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EVALUATION OF IRRADIATED CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) LARVAE FOR LABORATORY REARING OF *DORYCTOBRACON AREOLATUS* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

We report here that it is possible to rear *D. areolatus* on irradiated *A. suspensa* larvae without adversely affecting sex ratio and overall parasitoid emergence and with no adult *A. suspense* emergence. There was no difference in emergence of *D. areolatus* adults from irradiated versus non-irradiated hosts (72.4 ± 1.9% vs. 73.0 ± 1.9%), and no difference in sex ratio of parasitoids obtained from irradiated and non-irradiated hosts (50.0 ± 1.6 and 47.0 ± 1.4% female, respectively). The successful use of *A. suspensa* larval hosts can greatly ease the process of rearing, transporting, and releasing fruit fly parasitoids while eliminating the need to separate flies from parasitoids. Further improvements in the laboratory rearing process of *D. areolatus*, including irradiating late *A. suspensa* larvae at a lower dosage and irradiating *A. suspensa* as egg or early instars, are discussed.

RESUMEN

Reportamos que es posible criar *D. areolatus* sobre larvas de *A. suspensa* irradiadas sin afectar adversamente la proporción de machos y hembras y la emergencia total de los parasitoides y sin desarrollo de adultos de *A. suspensa*. No hubo diferencia en la emergencia de adultos de *D. areolatus* de hospederos irradiados versus no irradiados (72.4 ± 1.9% vs. 73.0 ± 1.9%), y no hubo diferencia en la proporción de machos y hembras obtenida de hospederos irradiados versus no irradiados (50.0 ± 1.6 y 47.0 ± 1.4% hembras, respectivamente). El uso exitoso de la larva hospedera *A. suspensa* puede facilitar mucho el proceso de cría, transporte y liberación de parasitoides de moscas de la fruta con la eliminación en la necesidad de separar las moscas de sus parasitoides. Se discuten otras mejoras en el proceso de criar *D. areolatus* en el laboratorio incluyendo la irradiación de los últimos estadios de las larvas de *A. suspensa* a una dosis menor y la irradiación los huevos y estadios tempranos de *A. suspensa*.

Mass-rearing and augmentative release of hymenopterous parasitoids has been a component of area-wide management programs for several tephritid fruit flies, including pestiferous species of the genus *Anastrepha* (Cancino & Montoya 2008). Laboratory rearing of *Doryctobracon areolatus* (Szepligeti), a braconid larval-prepupal parasitoid of *Anastrepha* fruit flies, was first done in the United States in Florida in the late 1960s as part of an effort to biologically control the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Baranowski et al. 1993; Cancino et al. 2008). Although releases of *D. areolatus* for management of the *A. suspensa* in Florida have ended, laboratory rearing of *D. areolatus* is needed to produce parasitoids for establishment in Caribbean locations with pest fruit flies (Holler, unpublished data).

The process of rearing, transporting, and releasing parasitoids can be simplified if irradiated fruit fly larvae are used as hosts (Sivinski & Smittle 1990). Larvae irradiated at an appropriate dose will not develop into adult flies, but are capable of supporting the development of a number of fruit fly-specific braconid parasitoids, including *Doryctobracon crawfordi* (Viereck) (Aluja et al. 2008; Cancino et al.2008). In addition, parasitoids can be moved as pupae without transferring the pest. Therefore, tests were conducted to determine the effects of gamma irradiation of host larvae at a single dose on *D. areolatus* production and sex ratio, and on the ability of the host to complete development to adult.

MATERIALS AND METHODS

Laboratory tests were initiated 18 Jun 2007 and all studies were completed 23 Aug 2007 when parasitoid and fly emergence had ceased. *Dorycto-*

bracon areolatus adults were hand aspirated from F26 or F27 generation stock cages maintained by the USDA-APHIS-PPQ-CPHST Station, in Gainesville, FL. Fifty females and 15 males per cage were placed into 10 oviposition Plexiglas cages (30 cm^3), with fine mesh fabric (organza) tops and provisioned with a water source and a food source (honey on a moistened paper towel). Food was replaced weekly, water was replaced every 2 weeks and cages were washed after 4 weeks. To replace parasitoids that had died, 50 females and 5 males were added to each cage after 2 weeks, 50 females and 15 males were added after 4 weeks, and 8 females and 6 males were added after 5 weeks. Positions of the cages in the room were rotated weekly. All studies were conducted in a room maintained at 24.4-26.7°C, 60-85% relative humidity, and with a 12:12 h light:dark cycle.

Host larvae were raised on a sterilized corncob-grit-based diet at the Biological Control Rearing Facility, FDACS—Division of Plant Industry in Gainesville, Florida. Weekly, 140 g of diet containing second instars were placed in 550 mL irradiation tubes and irradiated at 70 Gray for 7.5 min with a cesium source (Isomedix Gamma Cell 1000) (Sivinski & Smittle 1990; Aluja et al. 2008). After 5-6 d, irradiated and non-irradiated larvae were placed separately in sting rings and were presented to the parasitoids (Eitam et al. 2003). The sting rings contained 13.5 g of larvae and diet (approximately 107 insects) sandwiched between a piece of organza on the inside bottom of an embroidery ring and a piece of Parafilm on top. One sting ring with either irradiated or non-irradiated larvae was set in each cage on the top of an upside down cup for 24 h. The entire procedure was repeated the following day with fresh larvae. This procedure began 1 week after the oviposition cages were set up and continued for 4 weeks, with 2 oviposition periods tested per week for a total of 10 host exposure periods.

When removed from a cage, the sting ring was disassembled and the contents were placed in a plastic cup (215 mL) filled with fresh diet. The cup was placed in a plastic container (650 mL) with vermiculite (60 g) moistened with 1% sodium hypochlorite. The plastic containers were placed in a plastic storage box (25.5 L, Sterilite Corp., Birmingham, AL) with 2 organza-covered holes in the lid to provide ventilation. After 5-7 d, the pupae were sieved from the vermiculite and moved to fresh vermiculite, again moistened with 1% sodium hypochlorite, and kept for approximately 1 week. At 24- 48 h prior to emergence, the pupae were transferred to 10×10-celled emergence lids with louvered florescent light cover with a solid white acrylic bottom and clear acrylic top. After 12 d, approximately 25 d after the oviposition period, numbers of adult flies and parasitoids were recorded. Adult emergence was complete by that time.

Statistical Analyses

Analysis of variance with PROC ANOVA (SAS Institute1989) was used to test the effect of radiation treatment, host exposure period and cage on parasitoids. The numbers of host pupae per cage per d varied due to availability, so emergence data for both flies and parasitoids were converted to proportion parasitized, which were arcsine (square root)-transformed prior to analysis. Transformed data were compared by Wilcoxon paired-sample test. Sex ratio, as indicated by percentage of the offspring that were female, was compared by Student's *t*-test (SAS Institute 1989). All summary statistics are presented as mean and standard error, and emergence rates as percentages.

Results

There was a significant effect of day of exposure on emergence rates ($F = 19.5$; $df = 1, 9$; $P < 0.0001$) but no effect of cage ($F = 1.6$; $df = 1, 4$; $P = 0.19$). Parasitoid emergence in the last 2 exposure periods was lower than in previous exposure periods, most likely because fewer parasitoids were added to the oviposition cages prior to these periods. There was no difference in emergence of *D. areolatus* adults from irradiated versus non-irradiated hosts (72.4 ± 1.9% vs. 73.0 ± 1.9%; $t = 21.5$; $n = 10$; $P > 0.25$). Nor was there a difference in sex ratio of parasitoids obtained from irradiated and non-irradiated hosts (50.0 ± 1.6 and 47.0 ± 1.4% female, respectively; $t = 1.18$; $df = 1, 88$; $P = 0.24$). *Anastrepha suspensa* were affected by irradiation, with 0% emergence from irradiated larvae versus 15.3 ± 1.9% emergence from non-irradiated, parasitized larvae, ($t = 0$; $n = 10$; $P < 0.003$).

It was assumed that the mortality rates of the parasitoids in oviposition cages that were provided with non-irradiated larvae versus irradiated larvae were similar over time. The actual numbers of parasitoid females per cage at host exposure period were not recorded, so data on offspring per female were not available for analysis. Parasitoid numbers in the last 2 exposures, following a smaller than usual addition of parasitoids, were lower than in previous exposures, which resulted in lower parasitoid emergence and higher fly emergence.

Larvae of *A. suspensa* irradiated at the dose used herein can be utilized to rear *D. areolatus* successfully in the laboratory. No *A. suspensa* adults emerged from irradiated larvae, and there was no difference in the percent parasitism of irradiated and non-irradiated hosts. Use of irradiated larvae as rearing hosts would streamline the rearing process and result in both increased savings and greater safety when *D. areolatus* are shipped overseas for biological control programs.

Future studies might further improve use of irradiated host larvae for production of *D. areolatus*. Cancino et al. (2008) showed that irradiating *A. ludens* larvae at dosages as low as 20 Gray was just as effective as higher dosages in preventing the emergence of adults. Earlier irradiation might be more convenient and could expedite parasitoid production. *Anastrepha suspensa* that are used for rearing parasitoids are placed as eggs on artificial diet several days prior to stinging. It would be desirable to irradiate *A. suspensa* at the egg or earliest larval instar because it would not require handling later instars to obtain hosts for oviposition, which would avoid any concomitant mechanical damage. Preliminary tests by the authors suggest that irradiating the entire pan of diet shortly after the egg strips are added is successful in preventing emergence of *A. suspensa* adults, but will not prevent *A. suspensa* from pupating. Further work is needed to determine if irradiating *A. suspensa* as eggs or early instars could be incorporated into the rearing of *D. areolatus*.

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