Mortality by Parasitization in the Association between *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) and *Ceratitis capitata* (Diptera: Tephritidae) under Field-Cage Conditions

Patricia Albornoz Medina, Guido Van Nieuwenhove, Laura Patricia Bezdjian, Pablo Schliserman, Claudia Fidelis-Marinho, Sergio Marcelo Ovruski*

Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y sus Enemigos Naturales (LIEEMEN), División Control Biológico de Plagas, Planta Piloto de Procesos Industriales Microbiológicos y Biotecnología (PROIMI)—CCT Tucumán—CONICET, Tucumán, Argentina

Email: palbornozmedina@gmail.com, gavn12004@yahoo.com.ar, laurabezdjian@yahoo.com.ar, cfmarinhoo@gmail.com, schliserman73@yahoo.com.ar, ovruskisergio@yahoo.com.ar

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Abstract

*Ceratitis capitata* (Wiedemann) is one of the major pests currently affecting world fruit production. In Argentina’s northern *Citrus*-producing regions, *C. capitata* is actively multiplying in large exotic host fruits, such as *Citrus paradisi* Macfadyen (grapefruit), *Citrus aurantium* L. (sour orange) and *Citrus sinensis* L. (Osbeck) (sweet orange). Faced with this situation, the use of parasitoids as biocontrol agents is currently receiving renewed attention as a new biological tool for controlling pestiferous fruit flies within the Argentinean National Fruit Fly Control and Eradication Program (ProCEM). Consequently, a viable approach to controlling *C. capitata* involves the use of exotic parasitoids such as *Diachasmimorpha tryoni* (Cameron). In this study, the effectiveness of *D. tryoni* females to find and successfully parasitize *C. capitata* larvae infesting all *Citrus* species mentioned earlier was assessed. Parasitoids were allowed to forage for 8 h on grapefruits and oranges artificially infested with laboratory-reared *C. capitata* larvae under natural environmental conditions (field cage). Parasitoid emergence, parasitism, overall effectiveness, and sex ratio of parasitoid offspring were estimated as response variables. The higher effectiveness of *D. tryoni* females recorded from *C. sinensis* would be mainly a result of both increased host density per unit of fruit surface area and fruit physical features. The study provides evidence that *D. tryoni* contributed to

*Corresponding author.

C. capitata mortality in all Citrus species assessed. However, the mortality values recorded from C. sinensis, C. aurantium, and C. paradisi did not exceed 10%, 1.5%, and 1.7%, respectively. Nonetheless, D. tryoni might be selected to forage under both high and low host density conditions.

Keywords
Fruit Fly, Citrus, Parasitoid, Biological Control, Argentina

1. Introduction
The Mediterranean fruit fly (Medfly), Ceratitis capitata (Wiedemann), is one of the major pests of commercial fruit crops in Argentina. This tephritid species is widely distributed throughout Argentina, and severely limit the export of fresh fruit as a result of quarantine restrictions in countries free of this pest [1]. In Argentina’s northern Citrus-producing regions, C. capitata is actively multiplying in large exotic host fruits, such as Citrus paradisi Macfadyen (grapefruit), Citrus aurantium L. (sour orange) and Citrus sinensis L. (Osbeck) (sweet orange) (Rutaceae), all originated in Southeast Asia [2]. These Citrus species are commonly found in abandoned crop fields or in disturbed wild vegetation areas [3].

In Argentina, there is an increasing interest in combating C. capitata through ecologically acceptable practices, including both the use of natural enemies and the sterile insect technique, aimed towards the conservation of biodiversity in agroecosystems [1] [4]. Fortunately, the use of hymenopterous parasitoids as biocontrol agents is currently receiving renewed attention as a new biological tool for controlling pestiferous fruit flies within the Argentinean National Fruit Fly Control and Eradication Program (ProCEM) [5]. The most recent introduction of parasitoids in Argentina for fruit fly biological control took place in 1999. Two Indo-Pacific parasitoid species, Diachasmimorpha longicaudata (Ashmead) and D. tryoni (Cameron), were introduced in Argentina via Mexico for augmentative releases against C. capitata [6]. Both braconid species are solitary, koinobiont endoparasitoids, which attack late instar larvae of several fruit-infesting tephritid flies [7]. However, only D. longicaudata is currently being mass-reared at the “BioPlanta San Juan” facility from ProCEM-San Juan [4], and it is also being massively released against C. capitata in several fruit-producing valleys in the province of San Juan [8]. Although D. tryoni was successfully colonized at the laboratory on larvae of C. capitata, it was reared on a small scale and was not released [6].

Diachasmimorpha tryoni has been used in classical and augmentative biological control programs in Central America and Hawaii against tephritid pests such as Ceratitis capitata (Wiedemann), Anastrepha suspensa Loew, A. obliqua (Macquart), and Bactrocera dorsalis (Hendel) [7] [9]-[14]. In Hawaii, augmentative releases of D. tryoni against C. capitata showed that overall parasitism rates were increased by 48% [15]-[17]. In Guatemala, aerial mass-releases of D. tryoni into coffee crops affected by C. capitata achieved parasitism levels close to 84% [11].

Large exotic fruits highly infested by C. capitata larvae in northern Argentinian fruit-producing areas, such as Citrus spp., represent a vacant niche which could be exploited by exotic parasitoid species with suitable individual abilities to successfully attack the Mediterranean fruit fly [2]. Consequently, the prediction that females of D. tryoni would be most efficient in suppressing C. capitata larvae infesting C. paradisi, C. aurantium and C. sinensis fruits was assessed. This prediction was based on the good capacity of D. tryoni for successful development on the larvae of C. capitata [10] [17] and on its good performance in lowering Medfly populations in both Hawaii [15] [16] and Guatemala [11] by means of augmentative releases. Therefore, the purpose of this study was to assess the effectiveness of laboratory-reared D. tryoni females so as to find and successfully parasitize C. capitata larvae depending on the Citrus species under natural conditions. This research is part of a series of studies evaluating the efficacy of several exotic and native parasitoid species to kill C. capitata larvae infesting Citrus species in Argentina [2] [5].

2. Material and Methods
2.1. Importation of Parasitoid
The parasitoid D. tryoni was obtained from a colony that had been maintained on larvae of C. capitata at the Bi-
ological Control Laboratory of Mexico’s Moscamed-Moscafruit National Program and imported to Argentina within irradiated *C. capitata* pupae in 1999 [6]. The shipment was immediately brought to the quarantine facility at the Research Center for the Control of Harmful Organism Populations (CIRPON) in San Miguel de Tucumán, Argentina. Several generations later, the *D. tryoni* colony was transferred to the Ecological Research Laboratory of Fruit Flies and their Natural Enemies (LIEMEN) from the Biological Control Division of the Pilot Plant of Industrial Microbiological Processes and Biotechnology (PROIMI) in San Miguel de Tucumán.

2.2. General Insect Rearing Conditions

The parasitoid *D. tryoni* was reared on third-instar larvae of a wild *C. capitata* strain in the LIEMEN at PROIMI institute. Adult parasitoids were kept in cubical plexiglas cages (30 cm) holding 500 pairs per cage at 25°C ± 1°C; 75% ± 5% RH and a photoperiod of 12:12 (L:D). Parasitoids were daily provided with honey, soaked in paper towels on the top of a Petri dish, and with a small glass that held wet cotton. Every day, each cage was provided with one oviposition unit composed of an organdy screen-covered dish (10 cm diameter and 1 cm deep) containing about 750 host larvae. This unit was placed inside of the cage. After exposure to the parasitoids, each oviposition unit was removed from the cages. Parasitized *C. capitata* larvae were then placed in emergence cups (50 cm diameter, 65 cm deep) with mesh-screen covers and containing sterilised Vermiculite® as the pupation medium on the bottom. The cups were kept under the above mentioned laboratory conditions until the emergence of adult parasitoids or flies. The larvae of wild *C. capitata* strain were also reared at LIEMEN on wheat germen-based diet fortified with brewer’s yeast, sugar, agar-agar, citric acid, sodium benzoate, nipagin and water.

2.3. Field-Cage Test

The assay was carried out to assess the capability of *D. tryoni* in parasitising *C. capitata* larvae infesting three different species of *Citrus*, such as *C. paradisi* (“March” cultivar), *C. aurantium* (rootstock, wild cultivar), and *C. sinensis* (“tanjarina” cultivar) fruits. The experiment was a multiple-choice test performed under no controlled environmental conditions inside a field-cage at the experimental yard of the Research Centre for Control of Harmful Organisms Populations (CIRPON) in San Miguel de Tucumán, Argentina. The study site is located at 26°50'S, 65°13'W, and 426 m above sea level and has a mean annual rainfall of 945 mm and a mean annual temperature of 25.3°C [2]. The assay was performed on October, and the temperature fluctuated between 19.2 and 31.0°C (mean 21.9°C), and the relative humidity fluctuated between 49.7% and 83.2% (mean 63.2%).

Sweet oranges, sour oranges, and grapefruits used in the assay were collected from unsprayed trees grown in backyard gardens located in the outskirts of Tafí Viejo district (Tucumán). Several branches of those *Citrus* trees, each containing 3 - 7 unripe fruits, were covered with a cloth mesh in order to avoid fly infestation. Once the fruit reached maturity, they were harvested and transported to LIEMEN. Overall, 24 similar-size fruit per *Citrus* species were selected and individually weighed, and the rind thickness and pulp depth were measured. The rind thickness was determined by measuring the exocarp and mesocarp, while the pulp depth was determined by measuring the endocarp. In addition, the surface area of each *Citrus* fruit was calculated using the formula: sphere surface area = \(4\pi r^2\). Weights and measurements are detailed in Table 1.

Each ripe grapefruit or sweet orange or sour orange fruit was artificially infested by removing its pulp and replacing it with 120 laboratory-reared third-instar *C. capitata* [2]. Later, inoculated *Citrus* fruits were transported to CIRPON. In this place, experiment was conducted inside a cylindrical nylon field cage (2 m diameter, 3 m height) surrounded by willow trees that provided shade. The cage was protected from rain by a translucent fibre glass roof that allowed natural light to go through. The fruits were distributed inside cage using a similar method to that described by [2]. One inoculated fruit of each *Citrus* species was individually hung from the ceiling of

<table>
<thead>
<tr>
<th>Host <em>Citrus</em> species</th>
<th>Weight (g)</th>
<th>Rind thickness (cm)</th>
<th>Pulp depth (cm)</th>
<th>Surface area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. paradisi</em></td>
<td>432.9 ± 4.7</td>
<td>0.69 ± 0.02</td>
<td>9.03 ± 0.05</td>
<td>276.3 ± 1.7</td>
</tr>
<tr>
<td><em>C. aurantium</em></td>
<td>330.4 ± 3.3</td>
<td>0.73 ± 0.01</td>
<td>7.56 ± 0.05</td>
<td>231.2 ± 1.9</td>
</tr>
<tr>
<td><em>C. sinensis</em></td>
<td>96.3 ± 2.1</td>
<td>0.28 ± 0.01</td>
<td>4.32 ± 0.07</td>
<td>133.1 ± 1.4</td>
</tr>
</tbody>
</table>
the field cage and positioned to form a central circle (50 cm diameter) 2 m above ground level. A small potted lemon tree (1 m height) was placed below each test fruit to simulate a natural environment. All of the fruits were equidistant from each other, and their positions were randomised at each replication. Twenty naïve, 5-day-old, mated D. tryoni females were released inside the field cage at the central point of the circle formed by the test Citrus fruits at 2 m above ground level. Parasitoid females were allowed to forage freely for 8 h starting at 09:00 h. After exposure time to parasitoids, the fruits were removed from the cage. In the laboratory all fruits were dissected to retrieve C. capitata larvae, and the number of dead larvae per fruit was recorded. The living larvae were placed into plastic cups (7 cm diameter, 6 cm height) with sterilised Vermiculite® on the bottom as a pupation substrate. The top of each cup was tightly covered with a piece of organdy. The cups were placed in a room at 24°C - 26°C and 70% - 80% RH with a 12:12 (L:D) h regime. The pupae were moistened weekly to avoid desiccation and were held inside the cups until adult flies or parasitoids emerged. After the insect emergence was complete, the non-enclosed puparia were dissected to check for the presence or absence of immature parasitoid stages and/or fully developed pharate-adult parasitoids. The number and sex of the emerged parasitoids and flies, as well as the number of non-enclosed puparia were recorded. Control tests (inoculated fruit not exposed to parasitoids) were also conducted to determine natural C. capitata mortality and adult emergence rates. The assay and the control test were replicated 12 times. For each replicate, a new parasitoid cohort was released into the cage, and new inoculated fruits were hung from the cage roof.

2.4. Data Analysis
For data analysis, the dependent variables “parasitoid emergence”, “parasitism”, “overall effectiveness”, and “sex ratio of parasitoid offspring” were estimated. The adult parasitoid emergence was calculated as the number of emerged adult parasitoids divided by the total number of recovered pupae ×100. The parasitism percentage was calculated as the number of emerged adult parasitoids plus the number of unemerged parasitoids divided by the total number of pupae recovered from the test fruit ×100. The Abbot’s corrected formula was used to determine the overall effectiveness of the parasitoid species for killing the host [18]. The overall effectiveness involves both parasitism and additional host mortality rates [2]. Sex ratio was estimated as the proportion of female offspring. Pearson Product Moment Correlations (P = 0.05) were calculated to determine the degree of association between dependent variables before to compare data through univariate or multivariate General Linear Models (GLM) at P = 0.05 [19]. Since the fruit size among Citrus species was different, but the host density per fruit species was the same, a rate of “host density/cm² of fruit surface” was used as a covariate to assess a possible effect of host density on the four dependent variables above listed. Mean comparisons were analysed by Tukey’s Honesty Significant Difference (HSD) test at P = 0.05. The proportion data were transformed to arcsine square root before analysis. All untransformed means (±SE) were presented in the text.

3. Results
There were significant correlations between the parasitoid emergence, parasitism, and the effectiveness (Table 2). Therefore, these response variables were jointly analyzed using a multivariate analysis. The multivariate GLM showed significant differences among Citrus species regarding the parasitoid emergence, parasitism, and the effectiveness, but covariate was negligible (categorical factor: Wilks’ λ = 0.57875, $F_{4,62} = 4.87429$, $P = 0.00175$, covariate: Wilks’ λ = 0.57875, $F_{2,31} = 0.10403$, $P = 0.90150$). Because the multivariate GLM was significant, we then followed with univariate GLMs, and its results are detailed in Table 3.

**Table 2. Summary of Pearson Product Moment Correlations calculated to determine the degree of association between dependent variables (parasitoid emergence, parasitism, effectiveness, and sex ratio of parasitoid offspring).**

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Parasitoid Emergence</th>
<th>Parasitism</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 36</td>
<td>-</td>
<td>$r = 1.00$, $P &lt; 0.001^*$</td>
<td>$r = 0.47$, $P = 0.003^*$</td>
</tr>
<tr>
<td>N = 36</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N = 36</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N = 36</td>
<td>$r = 0.34$, $P = 0.132$</td>
<td>$r = 0.35$, $P = 0.131$</td>
<td>$r = 0.25$, $P = 0.127$</td>
</tr>
</tbody>
</table>

*Significant variables.
and attack *A. ludens* larvae at canopy level in guava trees [12]. This contrasting information will be useful in transferring braconid species into low and high host-density environments.

Augmentative releases of parasitoids may be mostly successful when combined with the Sterile Insect Technique [14] [16] [28]. A low-density forager, such as *D. tryoni*, might be a convenient candidate for releases because it might continue producing mortality at low host densities [12] [25].

### 4. Discussion

The field cage experiment yielded interesting results, showing that *D. tryoni* females had a better performance in killing *C. capitata* larvae infesting *C. sinensis* than in both *C. paradisi* and *C. aurantium*. Moreover, the effectiveness of *D. tryoni* females on *C. capitata* larvae infesting *C. aurantium* was similar to that recorded from *C. paradisi*. The relatively poor performance of *D. tryoni* at *C. paradisi* and *C. aurantium* appears to support the hypothesis that tephritid larvae infesting larger fruits containing a deep pulp could be more protected from parasitoid attack [20]-[22]. From all *Citrus* species tested in this study, *C. sinensis* had more advantageous physical characteristics to ease parasitoid success, such as a thinner rind and a shallower pulp. As previously suggested for *D. longicaudata* [2] [23]-[25], latter two physical features may have helped *D. tryoni* females better distinguish the vibrations and/or sound caused by *C. capitata* larvae [26] once the parasitoids landed onto the larva. *C. capitata* had the greater number of larvae infesting larger fruits than those found in both sour orange and grapefruit, respectively (Figure 1). There was no significant difference between sour orange and grapefruit regarding the parasitoid emergence, parasitism, and the effectiveness (Figure 1). Furthermore, *D. tryoni* exhibited a male-biased sex ratio. The univariate GLM revealed no significant difference in female offspring recovered from the three *Citrus* species (categorical factor: *F*$_{2,32}$ = 2.6325, *P* = 0.0874, covariate: *F*$_{1,32}$ = 0.9689, *P* = 0.3323) (Figure 2).

### Appendix A

#### Table 3. Summary of univariate one-way ANOVAs on the Diachasmimorpha tryoni females performance in killing Ceratitis capitata larvae infesting Citrus paradisi, C. aurantium and C. sinensis fruits (dependent variables: parasitoid emergence, parasitism, effectiveness).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df/df Error</th>
<th>Parasitoid Emergence</th>
<th>Parasitism</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host density/cm$^2$ (covariate)</td>
<td>1, 32</td>
<td><em>F</em> = 0.001, <em>P</em> = 0.965</td>
<td><em>F</em> = 0.001, <em>P</em> = 0.965</td>
<td><em>F</em> = 0.002, <em>P</em> = 0.652</td>
</tr>
<tr>
<td><em>Citrus</em> species (categorical factor)</td>
<td>2, 32</td>
<td><em>F</em> = 7.261, <em>P</em> = 0.002$^a$</td>
<td><em>F</em> = 7.261, <em>P</em> = 0.002$^a$</td>
<td><em>F</em> = 4.172, <em>P</em> = 0.024$^a$</td>
</tr>
</tbody>
</table>

$^a$Significant variables.
Figure 1. Mean (±SE) percentage of emerged parasitoids, percent parasitism, and effectiveness recorded from artificially infested *C. sinensis*, *C. aurantium* and *C. paradisi* fruits with third-instar *C. capitata* larvae that were parasitised by *D. tryoni* females under natural environmental conditions inside a field cage. Bars followed by the same letter indicate no significant differences (Tukey HSD test, *P* = 0.05).

Figure 2. Mean (±SE) percentage of female offspring (sex ratio) recorded from artificially infested *C. sinensis*, *C. aurantium* and *C. paradisi* fruits with third-instar *C. capitata* larvae that were parasitised by *D. tryoni* females under natural environmental conditions inside a field cage. Bars followed by the same letter indicate no significant differences (Tukey HSD test, *P* = 0.05).

5. Conclusions

The study provides evidence that *D. tryoni* contributed to *C. capitata* mortality in all *Citrus* species assessed. However, the mortality values recorded from *C. sinensis*, *C. aurantium*, and *C. paradisi* did not exceed 10%, 1.5%, and 1.7%, respectively. Interestingly, *D. tryoni* might be selected to forage under both high and low host density conditions.

The findings from this study may serve as a preliminary basis for assessing the potential impact of *D. tryoni* on *C. capitata* in *Citrus* species. In future studies, evaluating other major *C. capitata* host plant species found in the fruit-growing areas of northern Argentina, such as peach, plum, fig, and walnut, would help accomplish deeper insight into the performance of *D. tryoni* females in lowering natural populations of *C. capitata* occurring in this Argentinean region.
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References


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