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Use of irradiated *Musca domestica* pupae to optimize mass rearing and commercial shipment of the parasitoid *Spalangia endius* (Hymenoptera: Pteromalidae)

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This paper examines the potential for using irradiated *Musca domestica* pupae as suitable hosts of the parasitoid *Spalangia endius* for its use in biological control programs. Prior to being exposed to parasitoids, *M. domestica* pupae were gamma irradiated at 500 Gy and maintained for up to 2 months in anoxia at 6°C. The parasitization percentage, estimated by parasitoid emergence, decreased 25% after 26.5 days, 50% after 53.2 days, and 58% after 60 days. This was compared to a control group of *S. endius* parasitoids reared on cold-stored non-irradiated pupae whose emergence percentage decreased by 25% after 7.7 days, 50% after 15.5 days, and 72% after 22 days. Fecundity and adult longevity of parasitoids emerging from irradiated pupae were evaluated as indicators of fitness. There were no significant differences in fitness between parasitoids raised on irradiated, cold-stored pupae and the standard, live pupae presently being used in biocontrol programs. If this procedure is implemented for the mass rearing process of *S. endius*, it could allow the production of surplus stocks of pupae, improved efficiency, reduced rearing costs, and allow commercial shipments of non-parasitized host pupae.

Keywords: gamma radiation; *Musca domestica*; *Spalangia endius*; mass-rearing; parasitism; host stockpiling; pupal storage

Introduction

The house fly, *Musca domestica* L. (Diptera: Muscidae), is an important pest worldwide and breeds in places where food, warmth, and moisture are present. This situation is common in places with intensive animal production, around manure and produce composting areas, and near dumps and industrial landfill sites. Urbanization close to these facilities has resulted in a gradual lowering of the tolerance threshold for nuisance flies. Unfortunately, the close proximity of humans and animals to these facilities also often makes it difficult to apply chemicals for fly control. In addition, because of frequent applications, which are necessary for effective control, insecticide resistance has become a serious problem (Meyer, Georghiou, and Hawley 1987; Scott, Roush, and Rutz 1989). Contamination and intoxication are also frequent problems on many farms. As a result, biological control using pupal parasitoids within the

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framework of an integrated management system can provide an economical and efficient alternative (Zapater, Martínez-Rey, and Mazzoli 1994).

Spalangia spp. (Hymenoptera: Pteromalidae) are pupal parasitoids of certain fly species distributed around the world (Boucek 1963). Natural levels of parasitism due to these wasps are generally low. Meyer, Mullens, Cyr, and Stokes (1990) reported that 4% and 6.2% of stable flies and house flies, respectively, were being parasitized by *Spalangia* spp. in California dairies. Natural levels of parasitism of house fly pupae in installations of caged chickens have been reported at 0.5% (Rutz and Axtell 1981) and 7.6% (Rueda and Axtell 1985). Parasitism levels of stable flies in feedlots are only around 0.1% (Smith, Hall, and Thomas 1987). However, inundative releases of *Spalangia endius* Walker against *M. domestica* have been shown to increase parasitism levels to 80–90% with considerable control; in some instances parasitism rates even reached 100% (Morgan, Weidhass, and Patterson 1981). In caged-layer poultry houses, Zapater (1997) demonstrated that weekly releases of four *S. endius* per chicken combined with adequate manure management reduced fly populations 13.2 times compared to those facilities without parasitoid releases. Once the use of house fly parasitoids was considered to be innocuous to the environment, seven species were released on Easter Island, which has a fragile ecosystem (Ripa 1980, 1986). *Spalangia endius* and other muscid fly parasitoids are presently commercialized in North America (Hunter 1994), Colombia (Vergara-Ruíz 1996), and Argentina (Zapater et al. 1994). Techniques for mass rearing *S. endius* have been reported by Morgan and Patterson (1978), Morgan, LaBrecque, and Patterson (1978) and Morgan (1981).

One of the most important issues for a commercial insectary is the ability to guarantee customers regular (e.g. weekly) shipments of parasitoids. In order to deal with normal variations in daily pupal production and occasional urgent or increased demands for parasitoids, more host pupae are generally produced than are needed. Because the optimal age of house fly pupae for parasitism by *S. endius* is 24–72-h-old, it is not possible to stockpile host pupae for any length of time or purchase non-parasitized pupae from another insectary. The short period of time that host pupae are suitable for parasitism further complicates insectary operations where different species/strains of parasitoids are being reared in different facilities to avoid contamination problems. Another issue for commercial insectaries is that not all of the pupae that are exposed to parasitoids are parasitized. Fortunately, adult flies emerge from non-parasitized pupae before the parasitoids begin to emerge. Therefore, to insure that only parasitized host material is shipped and released, exposed pupae must be held for an additional 6 days to allow adult flies and empty puparia to be eliminated.

A potential strategy to address the above mentioned problems is to try to prolong the suitability of house fly pupae for parasitism, and prevent the emergence of adult flies from non-parasitized pupae using a combination of gamma radiation, anoxia, and cold. Morgan, Smittle, and Patterson (1986) showed that no house fly adults emerged from pupae irradiated (gamma radiation) at a dose of 500 Gy, and that pupae irradiated at this dose were still good hosts for *S. endius*. They were also able to extend the suitability of pupae for parasitism to 8 weeks by storing them at 4.4°C and adequate humidity. In this paper, we extend this research and further describe the effect on *S. endius* fitness of irradiating house fly pupae and placing them in cold storage in anoxia for up to 2 months before using them as host material under mass rearing conditions.

Materials and methods

Insect strains

Musca domestica *strain*

A 20-year-old house fly laboratory stock originating from the Institute for Pesticide Research, Wageningen, The Nertherlands, was provided to the University of Buenos Aires in 1999. In August 2000, the colony was invigorated by out-crossing virgin laboratory females with wild males collected from a poultry house in Mendoza, Argentina. The resulting offspring from these crosses has been used as the parental stock in all succeeding experiments. A colony of around 30,000 adults is permanently maintained. The average number of pupae in 10 mL is 325 ± 24.

Spalangia endius *strain*

A colony of *S. endius* was established in March 2000 with wild insects collected from three different areas in Argentina: Mi Granja, Córdoba province; La Plata, Buenos Aires province; and Pergamino, Buenos Aires province. Parasitoids were collected from poultry facilities by the placement and retrieval of mesh bags containing laboratory-reared house fly pupae. Pupae were removed from the bags and held in Plexiglas cages for fly emergence. After adult flies and empty puparia had been eliminated, the remaining pupae were held until parasitoids emerged. Parasitoids are presently maintained on house fly pupae under mass rearing conditions at a weekly production level of about 100,000 adults.

Irradiation

Irradiation of the house fly pupae was conducted at 'IONICS', Ingenieros 2475, El Talar, Buenos Aires, Argentina, a commercial irradiation facility, using a $Cobalt^{60}$ irradiator with an activity level of $(1942.5 \times 10^{13}$ Bq (525,000 Ci). Because of the high dose rate, special procedures adjusting the exposure distance were developed by the staff at 'IONICS' to ensure that an effective dose of only 500 Gy was delivered at a dose rate of 20 Gy/min. The dose was calculated such that no fly emergence was observed from material irradiated at 500 Gy, while increasing fly emergence was detected at doses of 400 and 300 Gy similar to that reported by Morgan et al. (1986).

Experiment I: storage potential of irradiated pupae

Two hundred mL of 48-h-old (± 12 h) *M. domestica* pupae were placed in each one of 54 plastic bags. The bags were hermetically sealed, which caused anoxia to develop due to respiration of the pupae, and then irradiated with 500 Gy of gamma radiation. The bags of pupae were placed in a cooler before and after transportation to the irradiation facility. Later, the bags were maintained permanently at $6 \pm 0.5^{\circ}$C in a refrigerator. Every day for the first eight days (days 0–7) and every third day for days 9–63 (total of 27 sampling days), one sample of 50 pupae was extracted from each of two bags and the bags discarded. The pupae were placed in small plastic Petri dishes (2×0.5 cm, diameter $\times$ high) and introduced into the parasitization cage containing 25–30 pairs of parasitoids. The cage was $40 \times 60 \times 40$ cm (long $\times$ wide $\times$ high), made of Plexiglas with a fine mesh screening on two sides for ventilation. An approximate

ratio of one female parasitoid for every four pupae was maintained in the cage and parasitoids were replaced every 2 days. The parasitization cage was kept in an environmental chamber maintained at 25 ± 1°C, 14 h L:10 h D, and 70–85% RH. After 2 days, the pupae were removed and placed in small plastic tubes (1 × 3 cm, diameter × long) covered with mesh to allow for parasitoid emergence. The tubes with parasitized pupae were maintained as above. A total of nine repetitions were conducted. In addition, two bags of non-irradiated pupae were prepared and exposed to parasitoids to compare the percentage of non-irradiated pupae parasitized on day 0 with that of the irradiated pupae. Percent parasitism was calculated as the number of emerged parasitoids, divided by the number of exposed pupae, × 100.

Experiment II: storage potential of non-irradiated pupae

Similar to Experiment I, plastic bags were prepared consisting of 200 mL of 48-h-old (± 12 h) *M. domestica* pupae, but in this case they were not irradiated. The bags were again maintained at 6 ± 0.5°C in a refrigerator until needed. Every day during 22 days beginning on day 0 (23 treatments), one sample of 50 pupae was extracted from each of two bags and placed into Petri dishes and the bags discarded. The Petri dishes were then introduced into similar parasitization cages as in Experiment I and exposed to *S. endius* for 2 days. As before, parasitized pupae were placed in small plastic tubes to allow parasitoid emergence for determination of the percent parasitism. Fourteen repetitions were performed consisting of 46 bags of pupae prepared for each repetition.

In addition to the samples taken each day to assess the suitability of the pupae for parasitism, a second sample of 50 pupae was taken from each bag (one sample from each of two bags for days 0–22) to assess fly emergence. Each sample was placed in a separate Petri dish. The Petri dishes were then placed within a Plexiglas box 10 × 10 cm and 6 cm high that had a 3 cm hole in the top covered with fine mesh for ventilation and maintained at 25 ± 1°C, 14 h L:10 h D, and 70–85% RH. After 30 days, the numbers of fully emerged flies, half emerged flies, and dead pupae were counted.

Experiment III: fitness of parasitoids reared on irradiated pupae held in cold storage in anoxia

Two simple tests were carried out to evaluate the fecundity and longevity of parasitoid females using house fly pupae that had been irradiated and then held in cold storage and anoxia for 1, 20 and 40 days as in Experiment I. Pupae from the normal colony production that were 48 h old (± 12 h) and had not been subjected to irradiation and cold storage were used for the control treatment.

Fecundity

Fecundity was evaluated by placing 400 pupae from each of the four treatments into separate 3 cm Petri dishes and then keeping the dishes in the parasitization cage containing approximately one female parasitoid for every four pupae. The dishes were removed after 48 h and placed in separate 10 × 10 × 6 cm Plexiglas boxes kept in an environmental chamber 25 ± 1°C, 14 h L:10 h D, 70–85% RH to allow parasitoid emergence. Newly emerged parasitoids (< 24 h old) were removed and placed in

additional $10 \times 10 \times 6$ cm boxes with excess 24-h-old pupae to allow the parasitoids to host feed and mate for 48 h. Ten females from each of the four treatments were then selected and placed in individual 1×3 cm plastic tubes containing 25 normal colony pupae (48-h-old). The pupae were removed and 25 new pupae added every 24 h for 4 days. Exposed pupae were held separately in plastic tubes in the environmental chamber to allow parasitoid emergence and to determine the number of progeny produced per female per day. A total of three replicates were performed.

Longevity

Longevity was calculated by selecting 15 newly emerged females from each treatment and placing them in individual 1×3 cm tubes containing five normal colony pupae (24-h-old). The host pupae were removed every 4 days and replaced with new pupae until the female died. Dead insects were counted daily and recorded. Three repetitions were done for each treatment.

Statistical analyses

Data were analyzed through linear regression analysis (Neter, Kutner, Nachtsheim, and Wasserman 1996). Least square estimates were obtained for the relationship among response variables and predictive variables, and a measure of how much variance in the response variable was explained by the independent variables (R^2). In Experiment II, we also employed a curvilinear regression (exponential). In the analysis of Experiment III, we employed a non-parametric procedure: the Kruskal–Wallis test ANOVA by ranks (Conover 1980).

Results

Experiment I: storage potential of irradiated pupae

The effect of storing house fly pupae in anoxia in the cold for increasing lengths of time on percent parasitism by *S. endius* is presented in Figure 1. Results indicated that there was a decrease in the parasitism rate as the length of pupal storage time

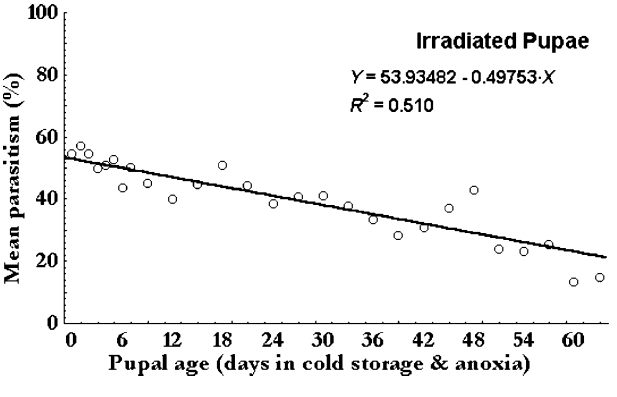

Figure 1. Mean percent parasitism by *Spalangia endius* on irradiated *Musca domestica* pupae that had been held in cold storage ($6 \pm 0.5^{\circ}$C) and anoxia for up to 63 days. $N = 9$ replications.

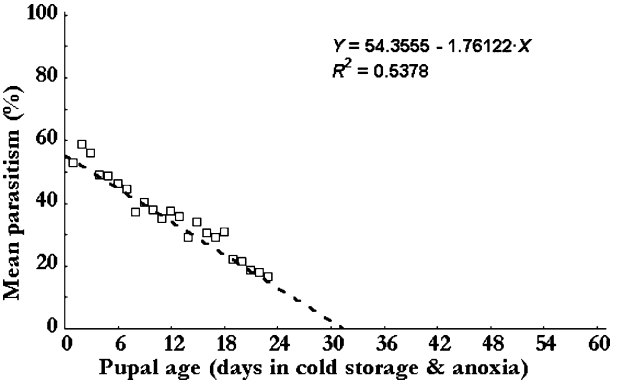

Figure 2. Mean percent parasitism by *Spalangia endius* on non-irradiated M*usca domestica* pupae that had been held in cold storage ($6 \pm 0.5^{\circ}$C) and anoxia for up to 22 days. $N = 14$ replications.

increased following the formula $Y = 53.93 - 0.50X$. The R^2 value for this equation was 0.51. In accordance with the equation, the rate of parasitism percentage decreased 25% after 26.5 days, 50% after 53.2 days and 58% near the end of the experiment 60 days later. This experiment was carried out under simulated mass rearing conditions, which probably accounts for the resulting variation in parasitism rates.

Experiment II: storage potential of non-irradiated pupae

In Experiment II, using non-irradiated pupae, the rate of parasitism (based on *S. endius* emergence) decreased much more rapidly than in irradiated pupae (Figure 2). The initial parasitism values were similar for both experiments, but the parasitism rate began decreasing much more rapidly with time, showing a linear regression between the average % parasitism and the number of days pupae were stored by: $Y = 54.35 - 1.76X$. This function had an $R^2 = 0.54$. In accordance with the regression formula, rates of parasitism decreased 25% after 7.7 days, 50% after 15.5 days and 72% at the end of the experiment at 22 days.

In the second part of Experiment II, emergence (full emergence and half emergence) of adult flies from non-irradiated pupae remained high following 2–3 days of storage, but decreased rapidly after the following curvilinear equation $Y = 1/0.01 + e^{-7.03 + 0.42X}$ (Figure 3). A 25% reduction in adult fly emergence was observed after 4 days, 50% after 6.2 days, 75% after 12.5 days and a 99% reduction in fly emergence was seen after 16.5 days of pupal storage.

The correlation between *S. endius* emergence and fly emergence was calculated and resulted in a coefficient of linear correlation, $r = 0.91$. The high correlation suggests that *S. endius* prefers to parasitize live or recently dead fly pupae.

Experiment III: fitness of parasitoids reared on irradiated pupae held in cold storage and anoxia

Fecundity

The results of the fecundity experiment comparing the number of progeny produced per female reared from normal (control) pupae versus females reared from irradiated

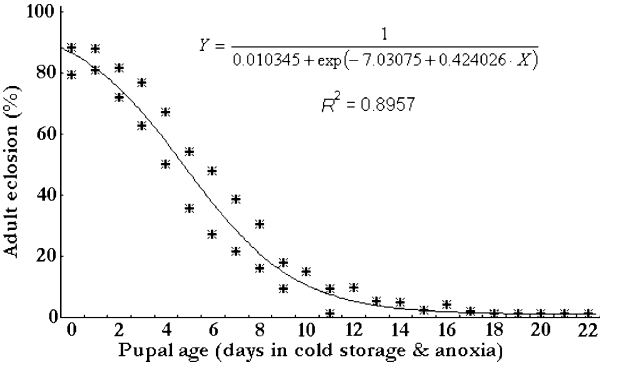

Figure 3. Mean percent adult emergence (=pupae that either fully or partially emerged as adults) of non-irradiated *Musca domestica* pupae that had been held in cold storage and anoxia for up to 22 days. $N =$ 14 replications.

pupae held in cold storage for 1, 20 or 40 days are presented in Figure 4. A Kruskal–Wallis ANOVA test for ranks was employed and no significant differences in fecundity were found among females from the different treatments (df = 3, $N = 179$, $H = 1.2792$, $P = 0.1692$). Females produced an average of 12–14 offspring on day 3, which decreased to 5–6 on day 6.

Longevity

The longevity of adult female parasitoids emerging from normal (control) pupae and pupae that were stored in anoxia and cold for 1, 20 or 40 days was monitored for 16 days and cumulative daily averages plotted in Figure 5. A Kruskal–Wallis ANOVA test for ranks was applied and no significant differences among the four treatments

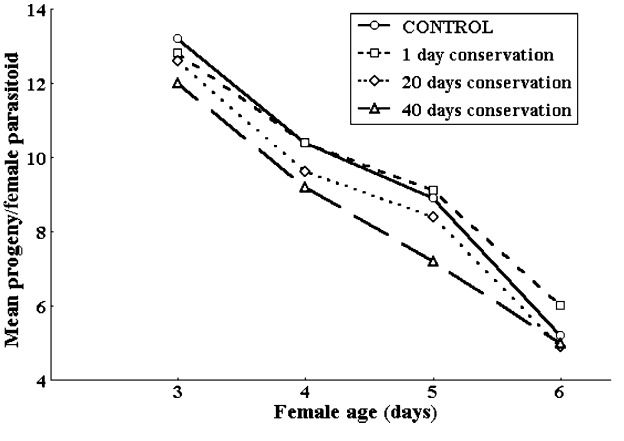

Figure 4. Mean daily adult progeny production (fecundity) of *Spalangia endius* females emerged from *Musca domestica* pupae that had been irradiated and refrigerated under anoxia (=conserved) for 1, 20, and 40 days and from non-irradiated/non-refrigerated (control) pupae. Newly emerged females were allowed to host feed and mate for 2 days and then were exposed to 25 fresh pupae each day for 4 days. $N = 3$ replications.

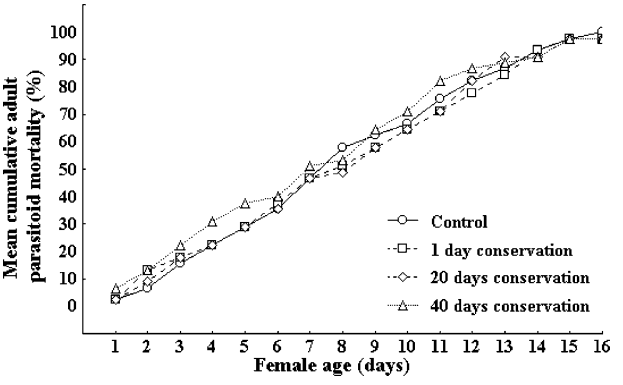

Figure 5. Mean cumulative percent mortality of *Spalangia endius* females emerged from *Musca domestica* pupae that had been irradiated and refrigerated under anoxia (=conserved) for 1, 20 and 40 days and from non-irradiated/non-refrigerated (control) pupae. Each female was provided five normal pupae that were replaced every 4 days until the female died. $N=3$ replications.

were discovered ($df=3$, $N=179$, $H=0.7899$, $P<0.8519$). Fifty percent of the females had died by day 8 and 100% by day 16.

Discussion

Our tests confirmed that 500 Gy irradiation of house fly pupae prevents adult emergence. Results from our experiments also indicated that the combined treatment of irradiation, anoxia and refrigeration can extend the suitability of house fly pupae for parasitism by *S. endius* to 30 days or more. For example, when irradiated pupae were used, the rate of parasitism based on progeny production decreased by 50% from about 60 to 30% after 53.2 days of pupal storage; when non-irradiated pupae were used, 50% fewer parasitoids were produced after only 15.5 days. And although the percentage of parasitized host pupae that produced viable adult parasiotids gradually declined with increased storage time, the parasitoids that were produced in this manner from pupae stored up to 40 days were of good quality and lived as long and produced as many offspring as parasitoids reared from normal pupae. Thus, from a commercial standpoint, it should be possible to guarantee customers a specified number of quality adult parasitoids by appropriately adjusting the number of parasitized pupae that are sent depending on how long the pupae had been stored.

The use of irradiated host material for mass rearing parasitoids has a number of advantages. First, in the case of *S. endius* rearing, developing house fly pupae produce a significant amount of biological heat. As a result, pupae must be well spread out on trays when they are exposed to the parasitoids. However, if the pupae are irradiated, development is stopped and more pupae could be placed per unit area. Similar concerns about the negative impact of metabolic heat, once parasitized pupae have been packaged for shipment, would also be minimized. Second, because not all of the exposed pupae are parasitized, current protocols require that the pupae must be held for four days to eliminate any flies that emerge before they can be shipped to customers. This costs both time and space. If irradiated pupae were used, parasitized pupae could be shipped sooner, which would free-up holding space and

create more flexibility in the system for orders to be prepared, shipments to arrive, and parasitoids to be delivered to the field. The use of irradiated host material has already become standard practice in the mass rearing of fruit fly parasitoids in support of sterile insect release programs (Sivinski and Smittle 1990; Cancino, Ruíz, Gómez, and Toledo 2002; see also articles by Cancino and Hepdurgun, this issue).

The ability to store/stockpile pupae for a period of time has a number of additional advantages. It would allow facility managers to better plan for the exact number of parasitoids needed and avoid the tendency to overproduce both host pupae and parasitoids. With proper management, a rotating stockpile of host pupae could be maintained such that when parasitoid demand was low, excess host material produced during that time could be put into storage. If parasitoid demand increased unexpectedly or an opportunity arose to develop new clients, pupae could be brought out of storage to quickly meet the need. The ability to store pupae could also reduce work, for example, during weekends and holidays.

Contamination of parasitoid strains can be a significant problem when rearing multiple species. If colony host pupae are parasitized by an unintended species prior to collection of the pupae for exposure to the intended parasitoid species (in this case *S. endius*), it is easy to contaminate stocks. However, if the host pupae are collected and then irradiated before exposure to a particular parasitoid, the window of opportunity for contamination by a competing parasitoid species is greatly reduced and much easier to manage. The use of gamma irradiation would also make it possible for insectaries to trade/sell irradiated host pupae amongst themselves instead of parasitized pupae, which occasionally occurs when demand exceeds production or problems with a colony develop. This would allow them to continue to provide their customers with the same strain they normally provide, rather than having to supplement an order with a strain from another facility.

The large commercial irradiator used in this study had a greater degree of dose error at low doses than most traditional Gammacell irradiators. Morgan et al. (1986) showed that a dose of 500 Gy was optimal for preventing fly emergence with 3-day-old pupae. However, for commercial mass-rearing, non-optimal doses between 250 and 750 Gy could probably be occasionally tolerated. Unfortunately, the primary limitation to the wide scale application of this technology is the availability of an irradiation source. Alternatives to irradiation, such as X-ray machines or linear accelerators, that would be more accessible, at more reasonable prices, are needed.

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