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Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713409232

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Jorge Cancino [a]; Lía Ruíz [a]; John Sivinski [b]; Fredy O. Gálvez [a]; Martín Aluja [c]
[a] Desarrollo de Métodos, Campaña Nacional Contra Moscas de la Fruta, Tapachula, Chiapas, México [b] Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, FL, USA [c] Instituto de Ecologia, Xalapa, Veracruz, México

First Published on: 10 October 2008

To cite this Article Cancino, Jorge, Ruíz, Lía, Sivinski, John, Gálvez, Fredy O. and Aluja, Martín(2008)'Rearing of five hymenopterous larval-prepupal (Braconidae, Figitidae) and three pupal (Diapriidae, Chalcidoidea, Eurytomidae) native parasitoids of the genus Anastrepha (Diptera: Tephritidae) on irradiated A. ludens larvae and pupae',Biocontrol Science and Technology,99999:1,

To link to this Article: DOI: 10.1080/09583150802377423

URL: http://dx.doi.org/10.1080/09583150802377423

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Biocontrol Science and Technology
2008, 1–16, iFirst article

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Rearing of five hymenopterous larval-prepupal (Braconidae, Figitidae) and three pupal (Diapriidae, Chalcidoidea, Eurytomidae) native parasitoids of the genus *Anastrepha* (Diptera: Tephritidae) on irradiated *A. ludens* larvae and pupae

Jorge Cancino[a], Lía Ruíz[a], John Sivinski[b], Fredy O. Gálvez[a] and Martín Aluja[c]*

[a]*Desarrollo de Métodos, Campaña Nacional Contra Moscas de la Fruta, Tapachula, Chiapas, México;*
[b]*Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, FL, USA;*
[c]*Instituto de Ecologia, Xalapa, Veracruz, México*

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The aim of this study was to ascertain if eight species of native larval-prepupal and pupal *Anastrepha* (Diptera: Tephritidae) parasitoids which have been recently domesticated and colonized (Aluja et al. in press) could be reared on irradiated larvae and pupae, and if such was the case, determine the optimal irradiation dose so that only adult parasitoids (not flies) would emerge. The species considered were: *Doryctobracon crawfordi*, *Utetes anastrephae*, *Opius hirtus* (all larval-prepupal braconids), *Aganaspis pelleranoi*, *Odontosema anastrephae* (both larval-prepupal figitids), *Coptera haywardi*, *Eurytoma sivinskii* and *Dirhinus* sp. (diapriid, eurytomid and chalcidoid pupal parasitoids). Eight-day-old *A. ludens* larvae or 3-day-old *A. ludens* pupae were irradiated with 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60 and 70 Gy under free oxygen and then subjected to parasitoid attack. Emergence of the unparasitized host was completely halted at 20–25 Gy but such was not the case with the three braconid parasitoids that emerged even if subjected to doses as high as 70 Gy. In the case of the figitids, the emergence of the host and the parasitoids was completely halted at 20 and 25 Gy, respectively. Some parasitoid emergence was recorded at 5–15 Gy but at this irradiation dose, fly adults also emerged rendering the fly/parasitoid separation procedures impractical. Finally, in the case of the pupal parasitoids, *A. ludens* adults emerged from unparasitized pupae irradiated at 15 Gy. Beyond this dose, only parasitoids emerged. With the exception of the figitid larval-prepupal parasitoids, irradiation did not negatively affect adult longevity or fecundity. Our results show that parasitoid mass rearing with irradiated hosts is technically feasible.

Keywords: fruit fly parasitoids; mass rearing; host irradiation; Tephritidae; Braconidae; Figitidae; Diapriidae; Eurytomidae; Chalcidoidae

Introduction

In the New World, some species of fruit flies in the genus *Anastrepha* (Diptera: Tephritidae) (e.g. *A. grandis* [Macquart], *A. fraterculus* [Wiedemann], *A. obliqua* [Macquart], *A. ludens* [Loew], *A. serpentina* [Wiedemann], *A. suspensa* [Loew]) represent important agricultural pests that also significantly hinder fruit exports (Aluja 1994). With increasing public resistance to widespread insecticide use (Clark, Steck, and Weems 1996), regional efforts are underway attempting to combine the use of the sterile insect technique (SIT) and augmentative releases of parasitoids. For example, in Mexican mango and citrus

*Corresponding author. Email: martin.aluja@inecol.edu.mx

ISSN 0958-3157 print/ISSN 1360-0478 online
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DOI: 10.1080/09583150802377423
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growing regions (e.g. Nayarit, Sinaloa, Nuevo León), sterile *A. obliqua* and *A. ludens* adults are being released in conjunction with the exotic parasitoid, *Diachasmimorpha longicaudata* (Ashmead) (Anonymous 2003). Despite the fact that this parasitoid has been proven effective at significantly lowering *A. suspensa* and *A. ludens* populations when repeatedly released in large numbers (Sivinski et al. 1996; Montoya et al. 2000) and that it is easily and cheaply mass-reared (Montoya and Cancino 2004), there has been a recent upsurge in interest at determining the potential of native parasitoids which had been so far neglected in fruit fly biological control programs. Native parasitoids, given their long-term evolutionary interaction with their host, could prove quite effective at lowering fly populations under certain circumstances (e.g. Sivinski, Aluja, and López 1997; Eitam, Sivinski, Holler, and Aluja 2004). For example, in fruit growing regions, officially declared as low fruit fly prevalence areas, a native parasitoid may be better suited at detecting and parasitizing the few larvae present. Furthermore, some authors have proposed that releasing large numbers of exotic parasitoids may be detrimental to native, non-target insects (e.g. Williamson 1996). In this sense, native parasitoids may represent a more environmentally friendly alternative.

There are three fundamental prerequisites to the use of native parasitoids in *Anastrepha* biological control programs. The first is to obtain basic knowledge of their natural history, ecology and behavior, and significant progress in this field has been made over the past 10 years (Sivinski et al. 1997; Aluja, López, and Sivinski 1998; Sivinski, Aluja, and Holler 1999; Sivinski, Vulinec, and Aluja 2001; Guillén, Aluja, Equihua, and Sivinski 2002; Ovruski and Aluja 2002; Aluja et al. 2003; Eitam et al. 2004; Guimarães and Zucchi 2004; Ovruski, Schliserman, and Aluja 2004; Ovruski, Wharton, Schliserman, and Aluja 2005). The second one is related to their domestication and colonization. Recently, Eitam et al. (2004) described some rearing techniques useful in the initial stages of the colonization of *D. areolatus* in Florida. Related to the work being reported here, we have successfully domesticated and colonized *D. areolatus*, *D. crawfordi*, *U. anastrephae*, *O. hirtus* (all larval-prepupal braconids), *A. pelleranoi* (Brèthes), *O. anastrephae* (both larval-prepupal figitids), *Coptera haywardi* (Oglobin), *E. sivinskii* and *Dirhinus* sp. at the Instituto de Ecologia, A.C. in Xalapa, Veracruz, Mexico (Aluja et al. in press). Thirdly, on top of having access to an established colony, parasitoids need to be mass-reared. Two efforts stand out in this respect. A fairly recent effort by Menezes et al. (1998) aimed at rearing the native pupal parasitoid *C. haywardi* in irradiated *A. suspensa* and *Ceratitis capitata* (Wiedemann) larvae. The other, is a yet unpublished but successful effort, directed at mass-rearing *D. crawfordi* in Mexico (L.R., unpublished data).

Our aim here was to ascertain if eight of the species of native larval-prepupal and pupal *Anastrepha* (Diptera: Tephritidae) parasitoids recently domesticated and colonized at the Instituto de Ecologia, A.C., in Xalapa, Veracruz, Mexico (Aluja et al. 2008) could be reared on irradiated larvae and pupae and if such was the case, to determine the optimal irradiation dose. Our approach was based on the pioneering effort by Sivinski and Smittle (1990), who successfully tested the idea of mass rearing the exotic parasitoid *D. longicaudata* on irradiated *A. suspensa* larvae. We also wanted to develop a technique that would facilitate the use of excess mass reared larvae that sometimes are left over in mass rearing facilities with the idea of finding an irradiation dose that would allow healthy adult parasitoids but not flies to emerge, as the latter would greatly facilitate handling procedures and reduce costs of production.

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Materials and methods

Study site

All experiments were carried out under controlled environmental conditions in facilities and laboratories belonging to the Subdirección de Desarrollo de Métodos and the Programas MoscaMed/MoscaFrut, Campaña Nacional Contra Moscas de la Fruta in Metapa de Domínguez, Chiapas, México. Mean temperature, relative humidity and illumination regime were as follows: 24 ± 2°C, 60–80% RH, and 12:12 h. Fly rearing and irradiation procedures took place in separate buildings.

Insects

All parasitoids were reared on *A. ludens* larvae stemming from a laboratory strain that had been kept for over 300 generations (Domínguez, Castellanos, Hernández, and Martínez 2000). *Doryctobracon crawfordi*, *U. anastrephae*, *O. hirtus*, *A. pelleranoi*, *O. anastrephae*, *C. haywardi*, and *E. sivinskii* colonies were obtained from the Instituto de Ecología, A.C. in Xalapa, Veracruz, Mexico and reared for over 25 generations in our laboratories in Metapa de Domínguez, Chiapas before being used for this study. *Dirhinus* sp. was discovered during a parasitoid survey in the Soconusco region (near the city of Tapachula, Chiapas) and subsequently domesticated and colonized in our laboratories.

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Irradiation procedures

Eight-day-old larvae and 3-day-old pupae of *A. ludens* were exposed to 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60 and 70 Gy, respectively. Experiments were replicated 30 (braconids), 50 (figitids), 25 (*C. haywardi*), 35 (*E. sivinskii*) and 20 (*Dirhinus* sp.) times (replication level determined on the basis of result variability (e.g. high in the case of the two figitids, low in the case of *Dirhinus* sp.)). We used a Gammacell 220 irradiator (γ radiation with a Co 60 source), applying a dose ranging between 2.5 and 3.0 Gy/min under free oxygen. Exposure times were determined by Fricke's dosimetry (IAEA 1977). Before being exposed to radiation, larvae were removed from their rearing medium (artificial diet in a plastic washbowl) and rinsed with tap water until all diet residues had been washed away. In the case of pupae, we removed excess vermiculite (pupation medium) with the aid of a sieve.

Exposure of A. ludens *larvae to parasitoids*

The method used to expose irradiated larvae or pupae to parasitism was tailored to the idiosyncrasies of the parasitoids. In the case of the braconids, 100 *A. ludens* larvae mixed with diet (same diet used for rearing them) were placed in a Petri dish that was covered with organza cloth kept in place with a rubber band. The parasitization unit was then placed in a Hawaii-type holding cage ($27 \times 27 \times 27$-cm wooden structure cage covered with 0.5-mm caliber mesh) (Wong, Ramadan, Herr, and McInnis 1992) into which 60 (30♀ and 30♂) 5–10-day-old parasitoids had been released. Exposure periods were 4, 6 and 8 h for *D. crawfordi*, *O. hirtus* and *U. anastrephae*, respectively. Given that not all species are equally adapted to the artificial rearing conditions, varying exposure times are required to, on the one hand avoid superparasitism (case of *D. crawfordi*) and on the other, secure minimally acceptable rates of parasitism (case of *U. anastrephae*). In the case of the two figitids that preferentially parasitize larvae in fallen fruit where they seek them out by penetrating the fruit, we did not cover the Petri dish to allow the female's direct access to the larvae. In this

case, 100 larvae were exposed to 100 adults (50♀:50♂) inside a 30 × 30 × 30-cm Plexiglass cage. Exposure periods were 4 and 6 h for *A. pelleranoi* and *O. anastrephae*, respectively. Finally, in the case of the three pupal parasitoids, 100 pupae were mixed with vermiculite after irradiation and placed in a Petri dish with a paper 'roof' to secure a darkened environment for the foraging females. In all cases (i.e. all three species), we released 100 parasitoids (50♀:50♂) and allowed them to parasitize pupae over a 24-h period.

Parasitoid developmental times and emergence

After exposure to parasitoid attack, larvae were again rinsed with tap water (to remove all diet residues) and placed in 4 × 8-cm plastic containers with moistened vermiculite as a pupation medium. Exposed pupae were also handled as described for larvae but were not rinsed with water. After 15 days had elapsed, the vermiculite was removed to facilitate emergence of fly and parasitoid adults, which varied among parasitoid species. After all insects had emerged, we counted the number of females and males, and transferred the insects into cages as described in what follows.

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Determination of parasitoid longevity and fecundity

After emergence, parasitoid adults were sorted out by species and irradiation treatment, and transferred to holding cages to determine their longevity and fecundity on a per treatment and replicate basis (three per species). Type of cage, parasitization unit and exposure period also varied according to species (details in section 2.4). Cohort size in each cage was 10♀: 5♂ in all cases (i.e. all species). Survival was measured over a 30-day period from the moment of emergence. Fecundity was measured over a 10-day period starting at age 5 days by offering females a parasitization unit that contained non-irradiated larvae and that was replaced daily after the exposure period was covered. Exposed larvae and pupae were then handled as described in Parasitoid developmental times and emergence. Parasitoids had *ad libitum* access to water and honey throughout the test period.

Statistical analyses

Mean number of flies and parasitoids that emerged, sex ratio, and number of offspring per female per day (i.e. fecundity; OFD), were subjected to a one-way ANOVA (each variable analyzed independently). Quadratic trends in OFD data were also ascertained but given extremely low r^2 values (<0.05), results are not reported. To compare means, we used Bonferroni's test (Snedecor and Cochran 1980). OFD values were obtained by dividing the number of offspring by the number of live mothers per day. The proportion of living parasitoids per day (i.e. longevity) was analyzed by means of a log-rank test (Francis, Green, and Payne 1993).

Results

Emergence patterns of irradiated and non-irradiated hosts

Developmental times (egg to adult) varied sharply among parasitoid species: 15 days for *U. anastrephae*, *O. hirtus*, *E. sivinskii*, 20 days for *D. crawfordi*, *A. pelleranoi*, *O. anastrephae* and *Dirhinus* sp., and 30 days for *C. haywardi*. Furthermore, we found that development of

Table 1. Mean proportion ($\pm$SE) of *A. ludens* adults emerging from unparasitized larvae and pupae that were subjected to irradiation.

Dose (Gy)	Larva	Pupa
0	82.58 $\pm$ 2.12 a	88.37 $\pm$ 2.52 a
5	81.51 $\pm$ 2.60 a	85.30 $\pm$ 2.46 a
10	38.51 $\pm$ 4.73b	2.80 $\pm$ 5.97b
15	5.95 $\pm$ 3.12c	0.30 $\pm$ 0.02c
20	0.13 $\pm$ 0.09c	0.11 $\pm$ 0.02c
25	0c	0c
30	0c	0c
35	0c	0c
40	0c	0c
50	0c	0c
60	0c	0c
70	0c	0c

Means within columns followed by the same letter are not significantly different (one-way ANOVA, followed by Bonferroni's test).

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irradiated *A. ludens* larvae or pupae not subjected to parasitism by any of the eight parasitoid species under study here was completely halted at 25 Gy (Table 1).

In the case of the braconid parasitoids and their host (exposed to parasitism), highly significant differences were found when comparing the effect of irradiation on emergence patterns of the host (*A. ludens*) but not the parasitoid (*A. ludens*, $F_{11} = 19.31$, $P = 0.0001$, *D. crawfordi*, $F_{11} = 0.6543$, $P = 0.781$; *A. ludens*, $F_{11} = 20.58$, $P = 0.0001$, *U. anastrephae*, $F_{11} = 0.7321$, $P = 0.7075$; *A. ludens*, $F_{11} = 15.74$, $P = 0.0001$, *O. hirtus*, $F_{11} = 1.7138$, $P = 0.0708$). Complete suppression of adult emergence for irradiated *A. ludens* larvae exposed to unsuccessful parasitism was achieved at doses of 20 Gy (Table 2). In the case of the parasitoids, over 30% emergence was recorded at doses as high as 70 Gy (Table 2). With respect to sex ratio, there were no statistically significant differences among any of the three parasitoid species under study (*D. crawfordi*, $F_{11} = 0.999$, $P = 0.447$; *U. anastrephae*, $F_{11} = 0.394$, $P = 0.957$; *O. hirtus*, $F_{11} = 0.6154$, $P = 0.815$). Despite the latter, sex ratio was consistently skewed towards females.

The two figitid species were much more susceptible to irradiation. As shown in Table 3, emergence was completely halted at 25 Gy, with a highly significant drop apparent at 20 Gy. At lower doses, even though emergence was observed in both species, a highly significant effect of irradiation was also detected, particularly in the case of *O. anastrephae* (*A. ludens*, $F_{11} = 8.91$, $P = 0.0003$, *A. pelleranoi*, $F_{11} = 20.89$, $P = 0.0001$; *A. ludens*, $F_{11} = 47.15$, $P = 0.0001$, *O. anastrephae*, $F_{11} = 33.72$, $P = 0.0001$). Sex ratios were highly skewed towards females in both species, with statistically significant differences detected when adult emergence was recorded ($<$25 Gy) (*A. pelleranoi*, $F_{11} = 8.26$, $P = 0.001$; *O. anastrephae*, $F_{11} = 134.64$, $P = 0.0001$).

Finally, in the case of the pupal parasitoids, emergence was observed at doses as high as 70 Gy, while the host exposed to unsuccessful parasitism (in this case irradiated in the pupal stage) totally ceased emerging at doses of 20 Gy (Table 4). The effect of irradiation dose was highly significant with respect to emergence of the host and in the case of *C. haywardi* (*A. ludens*, $F_{11} = 19.97$, $P = 0.0001$, *C. haywardi*, $F_{11} = 9.44$, $P = 0.0001$; *A. ludens*, $F_{11} = 128.4$, $P = 0.0001$, *E. sivinskii*, $F_{11} = 0.9568$, $P = 0.48$; *A. ludens*, $F_{11} = 38.89$, $P = 0.0001$, *Dirhinus* sp., $F_{11} = 1.148$, $P = 0.324$). With respect to sex ratios, significant differences were also only

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Table 2. Mean number (±SE) of flies and parasitoids and sex-ratio of three species of *Opiinae* parasitoids that emerged from irradiated fruit fly larvae that were subjected to parasitism (values are means±SE).

	Parasitoid species								
	D. crawfordi			*U. anastrephae*			*O. hirtus*		
	Mean number emerged		Sex-ratio	Mean number emerged		Sex-ratio	Mean number emerged		Sex-ratio
Dose (Gy)	Flies	Parasitoids	(♀: ♂)	Flies	Parasitoids	(♀: ♂)	Flies	Parasitoids	(♀: ♂)
0	20.37±1.91a	32.22±3.77a	2.70±0.44a	31.07±2.86a	40.30±3.23a	1.28±0.13a	36.31±3.37a	36.87±4.38a	1.00±0.16a
5	10.64±2.22b	27.66±2.51a	2.13±0.26a	27.92±3.24a	42.96±3.39a	1.04±0.11a	29.54±3.29a	42.00±0.91a	1.03±0.08a
10	3.75±1.20b	36.61±2.90a	2.23±0.45a	8.42±2.07b	41.96±4.03a	1.41±0.42a	14.20±2.03b	27.73±1.64a	1.22±0.16a
15	0.07±0.07c	35.68±3.02a	2.01±0.17a	0.46±0.26b	33.57±2.97a	1.51±0.47a	0.36±0.30c	31.76±1.70a	1.07±0.12a
20	0 c	36.68±4.04a	1.63±0.13a	0c	38.51±3.00a	1.13±0.15a	0	32.60±1.77a	1.27±0.16a
25	0 c	35.13±2.66a	1.85±0.15a	0c	35.68±2.93a	1.17±0.12a	0	33.32±1.61a	1.12±0.09a
30	0 c	31.25±2.70a	2.19±0.66a	0c	35.27±2.89a	1.13±0.17a	0	32.16±1.61a	1.31±0.12a
35	0 c	34.82±2.97a	2.17±0.23a	0c	38.84±3.24a	1.19±0.13a	0	32.44±1.45a	1.08±0.10a
40	0 c	35.39±3.06a	2.94±0.55a	0c	37.28±3.87a	1.01±0.14a	0	32.72±1.50a	1.17±0.13a
50	0 c	34.50±3.19a	2.71±0.60a	0c	39.64±3.60a	1.33±0.23a	0	30.48±1.36a	1.02±0.10a
60	0 c	33.86±3.21a	1.99±0.15a	0c	40.50±3.42a	1.31±0.12a	0	31.24±1.59a	1.06±0.13a
70	0 c	34.87±3.09a	1.92±0.29a	0c	40.84±3.06a	1.25±0.17a	0	30.13±1.73a	1.06±0.12a

Means within columns followed by the same letter are not significantly different (one-way ANOVA, followed by Bonferroni's test).

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Table 3. Mean number (±SE) of flies and parasitoids and sex-ratio of two species of Figitidae parasitoids that emerged from irradiated fruit fly larvae that were subjected to parasitism (values are means±SE).

	Parasitoid species						
	A. pelleranoi			*O. anastrephae*			
	Mean number emerged		Sex-ratio	Mean number emerged			
Dose (Gy)	Flies	Parasitoids	(♀: ♂)	Flies	Parasitoids	Mean no. [a] females	Mean no. [b] males
0	7.24±1.19a	22.68±2.08a	2.67±0.65a	32.11±2.08a	35.23±1.84a	35.08±1.85a	0.14±0.10a
5	8.84±1.29a	23.02±1.84a	2.36±0.27ab	16.67±1.81b	32.00±1.87a	31.93±1.87a	0.07±0.04a
10	3.09±0.57b	17.86±1.87a	2.82±0.49a	7.54±1.41c	15.42±1.73b	15.42±1.73b	0a
15	1.61±1.19b	5.15±1.45b	1.06±0.28bc	0.65±0.31d	2.82±1.10c	2.04±0.69c	0.02±0.02a
20	0 c	0.34±0.15c	0.10±0.05c	0 d	0.02±0.02d	0.02±0.02c	0a
25	0 c	0 d	0 c	0 d	0 d	0 c	0a
30	0 c	0 d	0 c	0 d	0 d	0 c	0a
35	0 c	0 d	0 c	0 d	0 d	0 c	0a
40	0 c	0 d	0 c	0 d	0 d	0 c	0a
50	0 c	0 d	0 c	0 d	0 d	0 c	0a
60	0 c	0 d	0 c	0 d	0 d	0 c	0a
70	0 c	0 d	0 c	0 d	0 d	0 c	0a

[a,b] Instead of sex-ratio, we provide actual emergence values for each sex to highlight fact that almost all emerged adults were females (apparently because we are dealing with a thelytokous strain). Means within columns followed by a common letter are not significantly different (one-way ANOVA, followed by Bonferroni's test).

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Table 4. Mean number (±SE) of flies and parasitoids and sex-ratio of three species of fruit fly pupal parasitoids that emerged from irradiated pupae that were subjected to parasitism (values are means±SE).

	Parasitoid species								
	C. haywardi			*E. sivinskii*			*Dirhinus* sp.		
	Mean number emerged		Sex-ratio	Mean number emerged		Sex-ratio	Mean number emerged		Sex-ratio
Doses (Gy)	Flies	Parasitoids	(♀: ♂)	Flies	Parasitoids	(♀: ♂)	Flies	Parasitoids	(♀: ♂)
0	22.21±1.22a	37.78±2.07a	1.35±0.10a	61.90±2.46a	24.88±1.38a	1.14±0.07a	49.32±2.74a	31.05±3.62a	0.92±0.11a
5	18.32±2.00a	38.70±1.39a	1.26±0.06a	63.10±2.46a	20.27±1.54a	1.29±0.14a	46.24±2.88a	27.05±3.46a	0.94±0.12a
10	8.95±1.30b	38.52±1.47a	1.32±0.09a	14.50±2.42b	20.08±1.89a	1.69±0.37a	17.05±2.86b	28.80±3.41a	0.87±0.10a
15	2.40±1.57c	36.07±7.30ab	1.74±0.13a	0.94±0.47c	22.48±1.55a	1.14±0.08a	0.20±0.2c	32.62±3.48a	0.88±0.10a
20	0d	31.25±1.99abc	1.78±0.22a	0d	22.19±1.74a	1.10±0.08a	0c	29.73±2.43a	1.06±0.12a
25	0d	35.72±1.50ab	1.42±0.07a	0d	23.00±2.20a	1.18±0.15a	0c	32.44±2.65a	0.98±0.09a
30	0d	30.90±1.4abcd	2.92±0.56b	0d	24.64±2.02a	1.01±0.07a	0c	33.52±2.91a	1.05±0.10a
35	0d	34.02±1.73ab	2.07±0.15ab	0d	24.69±1.88a	1.28±0.19a	0c	33.37±2.82a	1.52±0.34a
40	0d	34.19±1.47ab	1.79± 0.19a	0d	24.24±2.23a	1.14±0.08a	0c	36.20±2.85a	1.12±0.10a
50	0d	29.31±1.99bcd	2.26± 0.2ab	0d	25.74±2.22a	1.19±0.13a	0c	31.35±3.20a	1.11±0.09a
60	0d	23.73±2.04cd	1.89± 0.1ab	0d	23.18±1.97a	1.14±0.10a	0c	31.45±3.70a	0.96±0.14a
70	0d	23.25±1.18d	2.20± 0.2ab	0d	24.38±1.44a	1.16±0.11a	0c	23.35±2.78a	1.46±0.28

Means within columns followed by the same letter are not significantly different (one-way ANOVA, followed by Bonferroni's test).

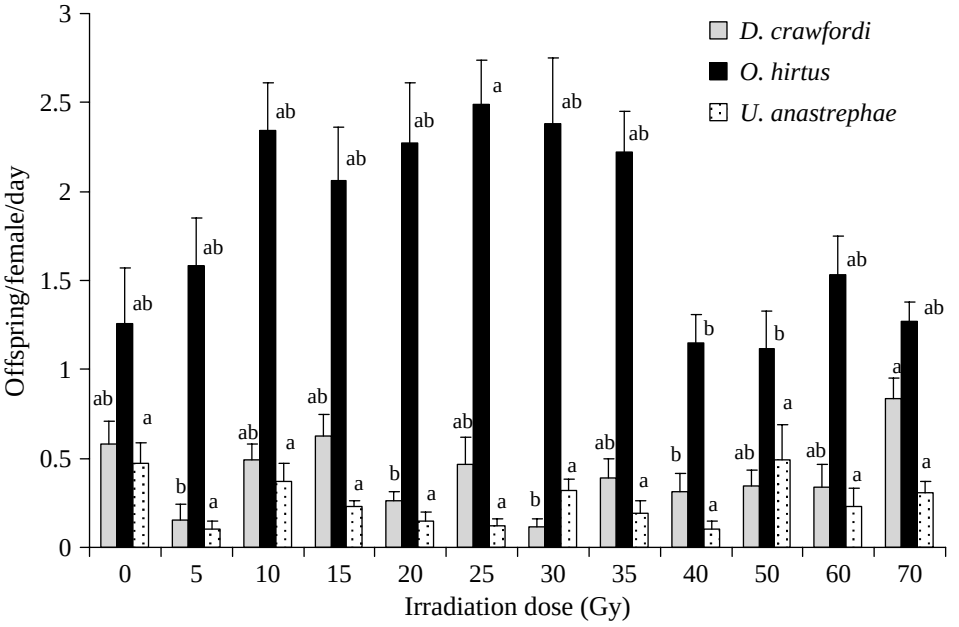

Figure 1. Fecundity of *D. crawfordi*, *O. hirtus* and *U. anastrephae* (Braconidae: Opiinae) stemming from larvae irradiated at varying gamma radiation doses. The larvae offered to the adult parasitoids were not irradiated.

detected in the case of *C. haywardi* (*C. haywardi*, $F_{11} = 4.11$, $P = 0.0001$; *E. sivinskii*, $F_{11} = 0.439$, $P = 0.937$; *Dirhinus* sp., $F_{11} = 0.849$, $P = 0.590$) (details in Table 4).

Fecundity and longevity of parasitoid offspring

Mean fecundity of the braconid species studied was significantly affected by irradiation (*D. crawfordi*: $F_{11} = 3.51$, $P = 0.0003$, *U. anastrephae*: $F_{11} = 2.32$, $P = 0.013$, *O. hirtus*: $F_{11} = 3.99$, $P < 0.0001$) (Figure 1). With respect to longevity, there was no statistically significant effect of irradiation in any of the three species (*D. crawfordi*, $\chi^2_{11} = 9.23$, $P = 0.60$, *U. anastrephae*, $\chi^2_{11} = 45.97$, $P = 0.001$, *O. hirtus*, $\chi^2_{11} = 16.32$, $P = 0.129$). Of the latter, *D. crawfordi* lived the longest (Figure 2).

In the case of *A. pelleranoi* and *O. anastrephae*, no statistically significant influence of irradiation on fecundity was detected in the few cases where adequate emergence was observed (up to 15 Gy) (*A. pelleranoi*, $F_{11} = 0.726$, $P = 0.542$; *O. anastrephae*, $F_{11} = 0.142$, $P = 0.934$; details in Figure 3). With respect to longevity, and particularly in the case of *A. pelleranoi*, irradiation had a marginally significant effect (*A. pelleranoi*, $\chi^2_3 = 7.54$, $P = 0.056$, *O. anastrephae*, $\chi^2_3 = 0.272$, $P = 0.965$) (Figure 4).

As for pupal parasitoids, fecundity was only influenced by irradiation in the case of *C. haywardi* and *E. sivinskii* (*C. haywardi*, $F_{11} = 5.595$, $P = 0.0001$; *E. sivinskii*, $F_{11} = 3.824$, $P = 0.0001$; *Dirhinus* sp., $F_{11} = 0.26$, $P = 0.99$). Remarkably, offspring was produced even at doses as high as 70 Gy (Figure 5). With respect to longevity, no statistically significant differences were detected when comparing the different irradiation doses in all three species (*C. haywardi*, $\chi^2_{11} = 4.58$, $P = 0.949$; *E. sivinskii*, $\chi^2_{11} = 11.74$, $P = 0.383$; *Dirhinus* sp., $\chi^2_{11} = 12.84$, $P = 0.303$). As can be seen in Figure 6, large numbers of adults were still alive after 30 days.

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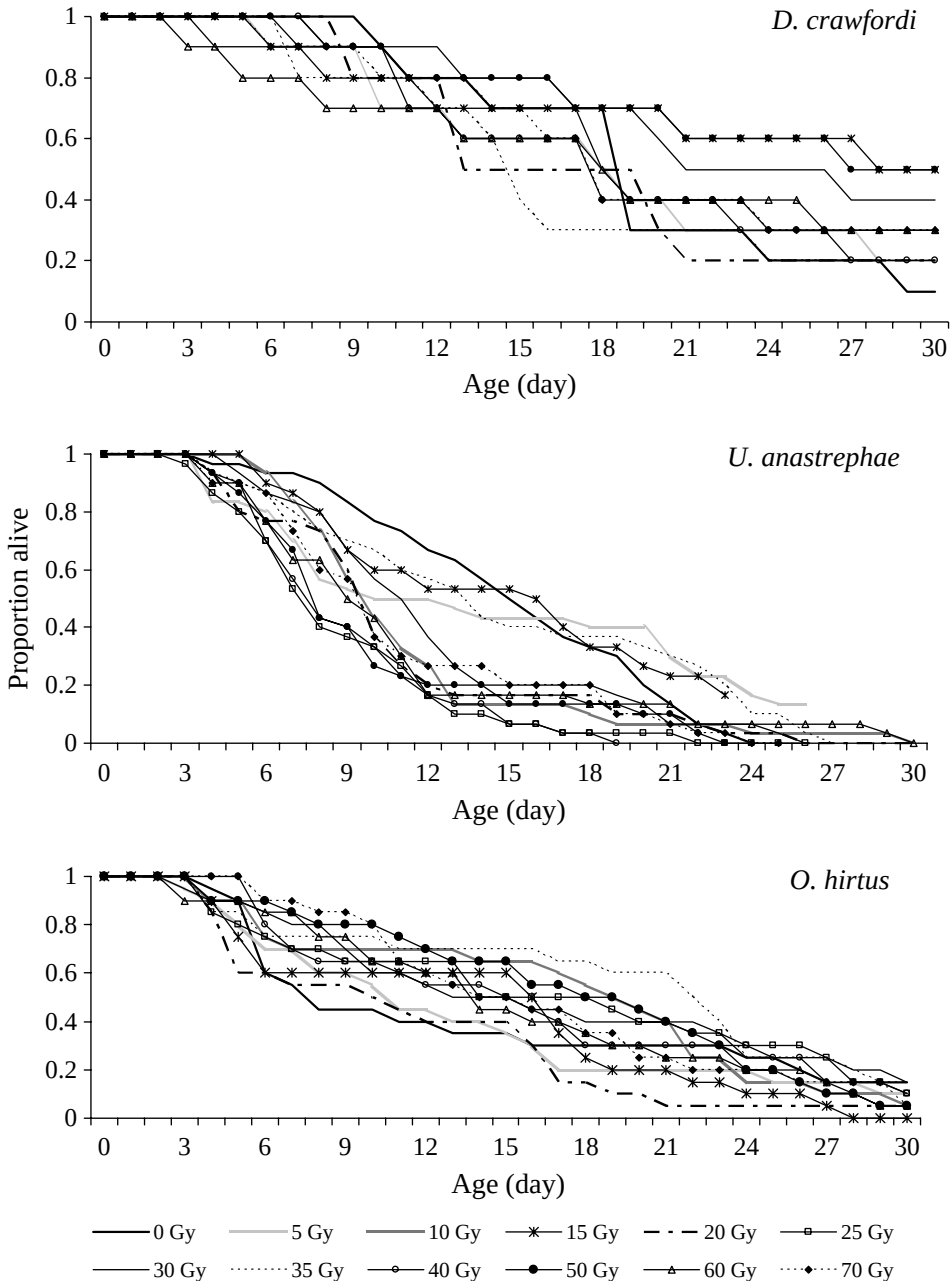

Figure 2. Longevity of *D. crawfordi*, *O. hirtus* and *U. anastrephae* (Braconidae: Opiinae) stemming from larvae irradiated at varying gamma radiation doses.

Discussion

Several points of basic physiological and applied significance emerged from our study: (1) irradiating larvae or pupae to mass rear native *Anastrepha* larval-prepupal and pupal parasitoids appears technically feasible in all but two of the species under study here. With the exception of *A. pelleranoi* and *O. anastrephae* (both figitids), host emergence was

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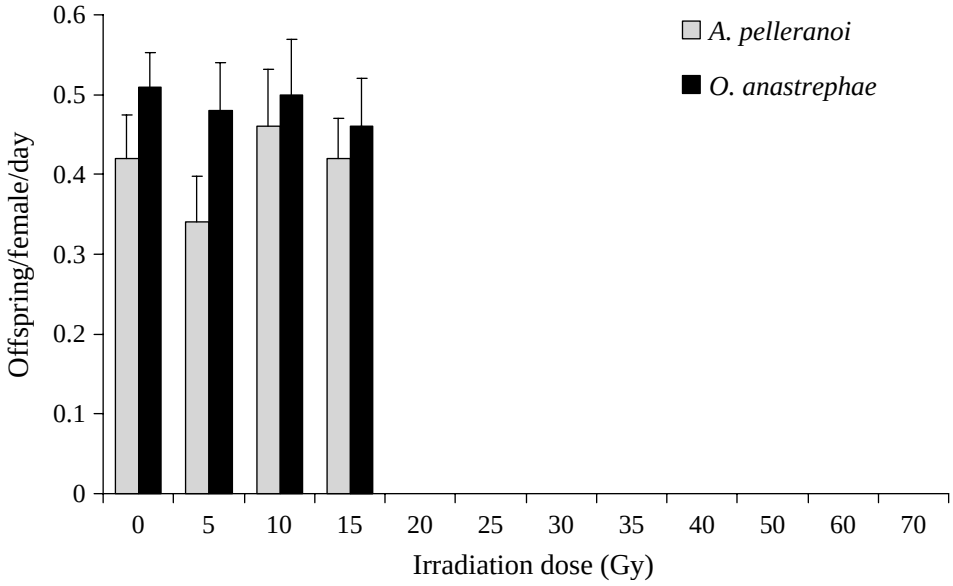

Figure 3. Fecundity of *A. pelleranoi* and *O. anastrephae* (Figitidae: Eucoilinae) stemming from larvae irradiated at varying gamma radiation doses. The larvae offered to the adult parasitoids were not irradiated.

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completely halted at doses that did not negatively affect parasitoid emergence, fecundity or survival. This capacity to develop in irradiated hosts is paralleled in certain Old World species such as *D. longicaudata* (Sivinski and Smittle 1990; Cancino, Ruiz, Gómez, and Toledo 2002). (2) Sex ratios were consistently (albeit not significantly) female biased, and did not vary when compared to the control. The latter adds significantly to the practical benefit of irradiation on native parasitoid mass rearing. (3) All three species of pupal parasitoids developed on irradiated hosts, although *C. haywardi* seemed the most sensitive to host irradiation, perhaps due to its unusual endoparasitic feeding habits and possible damage to host organs and physiology. (4) While *C. haywardi* is unable to develop in pupae resulting from irradiated larvae (Menezes et al. 1998), it was found to develop in irradiated pupae, suggesting some necessary early pupal development in the host. (5) Finally, it appears that *A. ludens* is highly susceptible to irradiation, as is *A. obliqua* (Toledo, Rull, Oropeza, Hernández, and Liedo 2004), highlighting the urgent need to reexamine currently used irradiation doses that seem unnecessarily high.

The opiine braconids contribute a number of important fruit fly biological control agents (Wharton and Marsh 1978; Wharton and Gilstrap 1983; Ovruski, Aluja, Sivinski, and Wharton 2000). Our present experiments found that, like the Old World species *D. longicaudata* and *D. kraussii* (Fullaway), the New World species *D. crawfordi*, *U. anastrephae* and *O. hirtus* develop as well or better in irradiated host-larvae (see Sivinski and Smittle 1990). However, this capacity is not universal in the subfamily. Attempts to rear *Psyttalia* spp. on irradiated hosts have been unsuccessful (E. Harris, unpublished data). It is possible that irradiation prevents some important developmental process in the host that subsequently prevents parasitoid development. For example, Thomas and Hallman (2000) documented that irradiating late third instar *A. ludens* larvae at ~20 Gy (gamma radiation), retarded protein metabolism and arrested development at the transition from cryptocepahlic to phanerocephalic pupa. Evidence of required host

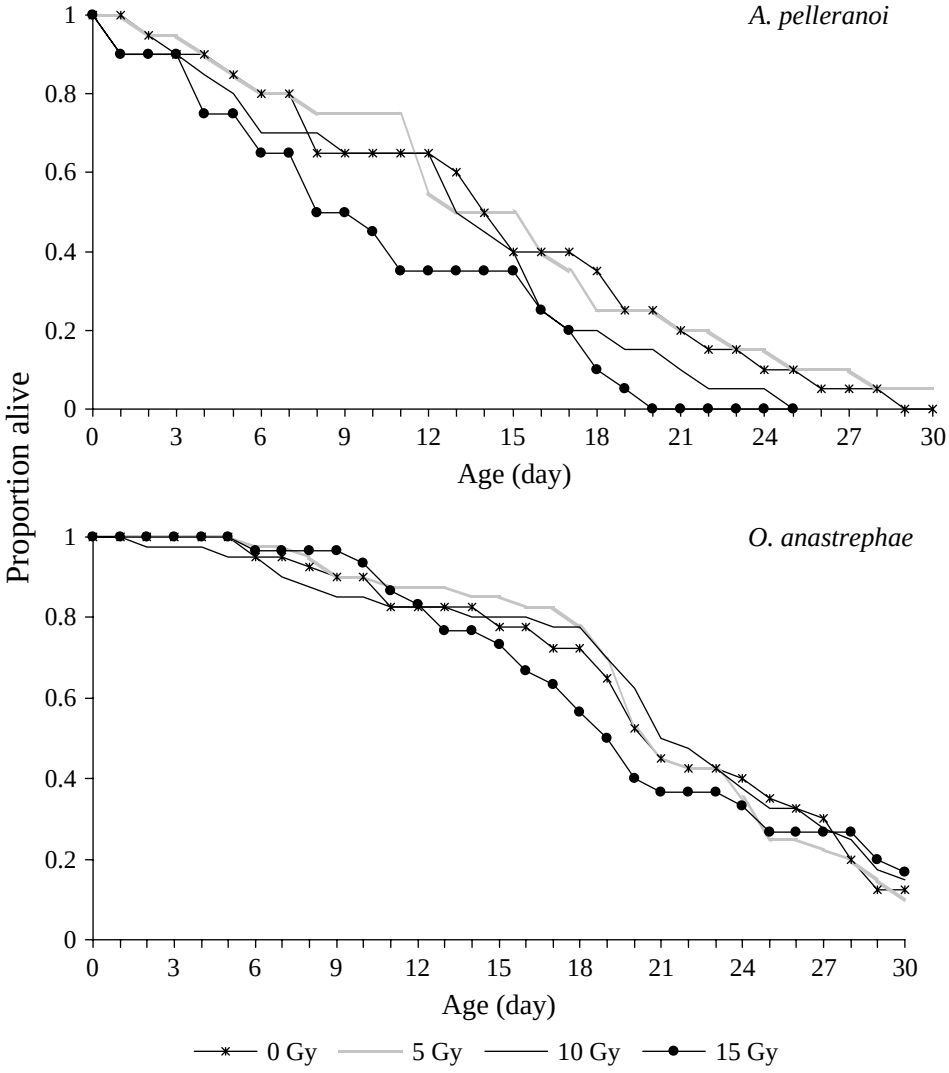

Figure 4. Longevity of *A. pelleranoi* and *O. anastrephae* (Figitidae: Eucoilinae) stemming from larvae irradiated at varying gamma radiation doses.

development for maturation of the endoparasitic pupal parasitoid *C. haywardi* can be obtained by comparing the capacity of the insect to develop in pupae derived from irradiated larvae and pupae. In the first instance, *C. haywardi* is unable to develop (Sivinski et al. 1999), while development is completed if radiation is applied after pupation (our data here).

In addition to retarding host development, irradiation might damage vital structures in the host required by the immature parasitoid. For example, radiation damages the nervous and endocrine systems of *Anastepha suspensa* (Loew) larvae (Nation, Smittle, Milne, and Dykstra 1995). None of the two figitid parasitoids emerged at doses above 20 Gy. These species have a longer developmental period, 20–25 days, than braconids, and this relatively slow development could be a disadvantage when irradiated hosts eventually begin to decompose. In addition, apparently larvae start as endoparasitoids but move outside the

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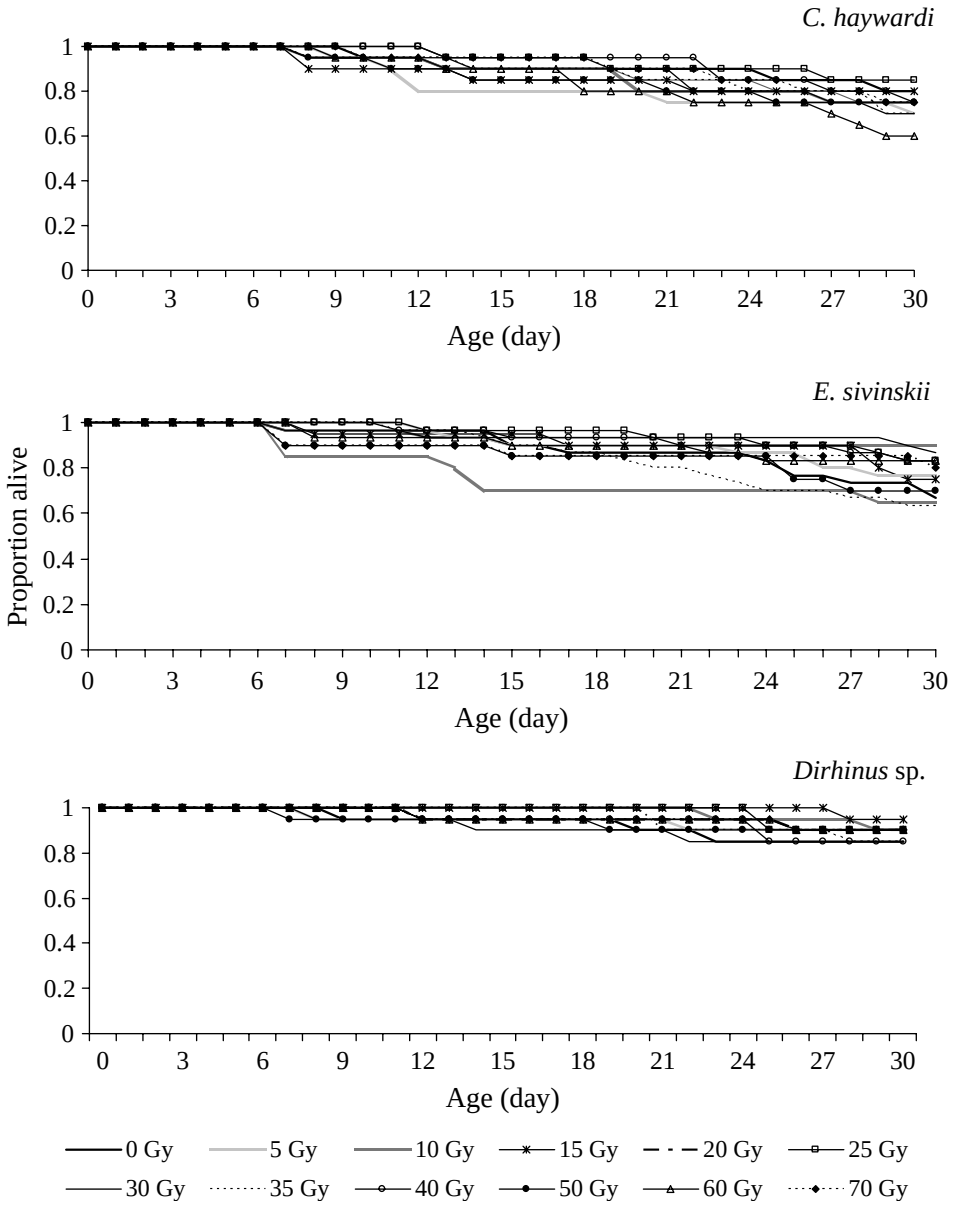

Figure 5. Longevity of *Dirhinus* sp., *C. haywardi* and *E. sivinskii* (Chalcidoidea, Diapriidae and Eurytomidae, respectively) stemming from pupae irradiated at varying gamma radiation doses. The pupae offered to the adult parasitoids were not irradiated.

host with increasing size. Given that parasitoid larvae may need to use the empty spaces between the host and the puparium (Ovruski 1994), unsatisfactory formation of the pupae might result from irradiation. However, damage to the host need not be detrimental to the developing parasitoid. Increasing levels of irradiation could possibly suppress the immune system of the host and inhibit its ability of for example, encapsulate the parasitoid developing inside.

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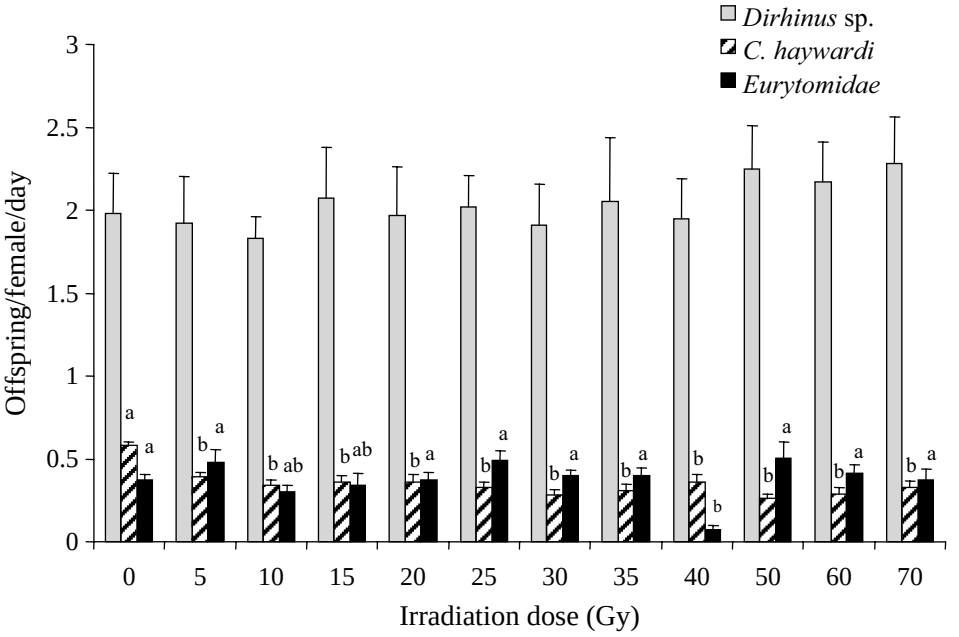

Figure 6. Fecundity of *Dirhinus* sp., *C. haywardi* and *E. sivinskii* (Chalcidoidea, Diapriidae and Eurytomidae, respectively) stemming from pupae irradiated at varying gamma radiation doses.

In conclusion, the results obtained here represent a significant step forward in the use of native parasitoids in fruit fly biological control. Although their augmentative release has to date not been formally tested, the use of irradiated hosts may provide various advantages in other activities. For example, tests to determine movement ability with artificial traps and studies of foraging behavior using irradiated hosts may be carried out under field conditions without the risk of releasing pests.

Acknowledgements

We thank two anonymous reviewers and the editor for helping us produce a more polished final product. Francisco Díaz-Fleischer (Subdirección de Desarrollo de Métodos, Campaña Nacional Contra Moscas de la Fruta [CNCMF]), Mariano Ordano, Juan Rull, Ricardo Ramírez and Larissa Guillén (all Instituto de Ecología, A.C. [INECOL]) also made many useful comments on an earlier draft. Javier Valle Mora (El Colegio de la Frontera Sur) and Francisco Díaz-Fleischer (CNCMF) made important suggestions on data analyses. We appreciate the technical support provided by Edelfo Pérez, Mario Pineda, Javier Robledo, Floriberto Pérez, Velizario Ribera and Marbel Monjaraz (all Subdirección de Desarrollo de Métodos, CNCMF). We are also grateful to Yeudiel Gómez and his staff (Programa MoscaMed) for having irradiated all the larvae and pupae used in this study. Thanks are due to Nicoletta Righini and Alberto Anzures (both INECOL) for formatting and final preparation of this manuscript. This work was financed by the International Atomic Energy Agency (IAEA) through contract No. 10848, the Mexican Campaña Nacional Contra Moscas de la Fruta (Secretaría de Agricultura, Ganadería, Desarrollo Rural y Pesca – Instituto Interamericano de Cooperación para la Agricultura [SAGARPA-IICA]) and the Instituto de Ecología, A.C. (INECOL).

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References

Aluja, M. (1994), 'Bionomics and Management of *Anastrepha*', *Annual Review of Entomology*, 39, 155–178.

Aluja, M., López, M., and Sivinski, J. (1998), 'Ecological Evidence for Diapause in Four Native and One Exotic Species of Larval-Pupal Fruit Fly (Diptera: Tephritidae) Parasitoids in Tropical Environments', *Annals of the Entomological Society of America*, 91, 821–833.

Aluja, M., Rull, J., Sivinski, J., Norrbom, A.L., Wharton, R.A., Macías-Ordóñez, R., Díaz-Fleischer, F., and López, M. (2003), 'Fruit Flies of the Genus *Anastrepha* (Diptera: Tephritidae) and Associated Native Parasitoids (Hymenoptera) in the Tropical Rainforest Biosphere Reserve of Montes Azules, Chiapas, Mexico', *Environmental Entomology*, 32, 1377–1385.

Aluja, M., Sivinski, J., Ovruski, S., Guillén, L., Cancino, J., López, M., Torres-Anaya, A., Gallegos-Chan, G., and Ruíz, L. 'Colonization and Domestication of Seven Species of Native New World Hymenopterous Larval-Prepupal and Pupal Fruit Fly (Diptera: Tephritidae) Parasitoids', *Biocontrol Science and Technology* (in press).

Anonymous (2003), Informe Anual Planta Moscafrut. Metapa de Domínguez, Chiapas, México. SAGARPA-DGSV, 56 p.

Cancino, J., Ruiz, L., Gómez, Y., and Toledo, J. (2002), 'Irradiación de larvas de *Anastrepha ludens* (Loew) (Diptera: Tephritidae) para inhibir la emergencia de moscas en la cría del parasitoide *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae)', *Folia Entomologica Mexicana*, 41, 195–208.

Clark, R., Steck, G., and Weems, H. (1996), 'Detection, Quarantine and Eradication of Exotic Fruit Flies in Florida', in *Pest Management in the Subtropics*, eds. D. Rosen, F.D. Bennett and J.L. Capinera, Andover, UK: Intercept Ltd, pp. 29–54,.

Domínguez, J., Castellanos, D., Hernández, E., and Martínez, E. (2000), 'Métodos de cría masiva de moscas de la fruta', *Memorias del XIII Curso Internacional sobre Moscas de la Fruta*. CICMF, Metapa de Domínguez, Chiapas, México, pp. 399–414.

Eitam, A., Sivinski, J., Holler, T., and Aluja, M. (2004), 'Biogeography of Braconid Parasitoids of the Caribbean Fruit Fly, *Anastrepha suspensa* (Diptera: Tephritidae) in Florida', *Annals of the Entomological Society of America*, 97, 928–939.

Francis, B., Green, M., and Payne, C. (1993), *Statistical System for Generalized Linear Interactive Modelling*, Oxford, UK: Clarendon Press.

Guillén, L., Aluja, M., Equihua, M., and Sivinski, J. (2002), 'Performance of Two Fruit Fly (Diptera: Tephritidae) Pupal Parasitoids (*Coptera haywardi* [Hymenoptera: Diapriidae] and *Pachycrepoideus vindemiae* [Hymenoptera: Pteromalidae]) under Different Environmental Soil Conditions', *Biological Control*, 23, 219–227.

Guimarães, J.A., and Zucchi, R.A. (2004), 'Parasitism Behavior of Three Species of Eucoilinae (Hymenoptera: Cynipoidea: Figitidae) Fruit Fly Parasitoids (Diptera) in Brazil', *Neotropical Entomology*, 33, 217–224.

IAEA (1977), 'Laboratory Training Manual on the Use of Isotopes and Radiation in Entomology', Technical Report Series No. 61, 2nd ed. Vienna, Austria, pp. 120.

Menezes, E., Sivinski, J., Holler, T., Aluja, M., Jeronimo, F., and Ramirez, E. (1998), 'Development of *Coptera haywardi* (Hymenoptera: Diapriidae) in Irradiated and Non-Irradiated Pupae of the Caribbean Fruit Fly and the Mediterranean Fruit Fly (Diptera: Tephritidae)', *Florida Entomologist*, 81, 567–570.

Montoya, P., and Cancino, J. (2004), 'Control biológico por aumento en moscas de la fruta (Diptera: Tephritidae)', *Folia Entomologica Mexicana*, 43, 257–270.

Montoya, P., Liedo, P., Benrey, B., Cancino, J., Barrera, J.F., Sivinski, J., and Aluja, M. (2000), 'Biological Control of *Anastrepha* spp. (Diptera: Tephritidae) in Mango Orchards through Augmentative Releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae)', *Biological Control*, 18, 216–224.

Nation, J.L., Smittle, B.J., Milne, K., and Dykstra, T.M. (1995), 'Influence of Irradiation on Development of Caribbean Fruit Fly (Diptera: Tephritidae) Larvae', *Annals of the Entomological Society America*, 88, 348–352.

Ovruski, S.M. (1994), 'Immature Stages of *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Cynipoidea: Eucoilidae), a Parasitoid of *Ceratitis capitata* and *Anastrepha* spp. (Diptera: Tephritidae)', *Journal of Hymenoptera Research*, 3, 233–239.

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Ovruski, S.M., and Aluja, M. (2002), 'Mating Behavior of *Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Figitidae, Eucoilinae), a Fruit Fly (Diptera: Tephritidae) Larval Parasitoid', *Journal of Insect Behavior*, 15, 139–151.

Ovruski, S.M., Aluja, M., Sivinski, J., and Wharton, R. (2000), 'Hymenopteran Parasitoids on Fruit-Infesting Tephritidae (Diptera) in Latin America and the Southern United States: Diversity, Distribution, Taxonomic Status and their Use in Fruit Fly Biological Control', *Integrated Pest Management Reviews*, 5, 81–107.

Ovruski, S.M., Schliserman, P., and Aluja, M. (2004), 'Indigenous Parasitoids (Hymenoptera) Attacking *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae) in Native and Exotic Host Plants in Northwestern Argentina', *Biological Control*, 29, 43–57.

Ovruski, S.M., Wharton, R.A., Schliserman, P., and Aluja, M. (2005), 'Abundance of *Anastrepha fraterculus* (Diptera: Tephritidae) and its Associated Native Parasitoids (Hymenoptera) in "Feral" Guavas Growing in the Endangered Northernmost Yungas Forests of Argentina with an Update on the Taxonomic Status of Opiine Parasitoids Previously Reported in this Country', *Environmental Entomology*, 34, 807–818.

Sivinski, J., and Smittle, B. (1990), 'Effect of Gamma Radiation on the Development of the Caribbean Fruit Fly *Anastrepha suspensa*, and the Subsequent Development of its Parasite *Diachasmimorpha longicaudata* (Ashmead)', *Entomologia Experimentalis et Applicata*, 55, 295–297.

Sivinski, J., Calkins, C., Baranowski, R., Harris, D., Brambila, J., Diaz, J., Burns, R., Holler, T., and Dodson, G. (1996), 'Suppression of a Caribbean Fruit Fly (*Anastrepha suspensa* (Loew) Population through Releases of the Parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae)', *Biological Control*, 6, 177–185.

Sivinski, J., Aluja, M., and López, M. (1997), 'Spatial and Temporal Distributions of Parasitoids of Mexican *Anastrepha* Species (Diptera: Tephritidae) Within Canopies of Fruit Trees', *Annals of the Entomological Society of America*, 90, 604–618.

Sivinski, J., Aluja, M., and Holler, T. (1999), 'The Distributions of the Caribbean Fruit Fly, *Anastrepha suspensa* (Tephritidae) and its Parasitoids (Hymenoptera: Braconidae) Within the Canopies of Host Trees', *Florida Entomologist*, 82, 72–81.

Sivinski, J., Vulinec, K., and Aluja, M. (2001), 'Ovipositor Length in a Guild of Parasitoids (Hymenoptera: Braconidae) Attacking *Anastrepha* spp. Fruit Flies (Diptera:Tephritidae) in Southern Mexico', *Annals of the Entomological Society of America*, 94, 886–895.

Snedecor, G.W., and Cochran, W.G. (1980), *Statistical Methods*, 7th ed., Ames, IA: The Iowa State University Press.

Thomas, D.B., and Hallman, G.J. (2000), 'Radiation-induced Pathology in the Metamorphosis of the Mexican Fruit Fly (Diptera: Tephritidae)', *Journal of Entomological Science*, 35, 267–278.

Toledo, J., Rull, J., Oropeza, A., Hernández, E., and Liedo, P. (2004), 'Irradiation of *Anastrepha obliqua* (Diptera: Tephritidae) Revisited: Optimizing Sterility Induction', *Journal of Economic Entomology*, 97, 383–389.

Wharton, R.A., and Gilstrap, F.E. (1983), 'Key to and Status of Opiinae Braconid (Hymenoptera) Parasitoids Used in Biological Control of *Ceratitis* and *Dacus s.l.* (Diptera: Tephritidae)', *Annals of the Entomological Society of America*, 76, 721–742.

Wharton, R.A., and Marsh, P.M. (1978), 'New World Opiinae (Hymenoptera: Braconidae) Parasitic on Tephritidae (Diptera)', *Journal of the Washington Academy of Sciences*, 68, 147–167.

Williamson, M. (1996), *Biological Invasions*, London: Chapman & Hall.

Wong, T.T.Y., Ramadan, M.M., Herr, J.C., and McInnis, D.O. (1992), 'Suppression of a Mediterranean Fruit Fly (Diptera: Tephritidae) Population with Concurrent Parasitoid and Sterile Fly Releases in Kula, Maui, Hawaii', *Journal of Economic Entomology*, 85, 1671–1681.

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