70\%$ of that seen with live hosts). After 4 mo of storage, the only significant treatment effect was higher production of progeny from heat-killed pupae (55.8) compared with freeze-killed hosts (42.2); neither treatment differed significantly from live hosts (51.4). Treatment effects on sex ratios were only observed after 4 mo of storage, when live pupae produced proportionally fewer females than killed pupae.

Table 3. Mean (SE) progeny production and sex ratio of *M. raptor* and *S. cameroni* on live house fly pupae and pupae killed by either irradiation (50-kR gamma irradiation), heat shock (15 min at 55°C), or cold (10 min at -80°C)

| Species | Pupal treatment | Pupal mortality | No. progeny produced | Percent females |
|---------------------------|-----------------|-----------------|----------------------|-------------------|
| <i>M. raptor</i> | Live | 62.5 (3.1) | 41.3 (1.8)ab | 63.8 (2.9) |
| | Irradiated | 99.9 (0.1) | 41.2 (2.8)ab | 62.3 (4.7) |
| | Heat-killed | 98.2 (0.9) | 49.0 (3.1)a | 64.5 (3.4) |
| | Freeze-killed | 98.9 (0.8) | 40.5 (2.7)b | 64.4 (2.8) |
| | ANOVA F^a | | 3.38 ^b | 0.2 ^d |
| <i>S. cameroni</i> | Live | 80.5 (2.0) | 64.4 (2.9)ab | 63.1 (2.6) |
| | Irradiated | 100.0 (0.0) | 69.9 (0.0)a | 65.2 (2.2) |
| | Heat-killed | 98.6 (0.8) | 61.0 (2.3)b | 65.8 (2.7) |
| | Freeze-killed | 99.9 (0.1) | 53.9 (2.8)c | 69.7 (2.1) |
| | ANOVA F | | 16.9 ^c | 2.09 ^d |
| No parasitoids (controls) | Live | 1.2 (0.2) | 0.0 (0.0) | — |
| | Irradiated | 100.0 (0.0) | 0.0 (0.0) | — |
| | Heat-killed | 98.8 (0.8) | 0.0 (0.0) | — |
| | Freeze-killed | 98.9 (0.8) | 0.0 (0.0) | — |

^a $df = 3,64$ (pupal treatment effect from two-way ANOVA that also included replication and treatment \times replication terms). Means within columns within species followed by the same letter are not significantly different at $P = 0.05$ (Tukey's studentized range test). $N = 4$ replicates of 5 sets of 100 pupae exposed to either 5 (*M. raptor*) or 10 (*S. cameroni*) female parasitoids for 24 h.

^b $P < 0.05$.

^c $P < 0.01$.

^d $P > 0.05$.

Table 4. Mean (SE) progeny production and sex ratio of *M. raptor* on live house fly pupae and pupae killed by irradiation (50-kR gamma irradiation), heat shock (15 min at 55°C), or cold (10 min at -80°C) and stored at 4°C in freezer bags for 2 wk to 4 mo

| Time | Pupal treatment | Pupal mortality | No. progeny produced | Percent females |
|-----------------------------|-----------------|-----------------|----------------------|-------------------|
| — | Live | 71.2 (5.2) | 45.2 (6.9) | 66.3 (2.2) |
| 2 wk | Irradiated | 100.0 (0.0) | 48.0 (5.5) | 64.6 (2.7) |
| 2 wk | Heat-killed | 100.0 (0.0) | 46.5 (4.4) | 66.4 (3.9) |
| 2 wk | Freeze-killed | 100.0 (0.0) | 38.2 (2.8) | 63.4 (4.6) |
| ANOVA <i>F</i> ^a | | | 0.89 ^b | 0.46 ^b |
| — | Live | 76.0 (7.3) | 54.2 (8.2) | 73.3 (3.6) |
| 4 wk | Irradiated | 100.0 (0.0) | 64.0 (8.4) | 68.4 (3.2) |
| 4 wk | Heat-killed | 100.0 (0.0) | 49.1 (6.4) | 72.9 (3.2) |
| 4 wk | Freeze-killed | 100.0 (0.0) | 53.5 (9.4) | 71.1 (3.8) |
| ANOVA <i>F</i> | | | 1.44 ^b | 0.49 ^b |
| — | Live | 63.2 (6.2) | 45.7 (6.4) | 63.6 (3.5) |
| 6 wk | Irradiated | 100.0 (0.0) | 53.3 (3.9) | 68.8 (3.4) |
| 6 wk | Heat-killed | 100.0 (0.0) | 49.4 (2.3) | 72.7 (1.7) |
| 6 wk | Freeze-killed | 100.0 (0.0) | 40.0 (2.2) | 69.6 (1.9) |
| ANOVA <i>F</i> | | | 2.27 ^b | 1.82 ^b |
| — | Live | 79.5 (5.3) | 58.9 (6.0) a | 69.9 (4.4) |
| 8 wk | Irradiated | 100.0 (0.0) | 57.1 (9.5) a | 73.5 (2.6) |
| 8 wk | Heat-killed | 100.0 (0.0) | 55.5 (7.4) a | 66.6 (5.5) |
| 8 wk | Freeze-killed | 100.0 (0.0) | 40.7 (5.8) b | 68.8 (3.9) |
| ANOVA <i>F</i> | | | 5.89 ^c | 0.57 ^b |
| — | Live | 78.0 (5.3) | 44.7 (4.3) a | 59.6 (4.8) |
| 3 mo | Irradiated | 100.0 (0.0) | 43.9 (5.8) ab | 73.3 (2.7) |
| 3 mo | Heat-killed | 100.0 (0.0) | 41.1 (4.9) ab | 74.8 (3.6) |
| 3 mo | Freeze-killed | 100.0 (0.0) | 31.8 (3.2) b | 65.9 (4.2) |
| ANOVA <i>F</i> | | | 3.15 ^d | 2.1 ^b |
| — | Live | 88.6 (3.2) | 45.4 (4.4) ab | 58.1 (5.4) a |
| 4 mo | Irradiated | 100.0 (0.0) | 51.4 (1.7) ab | 75.2 (1.0) b |
| 4 mo | Heat-killed | 100.0 (0.0) | 55.8 (1.5) a | 73.4 (1.9) b |
| 4 mo | Freeze-killed | 100.0 (0.0) | 42.2 (2.9) b | 77.2 (2.5) b |
| ANOVA <i>F</i> | | | 3.91 ^d | 6.89 ^c |

Pupal mortality of no-parasitoid controls = 4.7% for live pupae and 99–100% for all others.

^a *df* = 3,32 (pupal treatment effect from two-way ANOVA that also included replication and treatment × replication terms). Means within columns at the same storage time followed by the same letter are not significantly different at *P* = 0.05 (Tukey's studentized range test). *N* = 2 replicates of 5 sets of 100 pupae exposed to 5 female parasitoids for 24 h.

^b *P* > 0.05.

^c *P* < 0.01.

^d *P* < 0.05.

Progeny production by *S. cameroni* on heat-killed and irradiated pupae did not differ significantly from fresh live hosts during the first 8 wk of storage of killed pupae, after which both of these killed-host treatments produced ≈63% as many progeny as live hosts (Table 5). Freeze-killed pupae were never as effective as live hosts for producing progeny of this species, but their suitability decreased markedly after prolonged storage. During the first 8 wk of storage, progeny production on freeze-killed pupae was 65–81% of that observed using fresh live hosts. This proportion dropped to 41 and 28% after 3 and 4 mo of storage, respectively. Treatment effects on sex ratios were only significant on weeks 4 and 6 of storage, but the differences were small and not consistent (Table 5).

Discussion

The ability to use high-quality killed hosts has several applications and even some advantages over the use of live house fly pupae. For field surveys of parasitoid activity using sentinel pupae, live pupae are only susceptible to parasitism by most species for 2–3 d, and this period of suitability varies because of temperature effects on the rate of physiological aging of the de-

ployed sentinels. Because sentinel pupae are usually replaced weekly, field data using this method represents a snapshot of parasitism that occurs during this brief and variable window of host vulnerability. Using high-quality killed hosts for field surveys would expand the window to the full 7 d of pupal placement and thus provide greater sensitivity during times of relatively low parasitoid activity. Killed pupae can also be used in foreign exploration efforts to establish colonies of exotic parasitoids in locales where live hosts are not available. For example, irradiated pupae were used to obtain several species and strains of parasitoids for evaluation from dairy farms in Russia and Kazakhstan (Geden et al. 2006).

Killed pupae also offer advantages over live hosts in routine parasitoid rearing programs because hosts can be collected and killed at the time postpupation of maximum suitability for parasitoid production, which can vary depending on the species being produced (Petersen and Matthews 1984, Morgan et al. 1989, 1991). Use of killed hosts also eliminates the need to accommodate the handling of emerging adult flies and empty puparia of hosts that escape parasitism in cultures and permits the accumulation of pupae to respond quickly to meet fluctuating rearing demands for hosts. For these reasons, the USDA parasitoid rearing

Table 5. Mean (SE) progeny production and sex ratio of *S. cameroni* on live house fly pupae and pupae killed by irradiation (50-kR gamma irradiation), heat shock (15 min at 55°C), or cold (10 min at -80°C) and stored at 4°C in freezer bags for 2 wk to 4 mo

| Time | Pupal treatment | Pupal mortality | No. progeny produced | Percent females |
|-----------------------------|-----------------|-----------------|----------------------|-------------------|
| — | Live | 78.9 (3.1) | 59.6 (3.5)a | 63.0 (4.2) |
| 2 wk | Irradiated | 100.0 (0.0) | 56.2 (2.6)a | 61.7 (3.7) |
| 2 wk | Heat-killed | 100.0 (0.0) | 52.7 (1.6)ab | 61.0 (3.5) |
| 2 wk | Freeze-killed | 100.0 (0.0) | 47.1 (2.2)b | 61.3 (4.8) |
| ANOVA <i>F</i> ^a | | | 5.99 ^b | 0.05 ^c |
| — | Live | 72.6 (6.3) | 60.3 (4.8)ab | 59.4 (3.0)b |
| 4 wk | Irradiated | 100.0 (0.0) | 69.0 (4.4)a | 73.8 (2.6)a |
| 4 wk | Heat-killed | 100.0 (0.0) | 58.4 (3.8)ab | 63.5 (2.5)b |
| 4 wk | Freeze-killed | 100.0 (0.0) | 49.4 (3.0)b | 67.5 (2.0)ab |
| ANOVA <i>F</i> | | | 6.18 ^b | 5.78 ^b |
| — | Live | 78.0 (3.4) | 67.5 (3.2)a | 64.9 (3.3)a |
| 6 wk | Irradiated | 100.0 (0.0) | 67.5 (3.2)a | 59.8 (3.7)ab |
| 6 wk | Heat-killed | 100.0 (0.0) | 57.5 (2.3)a | 51.8 (3.3)b |
| 6 wk | Freeze-killed | 100.0 (0.0) | 43.8 (2.7)b | 71.3 (2.2)a |
| ANOVA <i>F</i> | | | 13.96 ^b | 7.60 ^b |
| — | Live | 62.7 (4.4) | 49.2 (3.9)a | 47.9 (3.8) |
| 8 wk | Irradiated | 100.0 (0.0) | 46.2 (4.7)ab | 50.5 (5.2) |
| 8 wk | Heat-killed | 100.0 (0.0) | 40.6 (4.9)ab | 50.6 (4.7) |
| 8 wk | Freeze-killed | 100.0 (0.0) | 36.0 (2.8)b | 56.54 (4.7) |
| ANOVA <i>F</i> | | | 3.5 ^d | 0.99 ^c |
| — | Live | 78.5 (4.4) | 54.0 (3.7)a | 54.5 (5.1) |
| 3 mo | Irradiated | 100.0 (0.0) | 34.2 (3.9)b | 54.4 (6.6) |
| 3 mo | Heat-killed | 100.0 (0.0) | 33.9 (2.3)b | 58.2 (4.6) |
| 3 mo | Freeze-killed | 100.0 (0.0) | 22.2 (2.7)c | 62.8 (7.3) |
| ANOVA <i>F</i> | | | 42.0 ^b | 1.3 ^c |
| — | Live | 90.8 (1.8) | 61.2 (3.6)a | 68.8 (0.7) |
| 4 mo | Irradiated | 100.0 (0.0) | 37.8 (1.7)b | 76.6 (3.2) |
| 4 mo | Heat-killed | 100.0 (0.0) | 39.2 (1.7)b | 70.9 (2.5) |
| 4 mo | Freeze-killed | 100.0 (0.0) | 17.4 (2.4)c | 76.2 (2.6) |
| ANOVA <i>F</i> | | | 46.3 ^b | 2.3 ^c |

Pupal mortality of no-parasitoid controls = 4.7% for live pupae and 99–100% for all others.

^a *df* = 3,32 (pupal treatment effect from two-way ANOVA that also included replication and treatment × replication terms). Means within columns at the same storage time followed by the same letter are not significantly different at *P* = 0.05 (Tukey's studentized range test). *N* = 2 replicates of 5 sets of 100 pupae exposed to 10 female parasitoids for 24 h.

^b *P* < 0.01.

^c *P* > 0.05.

^d *P* < 0.05.

program in Gainesville has used gamma-irradiated pupae for routine parasitoid propagation for >20 yr (Morgan et al. 1986).

Irradiation is not a practical option for most parasitoid producers because few have access to sources of gamma radiation. For this reason, there have been several evaluations of freeze-killed pupae. Results using such hosts have been mixed, but, in general, have shown that storing hosts under freezing conditions is satisfactory for production on *Muscidifurax* spp but not *Spalangia* spp., and this topic was recently reviewed by Floate (2002). In this study, pupae were subjected to very low temperatures for a minimally effective exposure time (10 min at -80°C) and kept stored under refrigeration rather than freezing temperatures. Although production of *S. cameroni* on such hosts was indeed significantly lower than on live hosts, freeze-killed hosts in our tests generally produced ≈80% as many *S. cameroni* progeny as live hosts, with no additional deterioration in host suitability for up to 2 mo of storage at 4°C.

Results of this study also indicate that heat can be a highly effective alternative to irradiation for producing hosts with high suitability for parasitoids. Progeny production by *S. cameroni* and *M. raptor* on such hosts was as high as on live hosts after 2 and 4 mo of

storage, respectively, and were as suitable as irradiated hosts in every test. This method allows researchers and commercial insectaries to produce and stockpile high-quality hosts with acceptable storage stability at low cost using readily available equipment.

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