Host Suitability and Fitness-Related Parameters in *Coptera haywardi* (Hymenoptera: Diapriidae) Reared on Irradiated *Ceratitis capitata* (Diptera: Tephritidae) Pupae Stemming From the *tsl* Vienna-8 Genetic Sexing Strain

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Abstract

*Coptera haywardi* (Ogloblin) is a pupal endoparasitoid of tephritid flies with great potential as a biological control agent worldwide as it does not attack other Diptera. To reach its full potential, its mass rearing needs to be enhanced lowering costs. Here, we focused on the use of irradiated pupae of *Ceratitis capitata* (Wiedemann) stemming from the temperature-sensitive lethal (tsl) Vienna-8 genetic sexing strain (= *Cc* Vienna-8), which is mass-produced in the San Juan Medfly and Parasitoid Mass Rearing Facility in Argentina. Exposure of 1- to 2-d-old *Cc* Vienna-8 pupae irradiated at 90 Gy to 6- to 8-d-old *C. haywardi* females at a 10:1 host/parasitoid ratio for 24 h turned out to be highly successful for the rearing of this parasitoid. High radiation doses (90–100 Gy) did not adversely influence fitness parameters of *C. haywardi* offspring F1, namely lifetime reproductive rates, adult life expectancy, and survival time. Demographic parameters in *C. haywardi* F1 from irradiated *Cc* Vienna-8 pupae improved compared to those values recorded from parasitoid originated from nonirradiated *Cc* Vienna-8 pupae. These findings will help to enhance parasitoid mass rearing for augmentative releases against medfly in Argentinean fruit-producing regions.

Graphical Abstract

**Key words:** biological control, pupal parasitoid mass rearing, radiation, temperature-sensitive lethal mutation
Augmentative biological control has been an ecologically and economically suitable method for pest control in fruit orchards, vineyards, and in various other field crops, as well as in greenhouses (Van Lenteren and Bueno 2003). Thus, augmentative fruit fly biological control through the use of parasitoids has received wide attention in the last three decades (Messing et al. 1993, Montoya et al. 2007, Sivinski and Aluja 2012, Sánchez et al. 2016). Improvements to fruit fly parasitoid mass-rearing techniques have allowed industrial parasitoid production levels to be reached, enhancing the viability of augmentative biological control and facilitating its implementation (Cancino et al. 2012a). As a result, several area-wide integrated fruit fly management programs included biological control as a complementary, economically feasible, environmentally friendly strategy, mainly in rural/urban areas harboring high densities of alternate host plants replacing the chemical control (Montoya et al. 2007, Aluja and Rull 2009).

Currently in Argentina, augmentative biological control is being implemented by the San Juan Fruit Fly Control and Eradication Program (ProCEM-San Juan) through mass releases of the exotic larval parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) as a compatible strategy with the sterile insect technique (SIT) to control/eradicate *Ceratitis capitata* (Wiedemann) (= medfly) populations in fruit-growing areas of San Juan (Suarez et al. 2019). This exotic, globally invasive fruit fly severely hinders foreign trade and impedes agricultural development throughout Argentina (Guillén and Sánchez 2007, Alós et al. 2014). Based on a renewed interest to incorporate new and complementary parasitoid species for medfly control in Argentina, the pupal parasitoid *Coptera haywardi* (Ogolbin) is currently being tested as a candidate species for mass releases.

Successful egg- or larval–pupal parasitoid mass-rearing processes have been achieved particularly with opine braconids such as *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) (Manoukis et al. 2011) and *D. longicaudata* (Montoya et al. 2007, Cancino et al. 2009a), among other species. However, some chalcidid, pteromalid, and diaprid pupal parasitoids have also been lab-reared and released against pestiferous tephritid species (Sivinski et al. 1998, Wang and Messing 2004, Aluja et al. 2009). Unlike other widely tested fruit fly pupal parasitoids, the Neotropical-native Diapriidae *C. haywardi*, which was originally found in northwestern Argentina (Loiácono 1981), represents a very promising fruit fly biological control agent, because of: 1) its high specificity on tephritid fruit flies (Sivinski et al. 1998, Guillén et al. 2002), thus greatly reducing the impact of mass releases on other native dipterans, 2) its capacity to discriminate host pupae previously parasitized by *D. longicaudata* (Cancino et al. 2012b, 2019) and to dig deep into the ground in search of pupae (Guillén et al. 2002), 3) its capability to exert complementary parasitism when it is used together with the aforementioned braconid species (Cancino et al. 2014, Van Nieuwenhove et al. 2016), 4) its appropriate population parameters for mass rearing on *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) (Núñez-Campero et al. 2012), and 5) its suitability to be produced on irradiated *Anastrepha ludens* (Loew) (Diptera: Tephritidae) host pupae (Aluja et al. 2009, Cancino et al. 2012a). The first three biological features minimize attack on nontarget hosts once *C. haywardi* adults are field-released (Baeza-Larios et al. 2002). The last two attributes are essential to achieve efficient mass-rearing procedures for *C. haywardi*. Thus, the use of irradiated hosts mainly guarantees both no fly emergence and high parasitoid quality (Cancino and Montoya 2008). In addition, radiation techniques facilitate the shipment and release of mass-reared parasitoids without having to wait for the host’s emergence and to undergo tedious procedures to separate adult flies from wasps (Cancino et al. 2017).

Here, we aimed at determining the most favorable host age and suitable host radiation dose using *C. capitata* pupae from the temperature-sensitive lethal (tsl) Vienna-8 genetic sexing strain as a host for *C. haywardi* mass production. Genetic sexing strains based on the tsl mutation are used to mass-produce sterile male medflies for SIT programs against this pestiferous tephritid species (Cáceres 2002). Furthermore, we also wanted to evaluate the consequence of host radiation on fitness parameters in *C. haywardi* progeny. These objectives are essential to assess the use of irradiated pupae to produce high-quality adult parasitoids for field augmentative release programs (Cancino et al. 2012a) in Argentina and in other places in America and the world.

**Materials and Methods**

**Insect Sources**

Experiments 1 and 2 were performed at the Medfly and Parasitoid Mass Rearing Facility of the San Juan government (hereafter San Juan facility), located in central-western Argentina. *Coptera haywardi* adults were reared at 25 ± 1°C, 70 ± 5% RH, 12:12 (L:D) h photo-period on nonirradiated pupae of the tsl Vienna-8 *C. capitata* strain (hereafter *C. capitata*-Vienna-8). The *C. haywardi* colony was initiated in December 2017 from individuals recovered from infested feral peaches in a protected wildlife area in Yerba Buena, northern Tucumán. The *C. haywardi* cohorts used in the experiments were the 9th and 10th generation under lab-rearing.

Experiments 3 and 4 were carried out at the PROIMI’s laboratory, located in Tucumán, Argentina, where a *C. haywardi* colony is currently kept at 25 ± 1°C, 70 ± 5% RH, 12:12 (L:D) h photoperiod on pupae of a biparental wild *C. capitata* strain (hereafter *C. capitata*-wild). The wild medfly colony was initiated in December 2017 from individuals recovered from infested feral peaches in a protected wildlife area in Yerba Buena, northern Tucumán. The *C. haywardi* cohorts used in the experiments were the 9th and 10th generation under lab-rearing.

**Experimental Setup**

**Experiment 1—Suitability of medfly pupae originated from irradiated larvae for the development of *C. haywardi***

This experiment was carried out to determine if *C. haywardi* may adequately and efficiently develop in pupae of *C. capitata*-Vienna-8 that originated from third instar larvae irradiated at different gamma radiation doses. We exposed 6- and 7-old day larvae of the *C. capitata*-Vienna-8 to 85, 90, 95, and 100 Gy (absorbed radiation doses). According to Suárez et al. (2019), no flies emerge when *C. capitata*-Vienna-8 larvae are irradiated at those four radiation doses. Only host male larvae stemming from the first batch were used in the trials. A quarter liter of naked larvae (= 15,000 larvae) were irradiated for each treatment. To irradiate larvae, an IMO-1 mobile irradiator with a Co-60 gamma radiation source was used. Radiation doses were applied at a rate of 23.6 Gy/min under anoxia, which was determined with Fricke dosimeters (IAEA, 2001). The IMO-1 irradiator is currently located at the San Juan facility. Approximately 500 irradiated larvae were placed into plastic containers with poplar sawdust and remained until the formed puparia were 1- to 2-old. Then, 100 naked puparia (without pupariation substrate) of each larval age/radiation dose treatment were extracted. Puparia were exposed to 10 mated, 6- to 8-old *C. haywardi* females in a 10-× 1-cm (diameter × height) plastic dish placed inside a voile-covered plastic cage (experimental cage). In total, 1- to 2-old naked puparia stemming from nonirradiated larvae from...
the C. haywardi were also exposed to parasitoids as controls. After a 24-h exposure period, host pupae were transferred to disposable plastic cups with sterilized poplar sawdust, and kept at 25°C and 80% RH. Once parasitoids emerged, both the number and sex were documented. Treatments and the control were replicated 20 times. In total, 200 adult parasitoids and 2,000 host puparia were used for each treatment. The weights of 500 2-d-old C. Vienna pupae originated from 6-d-old larvae irradiated at 90 Gy were compared with the weights of 2-d-old nonirradiated pupae from both C. Vienna and C. Wild-Tuc. This was done to evaluate a possible effect of radiation on the quality of the tephritid larvae (Cancino et al. 2012a). Nonemerged puparia were dissected as was mentioned in Experiment 1. These data plus those on emerged parasitoids were used to calculate the percentage of parasitism, that is, the percentage of the total parasitized host pupae.

Experiment 2—C. haywardi development on irradiated medfly pupae at different gamma-radiation doses

This experiment was carried out to evaluate the suitability of irradiated young C. Vienna pupae for C. haywardi mass rearing. For this, 1,000 1- to 2-d-old puparia of the C. Vienna strain were exposed separately to 60, 70, 80, 90, and 100 Gy inside 1,000-ml transparent hard plastic vials. The pupal radiation process was like the one described for C. Vienna larvae in Experiment 1. Batches of 10 mg-weight pupae were used in radiation tests. After irradiation, samples of 100 irradiated puparia were removed from vials and exposed to 10 mated, 6- to 8-d-old C. haywardi females in oviposition devices inside an experimental cage. Nonirradiated 1- to 2-d-old puparia of the C. Vienna were used as controls. Puparia were processed and kept as described under Experiment 1. Treatments and the control were replicated 20 times. In total, 200 adult parasitoids and 2,000 host puparia were used for each treatment adult flies and parasitoids emerged were recorded. Nonemerged puparia were dissected as was mentioned in Experiment 1.

Experiment 3—Optimal host age to rear C. haywardi and parasitoid performance on two medfly strains

This experiment was first carried out to determine the optimal host age of C. Vienna pupae irradiated at 90 Gy to obtain the highest C. haywardi yield and largest female offspring proportion. In addition, the performance of C. haywardi when parasitizing pupae of both C. Wild-Tuc and C. Vienna was also compared. For this purpose, irradiated puparia of C. Vienna were sent to the PROIMI facilities in Tucumán from the San Juan facility in November 2017. In Tucumán, 100 nonirradiated puparia of the C. Wild-Tuc and 100 puparia of the C. Vienna irradiated at 90 Gy divided in three age groups (young, 1- to 2-d-old; mid, 3- to 4-d; and old, 5- to 6-d-old) were individually exposed to 10 mated 6- to 8-d-old C. haywardi females, for 24 h in an oviposition device inside an experimental cage. That is, six pupal age range/medfly strain combinations were assessed. After exposure, host puparia of each treatment were removed from oviposition device and placed in containers with vermiculite as pupariation medium and maintained at 26°C, 70 ± 5% RH inside a room until all adult insects emerged. Batches of 10 mg-weight puparia of both C. capitata strains were used in the tests. As was the case in the two previous experiments, treatments were replicated 20 times. In total, 200 adult parasitoids and 2,000 host puparia were used for each of the six treatments. The number of emerged adult flies and parasitoids was recorded and nonemerged host puparia were dissected as was mentioned in Experiment 1.

Experiment 4—C. haywardi F1 generation fitness-related parameters

This experiment was carried out to evaluate fitness-related parameters of F1 generation C. haywardi offspring recovered from both 1- to 2-d-old 90 Gy-irradiated (hereafter Ch IrrV-8) and 1- to 2-d-old nonirradiated puparia of C. Vienna (hereafter Ch nonirrV-8). Survival, developmental time, sex ratio, and lifetime reproductive rates of parasitoid offspring were assessed to construct life tables of Ch IrrV-8 and Ch nonirrV-8. Irradiated and nonirradiated puparia from the C. Vienna came from the San Juan facility were sent to PROIMI on February 2018. For the group-reared life table, 20 groups (10 of Ch IrrV-8 and 10 of Ch nonirrV-8), each one composed by five females and five males of <1-d-old C. haywardi adults of the F1 generation were used in the study. Each group was placed into experimental cages. Every other day 100 naked, 1- to 2-d-old irradiated and nonirradiated puparia of the C. Vienna were exposed to Ch IrrV-8 and Ch nonirrV-8 lines, respectively, for a period of 24 h. Puparia exposure to parasitoids was conducted for 21 d at which time the experiment was ended as the reproductive rates and survival of lab-reared C. haywardi dropped sharply (Núñez-Campero et al. 2012). Medfly puparia were processed as described above (Experiment 3). Each experiment was run under controlled conditions (25 ± 1°C, 75% RH) inside an air-tight chamber for insect rearing (Percival, Model DR36VL, Percival Scientific Inc., Perry, IA). Emerged adult flies and parasitoids were recorded. Nonemerged host puparia were dissected as was mentioned in Experiment 1.

Data Analyses

For data analyses, parasitoid emergence percentage (emerged adult parasitoids), a fly emergence percentage (emerged adult medflies), parasitism percentage (emerged parasitoids plus nonemerged parasitoids), and female offspring ratio (females/females plus males) were estimated (Núñez-Campero et al. 2012). These response variables were analyzed by one- or two-way univariate GLMs with a normal distribution at α = 0.05. Fixed factors were radiation doses and larval host age in Experiment 1, radiation doses in Experiment 2, and both medfly strains and pupal host age in Experiment 3. Prior to analyses, all percentage values were transformed by using an arcsin square root function. Pupal weights of C. Vienna pupae from irradiated larvae and pupae of both C. Vienna and C. Wild-Tuc were compared by a one-way univariate generalized linear model (GLM). Pupal weight data were rank-transformed. Mean comparisons were evaluated by Tukey’s HSD test at α = 0.05. Group-reared life tables were built to estimate the ratio of individuals surviving at the start of the age interval (lx) and the life expectancy (ex) of the cohort at each age interval (Carey 2001). Population parameters were calculated according to Chang et al. (2016). Net reproductive rate (R0), intrinsic rate of natural increase (r), finite rate of increase (λ), mean generation time (T), mean fecundity per female (F), and gross reproductive rate (GRR) were estimated by means of ‘TWOSEX-MS Chart’ software, based on the age-stage two-sex life table analysis (Chi 2018). Means and standard errors of all reproductive parameters were calculated by using 100,000 effective bootstraps as suggested by Huang and Chi (2012). Kaplan–Meier method and log-rank test were used to analyze and compare parasitoid adults’ survival time (lx). One-way ANOVAs (α = 0.05) were used to contrast R0, r, λ, T, F, and GRR between C. haywardi population lines. A Student’s test for independent samples was used to compare the life expectancy (ex) between the two parasitoid lines studied. The ‘base’ and ‘stars’ R software (R Core Team 2020) packages were used for the statistical analyses.
Results

Experiment 1—Suitability of Medfly Pupae Originated From Irradiated Larvae for the Development of C. haywardi

Parasitism and parasitoid emergence percentages were recorded on host puparia formed by nonirradiated larvae of Cc Vienna-8, whereas puparia from host larvae irradiated at 85–100 Gy did not produce any parasitoids. Larval host age had a significant influence on parasitism (F = 16.31, df = 1, 38, P = 0.0003) and parasitoid emergence (F = 26.16, df = 1, 38, P < 0.0001), but not on female offspring ratio (F = 1.76, df = 1, 38, P = 0.1938). The highest percentage of both parasitism and emerged parasitoids was recorded on host puparia of Cc Vienna-8 6-d-old larvae that were not irradiated (parasitism: 10.4 ± 1.1 in 6-d-old larvae vs 5.2 ± 0.6 in 7-d-old larvae; parasitoid emergence: 7.5 ± 1.0 in 6-d-old larvae vs 2.4 ± 0.3 in 7-d-old larvae). Similar male-biased sex ratios were recorded in both larval host ages (1.6:1 and 2.5:1 male/female in 6-d-old and 7-d-old larvae, respectively).

Nonirradiated puparia of both Cc Vienna-8 and Cc Wild-Tuc exhibited significantly higher weights than those Cc Vienna-8 puparia originated from irradiated larvae (F = 1740.31, df = 2, 1497, P < 0.0001) (Fig. 1).

Experiment 2—C. haywardi Development on Irradiated Medfly Pupae at Different Gamma-Radiation Doses

The radiation doses significantly influenced medfly emergence (F = 240.56, df = 5, 114, P < 0.0001), parasitism (F = 31.79, df = 5, 114, P < 0.0001), and parasitoid emergence (F = 16.26, df = 5, 114, P < 0.0001), but not on female offspring ratio (F = 1.59, df = 5, 114, P = 0.167). The highest percentages of emerged medflies were recorded at 60 and 70 Gy, whereas the lowest percentage was found on host puparia irradiated at 80 Gy; no flies emerged at 90–100 Gy (Fig. 2). Percentages of emerged parasitoids recorded on Cc Vienna-8 puparia irradiated at 90 Gy were 2.4, 2.3, 2.5, and 1.5 times higher than those recorded on nonirradiated puparia, and on irradiated pupa at 60, 70, and 80 Gy, respectively (Fig. 2). Significantly higher parasitism levels were found in irradiated puparia at 90 and 100 Gy (Fig. 2). No significant differences were found in female offspring emerged from both control pupae and irradiated pupae. Only male-biased sex ratios were recorded (Fig. 2).

Experiment 3—Optimal Host Age to Rear C. haywardi and Parasitoid Performance on Two Medfly Strains

Medfly strains and pupal host age, and their interaction influenced both parasitism and parasitoid emergence (Table 1). Significantly higher parasitism and parasitoid emergence percentages were recorded from irradiated young puparia (1- to 2-d-old) of the Cc Vienna-8 (Fig. 3). The proportion of adult parasitoids that emerged from 3- to 4-d-old puparia stemming from the Cc Vienna-8 was similar to that recorded in nonirradiated young puparia of the Cc Wild-Tuc (Fig. 3). The pupal host age significantly influenced the emerged parasitoid female ratio (Table 1). Both, young- and mid-age host puparia produced 1.3 times more C. haywardi female offspring than older puparia. However, pupal host age and medfly strains interaction in this response variable was not significant (Table 1). Only male-biased sex ratios were recorded (Fig. 3).

Experiment 4—C. haywardi F1 Generation Fitness-Related Parameters

All lifetime reproductive parameters estimated differed significantly in both C. haywardi population lines (Table 2). The contribution of newborn females to the next generation (R), and the intrinsic rate of natural increase (r) recorded for the Ch Vienna population line, was 1.3 and 1.5 times higher than those found in Ch Wild-Tuc, respectively. Highly significant differences were found in the survival time between males and females within both Ch Vienna and Ch Wild-Tuc population lines. The cumulative survival curves did not differ significantly for males (χ² = 1.35, df = 2, P = 0.245) (Fig. 5A) and females (χ² = 1.57, df = 2, P = 0.209) (Fig. 5B) between C. haywardi population lines. The offspring cohort’s lifespan was similar in both parasitoid population lines, 46.1 ± 1.3 and 46.6 ± 1.2 d for Ch Vienna and Ch Wild-Tuc, respectively. A very similar female offspring ratio was found in both parasitoid population lines: 0.44 ± 0.03 and 0.46 ± 0.03 for Ch Vienna and Ch Wild-Tuc, respectively.

Life expectancy was not significantly different when comparing Ch Vienna and Ch Wild-Tuc (t = 0.47, df = 39,75, P = 0.638). The 60 survival days (~39 d for immature stages and ~21 for adults) shown in Fig. 6 evidences that survival was higher than expected by the life expectancy function. The mean daily percent parasitism was 4.14 ± 0.32% and 3.28 ± 0.22%, respectively, in the Ch Vienna and Ch Wild-Tuc (H = 3.46, df = 1, P = 0.06).

Discussion

Parasitoid emergence and offspring sex ratio greatly affect the viability of mass-rearing processes needed for augmentative biological control programs (Wong and Ramadan 1992, Cancino and Montoya 2004). A suitable host stage and age in relation to an adequate host radiation dose are critical prerequisites in fruit fly parasitoid-rearing processes to achieve optimal adult mass production (Hendrichs et al. 2009, Cancino et al. 2012a). In this regard, here we found that young host puparia stemming from irradiated 6- and 7-d-old larvae of the Cc Vienna were not suitable to mass-produce C. haywardi. Previous studies on C. haywardi at the ‘Aurora’ USDA-APHIS/
MOSCAMED rearing facility, Guatemala, also documented the inadequacy of pupae of a biparental *C. capitata* strain stemming from irradiated larvae as host for parasitoid production (Menezes et al. 1998). Similarly, as was the case with irradiated *C. capitata* larvae, no emergence of *C. haywardi* was recorded when *Anastrepha suspensa* (Loew) puparia stemming from irradiated larvae produced at the Florida Division of Plant Industry, Gainesville, FL (Menezes et al. 1998, Sivinski et al. 1999) were exposed to this parasitoid. In pupal endoparasitoids, as in the case with *C. haywardi*, host unsuitability may be related to internal biochemical and physiological changes produced in the host as a result of the ionizing radiation effect (Nussbaumer and Schopf 2000, Thomas and Hallman 2000, Harvey and Strand 2002, Cancino et al. 2009a). Compared to fruit fly pupal ectoparasitoids, such as *Dirhinus* sp. and *Eurytoma sivinskii* Gates and Grissell, *C. haywardi* is far more sensitive to the effects produced in the host by radiation, most likely because of its endoparasitic feeding habits (Cancino et al. 2009b). Nevertheless, another possibility is that irradiated host larvae never reached the true pupal stage.

Host quality, particularly host pupal weight, influence the adults fruit fly parasitoids in mass-rearings (Purcell et al. 1994, Cancino and Montoya 2004). In turn, radiation can reduce the quality of the host pupa as well as fly emergence (Mahmoud and Barta 2011). Here, we were able to determine that radiation caused weight loss in young host puparia (1- to 2-d-old) stemming from irradiated larvae of the *Cc Vienna-8*. These puparia weighed 1.4 times less than same-aged ones from the same strain that were irradiated as puparia. But importantly, the weight of irradiated *Cc Vienna-8* puparia remained stable compared with the mean weight of nonirradiated *Cc Wild-Tuc* puparia.

In this study we were able to show that 1- to 2-d-old *Cc Vienna-8* puparia irradiated at 90 Gy, at a high host to parasitoid ratio (10:1), and exposed to 6- to 8-d-old *C. haywardi* females for a long time (24 h), were suitable to obtain the highest mean adult emergence (~21%). Furthermore, medfly emergence was censored, and parasitoid emergence was significantly improved regarding values from nonirradiated 1- to 2-d-old puparia of both *Cc Wild-Tuc* and *Cc Vienna-8*. These findings indicate that *C. haywardi* can be successfully reared by using irradiated young puparia of the *Cc Vienna-8* at

**Table 1.** Summary of two-way univariate ANOVA on the effect of medfly strain, pupal host age, and their interactions on *C. haywardi* adult emergence, female offspring ratio, and parasitism recorded from both irradiated puparia of Vienna-8 *C. capitata* strain and nonirradiated puparia of wild biparental *C. capitata* strain

| Source of variation | Parasitism | Parasitoid emergence | Female offspring ratio |
|---------------------|------------|----------------------|------------------------|
|                     | df | Error df | F | P | F | P | F | P |
| Medfly strain (MS)  | 1  | 114      | 0.01 | = 0.899 | 62.79 | < 0.0001 | 129.06 | < 0.0001 |
| Pupal host age (PHA)| 2  | 114      | 3.29 | = 0.041 | 64.20 | < 0.0001 | 100.06 | < 0.0001 |
| PHA × MS            | 2  | 114      | 0.39 | = 0.677 | 6.14  | < 0.0029 | 15.26  | < 0.0001 |

Significant value.

**Fig. 2.** Mean (± SE) percentages of emerged flies (lowercase letter), parasitoid emergence (uppercase letter), parasitism (italic lowercase letter), and parasitoid female offspring (italic uppercase letter) recorded from nonirradiated and irradiated 1- to 2-d-old puparia stemming from a *C. capitata* Viennas-8 tsl genetic sexing strain (= *Cc Vienna-8*) exposed to *C. haywardi* females. Bars with the same letter indicate no significant differences (Tukey’s HSD test, *P* = 0.05).
high radiation doses. Similarly, *C. haywardi* was successfully reared in 3–d-old irradiated *A. ludens* puparia (Cancino et al. 2009b). In contrast to our data here, these authors report the highest parasitoid emergences when reared on puparia irradiated at low doses (20–50 Gy). These differences may be due to differential sensitivity to radiation of the host species (i.e., *C. capitata* vs *A. ludens*) or they may also be due to the fact that low irradiation doses do not kill the prepupa, which in turn allows it to develop as a pupa. *Anastrepha ludens* pupae were likely more sensitive to radiation than *C. capitata* pupae, which may be related to the larger host size compared to *Cc Vienna-8* pupae as Cancino et al. (2009b) showed that larger fruit fly larvae or pupae are usually more susceptible to radiation. In addition, the mechanism underlying radiosensitivity in the different fruit fly species may be associated to other factors, such as antioxidant defenses, chromosome structure, and time to develop from larvae to true pupae. Higher radiation doses are needed to interfere/harm physiological development in the smallest fruit fly larvae, such as in the tsl genetic sexing *C. capitata* strain (Mastrangelo and Walder 2011).

Progeny sex ratio is a quality control parameter commonly used in fruit fly parasitoid rearing, and it is affected by host age (Cancino et al. 2009a) and irradiation doses (Cancino et al. 2012a). Here, female offspring ratio (0.75:1; female:male) recorded from 1– to 2-d-old puparia irradiated at 90 Gy did not improve in relation to the control (nonirradiated puparia), pupal host age, and other irradiation doses tested. Similarly, Cancino et al. (2009b) found no substantial differences in *C. haywardi*’s offspring sex ratio at different radiation doses when 1–, 2–, and 3–d-old *A. ludens* puparia were used as host, but sex ratio was always female-biased. In turn, Aluja et al. (2009) found that the largest *C. haywardi*’s offspring with the highest female ratio was only yielded by young *A. ludens* pupae (0– to 5-d-old). Differences related to female offspring ratio in fruit fly parasitoids may be due to host body size (Cancino and Yoc 1993, López et al. 2009); e.g., smaller larvae usually facilitate

### Table 2. Population parameters of *C. haywardi* F1 offspring from the two parasitoid population lines reared on nonirradiated and irradiated 1- and 2-d-old puparia of Vienna-8 *C. capitata* strain

| C. haywardi population lines | Reproductive parameters (mean ± SE) |
|-------------------------------|-------------------------------------|
|                              | r   | λ    | R₀  | T   | F    | GRR  |
| *Ch irrV8*                   | 0.032 ± 0.003 | 1.033 ± 0.003 | 4.725 ± 0.699 | 47.576 ± 0.318 | 11.340 ± 1.165 | 9.460 ± 1.186 |
| *Ch nonirrV8*                | 0.027 ± 0.002 | 1.027 ± 0.002 | 3.875 ± 0.528 | 49.336 ± 0.298 | 9.300 ± 0.733 | 7.650 ± 0.749 |
| df = 1                       |     |     |     |     |     |     |
| F = 146,962                  |     |     |     |     |     |     |
| P = <0.001                   |     |     |     |     |     |     |

*a* Mean values followed by the same letter within a column indicate no significant differences (one-way ANOVA test, *P* = 0.05).
high proportions of males when *D. longicaudata* is reared (Van Nieuwenhove and Ovruski 2011). Pupae of both *Cc* Vienna-8 and *Cc* Wild-Tuc, which yielded male-biased *C. haywardi* sex ratios in the present study, are much smaller than pupae of both *A. ludens* and *A. fraterculus*. Based on results here, offspring sex ratio needs to be addressed in future studies if *C. capitata* is used as a host for *C. haywardi*’s mass-producing.

Radiation doses at 90–100 Gy influenced host pupal development. However, fitness parameters of *C. haywardi* F1 progeny, such as population and reproductive rates, life expectancy, and survival time, were not affected. Values recorded for demographic parameters in *C. haywardi* F1 originated from irradiated *Cc* Vienna-8 puparia were improved when they were compared with those of nonirradiated *Cc* Vienna-8 puparia. Furthermore, survival parameters were similar in both *C. haywardi* population lines tested. The most important lifetime reproductive parameters measured in this study, that is, $R_0$, $\lambda$, $r$, and $T$, had considerably lower values than those found for *C. haywardi* reared on pupae of both *A. ludens* (Aluja et al. 2009) and *A. fraterculus* (Núñez-Campero et al. 2012). These differences may be due to three factors: 1) fruit fly species used as hosts, 2) host pupae quality, and 3) parasitoid experimental handling procedures (Cancino and Montoya 2004, 2008). Nevertheless, data on survival and population growth parameters recorded for *C. haywardi* here provide useful insights for the optimization of mass-rearing processes, pointing to the maximum reproductive capacity of this parasitoid species reared on *Cc* Vienna-8 pupae.

In summary, our findings indicate that irradiated *Cc* Vienna-8 puparia are conducive for *C. haywardi* rearing when a suitable radiation dose is used. Notably, critical fitness parameters of F1 parasitoid offspring were significantly improved with regards to the use of nonirradiated *Cc* Vienna-8 young pupae. This will facilitate the implementation of augmentative biological control programs against *C. capitata* because of no release of flies. Overall, data presented here, together with previously detailed biological qualities, suggest that the use of *C. haywardi* for augmentative releases in Argentina and many other regions in the world holds great promise. However, field cage tests, such as those carried out in coffee plantations in Guatemala by Baeza-Larios et al. (2002) and in fruit orchards in Chiapas, México by Cancino et al. (2014), are necessary to test the performance of *C. haywardi* as a *C. capitata* biocontrol agent under the contrasting environment conditions of the fruit-growing Argentinean regions, such as Cuyo and NOA. The former exhibits semiarid environmental conditions, while the latter is a subtropical region.

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**Fig. 4.** Survival time differences between (A) males and females *C. haywardi* cohorts stemming from nonirradiated *Cc* Vienna-8 puparia and (B) males and females *C. haywardi* cohorts stemming from irradiated *Cc* Vienna-8 puparia.

**Fig. 5.** Survival time differences between (A) males and (B) females of *C. haywardi* cohorts stemming from irradiated and nonirradiated *Cc* Vienna-8 puparia.
Data Availability Statement
Data from this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.xgxd254c7 (Nuñez-Campero et al. 2019).

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