Journal of Economic Entomology, XX(XX), 2020, 1–11
doi: 10.1093/jee/toaa062
Research

Radiation on Medfly Larvae of *tsl*Vienna-8 Genetic Sexing Strain Displays Reduced Parasitoid Encapsulation in Mass-Reared *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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Subject Editor: Nikolaos Papadopoulos

Received 10 September 2019; Editorial decision 9 March 2020

Abstract

Improvements in the mass rearing of *Diachasmimorpha longicaudata* (Ashmead) on larvae of the Vienna-8 temperature-sensitive lethal genetic sexing strain of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (= GSS Vienna-8) at the San Juan biofactory, Argentina, are currently under way. Lowering cost production is a key factor regarding parasitoid rearing. Thus, the variation in mass-reared parasitoid encapsulation levels and the incidence of superparasitism were determined; also, the gamma radiation dose-effect relation on host larvae and the influence of Mediterranean fruit fly strain were considered. Naked Mediterranean fruit fly larvae of both GSS Vienna-8 and a wild bisexual strain (= WBS) aged 6-d-old were irradiated at 0, 20, 40, 60, 80, 100, and 120 Gy, and exposed to parasitoid females. Melanization level was tested for encapsulated parasitoid larval first-instars (= L_1). Non-irradiated and irradiated WBS larvae at 20–40 Gy displayed a significantly higher incidence of encapsulation when compared with GSS Vienna-8 larvae. The low melanized level in encapsulated parasitoid L_1 was the most common melanization process at 72 h puparium dissection. A high melanized level was only found in non-irradiated WBS larvae. Irradiated GSS Vienna-8 larvae can neutralize the host immunological reactions over irradiated WBS larvae much more quickly. Superparasitism intensity in both Mediterranean fruit fly strains was not affected by radiation doses. High levels of superparasitism seemingly helped to overcome the host's immune reaction by the surviving parasitoid larva. Parasitoid emergence increased from 60 Gy onwards in both Mediterranean fruit fly strains. Radiation in GSS Vienna-8 larvae may favor host's antagonistic reactions decrease in relation with *D. longicaudata* development.

Key words: parasitoid encapsulation, radiation, parasitoid mass rearing, superparasitism, Mediterranean fruit fly

Defense mechanisms displayed by a host insect play an essential role in a parasitoid-host interaction system for it to survive. Different kinds of innate immune responses can be exerted by the host when an endoparasitoid, i.e., the one whose larva develops inside the host body, is involved in the interaction (Blumberg 1990, Nappi et al. 1995, Nappi and Ottaviani 2000, Schmidt et al. 2001, Schmid-Hempel 2005). One of the most important defense strategies is encapsulation (Dubovskiy et al. 2016), which occurs when blood cells, namely hemocytes, surround and strongly adhere to an invading parasitoid body, and shape a multicellular-layered sheath capsule-like around it (Blumberg 1997). Regularly, innermost layers of hemocytes undergo melanization due to a massive deposition of melanin on the surface of the parasitoid body, a process which prevents its growth (Nappi and Christensen 2005). Melanogenesis is,

therefore, extremely important for the internal defense of the host, among other insect survival functions (Nappi 2010, Sugumaran and Barek 2016). Internal parasitoids may be eliminated by suffocation, starvation, and/or physical obstruction that hinder development by effect of encapsulation (Pennacchio and Strand 2006). As a consequence, encapsulated parasitoid may suffer egg deformation or growth neutralization, larval growth inhibition or development time extension, and adult offspring reduction (Blumberg 1997, Carton et al. 2008).

The relationship host-parasitoid may be negatively affected by parasitoid encapsulation. This entails the host reactions against the parasitoid development, which could be an important factor preventing biological control. Thus, high encapsulation rates may hinder parasitoid mass production under laboratory condition and/or would indicate low effectiveness of field-released parasitoids (Blumberg 1997). Nevertheless, the survival of many parasitoids within their hosts may be attributed to the injection of immune-suppressors, e.g., venoms and/or symbiotic viruses, which may play a crucial role in preventing encapsulation and ensuing damage of the parasitoid offspring by impairing both the host's humoral and cellular defense mechanisms (Beckage 1998, Shelby and Webb 1999, Tillinger et al. 2004, Renault 2012). In addition, superparasitism has been considered a further essential means by which parasitoids 'cheat' the host defense system (Blumberg 1997, Luna et al. 2016). Self-superparasitism is a strategy in which a solitary parasitoid female herself lays additional eggs in a previously parasitized host (Montoya et al. 2011). Encapsulation in a superparasitized host may not affect all the parasitoid eggs; thus allowing one or more eggs to develop normally (Vinson 1990, 1993). Some other important factors may also affect the frequency of parasitoid encapsulation; such is the case of host`s age and physiological condition, host and parasitoid species, host strain or origin, laboratory and/or room temperature (Blumberg 1997), as well as ionizing radiation (Nation et al. 1995, Tillinger et al. 2004, Mastrangelo and Walder 2011).

Holometabolous insects are mainly subjected to strong morphological and functional changes during metamorphosis, a fact which facilitates susceptibility to radiation (Seong et al. 2012, Gabarty et al. 2013, Kheirallah and El-Samad 2016). Thus, radiation applied at both an adequate dose and at a host critical growth stage may result in sterility, developmental suppression and/or physiological alterations, with the host immune system probably highly affected (Hasan et al. 2009, Hendrichs et al. 2009, Mastrangelo and Walder 2011, Cai et al. 2017, Tariq et al. 2017, Naveed and Khan 2019). Therefore, the use of irradiated hosts in mass-reared parasitoids may be an important additional support to further elude host's defensive mechanisms, not to mention other features that mostly improve natural enemy lab-production; in turn, open-field augmentative releasing procedures can also be facilitated (Fatima et al. 2009, Cancino et al. 2012, Yokoyama et al. 2012, Sarwar et al. 2015, Cancino et al. 2016, Costa et al. 2016, Hasan et al. 2019). *Ceratitis capitata* (Wiedemann), the Mediterranean fruit fly or medfly, is one of the most severe agricultural invasive pests in the world (CABI 2019), and strongly affects Argentine fruit production, marketing, and export (Alós et al. 2014, SENASA 2017). Because of this, augmentative biological control was added into biorational strategies of the Argentinian National Fruit Fly Control and Eradication Program (ProCEM) as an integrated tool to mass trapping, sterile insect technique (SIT), and cultural methods to Mediterranean fruit fly control in a healthier and environmentally friendly approach (Suárez et al. 2014, Sánchez et al. 2016, Suárez et al. 2019). The Southeast Asian-native parasitoid *Diachasmimorpha longicaudata* (Ashmead) is currently mass-reared on irradiated larvae of the Vienna-8 temperature-sensitive lethal (*tsl*) genetic sexing strain (GSS) of *C. capitata* (from now on: GSS Vienna-8) at the San Juan Mediterranean fruit fly and Parasitoids Mass Rearing Facility (from now on: San Juan biofactory), located in San Juan, central-western Argentina. Aiming to improve parasitoid mass production and to attain high-quality open-field released individuals, considerable progress has been made in *D. longicaudata* mass rearing at the San Juan biofactory (Suárez et al. 2019). However, further efforts are still needed to reach a more satisfactory cost–benefit ratio by using irradiated larvae of GSS Vienna-8 in *D. longicaudata* mass production. Some evidences point out that emergence rate of the mass-reared exotic opiine parasitoid *D. longicaudata* particularly increased following exposure of irradiated fruit fly larvae (Cancino et al. 2009a, b). Nevertheless, more detailed studies on radiation doses are required to attribute increases in *D. longicaudata* adult emergence performance due to a host immune-depressive effect (Cancino et al. 2012).

The following objectives were set up for the present study: 1) to assess variation in mass-reared parasitoid encapsulation levels and 2) to determine the incidence of superparasitism. For both purposes, two main factors were reviewed: 1) radiation dose-effect relation on host larvae, and 2) influence of *C. capitata* strain used for parasitoid mass rearing. It is therefore hypothesized that an external factor such as gamma radiation, added to a *D. longicaudata* innate response, such as superparasitism (Montoya et al. 2012), may together lead to overcome host immune defense. Both factors foster an increased parasitoid emergence rate regardless of tested host strains under mass-rearing conditions. The following issues were particularly examined: 1) variation in melanization degrees for those encapsulated parasitoid eggs and first-instar larvae with regards to both radiation doses and Mediterranean fruit fly strains; 2) parasitoid efficiency to survive inside the host of *C. capitata* strain in terms of parasitoid emergence proportion; 3) potential effect of both radiation doses and superparasitism on parasitoid offspring sex ratio; and 4) practical evidence of the decrease in the host immune response due to increase of gamma-radiation doses. The relevance of these findings is discussed within the framework of tephritid fruit fly parasitoid mass rearing and their use in open-field augmentative biological control.

Materials and Methods

Source and Rearing of Insects

The study was performed at the Parasitoid Rearing Laboratory (PRL) at the San Juan biofactory, in Rivadavia, San Juan, Argentina. Adults of *D. longicaudata* were mass-reared on third-instar larvae of the GSS Vienna-8. The parasitoid colony was kept in rectangular iron-framed mesh-covered cages (60 × 60 × 30 cm), as was described by Suárez et al. (2019). A second *D. longicaudata* colony was reared on third-instar larvae of a *C. capitata* wild bisexual strain (also called WBS). The Mediterranean fruit fly colony was initiated from wild *C. capitata* individuals recovered by harvesting figs from trees at different farms in San Juan. Mediterranean fruit fly larvae of both GSS Vienna-8 and WBS were reared in the Mediterranean fruit fly Rearing Laboratory at the San Juan biofactory on a wheat-based diet containing yeast, sugar, hydrochloric acid, sodium benzoate, Nipagin, water, and poplar chips as substrate. Batches of Mediterranean fruit fly larvae with fly emergence percentages < 95% were discarded and not used in the assays. Colonies of *D. longicaudata*, GSS Vienna-8, and WBS used in the experiments were at their 20th, 30th, and 1st generations under rearing conditions, respectively.

Experimental setup

Trials were performed under controlled environmental conditions (24 ± 1°C; 65 ± 5% RH and 12:12 (L:D) h) at the PRL, San Juan biofactory. Mediterranean fruit fly larvae of both strains aged 6 d-old (middle third-instars) were exposed to 20, 40, 60, 80, 100, and 120 Gy. Non-irradiated host larvae were also included in the experiments as control test. A quarter of a liter of naked, without artificial rearing diet, host larvae of both strains separately placed into a 3,000 ml-hard plastic flask were irradiated with each radiation dose aforementioned. Larvae of both Mediterranean fruit fly strains were washed with fresh water only to completely eliminate the rearing diet. The depth of the irradiated host larvae in each flask was 21 cm, which was equivalent to 15,000 Mediterranean fruit fly larvae. Host irradiation was performed in an IMO-1 mobile irradiator with a Co-60 source of γ radiation, located in San Juan biofactory and belongs to the National Atomic Energy Commission (Argentina). The radiation doses were applied at a rate of 23.6 Gy/min under oxygen-free. Exposure times were determined with Fricke dosimeters (IAEA 2001). For each treatment, equivalent to a certain irradiation dose, 1,000 naked Mediterranean fruit fly larvae, either the WBS or GSS Vienna-8, were exposed to 100 naïve, mated, 6- to 8-d-old *D. longicaudata* females inside a 10 × 1 cm (diameter by height) organdy screen-covered dishes on top of a cubical Plexiglas cage (30 × 30 × 30 cm). A constant 10:1 host/parasitoid ratio was used. Host exposure time was 90 min. The larval host age and host exposure time were based on results previously published by Suárez et al. (2019). The larval mean weight (±ES) of both GSS Vienna-8 and WBS used in the study was 10.59 ± 0.04 mg (n = 100) and 12.56 ± 0.05 mg (n = 100), respectively. After exposure, larvae were transferred into 10 × 13 cm (diameter × height) plastic cups with 2 cm layer of sterilized poplar shavings and covered with organdy. Treatments and controls were replicated 10 times. Separate batches of host larvae and parasitoid females were used in each replicate.

Of the total puparia number found inside the plastic cups, 20 were taken per replicate and per treatment and not allowed to develop until adult emergence, but 5 puparia were dissected 24, 48, 72, and 96 h after last larval exposure to parasitoid females, respectively. Dissections were made by using a stereomicroscope Leica EZ4 40x (Bio-Optic S.R.L., Vicente López, Buenos Aires, Argentina). In some host puparia, both parasitoid larval first-instars (= L_1) and second-instars (= L_2) were found. Eggs and larval instars of *D. longicaudata* were determined as described by Ibrahim et al. (1994). Size and shape of mandibles were used as basic features to separate instars. The presence, number, and condition of parasitoid eggs, L_1 and L_2 larvae, such as alive, dead without encapsulation, and dead with encapsulation process, were determined. A larva was considered dead without encapsulation when it either did not move or was damaged. Eggs were considered dead without encapsulation either when no embryo was observed or when the egg was collapsed without having hatched. The degree of melanization was also evaluated for those encapsulated *D. longicaudata* L_1. Three levels of melanization in parasitoid larvae were determined as follows: low melanized level (= %LML), when the host larva body was melanized between 1 and 25%; medium melanized level (= %MML), corresponding to 26–50% of melanized host body; and high melanized level (= %HML), equivalent to 51–100% of melanized host body (Fig. 1). The remaining pupae were kept in the plastic cups at 24 ± 1°C and 65 ± 5% RH until fly and/or parasitoid emergence. Both

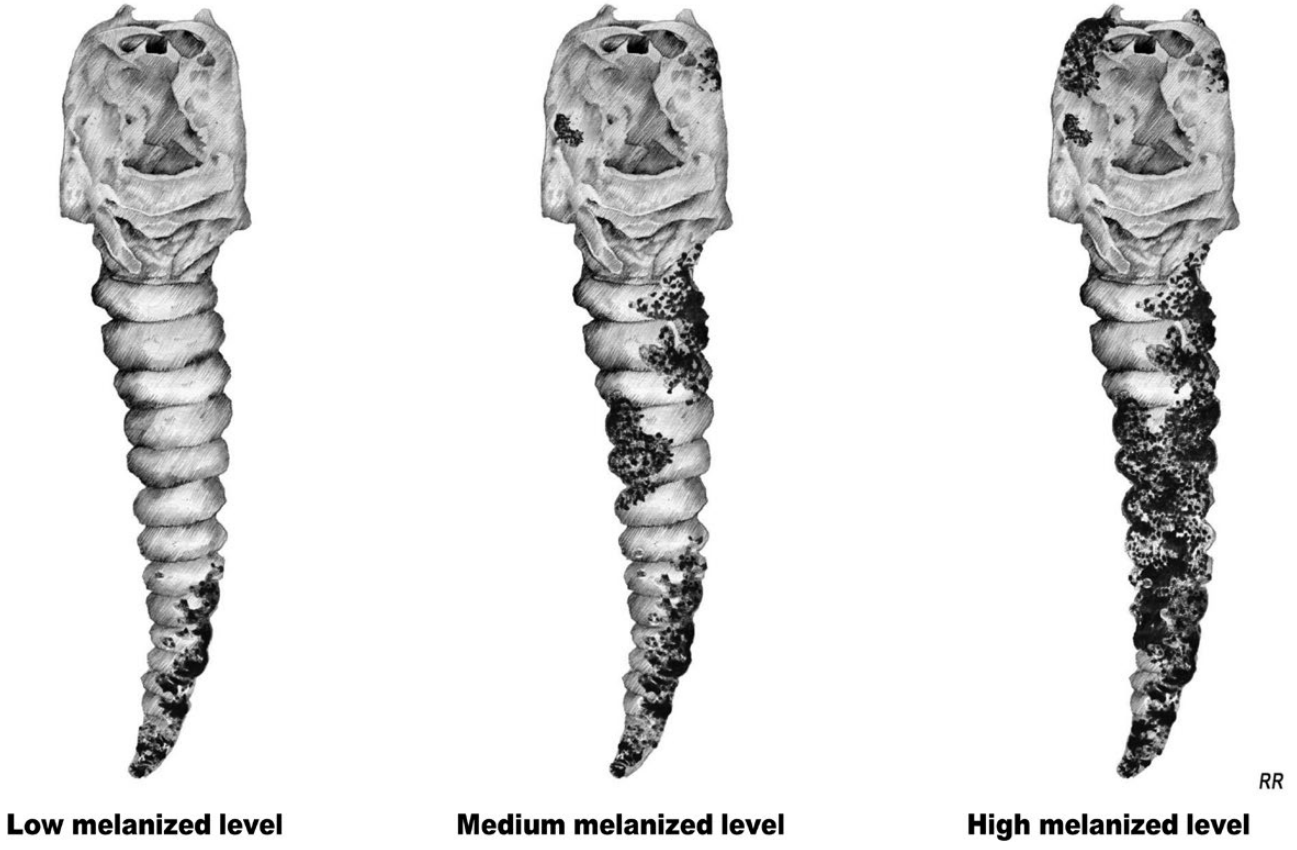

Fig. 1. Schematic representation of the gradual process of melanization in an encapsulated first-instar larva of *Diachasmimorpha longicaudata*. Melanization levels: low melanized level = when the host larva body was melanized between 1 and 25%; medium melanized level = 26–50%; high melanized level = 51–100%.

the number and sex of parasitoid offspring, the number of flies, and the number of non-emerged puparia were recorded. After the insect emergence was completed, the non-emerged puparia were dissected to check for the presence or absence of recognizable immature parasitoid stages and/or fully developed pharate-adult parasitoids. For data assessment, adult fly and parasitoid emergences, parasitism, parasitoid offspring sex ratio, and host mortality were estimated. The percentage of fly emergence was calculated as the number of emerged adult divided by the total number of offered pupae × 100; parasitoid emergence was calculated as the number of emerged adult divided by the total number of offered pupae × 100; parasitism percentage was calculated as the number of emerged adult parasitoids plus the number of non-emerged parasitoids divided by the total number of pupae recovered from the test fruit × 100; offspring sex ratio was calculated as the fraction of daughter over total parasitoid offspring; the host mortality was determined as the number of dead host larvae plus the number of puparia that did not yield insects divided by the total number of host larvae exposed to parasitoids × 100.

Data Analysis

The relationships between the percentage of encapsulated parasitoid larvae (%EPL) and the different levels of melanization processes (LML, MML, and HML) in L_1 parasitoid larvae, as well as between the mean number of both parasitoid L_1 and L_2 per Mediterranean fruit fly puparium, and the superparasitism were analyzed by the Pearson product–moment correlation test ($P = 0.05$). The %EPL was also correlated with percentages of fly and parasitoid emergence, as well as parasitism. Furthermore, the relationships between the percentage of fly emergence with percentages of parasitoid emergence, parasitism, offspring parasitoid females, and host mortality were also analyzed by the Pearson product–moment correlation test ($P = 0.05$). Response variables with significant correlations between them were evaluated by two-way multivariate general linear models (GLMs) with type III error at $P = 0.05$. Response variables with no significant correlations were evaluated by two-way univariate GLMs. These analyses allowed the identification of significant effects of the two categorical factors of the models, namely, radiation doses (= RD) and Mediterranean fruit fly strains (= MS), and their interaction. Mean comparisons were analyzed by Tukey's honestly significant difference (HSD) test ($P = 0.05$). Given the lack of normality, data were rank transformed prior to analyses (Conover and Iman 1981), but untransformed means (±SE) were used in figures. Statistical analyses were performed by using STATISTICA software, version 10.0 (StatSoft 2011).

Results

Mediterranean Fruit Fly Puparia Dissection: Determination of *D. longicaudata* Parasitoid Encapsulation

Significant positive correlations were found between the %EPL and the different levels of melanization processes in parasitoid L_1, as well as between the mean number of parasitoid L_1 per Mediterranean fruit fly puparium (LML, $r = 0.6265$; $n = 140$; $P < 0.0001$; MML, $r = 0.5708$; $n = 140$; $P < 0.0001$; HML, $r = 0.4121$; $n = 140$; $P < 0.0001$; N° L_1, $r = 0.5394$; $n = 140$; $P < 0.0001$). A significant negative correlation was found between %EPL and the mean number of parasitoid L_2 per Mediterranean fruit fly puparium ($r = -0.2932$; $n = 140$; $P < 0.0001$). No correlation was found between %EPL and superparasitism ($r = -0.1545$; $n = 140$; $P < 0.068$).

A very low number of parasitoid eggs in encapsulation process was only found in WBS, both 48 and 72 h after host puparia dissection in the control test, as well as in 20 and 40 Gy-irradiated larvae (Table 1). Dead *D. longicaudata* L1 were essentially found by partial or total encapsulation in both WBS (Table 1) and GSS Vienna-8 (Table 2). A smaller number of encapsulated L_1 were found in GSS Vienna-8 (Table 2).

The multivariate GLMs showed significant effects of both fixed factors and the interaction between them on the tested response variables, named %EPL, %LML, %MML, and %MML (RD, Wilks' $\lambda = 0.2454$; $F = 5.5429$; df = 36,534; $P < 0.0001$; MS, Wilks'$\lambda = 0.5995$; $F = 13.4697$; df = 6,121; $P < 0.0001$; RD × MS, Wilks' $\lambda = 0.4252$; $F = 3.1895$; df = 36,534; $P < 0.0001$). Higher %EPL values were found in non-irradiated host larvae in both *C. capitata* strains when both fixed factors interacted ($F = 3.3449$; df = 6,126; $P = 0.0043$). The WBS showed the highest %EPL values regarding GSS Vienna-8 when 0, 20 and 40 Gy were compared to each other (Fig. 2). The %EPL values recorded for 60, 80, 100, and 120 Gy were similar in both Mediterranean fruit fly strains (Fig. 2). The three evaluated melanization levels varied significantly due to the interaction between RD and MS (LML, $F = 4.8395$; df = 6,126; $P = 0.0001$; MML, $F = 3.8754$; df = 6,126; $P = 0.0014$; MML, $F = 4.6914$; df = 6,126; $P = 0.0002$). LMLs were mainly found in *D. longicaudata* L_1 in both Mediterranean fruit fly strains. Approximately 85% of encapsulated larvae were at LML, while the remaining 13 and 2% were at MML and HML, respectively. A higher LML value was recorded in the control test when the GSS Vienna-8 was evaluated, whereas no differences were found between the control and 20, 40, and 80 Gy in the WBS (Fig. 3). A higher MML occurred in the control tests in both Mediterranean fruit fly strains (Fig. 3). A HML was only found in non-irradiated WBS larvae (Fig. 3).

A high percentage of superparasitized host pupae was found in both Mediterranean fruit fly strains (Fig. 4). The RD factor and the interaction between RD and MS had a significant effect on superparasitism (RD, $F = 2.4730$; df = 6,126; $P = 0.0269$; RD × MS, $F = 2.1880$; df = 6,126; $P = 0.0483$), while the MS factor did not ($F = 0.6690$; df = 1,126; $P = 0.4148$). A lower percentage of superparasitism was recorded in the control test of the WBS, although it was similar to the percentage values of 40, 60 and 80 Gy in both Mediterranean fruit fly strains (Fig. 4). The interaction between RD and MS was not a significant predictor of the mean number of *D. longicaudata* L_1 and L_2 found in dissected pupae of both GSS Vienna-8 and WBS (L_1, $F = 1.9560$; df = 6,126; $P = 0.0768$; L_2, $F = 2.0710$; df = 6,126; $P = 0.0613$).

Adult Fly and Parasitoid Emergences, Parasitism, Offspring Sex Ratio and Host Mortality

A positive correlation was found between the %EPL and the percentage of adult fly emergence ($r = 0.4194$; $n = 140$; $P < 0.0001$), but not in relation to both percentages of adult parasitoid emergence and parasitism ($r = -0.1389$; $n = 140$; $P = 0.102$, and $r = -0.1432$; $n = 140$; $P = 0.091$, respectively). Negative correlations were found between fly emergence and both parasitoid emergence ($r = -0.4232$; $n = 140$; $P < 0.0001$) and parasitism ($r = -0.4302$; $n = 140$; $P < 0.0001$). No correlation was found between fly emergence and percentages of both offspring females ($r = -0.1564$; $n = 140$; $P = 0.065$) and host mortality ($r = -0.1147$; $n = 140$; $P = 0.177$).

The multivariate GLMs showed significant effects of the RD (Wilks' $\lambda = 0.0798$; $F = 28.181$; df = 18,351.2; $P < 0.0001$) and the interaction between both RD and MF on evaluated response variables (Wilks' $\lambda = 0.6178$; $F = 3.6210$; df = 18,351.2; $P < 0.0001$).

Table 1. Mean number (±SE) of alive or dead *D. longicaudata* immature stages hosted inside *C. capitata* puparia originated from non-irradiated and irradiated larvae of a wild bisexual strain (WBS) dissected at different levels of host development

		Status of parasitoid immature stages with or without encapsulation (= E)								
		Egg			1st instar			2nd instar		
Radiation dose	Dissected puparium age	Alive	Dead-no E	Dead-with E	Alive	Dead-no E	Dead-with E	Alive	Dead-no E	Dead-with E
0 Gy	24 h	1.8 ± 0.4	0.2 ± 0.2	0	0	0	0	0	0	0
	48 h	1.4 ± 0.2	0.8 ± 0.2	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0	0	0	0
	72 h	0.4 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	1.4 ± 0.5	0.6 ± 0.4	1.0 ± 0.0	0	0	0
	96 h	0	0	0	0.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.5	1.2 ± 0.2	0	0
20 Gy	24 h	1.6 ± 0.2	0.2 ± 0.2	0	0	0	0	0	0	0
	48 h	2.0 ± 0.3	0.5 ± 0.2	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0	0	0	0
	72 h	0.2 ± 0.2	0	0	1.2 ± 0.5	0.8 ± 0.4	0.8 ± 0.2	0.4 ± 0.2	0	0
	96 h	0	0	0	0.2 ± 0.2	0.6 ± 0.2	0.8 ± 0.4	0.8 ± 0.2	0.2 ± 0.2	0
40 Gy	24 h	1.4 ± 0.4	0	0	0	0	0	0	0	0
	48 h	1.8 ± 0.4	0.6 ± 0.2	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0	0	0
	72 h	0.2 ± 0.2	0	0	1.4 ± 0.2	0.8 ± 0.4	1.4 ± 0.4	0	0	0
	96 h	0	0	0	0	1.4 ± 0.2	1.6 ± 0.5	1.2 ± 0.2	0.2 ± 0.2	0
60 Gy	24 h	1.2 ± 0.2	0	0	0	0	0	0	0	0
	48 h	1.4 ± 0.2	0.4 ± 0.2	0	0	0.2 ± 0.2	0	0	0	0
	72 h	0.2 ± 0.2	0	0	1.6 ± 0.5	1.4 ± 0.7	1.0 ± 0.3	0.2 ± 0.2	0	0
	96 h	0	0.2 ± 0.2	0	0	1.6 ± 0.2	1.2 ± 0.5	1.4 ± 0.2	0	0
80 Gy	24 h	1.6 ± 0.2	0	0	0	0	0	0	0	0
	48 h	1.6 ± 0.2	0.2 ± 0.2	0	0	0	0	0	0	0
	72 h	0	0.4 ± 0.4	0	2.0 ± 0.5	1.6 ± 0.7	0.6 ± 0.2	0.2 ± 0.2	0	0
	96 h	0	0.2 ± 0.2	0	0.2 ± 0.2	2.6 ± 0.7	0.8 ± 0.4	1.2 ± 0.2	0.2 ± 0.2	0
100 Gy	24 h	1.2 ± 0.2	0	0	0	0	0	0	0	0
	48 h	1.6 ± 0.4	0	0	0	0	0	0	0	0
	72 h	0	0.2 ± 0.2	0	2.6 ± 0.7	1.2 ± 0.5	0.2 ± 0.2	0	0	0
	96 h	0	0	0	0	1.4 ± 0.2	0.2 ± 0.2	1.0 ± 0.0	0	0
120 Gy	24 h	1.4 ± 0.4	0	0	0	0	0	0	0	0
	48 h	1.6 ± 0.2	0.4 ± 0.2	0	0	0.2 ± 0.2	0	0	0	0
	72 h	0	0.6 ± 0.2	0	2.2 ± 0.6	0.8 ± 0.8	0.2 ± 0.2	0.2 ± 0.2	0	0
	96 h	0	0	0	0	2.0 ± 0.3	0.2 ± 0.2	1.2 ± 0.2	0	0

The effect of the MS on all response variables was not significant (Wilks' λ = 0.9965; F = 0.1420; df = 3,124; P = 0.9346). Significant higher percentages of emerged adult medflies were found in both controls compared with 20–60 Gy (RD × MS, F = 7.9690; df =6,126; P < 0.0001) (Table 3). Percentages of parasitoid emergence and parasitism were influenced by the RD (F = 9.2712; df =6,126; P < 0.0001 and F = 9.0925; df = 6,126; P < 0.0001, respectively). The higher percentages of emerged parasitoid and parasitism rate were recorded at 80 and 100 Gy (Table 3). The sex ratio of the parasitoid offspring was not affected by any fixed factor (RD, F = 1.7240; df = 6,126; P = 0.1206; MS, F = 0.2740; df = 1,126; P = 0.6012) or by their interaction (RD × MS, F = 0.5980; df = 6,126; P = 0.7318). Both male-biased sex ratio (0.9 female: 1 male) at 20 and 120 Gy, and female-biased sex ratio (1.2:1) at 0, 40–100 Gy were found (Table 3). The host mortality percentage was only influenced by the RD (F = 4.5670; df = 6,126; P = 0.0003). The highest host mortality percentage was recorded at 120 Gy, but the value did not differ from those recorded for 20, 40, and 60 Gy (Table 3).

Discussion

The *D. longicaudata* first-larval instar was only found encapsulated by *C. capitata* larvae of both strains tested. Capsules that covered the body of parasitoid L_1 partially or totally were always found in a melanization process. Eggs and parasitoid second instar larvae probably escaped from host immune response. Parasitoid eggs were neither found encapsulated nor in a partial melanization process 24 h after host dissection. This fact may be due to a quick development of the egg inside the host larva. Ninety percent of *D. longicaudata* eggs that were examined 48 h after parasitism had already hatched, whereas less than 3% were encapsulated. Therefore, parasitoid L_1 was easily found 48 h later. This finding indicates that fast egg parasitoid hatching may be a strategy to avoid the host`s immune system response, which is a time-consuming process (Pennacchio et al. 2006). In turn, this fact may be accompanied by the effect of symbiotic viruses, virus-like particles, parasitoid ovarian proteins, and particles on the parasitoid egg surface working alone or in combination to prevent egg parasitoid recognition by the host`s immune system (Fedderson et al. 1986, Lawrence 2002, Lawrence and Matos 2005, Strand 2008, Renault 2012). The presence of a symbiotic entomopoxvirus in the venom apparatus of *D. longicaudata* has also been pointed out as preventing encapsulation in eggs of this braconid parasitoid. This virus is introduced into host larvae during oviposition (Lawrence 2005).

Studies with the larval-pupal neotropical parasitoids *Doryctobracon brasiliensis* (Szépligeti) and *Opius bellus* Gahan attacking larvae of a wild *C. capitata* strain showed that their eggs and first-instars were partially encapsulated at low rates under natural conditions, but the encapsulation ratio increased considerably under controlled laboratory conditions (Córdova-García 2008). A similar finding was recorded by Fellowes et al. (1998). These authors found low encapsulation rates in both parasitoids *Asobara tabida* (Nees)

Table 2. Mean number (±SE) of alive or dead *D. longicaudata* immature stages hosted inside of *C. capitata* puparia originated from non-irradiated and irradiated larvae of Vienna-8 temperature-sensitive lethal genetic sexing strain (GSS) dissected at different levels of host development

		Status of parasitoid immature stages with or without encapsulation (= E)								
		Egg			1st instar			2nd instar		
Radiation dose	Dissected puparium age	Alive	Dead-no E	Dead-with E	Alive	Dead-no E	Dead-with E	Alive	Dead-no E	Dead-with E
0 Gy	24 h	2.8 ± 0.5	0.2 ± 0.2	0	0	0	0	0	0	0
	48 h	1.6 ± 0.4	0.4 ± 0.3	0	0.6 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0	0	0
	72 h	0.8 ± 0.4	0.6 ± 0.4	0	2.4 ± 0.4	1.2 ± 0.2	0.8 ± 0.2	0.2 ± 0.2	0	0
	96 h	0	0	0	0.2 ± 0.2	1.4 ± 0.3	0.6 ± 0.4	0.8 ± 0.2	0.2 ± 0.2	0
20 Gy	24 h	2.6 ± 0.2	0.2 ± 0.2	0	0	0	0	0	0	0
	48 h	1.8 ± 0.4	0.6 ± 0.3	0	0.6 ± 0.4	0.2 ± 0.2	0	0	0	0
	72 h	0.4 ± 0.2	0.6 ± 0.4	0	2.2 ± 0.4	1.0 ± 0.2	0.6 ± 0.2	0.4 ± 0.2	0	0
	96 h	0	0	0	0.4 ± 0.2	1.6 ± 0.2	0.6 ± 0.4	1.2 ± 0.2	0	0
40 Gy	24 h	2.0 ± 0.4	0	0	0	0	0	0	0	0
	48 h	1.6 ± 0.2	0.2 ± 0.2	0	0.2 ± 0.2	0	0	0	0	0
	72 h	0.2 ± 0.2	0.6 ± 0.2	0	2.4 ± 0.7	1.2 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0	0
	96 h	0	0	0	0	2.2 ± 0.2	0.2 ± 0.2	1.0 ± 0.0	0.2 ± 0.2	0
60 Gy	24 h	1.6 ± 0.4	0	0	0	0	0	0	0	0
	48 h	2.6 ± 0.9	0.4 ± 0.2	0	0.2 ± 0.2	0.2 ± 0.2	0	0	0	0
	72 h	0.4 ± 0.2	0.2 ± 0.2	0	2.0 ± 0.3	0.8 ± 0.4	0	0.2 ± 0.2	0	0
	96 h	0	0	0	0	2.4 ± 0.5	0	1.2 ± 0.2	0.4 ± 0.2	0
80 Gy	24 h	1.4 ± 0.4	0	0	0	0	0	0	0	0
	48 h	1.6 ± 0.2	0.6 ± 0.2	0	0	0.2 ± 0.2	0	0	0	0
	72 h	0	0	0	2.6 ± 0.4	0.2 ± 0.2	0	0.2 ± 0.2	0	0
	96 h	0	0	0	0	1.6 ± 0.2	0	1.2 ± 0.2	0.2 ± 0.2	0
100 Gy	24 h	1.2 ± 0.4	0	0	0	0	0	0	0	0
	48 h	2.2 ± 0.2	0.4 ± 0.2	0	0	0	0	0	0	0
	72 h	0	0.4 ± 0.4	0	2.2 ± 0.7	1.2 ± 0.7	0	0.2 ± 0.2	0	0
	96 h	0	0	0	0	2.4 ± 0.4	0	1.0 ± 0.0	0	0
120 Gy	24 h	1.4 ± 0.2	0	0	0	0	0	0	0	0
	48 h	1.8 ± 0.2	0.2 ± 0.2	0	0	0	0	0	0	0
	72 h	0	0.4 ± 0.2	0	2.4 ± 1.0	0.8 ± 0.4	0	0.4 ± 0.2	0	0
	96 h	0	0	0	0	1.6 ± 0.2	0	1.0 ± 0.0	0	0

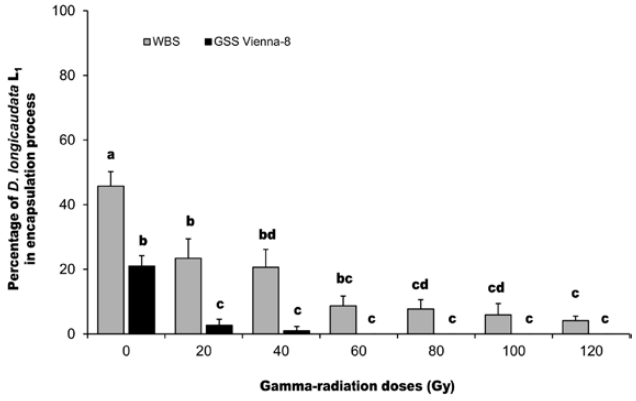

Fig. 2. Percentage of first-instars larvae of *Diachasmimorpha longicaudata* in encapsulation process related to both different gamma-radiation doses and Mediterranean fruit fly strains. Bars with the same letter indicate no significant differences (Tukey's HSD test, $P = 0.05$). Notations: WBS = *Ceratitis capitata* wild bisexual strain, GSS Vienna-8 = *C. capitata* Vienna-8 temperature-sensitive lethal genetic sexing strain.

and *Leptopilina boulardi* (Barbotin, Carton, and Kelner-Pillault) inside larvae of a wild strain of *Drosophila melanogaster* Meigen recently colonized under lab-conditions; from the 5th generation onwards, however, encapsulation ratios increased 12 and 45 times, respectively. Several rearing conditions, such as temperature and host physiological age and state, may have an effect on the incidence of encapsulation in lab-rearing parasitoids (Blumberg 1997). In the present study, both Mediterranean fruit fly strains tested were kept at the same environmental lab-conditions and host larvae used in assays were the same ages, but there were differences in the generational age between strains. The GSS Vienna-8 was 29 times older than WBS; however, non-irradiated WBS larvae displayed a higher incidence of encapsulation than GSS Vienna-8 larvae. Blumberg (1997) highlighted the host origin or strain as a feasible cause of variation in the encapsulation ratio.

The low melanized level in encapsulated *D. longicaudata* L_1 would be the most common melanization process at 72 h puparium dissection in both non-irradiated and irradiated GSS Vienna-8 larvae, as well as in irradiated WBS larvae. However, both low and medium melanized degrees were similarly abundant in non-irradiated WBS larvae. Besides, encapsulated parasitoid L_1 entirely melanized was only found in these host larvae. However, increasing radiation doses substantially reduced the melanization process in parasitoid L_1. In fact, melanization activity reduction was more noticeable in the GSS Vienna-8 than in WBS larvae from 20 Gy onwards. These data would indicate that irradiated GSS Vienna-8 larvae can neutralize the host immunological reactions over irradiated WBS larvae much more quickly. It was evident that WBS larvae were more sensitive to radiation. This may be related to the larger size of WBS larvae

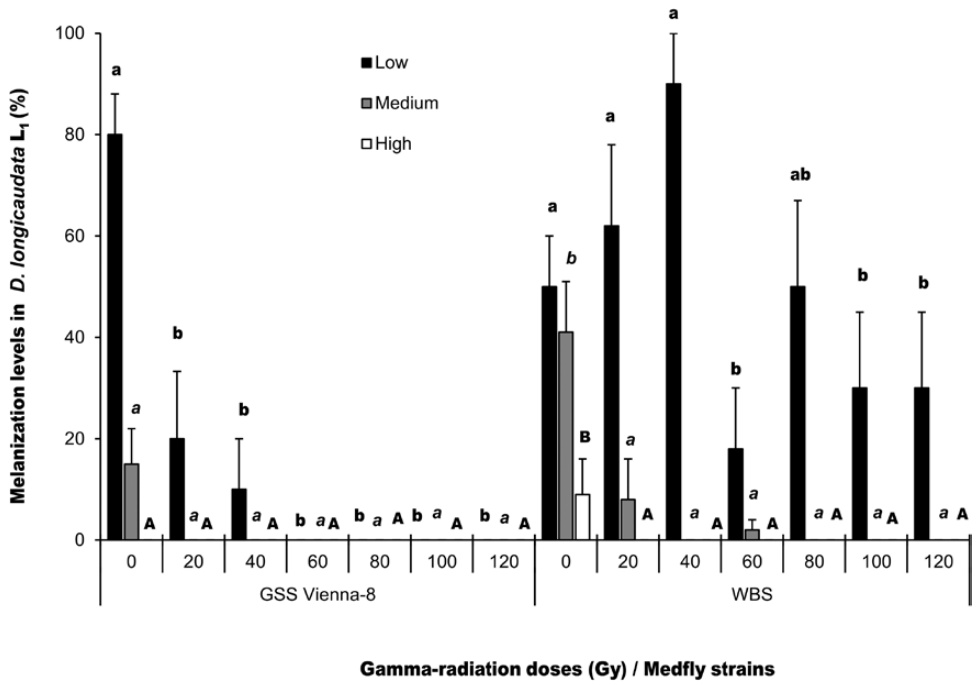

Fig. 3. Percentage of encapsulated first-instars larvae of *Diachasmimorpha longicaudata* with different melanization levels related to both gamma-radiation doses and Mediterranean fruit fly strains. Bars with the same letter indicate no significant differences (Tukey's HSD test, $P = 0.05$). Notations: WBS = *Ceratitis capitata* wild bisexual strain, GSS Vienna-8 = *C. capitata* Vienna-8 temperature-sensitive lethal genetic sexing strain.

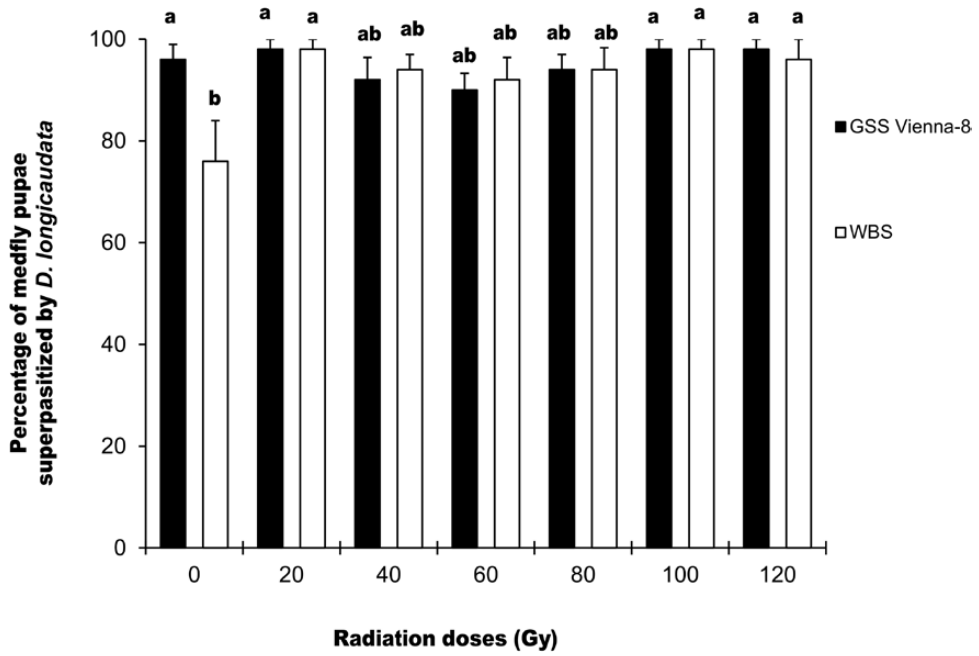

Fig. 4. Percentage of *Ceratitis capitata* pupae superparasitized by *Diachasmimorpha longicaudata* related to both different gamma-radiation doses and Mediterranean fruit fly strains. Bars with the same letter indicate no significant differences (Tukey's HSD test, $P = 0.05$). Notations: WBS = *Ceratitis capitata* wild bisexual strain, GSS Vienna-8 = *C. capitata* Vienna-8 temperature-sensitive lethal genetic sexing strain.

compared to GSS Vienna-8 larvae. Larger organisms are usually more susceptible to radiation (Cancino et al. 2009b, Mastrangelo and Walder 2011). Radiation may induce physiological changes that involve harmful alterations of the immune response of tephritid fruit fly larvae, such as a reduction in the action of phenoloxidase (Nation et al. 1995, Mansour and Franz 1996, Chang et al. 2016, Tariq et al. 2017), considered as a melanin-generating enzyme (Liu et al. 2007). Thus, in lab-reared irradiated *Anastrepha suspensa* (Loew) larvae, a greatly decreased phenoloxidase enzyme activity was verified at ≥20 Gy (Nation et al. 1995).

Although the largest decrease of immune activity in GSS Vienna-8 larvae contrasted with WBS larvae, both *D. longicaudata* emergence and parasitism percentages were remarkably similar in both Mediterranean fruit fly strains. Probably, there was a trade-off

Table 3. Mean percentages (± SE) of fly emergence, *D. longicaudata* adult emergence, parasitism, parasitoid female offspring, and host mortality recorded from irradiated and non-irradiated 6 d-old larvae of both Vienna-8 temperature-sensitive lethal genetic sexing (GSS Vienna-8) and wild bisexual (WBS) *C. capitata* strains

Mediterranean fruit fly strain/ radiation dose	Biological parameters (Mean ± SE)				
	Fly emergence (%)	Parasitoid emergence (%)	Parasitism (%)	Female offspring (%)	Host mortality (%)
GSS Vienna-8					
0 Gy	88.5 ± 5.2a	42.6 ± 3.7a	46,9 ± 3.8a	51.7 ± 2.3a	38.9 ± 3.1a
20 Gy	12.5 ± 2.3b	38.1 ± 4.8a	39.8 ± 5.6a	43.5 ± 5.2a	49.8 ± 3.5a
40 Gy	8.6 ± 1.5b	42.1 ± 2.8ab	45.7 ± 2.6ab	50.8 ± 4.8a	49.5 ± 2.6a
60 Gy	0.8 ± 0.5c	52.6 ± 5.1bc	56.1 ± 5.1bc	50.7 ± 4.2a	47.2 ± 5.0a
80 Gy	0.0 ± 0.0d	56.2 ± 3.9c	58.3 ± 3.9bc	51.7 ± 2.0a	43.8 ± 3.9a
100 Gy	0.0 ± 0.0d	57.7 ± 3.5c	59.9 ± 3.6c	57.5 ± 2.0a	42.3 ± 3.5a
120 Gy	0.0 ± 0.0d	41.8 ± 3.3a	44.5 ± 3.2a	46.0 ± 2.8a	60.2 ± 4.3a
WBS					
0 Gy	82.2 ± 6.2a	38.5 ± 2.7a	42.4 ± 2.7a	53.2 ± 2.1a	49.3 ± 2.8a
20 Gy	8.4 ± 1.2b	46.6 ± 1.2ab	48.5 ± 1.1ab	49.2 ± 2.9a	45.0 ± 2.5a
40 Gy	5.4 ± 1.5be	44.1 ± 1.9a	46.5 ± 1.9ab	53.1 ± 2.8a	50.5 ± 2.8a
60 Gy	3.2 ± 1.3ce	46.9 ± 3.8ab	48.7 ± 3.9ab	51.8 ± 6.3a	50.7 ± 3.9a
80 Gy	0.0 ± 0.0d	59.1 ± 3.6c	63.5 ± 3.8c	53.2 ± 2.1a	41.0 ± 3.8a
100 Gy	0.0 ± 0.0d	55.7 ± 3.1c	56.6 ± 3.0bc	51.0 ± 1.9a	44.3 ± 3.0a
120 Gy	0.0 ± 0.0d	45.9 ± 3.5ab	50.1 ± 3.4ab	48.1 ± 2.9a	54.1 ± 3.5a

Means followed by different letters in the same column differed significantly (Tukey's HSD test, $P = 0.05$).

occurring between larger sizes of WBS larvae and depressed host immune activity found in GSS Vienna-8 larvae, besides a good rearing quality of both host strains. The existence of trade-off occurring during host-parasitoid interactions according to host quality related to its size was demonstrated by Liu et al. (2011). In turn, high levels of superparasitism found in non-irradiated, as well as in irradiated larvae, of both GSS Vienna-8 and WBS seemingly help to overcome the host's immune reaction by the surviving parasitoid larvae. In addition, superparasitism intensity was not affected by radiation. Thus, self-superparasitism would be a recurrent innate strategy to successfully parasitize fruit fly larvae under field, as well as laboratory conditions (González et al. 2010, Montoya et al. 2011). Therefore, superparasitism may similarly benefit both parasitoid emergence and parasitism rates in both tested Mediterranean fruit fly strains.

Parasitoid emergence and parasitism increased from 60 Gy in regard to non-irradiated larvae of both Mediterranean fruit fly strains, with a considerably higher increase at 80 and 100 Gy. Similar outcomes with *D. longicaudata* have been recently reported by Suárez et al. (2019) using gamma-irradiated GSS Vienna-8 larvae under mass-rearing conditions. These authors demonstrated that radiation doses beyond 90 Gy neither increased the parasitoid yield nor improve the female offspring ratio. Related studies also showed increased *D. longicaudata* emergence rates following exposure of gamma-irradiated *C. capitata* larvae (Valle 2006); similar results were obtained for both *C. capitata* and *Anastrepha fraterculus* (Wiedemann) larvae with X-ray doses between 20 and 100 Gy under experimental lab-conditions (Viscarret et al. 2012, Bachmann et al. 2015). Gamma radiation studies on several lepidopterous species showed that parasitism rate and parasitoid production in the braconid *Habrobracon hebetor* (Say) were increased as the host larvae was irradiated with higher gamma-radiation doses (Hasan et al. 2019).

Interestingly, in both Mediterranean fruit fly strains, parasitoid emergence decreased while host mortality increased at 120 Gy relative to both 80 and 100 Gy doses. This finding may be due to the radiation exposure time to which the host larva is subjected. Higher radiation doses usually require prolonged exposure time, which may cause the body of the host larvae to overheat, with adverse implications for their survival (Cancino et al. 2012). In addition, the lowest and the highest radiation dose used in the trials, 20 and 120 Gy respectively, produced a slightly male-biased sex ratio. Increased host larval mortality and decreased parasitoid female offspring after exposure time are key parameters to evaluate both quality and health of the mass-reared parasitoid production (Messing et al. 1993, Cancino et al. 2002). Nonetheless, parasitoid offspring sex ratio was not affected either by the radiation dose or by the Mediterranean fruit fly strain, and it was practically 1:1 female per male. Concerning host larval mortality, current results, with host emergence wholly suppressed from 80 Gy onwards, match with data previously reported by Suárez et al. (2019), who recommended high radiation doses in mass-reared GSS Vienna-8 larvae to prevent fly emergence. However, Cancino et al. (2009b) reported lower radiation doses were necessary to completely suppress adult Mediterranean fruit fly emergence. In both the present study and that of Suarez et al. (2019) batches with a very high number of Mediterranean fruit fly larvae, were irradiated and used in the essays, while Cancino et al. (2009b) carried out tests with batches of only 100 larvae that were previously gamma-irradiated. Probably, the great difference between the current results and those by Cancino et al. (2009b) is due to the volume of irradiated larvae. It is well known that large volumes of biological material require a higher effective dose radiation (Cancino et al. 2002, Mastrangelo and Walder 2011, Cancino et al. 2012). Another explanatory factor of the aforementioned differences is the fly strain or the fly larval age used in the radiation process, which can also influence radiation dosage (Hepdurgun et al. 2009a, b; Hasan et al. 2009; Yokoyama et al. 2010; Cancino et al. 2012). Cancino et al. (2009b) carried out trials with irradiated 6- to 7-d-old larvae of a Mexico-native Mediterranean fruit fly bisexual strain, whereas in the current study, irradiated 6-d-old larvae of a wild bisexual strain from San Juan, as well as a GSS were used. Presence of water molecules on fly larvae or the larval rearing diet may also affect radiation dosage (Follett and Armstrong 2004). However, Mediterranean fruit fly larvae previously washed with fresh water to eliminate rearing diet scraps

were irradiated and used in both the present study and that of Cancino et al. (2009b).

In brief, to provide advantages to avoid non-parasitized host emergence and to stimulate the parasitoid emergence rate increase, radiation in GSS Vienna-8 larvae may favor the host's antagonistic reaction decrease towards *D. longicaudata* development. Results of the present study showed that batches with a high number of washed GSS Vienna-8 larvae, e.g., 15,000 larvae, aged 6 d old, and gamma-irradiated at 80–100 Gy significantly enhance parasitoid production. Therefore, radiation use, which is part of the *D. longicaudata* rearing managing process, may be added to biological strategies of the parasitoid to evade host immune response, such as a superparasitism trend (Montoya et al. 2012) and the injection of a symbiont virus at the time of egg insertion in the host larva (Lawrence 2005). These mechanisms may jointly favor *D. longicaudata* mass production. The suitability of irradiated GSS Vienna-8 larvae to rear *D. longicaudata* allows integration of SIT with the parasitoid mass production in the Biofactory. Whereas the first and second batches, meanly composed by males, are used for irradiation, GSS Vienna-8 larvae of the third batch that drop out of the larvae rearing tray are mostly female, and a waste product. Therefore, larvae coming from the third batch may be used for parasitoid mass rearing. From an area-wide integrated Mediterranean fruit fly management approach, the combination of augmentative release of parasitoids and sterile medflies (Wong et al. 1992, Rendon et al. 2006) may be a most effective strategy to achieve suppression of *C. capitata* in fruit-growing regions of Argentina.

Acknowledgments

Special thanks to Andrés Murúa Trincado for drawing the melanization degrees in parasitoid larvae, to Julian Ledesma (PROIMI Biotecnología) for improving graphics, to Alejandra Molina (DSVAA, San Juan) for the technical assistance and to Luisa Montivero for the English review. Financial supports were provided by the International Atomic Energy Agency (IAEA) - Research Contract No. 20562 'Use of Radiation to Reduce Host Antagonism Reaction to Fruit Fly Parasitosis Attacks', by the San Juan Eradication and Control Mediterranean fruit fly Program (ProCEM San Juan, Government of San Juan, Argentina), and by both the Secretariat of Science, Technology and Innovation of the Government of San Juan (SECITI) and the National Council for Scientific and Technical Research (CONICET, Argentina) - (grant PIO/2015–2016 No. 1502015 0100016 co, Res. No. 2584/16).

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