Augmentative releases of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) for *Ceratitis capitata* (Diptera: Tephritidae) control in a fruit-growing region of Argentina

Guillermo Sánchez, Fernando Murúa, Lorena Suárez, Guido Van Nieuwenhove, Gustavo Taret, Valeria Pantano, Mariana Bilbao, Pablo Schliserman, Sergio M. Ovruski

PII: S1049-9644(16)30138-4
DOI: http://dx.doi.org/10.1016/j.biocontrol.2016.08.002
Reference: YBCON 3473

To appear in: *Biological Control*

Received Date: 3 March 2016
Revised Date: 20 July 2016
Accepted Date: 2 August 2016

Please cite this article as: Sánchez, G., Murúa, F., Suárez, L., Van Nieuwenhove, G., Taret, G., Pantano, V., Bilbao, M., Schliserman, P., Ovruski, S.M., Augmentative releases of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) for *Ceratitis capitata* (Diptera: Tephritidae) control in a fruit-growing region of Argentina, *Biological Control* (2016), doi: http://dx.doi.org/10.1016/j.biocontrol.2016.08.002

Augmentative releases of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) for *Ceratitis capitata* (Diptera: Tephritidae) control in a fruit-growing region of Argentina

Guillermo Sánchez[1], Fernando Murúa[1,2], Lorena Suárez[1], Guido Van Nieuwenhove[3], Gustavo Taret[1], Valeria Pantano[4], Mariana Bilbao[1], Pablo Schliserman[5], Sergio M. Ovruski[6,*]

[1] Programa de Control y Erradicación de Mosca de los Frutos de San Juan (ProCEM)—Dirección de Sanidad Vegetal, Animal y Alimentos (DSVAA), Av. Benavides 8000 (O)5407, Rivadavia, San Juan, Argentina. Emails: sanguille33@gmail.com, fmurua80@gmail.com, lorenacsuarez@gmail.com, gustavotaret@yahoo.com.ar, marianabilbao@hotmail.com

[2] IMCN-Diversidad de Invertebrados, Departamento de Biología, UNSJ, Av. Ignacio de la Rosa 590 Oeste; 5402, Rivadavia, San Juan, Argentina

[3] Fundación Miguel Lillo, Miguel Lillo 251, S.M. de Tucumán, Tucumán, Argentina
Email: gavn12004@yahoo.com.ar

[4] Dirección de Sanidad Vegetal, Animal y Alimentos, Av. Benavides 8000 (O)5407, Rivadavia, San Juan, Argentina. E-mail: valeria_pantano@yahoo.com.ar

[5] Centro de Investigaciones y Transferencia de Catamarca (CITCA-CONICET-UNCA), Prado 366, K4700AAP. S.F.V.C., Catamarca, Argentina
Email: schliserman73@yahoo.com.ar

[6] PROIMI Biotecnología, CCT Tucumán CONICET, División Control Biológico de Plagas, Av. Belgrano y Pje Caseros, 4000, S.M. de Tucumán, Tucumán, Argentina. Tel. nº 54-381-4343817. Email: sovruski@proimi.org.ar

[*] Corresponding Author

Abstract

Field-open augmentative releases were conducted to assess the efficacy of *Diachasmimorpha longicaudata* (Ashmead) for the regulation of *Ceratitis capitata* (Weidemann) infesting *Ficus carica* (L.) in a commercial area located in a fruit-producing irrigated-valley of San Juan, central-western Argentina. Parasitoids were reared on Sensitive Lethal TemperatureVienna-8 strain of *C. capitata* at the BioPlanta San Juan facilities, and were weekly released throughout 9 weeks over two experimental plots of ca. 2.3 hectares each with a density of 5,200 wasps/plot. Host mortality and medfly emergence at the release plots were significantly 1.9-times higher and 1.5-times lower, respectively, than those recorded in the control plots. *D. longicaudata* females increase their effectiveness on medfly at both higher temperature (22-23ºC) and relative humidity (54-62%) values. Parasitoid females used in the study showed a good ability to spread once released in open-field. Between 16 and 75% of host mortality during the parasitoid release period was due to *D. longicaudata*, which appears to be promising for the control of medfly in San Juan as well as in other similar Argentinean fruit-growing semi-arid regions.

Keywords

Mediterranean fruit fly;Parasitoid release;Parasitoid effectiveness;Host mortality; Commercial fruit crop;Fruit fly biological control

1. Introduction

Argentina is one of the largest producers and exporters of fresh fruit and vegetables in the southern hemisphere. Argentina exports over 1.9 million tons of fruits and vegetables each year, generating revenues of around 1.7 billion dollars. Annually, fresh fruitexportation accounts for about 9% of total agricultural exports from Argentina (FundaciónExportAr, 2014). However, this value could be even higher except for the fact that the tephritid fruit flies *Ceratitis capitata* (Weidemann) and *Anastrepha fraterculus* (Weidemann) cause damage between 15% and 20% in the Argentinean annual production of fresh fruits and vegetables. Thus, direct crop losses by larval infestation represent a reduction of profit margins nationwide of up to approximately US$ 90 million per annum (Guillén and Sánchez, 2007).

In Argentina, the Mediterranean fruit fly (medfy), *C. capitata*, is a destructive pest of over 22 cultivated fruit species and it is a barrier to trade and a hindrance to agricultural development across the country (Guillén and Sánchez, 2007). Currently, *C. capitata*is found throughout all Argentinean fruit-growing regions, covering latitudes from 22° to 56°S. In the dry central-western fruit-producing region, namely, the provinces of San Juan and Mendoza, where grape, fig, pome fruits, and stone fruits are mainly grown, the only economically important tephritid species is *C. capitata* (Guillén and Sánchez, 2007). In this region, local governments, under the coordination of the National Fruit Fly Control and Eradication Program (ProCEM) from Argentina, have applied area-wide control/eradication actions against medfly for establishing pest free and low prevalence areas (Guillén and Sánchez, 2007). Biological control has recently been incorporated as a complementary toolfor maximizing the impact of the non-

chemical, biological components of the control measures currently deployed in the fruit-growing areas of San Juan.

Biological control is one of the most environmentally safe and economically profitable pest management method (van Lenteren, 2012), and it can be a practical and effective complementary tool in the fruit fly integrated management programs (Wang and Messing, 2004; Vargas et al., 2012). Augmentative release of parasitoids may be one of the most promising methods of suppressing fruit fly populations at the appropriate time and place (Knipling, 1992; Purcell et al., 1998; Montoya et al., 2011). Mass releases of *Diachasmimorpha tryoni* (Cameron) against *C. capitata* in Hawaii (Wong et al. 1991) and Guatemala (Sivinski et al., 2000) increased parasitism rates in release areas. Similarly, studies on the effectiveness of augmentative releases of *Psyttalia fletcheri* (Silvestri) against *Bactrocera cucurbitae* (Coquillett) in Hawaii (Vargas et al., 2004), and of both *Diachasmimorpha krausii* (Fullaway) and *Fopius arisanus* (Sonan) against *C. capitata* into field cages in Guatemala (Rendon et al., 2006) showed both reduced fly emergence rates and increased parasitism rates. Harris et al. (2010) demonstrated that simultaneous augmentative releases of both *F. arisanus* and *P. fletcheri* in Hawaii increased suppression of *B. cucurbitae* compared to releases of *P. fletcheri* alone. Establishingthe solitary larval endoparasitoid *Diachasmimorpha longicaudata* (Ashmead), native to Southeast Asia, into the American continent, has been important to augmentative biological control releases against pestiferous *Anastrepha* spp. (Ovruski et al., 2000; Cancino et al., 2014). Field-open augmentative releases have shown that *D. longicaudata* can substantially suppress populations of *Anastrepha suspensa* (Loew) in Florida (Sivinskiet al., 1996), *Anastrepha ludens* (Loew), *Anastrepha obliqua* (McQuart), *Anastrepha serpentina* (Wiedemann), and *Anastrepha striata* (Schiner) in the states of Chiapas, Michoacán, Sinaloa, Nayarit, and

Aguascalientes in Mexico (Montoya et al., 2000a, 2007). Faced with all this evidence, further evaluations are required to record the efficacy of biological agents prior to the development of this technology within action programs.

The braconid *D. longicaudata* was introduced in Argentina via Mexico in the 1990s to promote fruit fly biological control (Ovruski et al., 2000). Given this fact and due to several other reasons, *D. longicaudata* was considered to be suitable for augmentative releases in San Juan. The most relevant arguments to do so are the adaptability of *D. longicaudata* to the different environments into which it has been introduced (Ovruski et al., 2000), the development of efficient techniques for mass-rearing in Hawaii (Vargas et al., 2012) and México (Montoya et al., 2007), its capacity for successful development on the *C. capitata* larvae infesting fruit under field conditions (Ovruski et al., 2012), and its host-finding ability at different host-densities on a wide variety of fruit species and at canopy and ground levels (García-Medel et al., 2007). Therefore, *D. longicaudata* is being mass-reared at the BioPlanta San Juan facilities with the aim of using it for mass-releasing in organic growing areas and cultivated suburban locations in order to achieve suppression or selected eradication of medfly populations (Suárez et al., 2014).

Due to the semiarid environmental conditionsin San Juan, extensive fruit crops and backyard orchards are found in ecologically isolated vegetation patches subjected to artificial irrigation, which are ideal scenarios to test the effectiveness of *D. longicaudata* on medfly through open-field augmentative releases (Suárez et al., 2014). Consequently, *D. longicaudata* adults, reared on thegenetic sexing Sensitive Lethal TemperatureVienna-8 strain of*C. capitata,* were mass released over commercial crops of *Ficus carica* (L.) (fig) (Urticales: Moraceae) in San Juan. This research is part of a renewed effort to encourage broad use of a biological control with in a framework of

125 environment-friendly strategies to suppress both medfly and South American fruit fly
126 populations in Argentina (Van Nieuwenhove et al., 2016). In this regard, the relevance
127 of the findings is discussed bearing in mind the use of this parasitoid species in
128 augmentative biological control programs devised in Argentina.
129
130 **2. Materials and methods**
131
132 *2.1. insect rearing*
133
134 Parasitoids were obtained from the BioPlanta San Juan mass-rearing facility, located in
135 San Juan, Argentina. Adult parasitoids were reared on irradiated third-instar larvae (5-d
136 old) of Sensitive Lethal Temperature Vienna-8 *C. capitata* strain. Parasitoids were kept
137 in rectangular iron-framed mesh-covered cages (0.5 × 0.5 × 0.6 m) holding 2,000 pairs
138 per cage in a 25 m^2 room at 24˚C ± 1˚C; 65% ± 5% RH and a photoperiod of 12:12
139 (L:D). Light came from 1,000 lux daylight fluorescent tubes. Parasitoid rearing cages
140 with water and honey were provided every other day. The colony of *D. longicaudata*
141 was initiated with individuals from the Pilot Plant of Industrial Microbiological
142 Processes and Biotechnology (PROIMI) in Tucumán, Argentina.
143
144 *2.2. Experimental location and selected fruit species*
145
146 The study site was a commercial fig crop cultivated with the Kadota cultivar,
147 surrounded by native vegetation characterized by xerophytes shrubs. The fig trees were
148 ~3.5 m tall and separated from each other by 6m. The site was located in a rural area at
149 31°44´17" S and 68°18´51" W, and 600 m above sea level, in an irrigated fruit-

producing valley in 25 de Mayo, a rural village in the province of San Juan, in central-western Argentina (Fig. 1). The climate is continental-desert with a remarkable annual variation in temperature and atmospheric pressure. The mean annual temperature is 17.2˚C, and the mean annual rainfall is 110 mm. Rainfall is moderate, occurring mostly in summer, i.e., December through March. Maximum, minimum, and mean temperatures, relative humidity, cumulative rainfall, and wind speed recorded during each testing weeksin 2012 are detailedin Table 1. The environmental data were recorded with a wireless weather station (Automatic Agro-Meteorological Station NIMBUS, Model THP) located in the central sector of the crop.

High *C. capitata* population levels were recorded in the study area one year before parasitoid releases. This area is not under control actions by the ProCEM San Juan. Thus, the number of wild *C. capitata* captured per trap and per dayvaried from 0.4 to 12.4 during the fig fruiting period, i.e. mid December/2010-late April/2011 (Unpublished data, ProCEM San Juan). Fig was chosen because it is a key host plant for medfly proliferation throughout the fruit-growing central-western Argentinean region (Suárez et al., 2014).

2.3. Parasitoid releasing

Parasitized *C. capitata* pupae were packed in sulphite paper bags (17 cm width x 49 cm height) with a narrow strip of tissue filled with icing sugar as food, at a density of approximately 1,300 pupae per bag. The bags were closed at the top with six staples. Bagged pupae were kept in a dark room at 25 ± 1°C and 70 ± 5% RH for 3 days, until both males and females emerged. Ten bags were prepared weekly, with an average emergence of 40%, equivalent to about 520 parasitoids per bag, and a female:male sex

ratio of ca. 1:1. All release bags were taken to the study site in an air-conditioned vehicle every parasitoid release date. Releases of *D. longicaudata* were carried out over the fig postharvest stage, a period of 9 weeks between mid-March 2012 (week 12 to 18) and early-May 2012 (week 7 to 13). Parasitoids were released in two experimental plots (= release plots) of 23.400 m^2 (ca. 2.3 ha) each one, while another two plots of the same size were used as controls (without parasitoid releases). Each of the four plots, including control and release, contained ca. 522 commercial fig trees and was 500 m away from each other. All around control and experimental plots, dry traps were used to isolate *C. capitata* population that was in each study plot. McPhail traps (Susbin^{TM}, Guaymallen, Mendoza, Argentina) baited with both TMA lure cards (Trimethylamine) (Susbin^{TM}) and DDPV tablets (Diclorvos) (Susbin^{TM}) were used with a density of 1 trap per 200 m^2. A total of 1,000 traps were used in those crop sectors located among the different study plots. Of all these traps, 62 were used surrounding each study plot. The traps were separated from each other by 10 m , and placed parallel to the margins of the study plots 20 meters away from them**.** Parasitoids were released by ground on a weekly basis by using a system transects. Five 150 m-long line transects in a south-north direction were arranged in each experimental plot (Fig. 1A). One transect was located at the center of the plot, one near the western margin of the plot, another one close to the eastern margin, and two between the center and the margins of the plot.Transects were separated from each other by 25 m,15 m apart from both western and eastern edges of the plot and 15 m from both southern and northern edges of the plot. At each transect, ten release sectors, spaced 15 m apart, were marked (Fig. 1A). On the release date, two bags were opened along of each transect at two different release sectors and tissues filled with icing sugar were put on tree branches. In total, 10 bags were opened in the experimental plot and the release sectors were randomised by release date.

Approximately 5,200 parasitoids per experimental plot (~ 2,261 parasitoids/ha) were released every week.

2.4. Fly and parasitoid monitoring

Unsprayed and uninfested figs from Kadota cultivar were used during the parasitoid release tests. Figs were obtained from backyard gardens. Several branches of unsprayed fig trees, each containing 7–10 unripe fruits, were covered with cloth meshes. Once the fruit reached a commercial grade ripeness based on color and firmness, such as light green and 100% hard, they were harvested and taken to the study site.To guarantee consistency in the fruit size, the weight and diameter of each fruit was determined before starting tests. Fruit was between 3.9-4.1-cm diameter, and 34.4- 37.1-g weight. Artificial devices were specially designed to hold figs and expose them to natural infestation by oviposition of wild *C. capitata* females (Fig. 2). The device consisted in an inverted U-shaped galvanized wire frame, with two rings at the tip. A 200 ml-longitudinal plastic container with wheat bran inside to act as the pupation substrate was held between the rings. The central portion of the container had a rectangular hole of 45 cm long and 15 cm wideon the upper part. Three fig fruit were hung one beside the other from the top of the wire frame and positioned 5 cm above the central hole of the container. Each fruit was hung by means of a plastic string tied at the base of the long stalk. Each device was hung from a branch of a fig tree 1.5 m above ground level. 30 devices, containing 90 figs in total (3 per device),were placed into each release plot, as well as into each control plot, three days before each parasitoid release date. Devices were distributed in five longitudinal rows in a south-north direction, each row containing six devices (Fig. 1B). All exposure devices covered a 15,000 m^2–central

225 rectangular area within the plot. This area was distant from all margins of the plot by 15
226 m, respectively. In turn, the exposure devices were separated from each other by 25 min
227 west-east direction and by 30 m in south-north direction. Overall, taking into account
228 the two release plots and the two control plots, 360 uninfested similar-size figs were
229 used in each testing week as oviposition units.

230 The hanging fruit, as well as wheat bran in each exposure device, were weekly
231 replaced by new uninfested figs and a new pupation substrate. In the laboratory, infested
232 fruit was individually placed in 500 ml-plastic containers for 7 days with wheat bran in
233 the bottom to facilitate pupation. Subsequently, fruit were dissected to retrieve *C.*
234 *capitata* larvae, and the number of dead larvae per fruit was recorded. In addition, the
235 wheat bran from the containers was sieved to recover puparia originated from larvae
236 that fell from hanging figs. Then, *C. capitata* larvae and/or puparia were placed in 250-
237 ml plastic cups with new damp and sterilised wheat bran in the bottom. The top of each
238 cup was tightly covered with a piece of organdy. The puparia were moistened weekly to
239 avoid desiccation and were held inside the cups until adult flies or parasitoids emerged.
240 The cups were placed in a room at 25 ± 8 ºC and 70 ± 5% RH with a 12:12 (L:D) h
241 regime. Thus, the portion of *C. capitata* population located in each study plot, as well as
242 parasitism caused by *D. longicaudata* in both release plots, was monitored by using
243 adult emergence data from device-collected figs. No traps within either experimental
244 and control plots were used in order to avoid an external mortality factor of the target
245 pest. Both the number and sex of the parasitoids, the number of flies, and the non-
246 eclosed puparia were recorded. Control plots allowed determining natural rates of both
247 *C. capitata* larvae and pupae mortality and adult emergence.

248 Twelve McPhail traps baited with yeast plus borax pellets PBX (Susbin®) plus
249 water were used to monitor adult medfly population in the fig crop. These traps were

250 distributed inside two other plots of the same size and with the same number of
251 commercial fig trees detailed above for both release and control plots. In each trapping
252 plot, traps were located in two 100 m-long line transectsin south-north direction. Three
253 traps were located per transect, that is, six traps in each plot. Each trap was separated
254 from one another by 50 m in both south-north and west-east directions, and distanced
255 from both western and eastern margins, and from both southern and northern margins of
256 the plot by 40 m.The trapping plots were distanced from both control and release plots
257 by 500 m, and they were surrounded with dry traps, as described above, to isolate
258 medfly population present within the plot. Traps were serviced every 7 days for 9
259 weeks. Captured flies were identified and sexed in the laboratory. The FTD index (fly
260 per trap per day) was weekly calculated.
261
262 *2.5. Data analysis*
263
264 Infestation level in fruit, adult *D. longicaudata* and *C. capitata* emergences, and host
265 mortality were estimated for experimental and control plots. The infestation level was
266 calculated as the number of fly larvae that did not get to pupate, plus the number of
267 puparia recovered from fruits. The parasitoid and fly emergences were calculated as the
268 number of emerged adult parasitoids or flies. The host mortality was calculated as the
269 number of dead host larvae plus the number of puparia that did not yield insects.All
270 these variables were subjected to one-way univariate mixed-model ANOVAs with type
271 III error at $P = 0.05$. This type of analysis allowed the identification of significant
272 effects between the plots, i.e. experimental and control, on all response variables,
273 namely, infestation level, parasitoidand medfly emergences, and host mortality. The
274 fixed component of the models were plots, treated vs untreated, whereas the random

component, time, with 9 levels, days 1–9, was blocked. Mean comparisons were analyzed by Tukey's honestly significant difference (HSD) test at $P = 0.05$. Prior to analyses, data were checked for normality and homogeneity of variance by using Shapiro-Wilks test (Bolker et al., 2009).The real host mortality inflicted by the parasitoid (efficacy) was estimated through the Abbot's corrected formula (Rosenheim and Hoy, 1989).The *D. longicaudata* effectiveness was estimated for each parasitoid releasedate per experimental plot and expressed as percentage. The relationships between *D. longicaudata* effectiveness and mean temperature, as well as relative humidity were analyzed by Pearson's Product Moment correlation tests ($P < 0.05$).Sex ratio of parasitoid offspring was estimated as the ratio of female offspring over male offspring. Statistical analyses were performed using STATISTICA, version 10.0 software (StatSoft Inc., 2011).

3. Results

The mean (± SE) infestation levels, that is to say, dead host larvae plus recovered puparia per fruit, recorded in both control plots (1.4 ± 0.2 and 1.1 ± 0.3) and in bothrelease plots (1.6 ± 0.3 and 1.4 ± 0.2) were significantly similar ($F_{(3, 24)}$= 1.4400, P = 0.2558). Nevertheless, mean (± SE) host mortality at the experimental plots was notably 1.9-times higher than that recorded in the control plots (Fig. 3) ($F_{(3, 24)}$= 15.023, P< 0.0001), whereas mean (± SE) *C. capitata* emergence at the release plots was significantly 1.5-times lower than that found in the control areas (Fig. 4) ($F_{(3, 24)}$= 31.019, P< 0.0001). Around 67% of the fruit samples from the control plots yielded parasitoids; however, the *D. longicaudata* emergence in the control was substantially 6-times lower than that recorded in the release plots (Fig. 5) ($F_{3, 24}$= 18.943, $P < 0.0001$).

As regards *D. longicaudata* efficacy into the release plots, it ranged from 16 to 75% during the parasitoid release period (Fig. 6). Taking into account the nine release weeks and the two experimental plots, *D. longicaudata* caused 35.7 ± 4.0% (mean ± SE) of real mortality in the *C. capitata* population throughout the parasitoid release phase. Significant positive correlations were found between *D. longicaudata* effectiveness and both the mean temperature (r = 0.769, N = 18, P= 0.0002) and the relative humidity (r = 0.605, N = 18, P= 0.0077) recorded in the study area. A modestly female-biased sex ratio was exhibited by *D. longicaudata*; approximately 53% of parasitoid individuals recovered from fruit samples were females. Figure 7 shows adult medfly capture variations throughout testing weeks in the trapping plots. The percentage of females on total caught adult flies varied between 42.7 ± 4.3 and 61.1 ± 3.0%. The highest FTD indexes recorded occurred in March. Since that month on, medfly population decreased.

4. Discussion

Previous spot releasesshowed that *D. longicaudata* adults reared on Vienna-8 *C. capitata* strain of the BioPlanta San Juan were able to attack wild *C. capitata* larvae in several host fruit species in different irrigated fruit-producing valleys of San Juan (Suárez et al., 2014). Results of the present study carried out on a fig crop suggest that augmentative releases of that *D. longicaudata*'s lineage significantly reduced *C. capitata* adult emergence in the treatment plots, compared with that in the control plots. This was evident when comparing the similar fruit infestation levels recorded in both plots with the two variables host mortality and adult fly emergence. The host mortality was remarkably higher in the release plots, a fact which probably induced a lower numberof emerged adult medflies in that site. This marked decrease of viable host

puparia may have resulted from mortality inflicted by *D. longicaudata* on the developing host. In addition to host mortality caused by parasitoid emergence, other mortality factors, such as superparasitism and stinging activity without oviposition, may have increased host mortality rate. Several studies have demonstrated that *D. longicaudata* tends to superparasitize host larvae strongly not only under laboratory conditions (Montoya et al., 2011; González et al., 2010) but also under natural field conditions (Montoya et al., 2013). This may increase efficacy of the female parasitoid on the host (Ovruski et al., 2012). In addition, Montoya et al. (2000b) reported that mortality in the mass-reared *A. ludens* larvae may be due to damage caused by an excessive number of punctures caused by *D. longicaudata* females. In view of the future *D. longicaudata* mass releases in fruit-growing areas of San Juan, the efficacy of *D. longicaudata* against *C. capitata* should be properly estimated by evaluating the real host mortality, instead of basing field evaluation on the number of parasitoids that emerged from the host. This finding is in agreement with studies performed in different conditions. Montoya et al. (2000a) researched on *D. longicaudata* parasitizing *A. ludens* larvae under mass-rearing conditions; Ovruski et al (2012) analysed *D. longicaudata* parasitizing *C. capitata* larvae under field-cage, and Harris et al (2010) conducted a research on *F. arisanus* and *P. fletcheri* (Silvestri) parasitizing *B. cucurbitae* under open-field conditions.

Interestingly, a certain level of association between temperature and parasitoid effectiveness was detected. The two highest mean parasitoid efficacy values (63.6 ± 5.3% and 62.1 ± 2.6% real host mortality) recorded in the release weeks March 26-April 1and April 2-8, respectively,occurred at high mean temperatures (Table 1). In contrast, the lowest mean parasitoid effectiveness value (17.4 ± 0.7%) recorded in the release week April 23-39, coincided with the mean lower temperature than others

recorded throughout release period (Table 1). In addition, on the basis of correlation data recorded in the present study there is some indication that *D. longicaudata* females increase their oviposition activity at higher relative humidity values. However, this information on the effect of different climatic conditions on the performance of *D. longicaudata* on medfly must be consideredwith caution;more detailed studies on the bioclimatic requirements of this exotic parasitoid in San Juan are still needed, especially considering that it is a native species in a tropical region (Cancino et al., 2014). According to Sime et al. (2006) and Paranhos et al. (2007) *D. longicaudata* appears to have a poor performance at low winter temperature.

The presence of *D. longicaudata* in the control plot was probably due to natural dispersion of individuals from the release plot. This braconid species has a good ability to spread once released in open-field (Paranhos et al., 2007). Parasitoid dispersal might be influenced bymany factors, among which climatic conditions are important; the prevailing wind, as well as temperature and rainfall, may affect *D. longicaudata* displacement (Messing et al., 1997). The southeast location of experimental plots relative to the control plots, and the predominant wind from the south, could have facilitated dispersion of released parasitoids from one plot to the other. Dispersion of *D. longicaudata* individuals towards the control plotsmay have been facilitated by other factors such as density and continuity of host fruit treesbetween plots (Montoya et al., 2000a), olfactory stimuli predominant in host-habitat (Jang et al., 2000) coming from the control plot, density of the released parasitoids, as well as intra-specific competition inside release plot (Paranhos et al., 2007). Additionally the proximity between plots, which were separated from each other by 500 meters, is also worth considering. Thus, the incidence of *D. longicaudata* in the control plots would suggest a good capability of parasitoid individuals originated from Vienna-8 *C. capitata* strain to disperse, survive

and find hosts outside the experimental plots, potentially a valuable trait for using this parasitoid lineage in augmentative releases.

Results of this study demonstrated that *D. longicaudata* reached ca. 36% efficacy on mortality rate of *C. capitata* in the release plots, even though mean emergence percentages of this braconid parasitoid were less than 10%. These values are far from those reported for *D. longicaudata* by Montoya et al. (2007), who recorded 70% of control on pestiferous *Anastrepha* populations with a 50% of parasitism in some fruit-growing regions of México. Nevertheless, the data on the effectiveness of *D. longicaudata* recorded in the present study is encouragingfor further evaluation of this braconid, not only because parasitoid adults were reared on larvae of Vienna-8 *C. capitata* genetic sexing strain, but also because they were released in a semi-arid area of fluctuating environmental conditions to control wild *C. capitata* with a high population level throughout parasitoid release dates. In this regard, it is worth noticing that trap capture within the study area showed a medfly natural population with a mean (± SE) value of 22.7 ± 4.3, 15.1 ± 1.5, and 2.3 ± 0.5 flies/trap/day in March, April, and May, respectively.

To sum up, the findings from this study served as a preliminary basis for monitoring andassessing the potential impact of *D. longicaudata* mass-reared in the BioPlanta San Juan factory on a wild population of *C. capitata* occurring in afruit-growing area of San Juan. Thus, the study provided clear evidence that augmentative release of *D. longicaudata* significantly contributed to *C. capitata* mortality in the selected fig crop. Future studies manipulating different released parasitoid densities will surely achieve deeper insight into theperformance of *D. longicaudata* as a biocontrol agent against *C. capitata* in San Juan. However, its performance under augmentative releases in a fruit-producing semi-arid area, such as the Cuyo region, was

unknown until now. Even though it is yet too early to determine real control effect of *D. longicaudata* on *C. capitata,* the preliminary information provided from this parasitoid release assessment opens up the possibility of devising new control strategies for medfly in San Juan. One alternative is to consider combined mass releases of sterile medflies and parasitoids, as well as the use of selective toxic baits (Vargas et al., 2001). Such actions will greatly contribute to the objectives of ProCEM-San Juan, namely,to establish both low *C. capitata* prevalence areas and free medfly areas in fruit-growing irrigated-valleys of San Juan based on the concept of area-wide integrated pest management (Guillén and Sánchez, 2007).

Acknowledgments

Special thanks to Andrés Murúa Trincado (Universidad Nacional de La Plata, Argentina) for drawing the fruit exposure device. Financial supports were provided by the San Juan Eradication and Control Medfly Program (ProCEM San Juan, Argentina), and by the National Fund for Science and Technology from the Argentinean National Agency for Scientific and Technological Promotion (FONCyT-ANPCyT, Argentina) (grant PICT/2013 No. 0604).

450 **References**

451

452 Bolker, B.M., Brooks, M.E., Clark , C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H.,
453 White, J.S.S., 2009. Generalized linear mixed models: a practical guide for
454 ecology and evolution. Trends Ecol. Evol. 24, 127–135.

455 Cancino, J., Montoya, P., Barrera, J.F., Aluja, M., Liedo, P., 2014. Parasitism by
456 *Coptera haywardi* and *Diachasmimorpha longicaudata* on *Anastrepha* flies with
457 different fruits under laboratory and field cage conditions. BioControl 59, 287-
458 295.

459 Fundación ExportAr, 2014. Argentina partner country fruit logística. Hall 25 stand A14-
460 A17. http://issuu.com/exportar/docs/catalogo_fruit2014.

461 Garcia-Medel, D., Sivinski, J., Diaz-Fleischer, E., Ramirez-Romero, R., Aluja, M.,
462 2007. Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae)
463 released as single-or multiple-species cohorts in field cages: Influence of fruit
464 location and host density. Biol. Control 43, 12-22.

465 González, P., Montoya, P., Pérez-Lachaud, G., Cancino, J., Liedo, P., 2010. Host
466 discrimination and superparasitism in wild and mass-reared *Diachasmimorpha*
467 *longicaudata* (Hym.: Braconidae) females. Biocontrol Sci. Technol. 20, 137–148.

468 Guillén, D., Sánchez R., 2007. Expansion of the national fruit fly control programme in
469 Argentina, in: Vreysen, M.J.B., Robinson, A. S., Hendrichs, J. (Eds), Area-Wide
470 Control of Insect Pests: from research to field implementation. Springer,
471 Dordrecht, The Netherlands, pp. 653-660.

472 Harris, E.J., Bautista, R.C., Vargas, R.I., Jang, E.B., Eitam, A., Leblanc, L., 2010.
473 Suppression of melon fly (Diptera: Tephritidae) populations with releases of

Fopius arisanus and *Psyttalia fletcheri* (Hymenoptera: Braconidae) in North Shore Oahu, HI, USA. BioControl 55, 593-599.

Jang, E.B., Messing, R.H., Klungness, L.M., Carvalho, L.A., 2000. Flight Tunnel Responses of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) to olfactory and visual stimuli. J Insect Behav. 13, 525-538.

Knipling, E.F., 1992. Principles of insect parasitism analysed from new perspectives: Practical implications for regulating insect populations by biological means. USDA-ARS, Agriculture Handbook N° 693.

Messing, R.H., Klungness, L.M., Jang, E.B., 1997. Effects of wind on moviment of *Diachasmimorpha longicaudata*, a parasitoid of tephritid fruit flies, in a laboratory flight tunnel. Entomol. Exp. et Appl. 82, 147-152.

Montoya, P., Cancino, J., Pérez-Lachaud, G., Liedo, P., 2011. Host Size, Superparasitism and Sex Ratio in Mass-reared *Diachasmimorpha longicaudata*, a Fruit Fly Parasitoid. BioControl 56, 11-17.

Montoya, P., Cancino, J., Zenil, M., Santiago, G., Gutierrez, J.M., 2007. The augmentative biological control component in the Mexican national campaign against *Anastrepha* spp. fruit flies, in: Vreysen, M.J.B., Robinson, A. S., Hendrichs, J. (Eds), Area-Wide Control of Insect Pests: from research to field implementation. Springer, Dordrecht, The Netherlands, pp. 661-670.

Montoya, P., Ruiz, L., Pérez-Lachaud, G., Cancino, J., Liedo, P., 2013. Field superparasitism by *Diachasmimorpha longicaudata* attacking *Anastrepha* spp. Biol. Control 64, 160–165.

Montoya, P., Liedo, P., Benrey, B., Barrera, J.F., Cancino, J., Aluja, M., 2000b. Functional Response and Superparasitism by *Diachasmimorpha longicaudata*

(Hymenoptera: Braconidae), a Parasitoid of Fruit Flies (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 93, 47-54.

Montoya, P., Liedo, P., Benrey, B., Barrera, J.F., Cancino, J., Sivinski, J., Aluja, M., 2000a. Biological Control of *Anastrepha* spp. (Diptera: Tephritidae) in Mango Orchards Through Augmentative Releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 18, 212-224.

Ovruski, S.M., Aluja, M., Sivinski, J., Wharton, R.A., 2000. Hymenopteran Parasitoids on Fruit infesting Tephritidae (Diptera) in Latin America and the Southern United States: Diversity, Distribution, Taxonomic Status and Their Use in Fruit Fly Biological Control. Int. Pest Manag. Rev. 5, 81-107.

Ovruski, S.M., Van Nieuwenhove, G., Bezdjian, L., Albornoz-Medina, P., Schliserman, P., 2012. Evaluation of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) as a mortality factor of *Ceratitis capitata* (Diptera: Tephritidae) infesting Citrus species under laboratory and field-cage conditions. Biocontrol Sci. Technol. 22, 187-202.

Paranhos, B.A.J., Mendes, P.C.D., Papadopoulos, N.T., Walder, J.M.M., 2007. Dispersion patterns of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in citrus orchards in southeast Brazil. Biocontrol Sci. Technol. 17, 375-385.

Purcell, M.F., John, C., Messing, R.H., Wong, T.T.Y., 1998. Interactions between augmentatively released *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) and a complex of Opiine parasitoids in a commercial guava orchard. Biocontrol Sci. Technol. 8, 139-151.

Rendon, P., Sivinski, J., Holler, T., Bloem, K., López, M., Matinez, A., Aluja, M., 2006. The effects of sterile males and two braconid parasitoids, *Fopius arisanus* (Sonan)

and *Diachasmimorpha kraussii* (Fullaway) (Hymenoptera), on caged populations of Mediterranean fruit flies, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) at various sites in Guatemala. Biol. Control 36, 224–231.

Rosenheim, J.A., Hoy, M. A., 1989. Confidence intervals for the Abbott's formula correction of bioassay data for control response. J. Econ. Entomol. 82, 331-335.

Sime, K.R., Daane, K.M., Nadel, H., Funk, C.S., Messing, R.H., Andrews Jr., J.W., Johnson, M.W., Picket, C.H., 2006. *Diachasmimorpha longicaudata* and *D. kraussii* (Hymenoptera: Braconidae), potential parasitoids of the olive fruit fly. Biocontrol Sci. Technol. 16, 169-179.

Sivinsk., J., Jeronimo, F., Holler, T. 2000. Development of aerial releases of *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae), a Parasitoid that attacks the Mediterranean Fruit Fly, *Ceratitis capitata* (Weidemann) (Diptera: Tephritidae), in the Guatemalan Highlands. Biocontrol Sci. Technol. 10, 15-25.

Sivinski, J., Calkins, C.O., Baranowski, R., Harris, D., Brambila, J., Diaz, J., Burns, R.E., Holler, T., Dodson, G., 1996. Suppression of a Caribbean fruit fly (*Anastrepha suspensa*) (Loew) (Diptera: Tephritidae) population through augmentative releases of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol Control 6, 177-185.

StatSoft, Inc., 2011. STATISTICA (data analysis software system), version 10. Dell Software, USA.

Suárez, L., Murua, F., Lara, N., Escobar, J., Taret, G., Rubio, J., Van Nieuwenhove, G., Bezdjian, L.P., Schliserman, P., Ovruski, S.M., 2014. Biological control of *Ceratitis capitata* (Diptera: Tephritidae) in Argentina: Releases of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in fruit-producing semi-arid areas of San Juan. Natural Science 6, 664-675.

548 Van Lenteren, J.C., 2012. The state of commercial augmentative biological control:
549 plenty of natural enemies, but a frustrating lack of uptake. BioControl 57, 1–20.
550 Van Nieuwenhove, G., Bezdjian, L.P., Schliserman, P., Aluja, M., Ovruski, S.M., 2016.
551 Combined effect of larval and pupal parasitoid use for *Anastrepha fraterculus*
552 (Diptera: Tephritidae) control. Biol. Control 95, 94-102.
553 Vargas, R.I., Leblanc, L., Harris, E.J., Manoukis, N.C., 2012. Regional Suppression of
554 BactroceraFruit Flies (Diptera: Tephritidae) in the Pacific through Biological
555 Control and Prospects for Future Introductions into Other Areas of the World.
556 Insects 3, 727-742.
557 Vargas, R.I., Peck, S.L., McQuate, G.T., Jackson, C.G., Stark, J.D., Armstrong, J.W.,
558 2001. Potential for Area-Wide Integrated Management of Mediterranean Fruit Fly
559 (Diptera: Tephritidae) with a Braconid Parasitoid and a Novel Bait Spray. J. Econ.
560 Entomol. 94, 817-825.
561 Vargas, R.I., Long, J., Miller, N.W., Delate, K., Jackson, C.G., Uchida, G.K., Bautista,
562 R.C., Harris, E.J., 2004. Releases of *Psyttalia fletcheri* (Hymenoptera:
563 Braconidae) and sterile flies to suppress melon fly (Diptera: Tephritidae) in
564 Hawaii. J. Econ. Entomol. 97, 1531-1539.
565 Wang, X.G., Messing, R.H., 2004. Potential interactions between pupal and egg- or
566 larval-pupal parasitoids of tephritid fruit flies. Environ. Entomol. 33, 1313-1320.
567 Wong, T.T.Y., Ramadan, M.M., McInnis, D.O., Mochizuki, N., Nishimoto, J.J., Herr,
568 J.C., 1991. Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera:
569 Braconidae) to suppress a Mediterranean fruit fly (Diptera: Tephritidae)
570 population in Kula, Maui, Hawaii. Biol. Control 1, 2–7
571
572

573 **Table caption**

574

575 **Table 1**. Mean, minimum and maximum temperatures, relative humidity, cumulative
576 rainfall, and wind speed in the study area during testing weeks (mid-Summer – mid-
577 Autumn) in 2012.

578

579

580

581

582

583

584

585

586

587

588

589

590

591

592

593

594

595

596

597

598 **Figure legends**

599

600 Fig. 1. Location of the study site in San Juan, central-western Argentina. Distribution of
601 control and release plots (CP and RP, respectively) in the study site (fig crop). A.-
602 transects system for parasitoid releasing in the experimental plots; transects
603 (longitudinal lines) and release sectors (black circles distributed along lines). B.-
604 Distribution of fruit exposure devices (black circles) in five longitudinal rows in both
605 control and release plots.

606 Fig. 2. Artificial device designed to hold figs and expose them to natural infestation by
607 oviposition of wild *C. capitata* females and for subsequent attack by released
608 parasitoids. See detailed explanation of the device in text.

609 Fig. 3. Mean (± SE) percentage of host mortality (dead larvae plus dead pupae)
610 recorded from both release and control plots. Bars followed by the same letter indicate
611 no significant differences (Tukey HSD test, $P = 0.05$).

612 Fig. 4. Mean (± SE) percentage of *C. capitata* emergence recorded from both release
613 and control plots. Bars followed by the same letter indicate no significant differences
614 (Tukey HSD test, $P = 0.05$).

615 Fig. 5. Mean (± SE) percentage of *D. longicaudata* emergence recorded from both
616 release and control plots. Bars followed by the same letter indicate no significant
617 differences (Tukey HSD test, $P = 0.05$).

618 Fig. 6. Variation of the real host mortality inflicted by *D. longicaudata* (parasitoid
619 efficacy) during the parasitoid release period at the two experimental plots.

620 Fig. 7. Weekly adult *C. capitata* captures expressed as mean (± SE) flies/trap/day (FTD)
621 in the fig crop throughout all testing dates.

622

Fig. 1

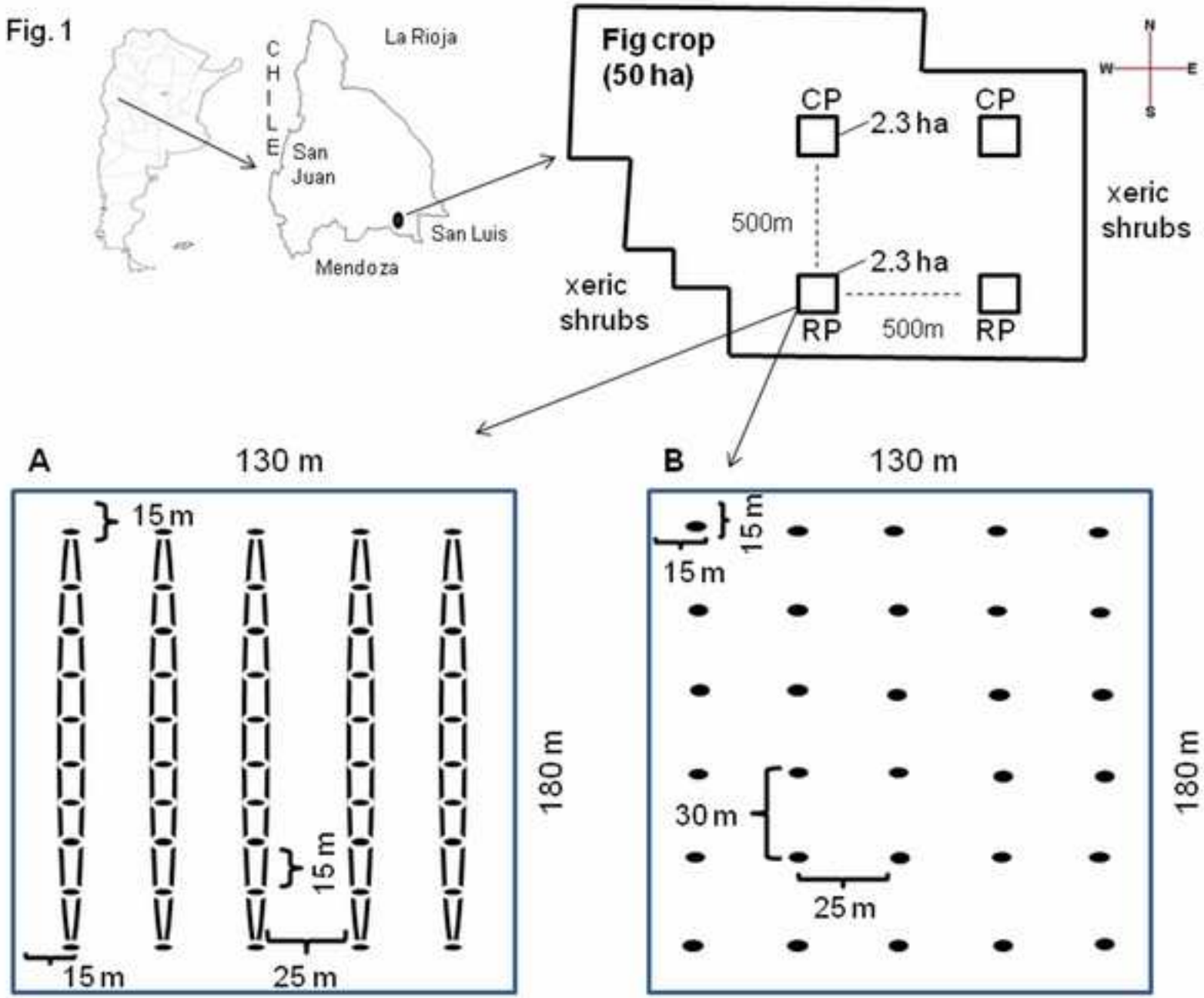
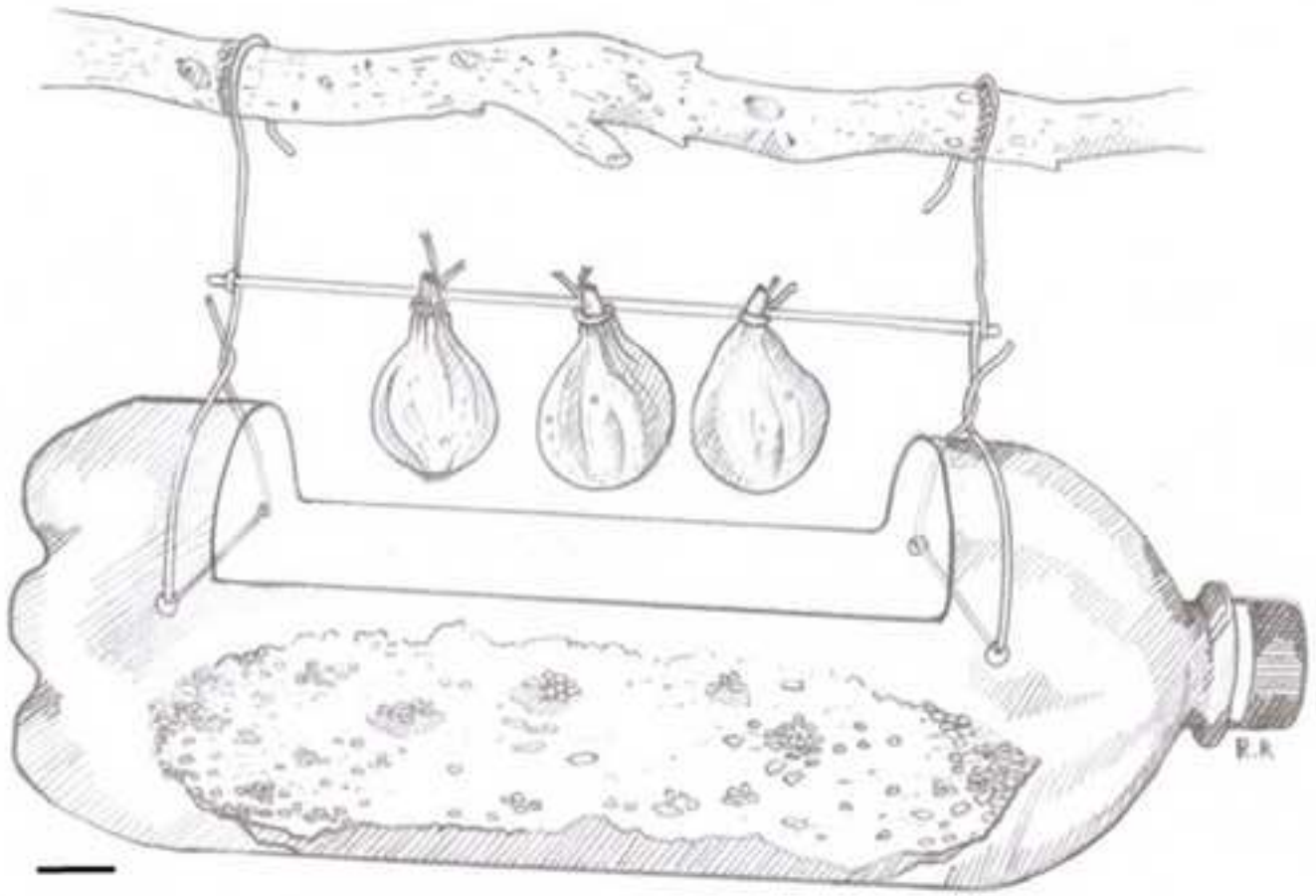
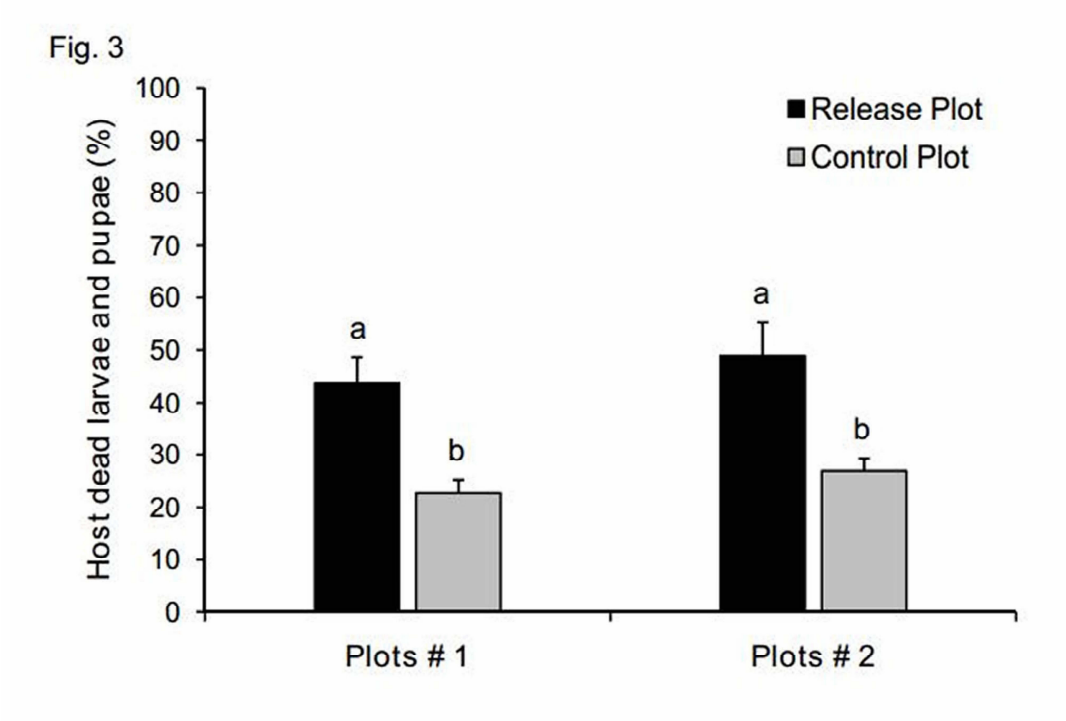

CHILE
La Rioja
San Juan
San Luis
Mendoza
Fig crop
(50 ha)
CP
2.3 ha
CP
500m
2.3 ha
RP
500m
RP
xeric shrubs
xeric shrubs
N
W
E
S
A
130 m
15 m
15 m
15 m
25 m
180 m
B
130 m
15 m
15 m
30 m
25 m
180 m

Fig. 2



2 cm

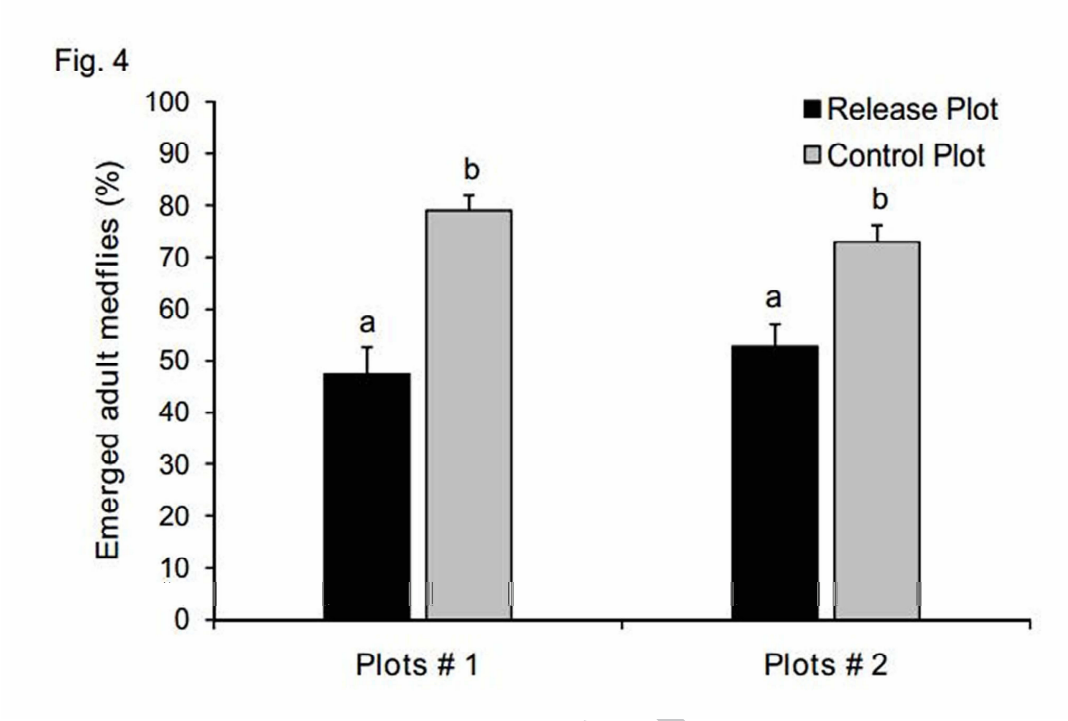
Fig. 3



627

628

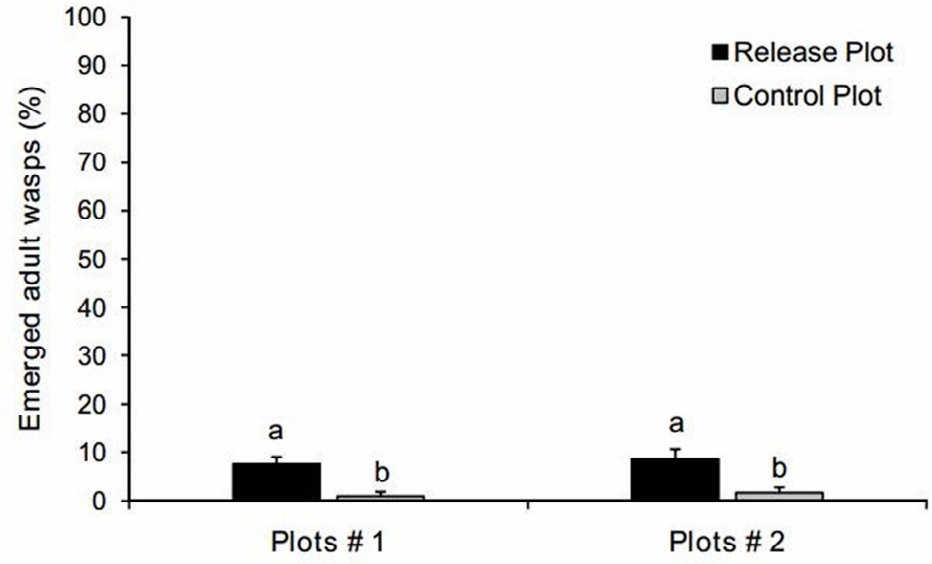
Fig. 4



629

630

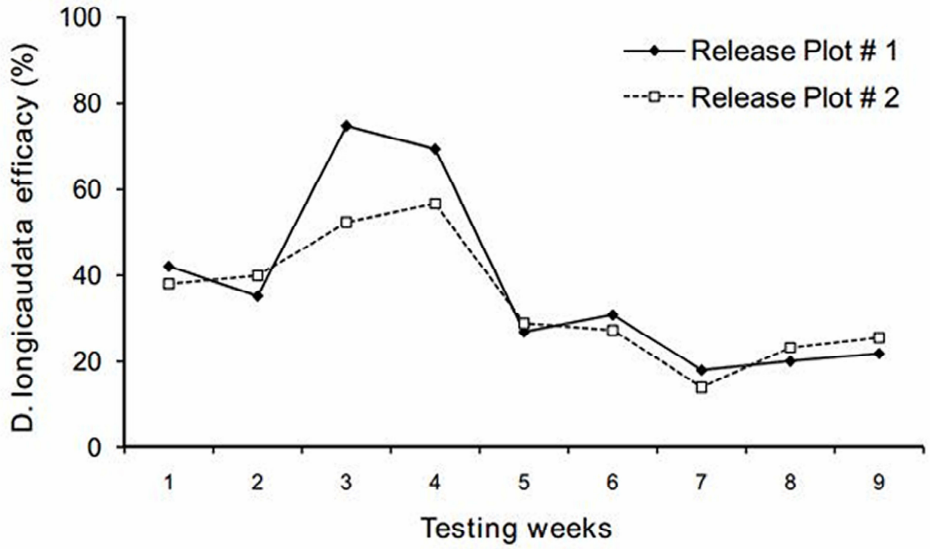
Fig. 5



631

632

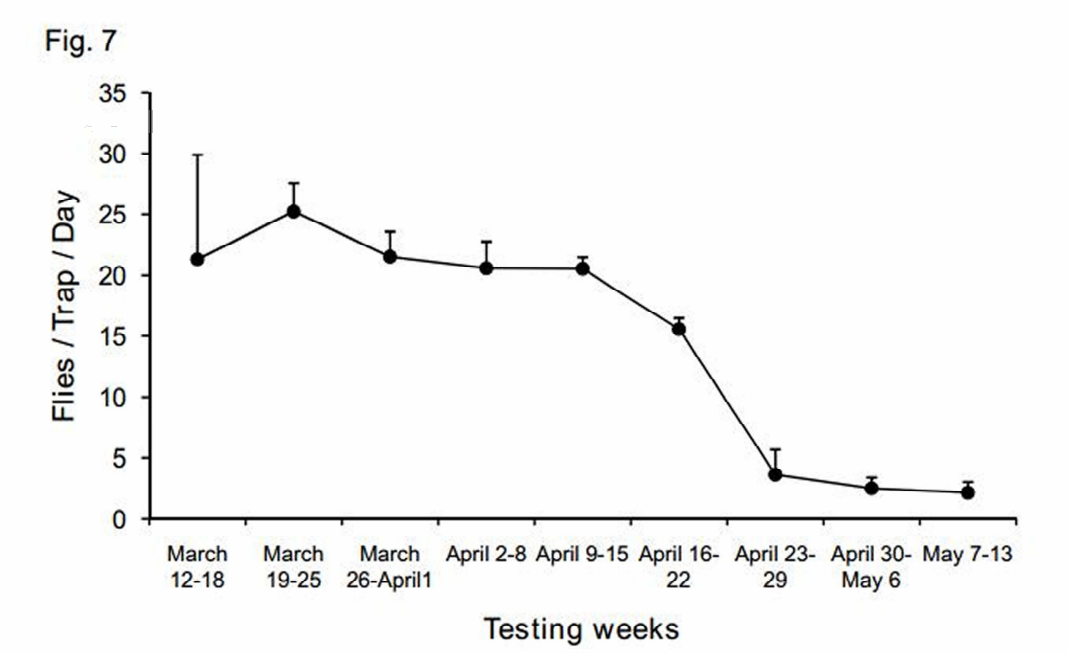
Fig. 6



633

634

Fig. 7



635

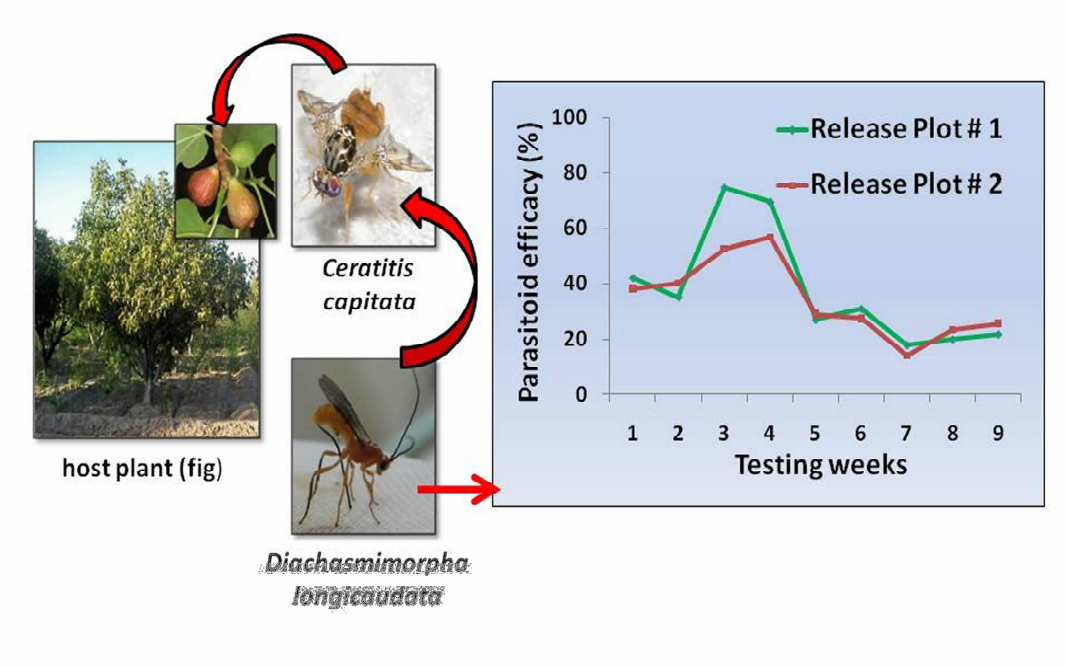
636

637 **Table 1**.

638

Testing weeks (month, day)	Max. Temp. (ºC)	Min. Temp. (ºC)	Mean Temp. (ºC)	Mean RH (%)	Rainfall (mm)	Wind speed (km/h)
March, 12-18	30.8	15.2	23.0	58,9	16.0	1.2
March, 19-25	28.3	14.3	21.3	51,4	0.0	5.4
March, 26-April, 1	28.8	16.6	22.7	62,4	0.0	9.4
April, 2-8	28.6	15.9	22.3	54,3	0.0	4.9
April, 9-15	25.6	12.6	19.1	49,9	0.0	4.1
April, 16-22	27.6	11.1	19.4	52,5	0.0	5.9
April, 23-29	15.9	6.3	11.1	48,1	2.4	5.3
April, 30-May, 6	21.2	7.5	14.4	54,0	0.0	5.4
May, 7-13	23.9	5.4	14.6	56,6	0.0	4.4

639
640
641



642

643

644 **Highlights**
645
646 - Field-open augmentative releases of *D. longicaudata* against *C. capitata* were assessed
647
648 - Parasitoids were reared on Sensitive Lethal Temperature Vienna-8 *C. capitata* strain
649
650 - Host emergence at the release plots was ca. 26% lower than that in the controls
651
652 - Between 16 and 75% of host mortality at the release plots was due to *D. longicaudata*
653
654 - Medfly biological control in Argentinean fruit-growing semi-arid areas is encouraged
655
656