El Colegio de la Frontera Sur

Viabilidad de la cepa sexada genéticamente de *Anastrepha ludens,* Tapachula-7, para la cría masiva de *Coptera haywardi*

TESIS

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Introducción

Las moscas de la fruta (Diptera:Tephritidae) son consideradas como plagas de importancia económica que afectan la fruticultura a nivel mundial (Aluja, 1994), limitando la comercialización y exportación de frutas. Para su control se han desarrollado diversos métodos, los cuales son aplicados de manera integrada. Entre los más importantes está el uso de atrayentes sexuales (feromonas, para-feromonas) y alimenticios para el monitoreo y detección de poblaciones, la aspersión de cebos tóxicos específicos, la aplicación de la Técnica del Insecto Estéril (TIE) y el control biológico por aumento. Diversos autores señalan que las liberaciones de parasitoides y moscas estériles tienen un efecto sinérgico y que por lo tanto puede ser considerada como una seria alternativa para la supresión de moscas de la fruta (Barclay, 1987; Knipling, 1992; Wong, et al., 1992; Sivinski, et al., 1996).

Diachasmimorpha longicaudata (Ashmead), endoparasitoide solitario de larvas de moscas de la fruta originario de la región Indoaustraliana, ha mostrado ser altamente efectivo cuando se libera contra poblaciones de moscas de la fruta del género *Anastrepha* (Ovruski, et al., 2000). Esta especie es criada masivamente en México en larvas de *Anastrepha ludens* Loew, y liberada en zonas específicas con alta densidades de hospederos (Montoya, et al., 2007), mostrando reducciones significativas en las poblaciones de moscas de la fruta (e.g., Sivinski, et al., 1996; Montoya, et al., 2000).

Sin embargo, se considera que la liberación de una sola especie de enemigo natural puede presentar limitaciones en la supresión de la población objetivo (Aluja, et al., 2008). Por ejemplo, para el caso de *D. longicaudata*, la

presencia de frutos de mayor tamaño en el medio ambiente representa refugios físicos para las larvas fitófagas (Montoya, et al., 2000), en donde, a pesar de que el parasitoide identifique la presencia de la larva, ésta puede escapar al alcance de su ovipositor y continuar con su ciclo biológico hasta convertirse en adulto. La adición de otra especie de parasitoide podría incrementar la acción supresora sobre las poblaciones de moscas de la fruta al atacar dos estados biológicos de la plaga (Aluja et al., 2008). Un parasitoide de pupa podría complementar la acción de parasitoides de larva (Sivinski, 1996). Una opción la representa el parasitoide de pupa *Coptera haywardi* (Ogloblin i.l.) (Diapriidae), endoparasitoide solitario de moscas del género *Anastrepha* Schinner, ampliamente distribuido en la región Neotropical (Aluja, et al., 2008; López, et al., 1999; Sivinski, et al*.,* 1998). Su elección se fundamenta en que este parasitoide es específico de tefrítidos y ha mostrado una importante capacidad para discriminar pupas previamente parasitadas por *D. longicaudata* (Cancino, et al., 2012). Además *C. haywardi* se caracteriza por ser de cría relativamente fácil en laboratorio, con altos porcentajes de parasitismo y emergencia (Aluja, et al., 2008). Sin embargo, la cría masiva de pupas hospederas implica altos costos de producción.

La eficiencia de la TIE se ha mejorado notablemente con el desarrollo de cepas sexadas genéticamente, que permiten la liberación exclusiva de machos, lo cual, además de mejorar la efectividad de la TIE representa importantes ahorros en la producción y dispersión de los insectos estériles (Franz, et al., 1994, 1996; Hendrichs, et al., 1995; Rendón, et al., 2004).

En algunos casos, la producción masiva de estas cepas genera subproductos que no se utilizan en las liberaciones, pero que pueden

representar un recurso importante para la multiplicación masiva de parasitoides de pupas (Ovruski, et al., 1999; Gómez, et al., 1998).

En la planta Moscafrut (SAGARPA IICA) se ha desarrollado una cepa de *A. ludens* sexada genéticamente, donde las pupas de color café corresponden a machos que se emplean para liberación en el campo, mientras que las pupas negras solo se requieren para el mantenimiento de la colonia (Orozco et al., 2013). Estas pupas negras podrían emplearse como hospederos para la producción masiva de *C. haywardi.*

El objetivo de esta investigación fue evaluar la viabilidad de las pupas negras (hembras) de la cepa Tapachula-7 de *A. ludens* como hospederos para la cría masiva de *C. haywardi.* Para ello se determinó: 1) la preferencia de estas pupas por las hembras parasitoides, 2) el efecto de la edad, la irradiación y la separación mecánica automatizada en el desarrollo y emergencia de *C. haywardi,* y 3) los parámetros de aptitud de los adultos de *C. haywardi* emergidos de la pupa negra.

BioControl

Female pupae from the genetic sexing strain "Tap-7" of Anastrepha ludens as hosts of Coptera haywardi

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Running Head: Black pupae as hosts of *C. haywardi*

Female pupae from the genetic sexing strain "Tap-7" of *Anastrepha ludens* as hosts of *Coptera haywardi*

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Abstract

The female black pupae from the genetic sexing strain Tapachula-7 *Anastrepha ludens* were evaluated as a host of *Coptera haywardi.* We studied the acceptance and effects of age, irradiation and automated mechanical separation of black pupae on the emergence, survival, fecundity and flight ability of *C. haywardi* adults. Our results indicated that the black pupa is a viable host of *C. haywardi*. Adult emergence was greater when the exposed pupae were 3 and 5 days old. The impact during mechanical separation reduced emergence by 16%. The tested irradiation doses (25, 35 and 45 Gy) did not affect significantly adult emergence. No differences in longevity, fecundity or flight ability were registered between the black pupa parasitoids and those emerging from the mass-reared standard strain.

Key words: Fruit flies, Pupal parasitoid, Hymenoptera: Diapriidae, Biological control, Host, Black pupa.

42 **1. Introduction**

43 Fruit flies (Diptera: Tephritidae) are considered economically important pests that damage
44 fruit and vegetable trade worldwide (Aluja 1994). These pests are controlled through
45 integrated pest management (IPM), which includes the use of attractants and specific lures
46 for the detection and monitoring of populations, the destruction of infested fruits, the
47 selective application of toxic baits, the Sterile Insect Technique (SIT) and biological control
48 through the augmentative release of parasitoids. To achieve the best results, parasitoids
49 must be released in large areas (Reyes et al. 2000; Enkerlin 2005; Montoya et al. 2007).

50 *Diachasmimorpha longicaudata* (Ashmead), a solitary endoparasitoid larva from the
51 Indo-Australian region, has been released throughout various regions of the Americas to
52 control flies of the genus *Anastrepha* Schinner (Ovruski et al. 2000). The augmentative
53 release of this parasitoid significantly reduced populations of *Anastrepha suspensa* (Loew) in
54 Florida, USA (Sivinski et al. 1996) and *Anastrepha obliqua* (McQuart) and *A. ludens* (Loew)
55 populations in Chiapas, Mexico (Montoya et al. 2000). However, this control method can be
56 improved through the simultaneous release of two or more natural enemies to increase
57 parasite levels in the field (Aluja et al. 2008). One option is the pupal parasitoid *Coptera*
58 *haywardi* (Ogloblin i.l.) (Diapriidae), a solitary endoparasitoid of *Anastrepha* spp. which is
59 widely distributed throughout the Neotropic ecozone (Aluja et al. 2008; Lopez et al. 1999;
60 Sivinski et al. 1998). *C. haywardi* is also a specific parasitoid of Tephritidae (Sivinki et al.
61 1998) that has the ability to discriminate pupae that were previously parasitized by *D.*
62 *longicaudata* (Cancino et al. 2012). This parasitoid is relatively easy to breed in the
63 laboratory, with high percentages of parasitism and emergence (Aluja et al. 2009).
64 Preliminary laboratory and field assays have shown that the simultaneous action of both
65 parasitoids (*D. longicaudata* and C. *haywardi*) increases the percentage of parasitism

66 compared with that of the two species separately (Cancino et al. personal communication).
67 Importantly, the use of *C. haywardi* in augmentative biological control programmes requires
68 massive pupal rearing of the host, implying high production costs.
69 The efficiency of the SIT has been greatly improved with the development of genetic
70 sexing strains, as these strains promote the exclusive production of males (Franz et al. 1994,
71 1996; Hendrichs et al. 1995; Rendón et al. 2004). Moscafrut (SAGARPA-IICA) developed a
72 genetic sexing strain of *A. ludens*, with brown male and black female pupae required to
73 maintain the colony (Orozco et al. 2013). These black pupae could be used as hosts for the
74 mass production of *C. haywardi.* The objective of this investigation was to study the viability
75 of the genetic sexing strain Tapachula-7 *A. ludens* black pupae (females) as hosts for the
76 mass production of *C. haywardi*. We evaluated the following: 1) the preference of these
77 pupae for female parasitoids; 2) the effect of age, irradiation and automated mechanical
78 separation on the development and emergence of *C. haywardi*; and 3) the fitness
79 parameters of *C. haywardi* adults emerging from black pupae.
80 **2. Materials and methods**
81 In this paper, we refer to the *A. ludens* mass-reared standard strain as SMR and to the
82 Tapachula-7 strain as TAP-7.
83 **2.1 Biological samples and study site**
84 This study was performed in the Biological Control Laboratory of the Moscafrut Program
85 managed by the Mexican Secretariat of Agriculture, Livestock, Rural Development, Fisheries
86 and the Food Inter-American Institute for Cooperation on Agriculture (SAGARPA-IICA) in
87 Metapa de Dominguez, Chiapas, Mexico. The laboratory conditions were 24 ± 2 °C and 60-
88 80% relative humidity (RH). The adults of *C. haywardi* were obtained from the colony
89 maintained in the Biological Control laboratory, and *A. ludens* pupae (SMR and TAP-7) were

produced at the Moscafrut plant according to the methods described elsewhere (Dominguez et al. 2010; Zepeda, 2010).

2.2 Host preference determination

In this experiment, TAP-7 black (females) and SMR brown pupae were used as hosts. The following treatments were applied: 1) The separate exposure of 20 pupae of each type to 10 males and 10 females of *C. haywardi* aged 6-8 days for 48 h in 5-cm Petri dishes containing a 3-mm layer of moist vermiculite. The Petri dishes were separately placed in 20x20x20 cm Plexiglas cages covered on one side with sleeve-shaped organza fabric to facilitate handling. 2) A total of 20 pupae of each type were exposed to 20 males and 20 females of *C. haywardi* within the same cage and Petri dish. 3) A total of 20 black TAP-7 and 20 brown SMR pupae were exposed to 20 males and 20 females *C. haywardi* within the same cage and Petri dish. Each treatment was repeated 10 times. In all treatments, the pupae were 3 days old, and a piece of cardboard was placed over the oviposition unit to provide 8-lux darkness and promote parasitoid activity (Cancino et al. 2012).

After exposure, twenty pupae were placed in plastic containers (4 cm height x 7.5 cm diameter) for 30 days until adult emergence. We determined the percentage parasitoid emergence and the sex ratio per treatment. Approximately 10% of the exposed pupae was sampled to analyse the relationship between the number of oviposition scars per pupa and the number of immature stages of the parasitoid at 72 h after exposure.

2.3 Effect of the Sortex-Buhler mechanical impact during the separation of pupae

The automated mechanical separation of brown pupae was achieved using a Sortex-Buhler sorter, which uses optical systems to select unwanted pupae (black pupae in this case), while the main product pupae was subjected to a minor operation. Eighty grams of pupae (3-11

113 days old) was exposed to the following treatments: 1) black pupae of the TAP-7 strain were
114 mechanically sorted, and 2) black pupae (control) were manually sorted.
115 One hundred black pupae were placed in a Petri dish (5.5 cm diameter) containing a
116 layer of wet vermiculite and exposed to 10 male and 10 female *C. haywardi* in a plastic
117 container (7.5 cm height x 11 cm diameter). Each treatment was replicated 10 times. After
118 exposure, the pupae were placed in plastic containers to determine the percentage of adult
119 emergence and the sex ratio.
120 **2.4 Irradiation of Tapachula-7-strain pupae**
121 Two hundred and fifty grams of TAP-7-strain pupae (3-11 days old) was separated by colour
122 using a Sortex-Buhler sorter. Subsequently, the black pupae (females) were irradiated at
123 doses of 25, 35 and 45 Gy using cobalt 60 gamma radiation in a Gamacell 220 irradiator
124 (Nordion Int., Ontario, Canada). SMR-strain pupae of the same age were irradiated at the
125 same doses as control. One hundred pupae of each strain were exposed to 10 females of *C.*
126 *haywardi* for 48 h. After exposure, the pupae were placed in plastic containers (as previously
127 described) to determine the percentage of emergence and the sex ratio. Each treatment
128 was repeated 10 times.
129 **2.5 Fitness tests of *C. haywardi* emerged from the Tapachula-7 strain**
130 Black pupae (5, 9, 10, and 11 days old) were irradiated at 45 Gy, and the survival, fertility and
131 flight ability were evaluated as indicators of *C. haywardi* fitness. The SMR-strain pupae (3
132 days old) were irradiated at 35 Gy and used as control (Cancino et al. 2008).
133 The individuals tested were obtained by placing 1,000 pupae of each treatment in a 15-
134 cm plastic dish containing wet vermiculite covered with a piece of cardboard in a Plexiglas
135 cage (20 x 20 x 20 cm). The flies were exposed to 100 males and 100 females of *C. haywardi*

136 (6-8 days old) for 48 h. Subsequently, the pupae were placed in plastic containers (11 cm
137 diameter x 7.5 cm height) containing wet vermiculite for 30 days until adult emergence.
138 **2.5.1 Survival.** Cohorts of 20 males and 20 females were placed in plastic cylindrical
139 containers (10 cm diameter x 16 cm height). The adults were subjected to a no-water and
140 no-food regime. The number of dead individuals of each sex was recorded until all
141 individuals died. This process was repeated 10 times.
142 **2.5.2 Fecundity.** Cohorts of 20 males and 20 females were placed in the same containers as
143 described above (2.5.1). The parasitoids were provided water and honey mixed with toilet
144 paper as a source of food (Montoya et al., 2012). At 5 days old, females were provided with
145 200 pupae of the SMR-strain *A. ludens*, irradiated at 35 Gy and placed in a 10 cm plastic dish
146 for 48 h. The females were exposed daily until they were 20 days old. After exposure, the
147 pupae were placed in plastic containers containing wet vermiculite until adult emergence.
148 The sex and number of emerged parasitoids were recorded. The emerged parasitoids were
149 associated with the number of live females each day. This process was repeated 10 times.
150 **2.5.3 Flight ability.** One hundred pupae samples of each selected age were placed at the
151 base of a black PVC pipe (10 cm diameter x 8 cm height) to evaluate the flight ability of the
152 emerged parasitoids. The inner walls of the tubes were impregnated with neutral talc to
153 prevent the escape of parasitoids by crawling, (FAO/IAEA/USDA manual /2003). The tubes
154 were placed in a cage (60 x 60 x 60 cm) with two light bulbs (75 Watts) placed 50 cm from
155 the top. The parasitoids flying out of the tubes were removed, and daily observations were
156 made. After a period of 10 days, the number of parasitoids remaining inside the tubes and
157 the number of empty pupae cases were recorded, and the percentage of parasitoids able to
158 fly was determined. The process was repeated 10 times.
159

160 **2.6 Statistical analysis**

161 The emergence percentages from the host preference test were analysed using a one-way
162 analysis of variance (ANOVA), and the mean values were compared using the post-hoc
163 Tukey's test (Zar 1984). The relationship between the number of oviposition scars per pupa
164 and the number of immature individuals per pupa was determined using Spearman's
165 correlation coefficient. The percentages of superparasitized pupae were analysed using the
166 chi-square test with contingency tables (Zar 1984). The effect of mechanical separation on
167 the TAP-7-strain pupae was analysed using bivariate analysis considering the type of
168 separation (mechanical and manual) and the pupae age (3-11 days) as factors. In the
169 irradiation tests, the emergence of *C. haywardi* was analysed using a three-way ANOVA
170 (type of pupa, age, and irradiation dose). The proportion of flying adults and the sex ratio
171 were analysed using a one-way ANOVA. Prior to analysis, the data were transformed to ln 10
172 + 1, x arcsine and box-cox where necessary. The survival and fecundity of *C. haywardi* adults
173 were analysed using demographic methods, and the survival curves were compared using
174 the log-rank test (Francis, et al 1993). A confidence level of 95% was used for all tests. The
175 data were analysed using the JMP statistical package (version 5.0.1.).

176 **3. Results**

177 **3.1 Host preference**

178 When pupae were exposure in separate cages, no significant differences in parasitoid
179 emergence were observed between treatments ($F_{1, 18}$= 0.04; P= 0.83) (Table 1). Significant
180 differences were detected when the pupae were exposed in different Petri dishes within the
181 same cage ($F_{1, 18}$= 6.08; P= 0.02) and when the black and the SMR pupae were exposed
182 together ($F_{1, 18}$= 6.10; P= 0.02). The sex ratio did not show any significant differences
183 between treatments; pupae exposure in separate cages ($F_{1, 18}$= 1.73; P= 0.20), pupae

184 exposed in different Petri dishes within the same cage ($F_{1, 18}$= 0.94; P= 0.34), pupae exposed
185 together ($F_{1, 18}$= 0.59; P= 0.44).
186
187 **3.1.1 Relationship between oviposition scars and number of immature individuals per**
188 **pupa.** A significant relationship between the number of oviposition scars and the first instar
189 larvae inside the pupae was observed for all treatments (Figure 1).
190 **3.1.2 Percentage of superparasitized pupae.** When pupae exposure occurred in either
191 separate cages or within the same cage but in separate Petri dishes, the percentage of
192 superparasitized pupae was not different between treatments (χ^2_1 = 3.53, P= 0.17 and χ^2_1 =
193 4.43, P= 0.10, respectively). However, when exposure was performed within the same Petri
194 dish, the percentage of superparasitized pupae was higher in the SMR (80%) than in the
195 black (40%) pupae (χ^2_1 = 6.74, P= 0.03).
196 **3.2 Impact during pupae separation**
197 The impact during pupae separation had a negative effect on *C. haywardi* emergence, which
198 was lower for mechanical (16%) than for manual (34%) separation ($F_{1, 162}$ = 128.7; P=
199 <0.0001). The separation method did not have a effect on the sex ratio ($F_{1, 162}$ = 1.50; P=
200 0.22).
201 **3.3 Irradiation of Tapachula-7-strain pupae**
202 No effect of the irradiance dose on the emergence of *C. haywardi* was observed in a 3-
203 factorial experiment combining irradiation dose, type of pupae and pupae age ($F_{3, 648}$= 0.04;
204 P= 0.98). The pupae type exhibited a significant difference in emergence ($F_{1, 648}$= 445.9; P=
205 <0.0001), with a 37% of emergence in the SMR pupae and a 20% in the black pupae. Age was
206 also a significant factor ($F_{8, 648}$ = 20.17; P= <0.0001), with the highest emergence rates
207 observed for 3- and 5-day-old pupae and the lowest emergence rates for 11-day-old pupae

208 (Figure 2). There was a significant interaction between type of pupa, dose and age ($F_{24, 648}$ =
209 1.6; P= 0.03), both types of pupae (black and SMR) used as hosts of *C. haywardi*, pupa age of
210 both strains (3-11 days) and different doses (25, 35, 45 Gy) had an effect on the emergence
211 of *C. haywardi*. Also there was a significant interaction of age and the type of pupa ($F_{8, 648}$ =
212 5.03; P= <0.0001), the pupa black and SMR and the different ages of pupae of both strains,
213 had an effect on the emergence of *C. haywardi*. The pupae type x irradiation dose and pupae
214 age x irradiation dose interactions were not significant (Table 2). Emergence was suppressed
215 in 3-5-day-old pupae, while the 6-11-day-old pupae emerged in both pupae type. None of
216 the irradiation doses affected parasitoid emergence, although the pupae that were not
217 irradiated showed slightly lower emergence percentages.

218 **3.4 Fitness tests**

219 *C. haywardi* emergence in SMR pupae was different from that in black pupae ($F_{4, 45}$ = 28.69;
220 P= <.0001). The adults that emerged from pupae of different ages were not different ($F_{3, 36}$ =
221 0.95; P= 0.42).

222 The flight ability of the adults emerging from both pupae types was not different ($F_{4,}$
223 $_{45}$ = 0.79; P= 0.53). The survival rate of male and female pupae of different ages was different
224 (χ^2_4 = 60.35, P= <0.0001, for males and χ^2_4 = 19.40, P= 0.0007 for females), with the highest
225 emergence rate registered for 11-day-old pupae (Figure 3). Adult fecundity did not show any
226 difference between black and SMR pupae ($F_{4, 450}$ = 0.41; P= 0.80) (Table 3).

227 **4. Discussion**

228 Our results show that it is possible to produce *C. haywardi* using the black TAP-7-strain
229 pupae as hosts and that the adults that emerged from this strain do not show differences in
230 their fitness parameters compared with adults that emerged from the SMR strain.

231 We first determined whether the colour differences (i.e., melanin content) between
232 the pupae types led to differences in host acceptance, because visual signals, such as colour,
233 size and shape, are of great importance for host selection in female parasitoids (Rousse et al.
234 2007; Henneman et al. 2002; Harris and Foster 1995).
235 Colour significantly affected host recognition and oviposition in the parasitoid
236 *Aphidius ervi* Haliday (Battaglia et al. 2000) and in the egg parasitoid *Trichogramma ostriniae*
237 Pang & Chen (Lobdell et al. 2005). These results showed that the black pupa was well-
238 accepted when there was no chance of choice, but this pupa strain was not preferred when
239 females could choose between both types of strains, a situation that would not arise under
240 mass rearing conditions.
241 Adult emergence percentages were lower in the black pupae than in the SMR pupae.
242 This result could potentially reflect the maximum expected survival of 50% in genetic sexing
243 lines due to the presence of non-viable zygotes produced by adjacent segregation during
244 meiosis (Robinson et al. 1999).
245 *Coptera haywardi* superparasitized both TAP-7- and SMR-strain pupae, with
246 percentages ranging between 30 and 80%. In parasitoids such as *D. longicaudata*, it has been
247 registered that the superparasitism is positively correlated with the proportion of females
248 and does not affect fitness (González et al. 2007; Montoya et al. 2011). However,
249 superparasitism has not been studied in *C. haywardi*, and its potential biological effects
250 remain unknown. Cancino et al. (2012) reported that under dual choice (parasitized/non-
251 parasitized pupae) conditions, *C. haywardi* discriminates between non parasitized pupa and
252 pupae parasitized by conspecifics, as well between non parasitized pupa from those
253 previously parasitized by larval *D. longicaudata*. The last does not imply that under specific

254 conditions (e.g., high competition from conspecifics), *C. haywardi* females cannot
255 superparasitize their hosts (see van Alphen and Visser 1990).
256 The mechanical impact produced during the separation of black pupae in the Sortex-
257 Buhler sorter further reduced the emergence of *C. haywardi* adults from black pupae. It has
258 been reported that the mechanical separation of pupae using sieves (i.e., "screening")
259 negatively affects the percentage of flying flies when *C. capitata* are between 3 and 7 days
260 old because their muscles are still forming (Little et al. 1981; Chang et al. 1982; Ozaki and
261 Kobayashi 1982). This result reflects the low hosting quality of impacted black pupae, with
262 repercussions on parasitoid emergence.
263 None of the tested irradiation doses adversely affected the parasitoid emergence. In
264 contrast, it was observed that the emergence of non-irradiated pupae was slightly lower,
265 reflecting the suppression of host defences through irradiation (Hooper 1989). There is
266 evidence that irradiation of eggs, larvae and pupae of tephritids favours the emergence of
267 parasitoids of the involved species (Cancino et al. 2009; Hepdurgun et al. 2009), particularly
268 in the case of young pupae, which are more susceptible to irradiation due to their metabolic
269 activity and morphological changes during metamorphosis. In older pupae, for which
270 metamorphosis is nearly complete, the irradiation effects are dramatically reduced, and
271 adult emergence might occur despite irradiation, depending on the dose applied. Our results
272 confirm these findings. Nonetheless, it is known that the eggs produced by flies that
273 emerged from pupae irradiated with 40 Gy are sterile (Hallman 2000). Therefore, all of the
274 ages evaluated in this study are theoretically viable for the mass production of *C. haywardi*.
275 Adults emerging from black pupae registered the same biological features as those
276 emerging from SMR strains, suggesting that the parasitoids have equivalent quality and
277 could be equally successful in biological control programmes. Unlike other fruit fly

parasitoids (e.g., *Pachycrepoideus vindemmiae* (Rondani), *Trichopria anastrephae* (Costa Lima), *Pachyneoµuron* sp., and *Spalangia* sp.) (Ovruski et al. 2000), *C. haywardi* specifically parasitizes Tephritidae pupae (Sivinski et al. 1998; Baeza-Larios et al. 2002; Guillen et al. 2002). Therefore, this species could be used as a complementary biological control agent to reduce fruit fly populations. Several authors (e.g., Paine et al. 2000; Denoth et al. 2002; Cusson et al. 2002; Snyder et al. 2004; Pedersen and Mills 2004) have reported successful Insect pest suppression using more than one parasitoid species.

In conclusion we found that 1) the TAP-7-strain black pupa is a viable host of *C. haywardi*, particularly those pupae irradiated at 3-5 days old, which produced the highest adult emergence and prevented fly emergence; 2) the tested irradiation doses did not affect parasitoid emergence; and 3) adults that emerged from black pupae exhibited the same biological features as those emerging from SMR strains. These results suggest that the TAP-7-strain black pupae can be used as hosts for the mass rearing of *C. haywardi.*

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Table 1. Average (± SE) of emergence and sex ratio of *C. haywardi* obtained in TAP-7 strain pupa and pupa of cepa SMR

Form of exposure of pupae	% *C. haywardi* emergence	Sex ratio (♀/♂)
a) Separate exposure		
Pupa of strain mass rearing (SMR)	62.0 ± 6.98 a	1.52 ± 0.25 a
Black pupa TAP-7 strain	59.5 ± 6.98 a	1.25 ± 0.25 a
b) Different dish within the same cage		
Pupa of strain mass rearing (SMR)	71.0 ± 5.21 a	1.74 ± 0.25 a
Black pupa TAP-7 strain	52.5 ± 5.21 b	1.45 ± 0.25 a
c) Mixed within the same cage		
Pupa of strain mass rearing (SMR)	71.5 ± 4.84 a	1.99 ± 0.29 a
Black pupa TAP-7 strain	54.5 ± 4.84 b	2.05 ± 0.29 a

Values followed by a different letter in the same column are statistically different Tukey-Kramer HSD test ($\alpha = 0.05$)

Table 2. Results from the 3-way analysis (pupa, dose, age) of *C. haywardi* emergence from TAP-7 and SMR

Source	Degrees of freedom	F ratio	Prob > F
pupa	1,648	445.92	<.0001
dose	3,648	0.04	0.98
age	8,648	20.17	<.0001
pupa*dose	3,648	2.13	0.09
pupa*age	8,648	5.03	<.0001
dose*age	24,648	0.95	0.52
pupa*dose*age	24,648	1.6	0.034

Table 3. Mean (± SE) emergence, flight ability, survival and fecundity of *C. haywardi* obtained from SMR and TAP-7 black pupae

Type host pupa	Pupal age (days)	(%) Emergence	(%) Flight ability	Survival (days) ♀	Survival (days) ♂	Fecundity offspring/♀/day
Pupa SMR	3	59.1 ± 2.79 a	92.4 ± 1.65 a	4.50 ± 0.06 a	4.21 ± 0.05 a	1.58 ± 0.08 a
Black pupa TAP-7 strain	5	29.7 ± 2.79 b	92.1 ± 1.65 a	4.46 ± 0.07 a	4.36 ± 0.07 a	1.56 ± 0.08 a
	9	27.3 ± 2.79 b	91.8 ± 1.65 a	4.65 ± 0.07 a	3.73 ± 0.08 a	1.54 ± 0.08 a
	10	23.7 ± 2.79 b	90.9 ± 1.65 a	4.44 ± 0.08 a	4.07 ± 0.09 a	1.58 ± 0.08 a
	11	23.7 ± 2.79 b	88.6 ± 1.65 a	4.85 ± 0.08 b	4.73 ± 0.14 b	1.52 ± 0.08 a

Average followed by a different letter in the same column indicate statistic significance Tukey-Kramer HSD test (α=0.05)

Figure legends

Figure 1. Relationship between the number of scars per pupa and the number of immature stages according to the number of pupae in the SMR and TAP-7 strains exposed in separate cages (a), in different Petri dishes within the same cage (b), and in mixed conditions (c).

Figure 2. Average ($\pm$ SE) of emergence percentage of *C. haywardi* from 3-11-day-old black TAP-7 and SMR (control) pupae exposed to irradiation. Average followed by a different letter in the same column indicate statistic significance between treatments.

Figure 3. Survival of *C. haywardi* males and females emerging from TAP-7 black pupae (5, 9, 10 and 11 days) and 3-day-old SMR pupae.

Figure 1

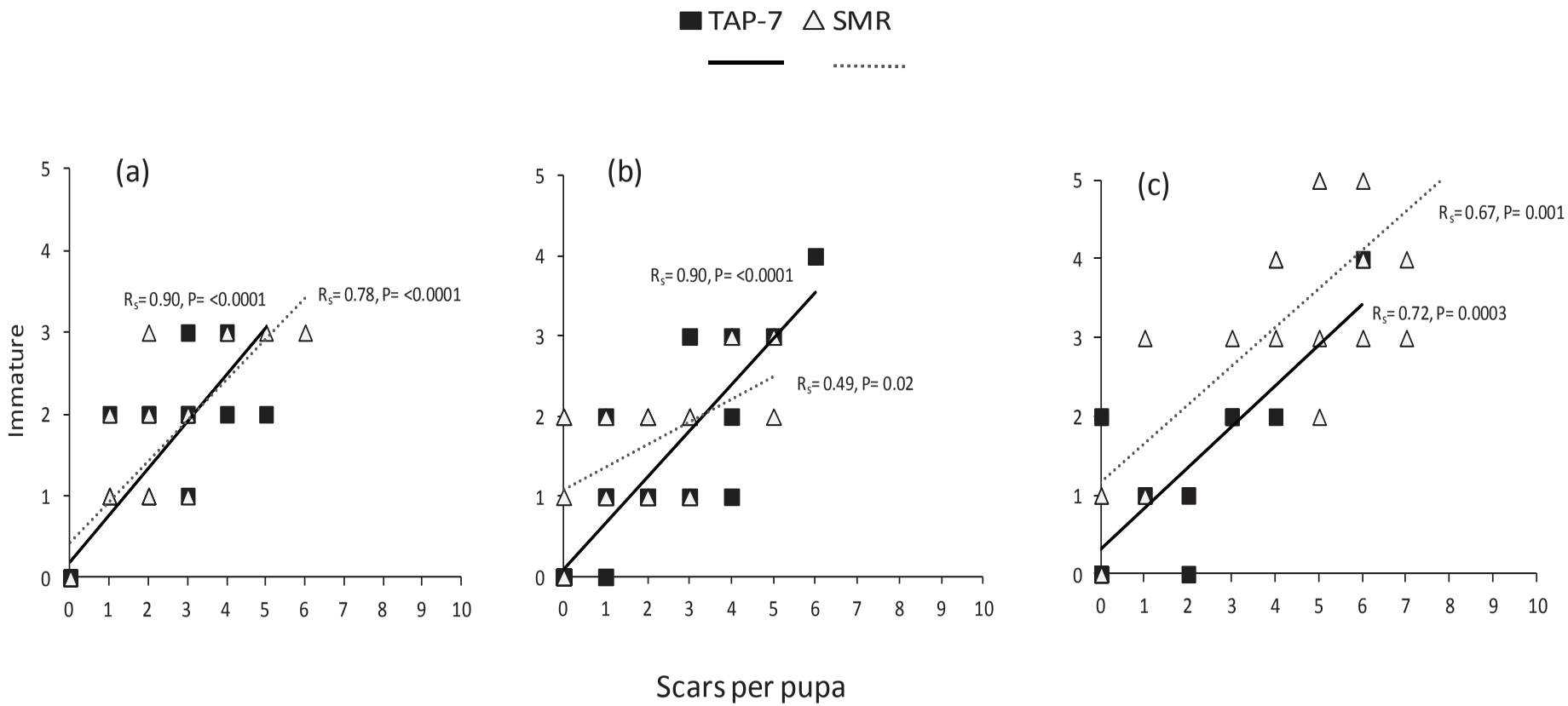
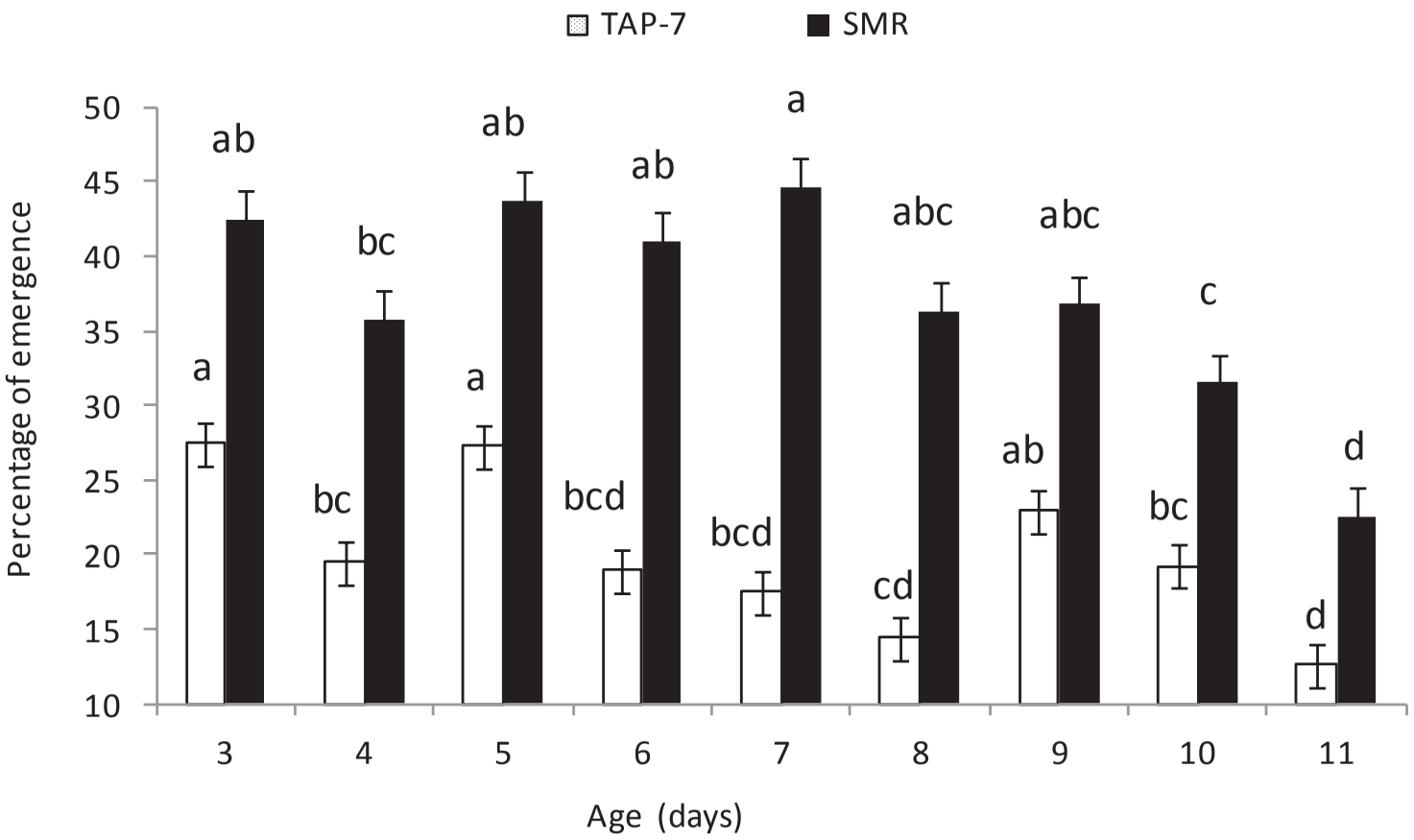

TAP-7
SMR
(a)
(b)
(c)
Immature
Scars per pupa
R_s= 0.90, P= <0.0001
R_s= 0.78, P= <0.0001
R_s= 0.90, P= <0.0001
R_s= 0.49, P= 0.02
R_s= 0.67, P= 0.001
R_s= 0.72, P= 0.0003

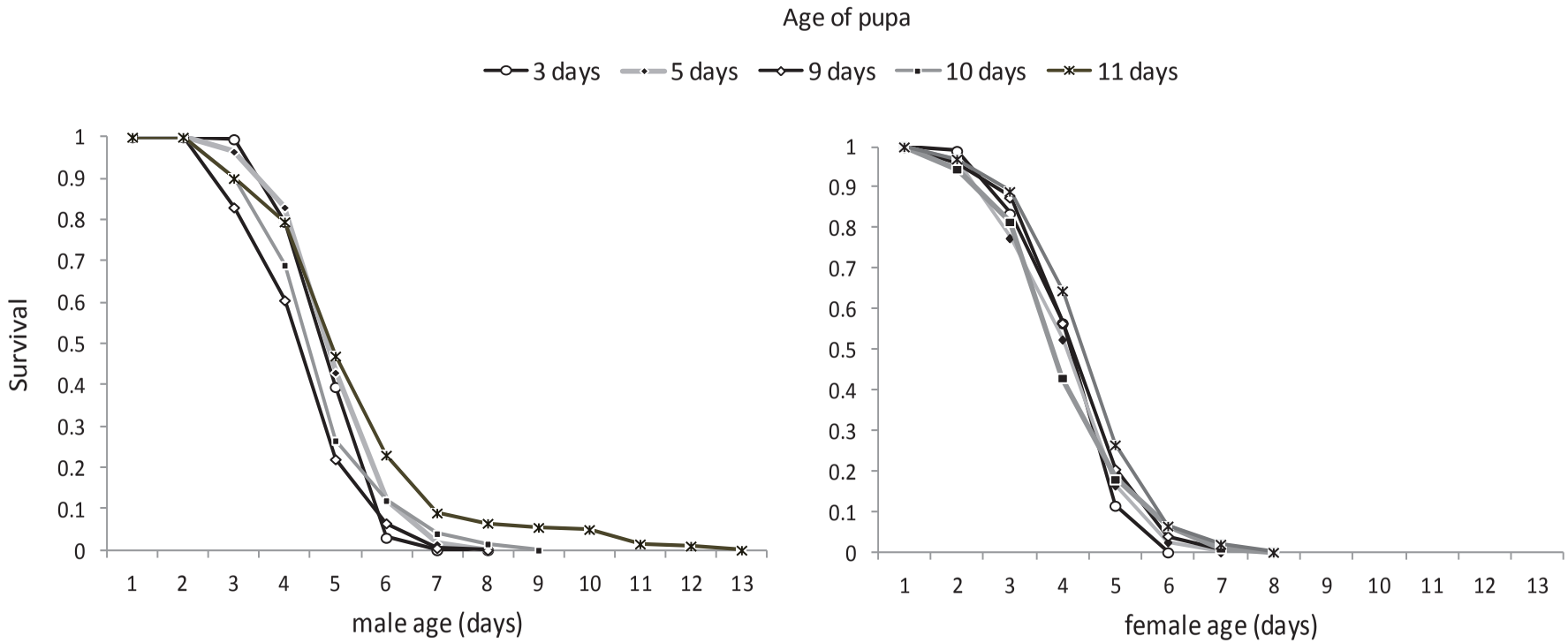
Figure 2


TAP-7
SMR
Percentage of emergence
50
45
40
35
30
25
20
15
10
ab
bc
ab
ab
a
abc
abc
c
a
bc
a
bcd
bcd
cd
ab
bc
d
d
3
4
5
6
7
8
9
10
11
Age (days)

(a)

(b)

Figure 3


Age of pupa
3 days
5 days
9 days
10 days
11 days
Survival
male age (days)
female age (days)

Conclusiones

1. *C. haywardi* aceptó como hospedero la pupa negra de la cepa Tapachula-7 separada como pupa de desecho (subproducto), aunque los porcentajes de emergencia obtenidos fueron bajos en comparación a los obtenidos en la pupa estándar de cría masiva.

2. *C. haywardi* se desarrolló en ambos tipos de cepas (pupa negra de cepa Tapachula-7 y pupa estándar de la cría masiva). cuando las hembras no tuvieron la opción de elegir, los porcentajes de emergencia fueron similares en los tipos de pupas ofrecidas, pero cuando tuvieron la opción de elegir, la pupa negra fue menos preferida que la de la cepa estándar, aunque el parasitoide se desarrolló adecuadamente.

3. El golpe recibido sobre la pupa negra durante el proceso de separación mecánica afectó la emergencia de *C. haywardi,* siendo significativa la diferencia en la emergencia de parasitoides entre pupa separada mecánicamente y manualmente.

4. Las dosis de irradiación empleadas en la pupa negra no afectaron la emergencia de *C. haywardi*, por lo contrario, esta se vio favorecida.

5. Los adultos de *C. haywardi* emergidos de pupa negra no mostraron diferencia en los parámetros de aptitud (supervivencia, fecundidad y habilidad de vuelo) comparados con los adultos que emergieron de la cepa estándar de cría masiva.

6. En ambas cepas se observó una relación significativa entre el número de cicatrices de oviposición y el número de larvas de primer estadio dentro de la pupa.

7. La pupa negra de la cepa Tapachula-7, que es un subproducto de la producción, puede ser utilizada como hospedero para la cría masiva de *C. haywardi*.

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