CARBON DIOXIDE ANESTHESIA OF *TAMARIXIA RADIATA* (HYMENOPTERA: EULOPHIDAE) PARASITOID OF *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE)

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Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae), is an arrhenotokous ectoparasite of the Asian citrus psyllid (ACP) *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae), vector of citrus greening disease or huanglongbing (HLB). The parasitoid is reported to have controlled ACP populations to low levels on the islands of Réunion, Guadeloupe and Puerto Rico (Aubert & Quilici 1984; Étienne et al. 2001; Pluke et al. 2008). *Tamarixia radiata* was first imported to Florida from Taiwan and Vietnam in 1998 and released in 1999-2001 (Hoy & Nguyen 2001). A survey conducted in 2006-07 determined that *T. radiata* was well distributed in citrus orchards throughout the state (Qureshi et al. 2009). However, incidence of parasitism was generally low, especially early in the growing season, suggesting a need for augmenting parasitoid populations at that critical time as component of an integrated management program (Qureshi et al. 2007, 2009). Studies of *T. radiata* biology and current efforts at mass rearing and release of this species might benefit from an ability inactivate adults by CO_2 anesthetization, including separation of emergent wasps and psyllids.

Carbon dioxide (CO_2) is widely used to anesthetize insects, but may also cause deleterious side effects on biology and behavior. Brooks (1957) found that development rate of German cockroach, *Blattella germanica* L. (Blattodea: Blattellidae), nymphs decreased 53% when exposed weekly to high CO_2 concentrations for 3 min. Crystal (1967) reported significantly decreased survival rates and fertility of screwworm, *Cochliomyia homini-vorax* (Coquerel) (Diptera: Calliphoridae), exposed to 100% CO_2 for 30 min. Sherman (1953) reported that CO_2 anesthesia of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), led to increased mortality.

This study was undertaken as a first step toward using anesthetization for mass rearing by evaluating the response of *T. radiata* to CO_2 exposure. The objective was to test the effectiveness of CO_2 anesthetization of *T. radiata* and to determine the incidence and severity of side effects of CO_2 anesthetization on longevity, parasitism rate and sex ratio.

A *T. radiata* colony was maintained at FDACS-DPI in Gainesville on ACP nymphs using orange jasmine, *Murraya paniculata* (l.) Jack (Sapindales: Rutaceae), as plant host (Skelley & Hoy 2004). Six newly trimmed plants with new growth were held in an acrylic 62 cm cubic cage and 600 *D. citri* adults were released for 72 h for oviposition a greenhouse under natural sunlight 25 ± 5 °C and 50% ~ 70% RH. Adults were removed and plants held for 10 d until 4th instar nymphs were available. Plants were moved into another clean cage of the same type for 20 days into which 100 *T. radiata* were released. Adult progeny were later collected daily until no more emerged.

A gas chamber was constructed consisting of a vial, 6.50 cm in diam and 12 cm in height (Fisher Scientific, Pittsburg Pennsylvania), provided with two 0.50 cm diam holes in the lid into each of which was fitted a 0.5 cm plastic tube inserted either 1 cm or 11 cm into the chamber for a gas outlet and inlet respectively. Plasticine modeling clay (Flair Leisure Products, Cheam Surrey, England) was molded around the openings of the lid to prevent leakage.

Flow time of gas at 3.8 kpa (2 psi) needed to displace all air in the chamber was assessed by filling the vial with water and then replacing with CO_2 through the inlet. All the water was displaced in 10 s. A CO_2 sensor (K-33 ICB 30% CO_2 Sensor, CO_2Meter Inc., Ormond Beach Florida, USA) was used to determine that 3 s of flow time were necessary to attain a 30% CO_2 concentration confirming the earlier result. The CO_2 sensor was also used to test for leaks by confirming that a given concentration remained constant over several min.

Five wasps having emerged within 24 h or less were placed in the chamber. The lower ¾ of the vial was covered with black cloth to induce the wasps to walk to the top and thus avoid injury from in rushing gas. Gas was introduced through an inlet from a CO_2 tank at 3.8 kpa for 15s to exchange all the air, and then the 2 tubes closed with metal clamps. Wasps were removed after a 5 min exposure and observed with the naked eye using a stop watch to record recovery time (normal movement). Males and females were treated separately, each with eight replications so that a total of 80 wasps were used.

Seventy percent of *T. radiata* females recovered from anesthetization with CO_2 within 4 min, males recovered about as quickly. Indeed, there was no significant difference between male and female recovery time ($\chi^2 = 13.04$, df = 7, $P = 0.071$, Fig. 1). It was noted that a wasp often would recover immediately after being crawled over by another recovering individual.

To evaluate survival, 5 anesthetized wasps were collected into each of 6 small glass vials (1.5 cm in diam, 5.3 cm high) and provided pure honey on a tissue paper strip. On the same day control wasps not anesthetized were placed in 6 other vials. Vials with wasps were held in a growth chamber at 25 °C, 14:10 h L:D and 60 ± 5% RH, and checked daily, noting sex of all cadavers until all had died.

Survival rate for the treated wasps was consistently lower than the control over the entire study period (Fig. 2). Insect-days, the area under the curve of insect numbers by time (Ruppel 1983), was significantly less for the CO_2 treatment (3445.3 ± 348.6) than the control (5610.5 ± 836.6) ($t = 2.39$, df = 7, $P < 0.05$).

Six newly trimmed plants were held in a ventilated 62 cm acrylic cubic cage until there were at least 3 new shoots 3 cm in length upon which to evaluate parasitism. Plants were infested by releasing 600 ACP adults for a 24-h oviposition period. ACP adults were removed and the plants were held for 9 days in a rearing room at 25 °C and 60 ± 5% RH. A small brush was used to remove nymphs until exactly 120 fourth instars remained on each plant. Each plant was then placed individually into a clear acrylic cylinder (12.5 cm diam, 43 cm high) into which 3 *T. radiata* females and 2 males were released. Cages were randomly selected to receive either anesthetized or untreated wasps (N = 8). Newly emerged *T. radiata* offspring were collected daily from day-7 until day-19 after which no more new progeny were found. Progeny were counted and sexed and parasitism rate calculated based on 120 original hosts.

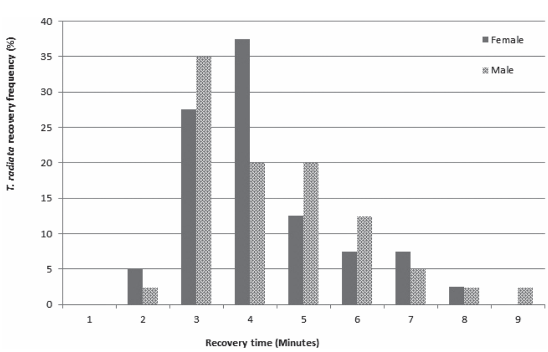

Fig. 1. *Tamarixia radiata* female and male recovery time frequency (%) distribution

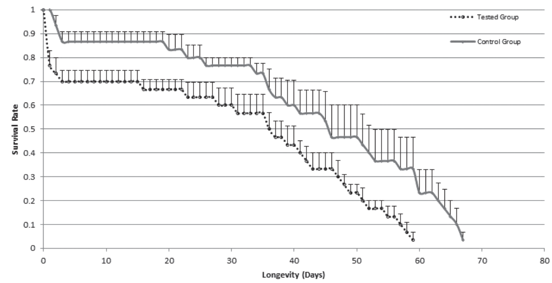

Fig. 2. Survivorship of CO_2 treated and untreated *Tamarixia radiata* adults.

A mean of 59.0 ± 3.8 wasps emerged over 12 days from 120 fourth instar hosts exposed to 3 female and 2 male *T. radiata* treated with CO_2 compared to a mean of 85.2 ± 4.5 in the control. This corresponded to a parasitism rate of 49.2 ± 3.2% for treated wasps compared to 71.0 ± 3.7% for untreated wasps ($t = 4.46$, df = 14, $P = 0.00054$). There was no significant difference in progeny sex ratio ($t = 1.03$, df = 14, $P = 0.32$) between the treated and the control.

Summary

Carbon dioxide anesthesia is a convenient tool for manipulating insects, but can cause deleterious side effects. In this case, a 5 min exposure of *Tamarixia radiata* adults to 100% CO_2 concentration caused a knockdown of about 4 min, significantly reduced survivorship and fecundity, but did not affect the sex ratio of progeny from treated adults. Future research will focus on using less concentrated doses or shorter exposure times to inactivate the wasps in order to improve survival and fecundity.

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