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Life Cycle and Larval Predation by Ceræochrysa valida (Banks) on Nymphs of Diaphorina citri Kuwayama

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Abstract. Area-wide management of Asian citrus psyllid, Diaphorina citri Kuwayama, might use predators abundant in citrus orchards, making necessary the assessment of local predatory species such as Ceræochrysa valida (Banks). The objective of the study was to determine the life cycle of C. valida under different rearing conditions; its fecundity, survival, and longevity; and its predatory capacity on different larval instars of D. citri, both in the laboratory and greenhouse. Predators and pests were reared from insects collected in citrus orchards in the central coastal area of Veracruz, Mexico. Developmental time for C. valida eggs; larvae 1, 2, and 3; and prepupae-pupae were 5.5 ± 0.7, 6.5 ± 0.7, 5.0 ± 1.0, 7.0 ± 1.0, and 14.5 ± 0.7 days, respectively. Male and female longevities were 74.0 ± 38.9 and 78.0 ± 28.9 days. Mean daily number of eggs per female was 10.0 ± 8.4. Predation in the laboratory and greenhouse was greater on older larvae (P < 0.0001) regardless of the nymphal stages consumed. In the laboratory, C. valida consumed as many as 368 prey in 48 hours. However, consumption in a greenhouse was not as much as in the laboratory during the first 6 hours.

Resumen. El manejo de D. citri en Áreas Regionales de Control podría incorporar el uso de depredadores abundantes en huertas de cítricos, lo que hace necesario evaluar especies locales, como Ceræochrysa valida (Banks). El objetivo de este estudio fue determinar el ciclo biológico de C. valida bajo diferentes condiciones de crio; su fecundidad, supervivencia, y longevidad; y su capacidad depredadora sobre diferentes instares larvales de D. citri, tanto en laboratorio como en invernadero. El depredador y la plaga se criaron de insectos colectados en huertas de cítricos en el área Central Costera de Veracruz, México. El tiempo de desarrollo del huevo; las larvas 1, 2, y 3; y la prepupa-pupae de C. valida fue de 5.5 ± 0.7, 6.5 ± 0.7, 5.0 ± 1.0, 7.0 ± 1.0, y 14.5 ± 0.7 días, respectivamente. La longevidad de machos y hembras fue de 74.0 ± 38.9 y 78.0 ± 28.9 días. El promedio diario del número de huevos por hembra fue de 10.0 ± 8.4. La depredación tanto en laboratorio como en invernadero, fue mayor en larvas de mayor edad (P < 0.0001), independientemente del estado ninfal consumido. En el laboratorio, C. valida consumió hasta 368 presas en 48 h. Sin embargo, el consumo en el invernadero no fue tan alto como en el laboratorio durante las primeras 6 horas.

1Neuroptera: Chrysopidae
2Hemiptera: Liviidae
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Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is one of the most important pests of citrus in the world. It feeds on the sap of citrus plants, but most damage occurs as a result of transmission of *Candidatus* Liberibacter spp., pathogenic agent of the disease Huanglongbing (Torres-Pacheco et al. 2013). Global estimation indicated Huanglongbing destroyed more than 60 million trees (Das et al. 2007). In the Americas, the disease was first observed in Brazil in 2004, and by 2005, was found in Florida (Alemán et al. 2007). In Mexico, Huanglongbing was first reported in 2009 on citrus trees in the state of Yucatan; it now occurs or has been detected in infected insects in all 23 states where citrus is produced. Most-affected states are Colima, Jalisco, Michoacan, Nayarit, and Sinaloa, but the disease continues to progress to other important citrus-growing areas along the Gulf of Mexico, such as Veracruz (Torres-Pacheco et al. 2013). National strategy for vector management in citrus orchards is based on use of insecticides in Regional Control Areas (Mora-Aguilera et al. 2013). However, improper use of chemicals can cause negative effects, such as elimination of natural enemies of insect pests, development of resistance to insecticides, and emergence of secondary pests, in addition to environmental pollution. Hence, effective methods such as biological control that reduce environmental impact are needed (González-Castillo et al. 2012).

Biology and consumption capacity must be known to incorporate a natural enemy into a program of area-wide integrated pest management (Salamanca-Bastidas et al. 2010). Predators of *D. citri* have great potential for regulating pest abundance, including larvae of various species of Chrysopidae: *Ceraeochrysa valida* (Banks), *C. sp. nr. cincta* (Schneider), *C. cubana* (Hagen), *C. claveri* (Navás), *C. everes* (Banks), *Chrysoperla rufilabris* (Burmeister), *C. carnea* (Stephens), and *C. comanche* (Banks) (Cortez-Mondaca et al. 2011, Lozano-Contreras and Jasso-Argumedo 2012). Larvae of the genus *Ceraeochrysa*, also called “junk loaders”, can be found feeding on aphids, thrips, whiteflies, mites, and lepidopterans in various agroecosystems (Albuquerque et al. 2001); they also are easy to rear in large numbers (López-Arroyo et al. 2000). In the Americas, *C. valida* is distributed in the U.S., Mexico, Guatemala, Honduras, El Salvador, Costa Rica, Dominican Republic, Haiti, Venezuela, and Peru (De Freitas et al. 2009). In Mexico, the species was originally described from a collection in the state of Baja California Sur. It is present throughout the year, inhabiting cotton, papaya, vegetables, maize, and citrus, with the larvae feeding on aphids and whiteflies (Tauber et al. 2000). The authors collected *C. valida* nymphs feeding on *D. citri* in grapefruit and orange orchards in the central zone of the state of Veracruz. Little information exists on the consumption capacity, and biology of the species is unknown. Thus, the objective of the study was to determine the life cycle of *Ceraeochrysa valida* under different rearing conditions; its fecundity, survival, and longevity; and its capacity to prey on different immature stages of *Diaphorina citri* in laboratory and greenhouse conditions.

Materials and Methods

Research was done at the Laboratory of Plant Health at Campus Veracruz, Colegio de Postgraduados. *C. valida* was identified using keys by Tauber and De
León (2001) and Valencia Luna et al. (2006) and the identify was corroborated by Iliana Pacheco-Rueda, specialist in Chrysopidae at the National Reference Center for Biological Control, SENASICA, Mexico. A growth chamber for the assay was maintained at 25 ± 0.6°C, 72 ± 9% relative humidity, and a photoperiod of 12:12 light:dark hours. *C. valida* was reared from 12 mating pairs collected from trees in a 'Río Red' grapefruit (*Citrus paradisi* Macf.) orchard at Tierra Colorada, municipality of Paso de Ovejas, Veracruz, Mexico. Insects were placed into a 2-liter rectangular plastic box covered with cheesecloth and provided with food and water. The food was a mixture of yeast (30 g), condensed milk (15 ml), fructose (20 g), egg (1 yolk + 2 egg whites), wheat germ (50 g), honey (30 g), and distilled water (45 ml) (Vogt et al. 2000). Larvae were fed eggs of *Sitotroga cerealella* (Olivier) from the Local Plant Protection Board of Navojoa, Sonora, Mexico.

A life cycle study was established with a cohort of 100 *C. valida* eggs within 24 hours of oviposition, obtained from four pairs of breeding adults. Eggs were transferred to 2-liter rectangular plastic boxes covered with cheesecloth; larval emergence was evaluated twice each day with the aid of a stereoscopic microscope. Each newly hatched larva was placed into an individual Petri dish to prevent cannibalism. All larval instars were fed *S. cerealella* eggs, until they reached the pupal stage. Observations at 0800 and 1800 hours estimated the duration of each biological stage, total number of eggs hatched, number of larvae developing into the next stage, number of pupae formed, and number of adults emerged, as well as adult survival.

Fecundity was evaluated from the 38 females newly emerged during the life cycle assay. Two males were provided per female, with 39 males from the cohort and 37 breeding males of the same age. Identification of sex was based on external genitalia (Valencia Luna et al. 2006). Adults were placed with 1.2 g of artificial diet, an 8-ml plastic container of potable water, and a wick sponge in 545-ml plastic containers covered with a cheesecloth lid. The cheesecloth was changed daily and the number of eggs laid per female was recorded. Adult fresh feed was placed into the containers every other day.

In the life cycle assay, the numbers of eggs, larvae, and pre-pupae-pupae were recorded every 24 hours. Mean adult longevity was determined using 39 males and 38 females from the initial cohort of 100 eggs. Time to emergence and numbers of males and females that died were recorded every 24 hours. Mean and standard deviation for adult longevity of both sexes were calculated.

A colony of *D. citri* was established from 30 adult females collected from orange jasmine plants (*Murraya paniculata* (L.) Jack) and 5-year-old Persian lime trees (*Citrus latifolia* Tanaka) at Colegio de Postgraduados, Campus Veracruz. Adults were maintained on shoots from Persian lime grafted onto *Citrus volkameriana* Tan. and Pasq. in entomological cages (1.10 m high x 0.95 m wide and deep) covered with cheesecloth and maintained in a greenhouse. Ten, 1- to 2-year-old citrus plants were maintained in 1-liter plastic pots, fertilized with 8 g of Triple 17 (N-P-K) (Vigoro®, Mexico), watered every 6 days, and pruned to stimulate sprouting. To obtain *C. valida* larvae of the same age, 10 pairs of breeding adults were placed into rectangular plastic boxes, and the eggs they laid were collected within 24 hours. Second- and third-instar larvae were individually maintained in Petri dishes and fed eggs of *S. cerealella* until the larvae molted.

The assay was established with newly hatched first-, second-, or third-instar larvae fed nymphs of different stadia of *D. citri* on the previous day. Subsequently, larvae were fasted for 8 hours (from 2200 to 0600 hours of the next day), emulating
a normal overnight fasting period. During the last 3 hours of the fasting period, Persian lime shoots 5 cm long were infested with *D. citri* nymphs of known age. Shoots were collected from plants maintained in a greenhouse, washed with running tap water, dipped in a 2% sodium hypochlorite solution, rinsed in sterile potable water, and placed on absorbent paper to dry. The shoot stem was put into an 8-ml vial containing Murashige and Skoog's nutrient solution (4.1 g L⁻¹, Sigma-Aldrich®, USA) and the aperture of the vial was sealed using Parafilm M (USA), with the shoot positioned vertically. Vials were fixed with plasticine Vinci® (Mexico) to the base of a Petri dish 10 cm in diameter, and enclosed with a 345-ml crystalline plastic container with two 2 x 5 cm ventilation apertures, covered with polyester organza. The design was completely randomized with three replications. Shoots were infested with stadia 1-2, 3-4, or 5 *D. citri* nymphs. To obtain enough prey during the assay, infested stems of each replication were re-infested every daylight hour, with as many as 50, first- to second-instar *D. citri* nymphs; 50, third- to fourth-instar nymphs; and 20, fifth-instar nymphs. *C. valida* prey consumption rate for each of the three larval-instar groups was obtained on each of the three stages of *D. citri* nymphs. At 0600 hours on the first day of the experiment, one predatory larva was released on each experimental unit. To determine the number of prey consumed by *C. valida*, the number of live prey remaining on each shoot was counted every hour with the aid of a stereoscopic microscope during the daily active period of two consecutive days.

Predation capacity of *C. valida* larvae was studied at a temperature of 24.9 ± 6.1°C and 82.5 ± 17.6% relative humidity in a greenhouse. To obtain *D. citri* nymphs of stadia 3 and 4, nine, 2-year-old Persian lime plants grafted onto *C. volkameriana* were pruned, watered, and fertilized in 1-liter pots and placed in entomological cages covered with polyester organza. Forty fecund *D. citri* females were collected from orange jasmine plants and placed on 1- to 2-cm shoots of Persian lime stems; females were removed 48 hours later to obtain a cohort of individuals of known age. Larvae of *C. valida* were obtained from a culture in a laboratory. Newly hatched first-, second-, and third-instar *C. valida* larvae at 1 day before the assay were fed nymphs of *D. citri* of various stadia, reared on Persian lime shoots in vials with 8 ml of Murashige and Skoog's solution. Subsequently, larvae fasted overnight for 8 hours (from 2200 to 0600 hours the next day).

A completely randomized design with three treatments and four replications was used. The consumption rate for each larval instar of *C. valida* (L1, L2, and L3) was determined based on a group of known age (N3-4) *D. citri*. In treatments with first- and second-instar larvae, predators were offered 80 prey repeatedly during the assay. Two hours before starting the assay, third-instar larvae were offered 100 prey on shoots of greenhouse plants. The amounts offered were based on the results of 6 hours of consumption in the laboratory. At 0600 hours, a fine brush was used to place one larva onto one shoot per plant; this was considered a replication. Each shoot was covered with a crystalline plastic container with ventilation apertures covered with polyester organza. The container was held in place with Parafilm®. Six hours later, larvae of *C. valida* were removed and the number of surviving prey was counted to calculate the number of prey consumed.

Mean and standard deviation were calculated for the variables: developmental time of *C. valida* larval instars, fecundity, and adult longevity of males and females. Analysis of variance and mean comparison (Tukey test) were done on prey consumption for the first 6 hours, and for the 1st and 2nd day of evaluation in a laboratory. Prey consumption during 6 hours under greenhouse
conditions was subjected to analysis of variance and Tukey test. Data were analyzed by using SAS/STAT® 9.4 software (SAS Institute 2014).

Results

From the cohort of 100 eggs observed, 38 females and 39 males reached adulthood (sex ratio 1:1.03). Mean development time from egg to adult emergence of *C. valida* under laboratory conditions was 38.5 ± 0.16 days. Eggs developed in 5.5 days, larvae in 18.5 days, and the pre-pupa to pupa period lasted 14.5 days (Table 1).

Fertile females began ovipositing approximately 10 days after emergence. Mean daily number of eggs per female was 10 ± 8.37. Fig. 1 illustrates oviposition accumulated every 5 days; greater values were accumulated on Days 10, 15, and 20, with 694, 764, and 834 eggs, respectively. Fecundity was greatest during the first 40 days, with greatest oviposition of 45 eggs per female on Day 15. A gradual decrease was observed in the number of eggs until 102 days. Mean fecundity per female during 102 days was 854.63 eggs. Numbers of eggs laid by each female *C. valida* ranged from a minimum of seven to a maximum of 1,923.

Table 1. *Ceraeochrysa valida* (Banks) Life Cycle Duration at 25 ± 0.6°C, 72 ± 9% Relative Humidity, and 12:12 Light:Dark Hours

<table>
<thead>
<tr>
<th>Development</th>
<th>N</th>
<th>Duration (days) ± SD</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>100</td>
<td>5.5 ± 0.7</td>
<td>98.0</td>
</tr>
<tr>
<td>Larva 1</td>
<td>98</td>
<td>6.5 ± 0.7</td>
<td>96.9</td>
</tr>
<tr>
<td>Larva 2</td>
<td>95</td>
<td>5.0 ± 1.0</td>
<td>98.9</td>
</tr>
<tr>
<td>Larva 3</td>
<td>94</td>
<td>7.0 ± 1.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Prepupa-pupa</td>
<td>94</td>
<td>14.5 ± 0.7</td>
<td>81.2</td>
</tr>
<tr>
<td>Period from egg to adult</td>
<td>77</td>
<td>38.5 ± 0.2</td>
<td>77.0</td>
</tr>
</tbody>
</table>

![Graph](image)

Fig. 1. Mean fecundity of *Ceraeochrysa valida* accumulated every 5 days during the oviposition period (25 ± 0.6°C, 72 ± 9% relative humidity, and 12:12 light:dark hours).
Mean *C. valida* adult longevity for both males and females was 76.59 ± 34.18 days (range 9 to 147 days). Females lived 78 ± 28.95 days, males 74 ± 38.92 days. Survival of adult males and females decreased gradually over time (Fig. 2).

![Survival curves for *Ceraeochrysa valida* adult males and females at 25 ± 0.6°C, 72 ± 9% relative humidity, and 12:12 light:dark hours.](image)

Table 2 shows *C. valida* consumption rate by larval instar during the first 6 and 24 hours on the 1st day and during 24 hours on the 2nd day (here referred to as 48 hours) of exposure to *D. citri* nymphs of known age. Analysis of variance for the consumption rate by the three larval instars of *C. valida* on three age groups of *D. citri* nymphs showed very significant differences (*P* < 0.0001) among larval and nymphal stages at 6, 24, and 48 hours. There also was significant interaction between larvae and nymphs (*P* ≤ 0.001).

In general, as a *C. valida* larva grew older, it consumed more *D. citri* nymphs, and the older the prey, the fewer the nymphs consumed by the larva. With analysis of variance, an interaction between the variables larva and nymph was observed because of less consumption of small nymphs (N1-2) by Larva 1, similar to the number of medium nymphs consumed (N3-4). This did not allow significant separation of the two groups for consumption by nymphs at 6 and 24 hours (Table 2), which was not the case at 48 hours. Such results might have been because small *C. valida* larvae tasted rather than consumed the prey whole. Interaction also was observed as reduced consumption of large nymphs (N5) by the three predatory larval instars, with a much lower consumption rate by larger instars, but this did not increase at the same rate in Larva 3 as with smaller nymphs.

The average rate of consumption of Nymphs 3 and 4 of *D. citri* by the three larval instars of *C. valida* increased positively with predator age (Fig. 3). Larva 3 of *C. valida* consumed significantly more prey (47.75 ± 4.57), followed by Larva 2 (40.75 ± 2.36), and Larva 1 (19.50 ± 3.31).
Table 2. Consumption Rate for Three Larval Instars of *Ceraeochrysa valida* on Three *Diaphorina citri* Nymphal Groups of Known Age during the First 6 Hours and On the First and Second Days after Fasting (*25 ± 0.6°C, 70 ± 10% Relative Humidity, and 12:12 Light:Dark Hours)*

<table>
<thead>
<tr>
<th>Treatment</th>
</tr>
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<tbody>
<tr>
<td>C. valida</td>
</tr>
<tr>
<td>Larva 1a</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Larva 2</td>
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<td></td>
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<td>Larva 3</td>
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*For each larval instar, means in the same column with different letters are significantly different (P < 0.0001).*

Discussion

Egg duration in *C. valida* was comparable under similar conditions to other species of Chrysopidae such as *C. everes*, *Ceraeochrysa paraguaria* (Navas), and *Chrysopodes lineafrons* (Adams and Penny) (Barbosa et al. 2002, De Bortoli and Murata 2007, Silva et al. 2007).
Larval stage duration (18.5 days) was shorter than that found by Serrano et al. (1988) for C. cubana and by Giffoni et al. (2007) for Chrysoperla externa (Hagen), and slightly greater than that for C. externa and C. cincta (Núñez 1988). However, Serrano et al. (1988) reported only 10 days for C. cubana. Results of Larva 1 and Larva 2 were similar to those found by Olazo and Heredia (2010) for Chrysopodes spinellus (Adams and Penny); Larva 3 was similar to that found by Alcantra et al. (2008) for C. cubana and by Olazo et al. (2009) for Chrysoperla asoralis (Banks). Mean development time from pre-pupa to pupa (14.5 days) was similar to that reported by Serrano et al. (1988), De Bortoli and Murata (2007), and Castro et al. (2009) for C. cubana, C. paraguaria, and Ceraeochrysa caligata (Banks). Smaller values were obtained by Giffoni et al. (2007) for C. externa and by Olazo and Heredia (2010) for C. spinellus.

Large viability (98%) was found in C. valida, similar to C. cubana (Silva et al. 1994) and C. externa (Ribeiro et al. 2011), but Barbosa et al. (2002) found less viability by C. everes. More C. valida died during the pupal stage, similar to findings by Ki-Sang and Jang-Hoon (2005) for Chrysopa pallens (Rambur). The development of C. valida from egg to adult (38.5 days) was similar to that obtained by Núñez (1988) for C. cincta and by Barbosa et al. (2002) for C. everes. Lower values were obtained by Serrano et al. (1988) for C. cubana. In general, McEwen et al. (2001) found the development time of different Chrysopidae species varied with temperature and prey species used to feed the immatures.

Mean fecundity of C. valida coincided with that found by Silva et al. (2007) and Ribeiro et al. (2011) for C. lineafrons with 8.7 and for C. externa with 8.9 eggs per female per day. Oviposition capacity for female predators was similar for Chrysoperla genanigra (De Freitas) (Bezerra et al. 2012). Ulhaq et al. (2006) indicated that diet composition (sugar, eggs, yeast) had a pronounced effect on egg production by chrysopids. Also, female-male proportion was important (Ramírez-Delgado et al. 2007). In our study, two males were provided per female, which might have shown fertility close to the species potential.

Mean C. valida female and male longevity were similar to that reported by Ribeiro et al. (2011) and that of Lavagnini and Freitas (2012) for C. externa. However, Carvalho et al. (2002) indicated greater longevities in Chrysoperla mediterranea (Hölzel) and less in C. carnea (Ulhaq et al. 2006). Overall, Ribeiro et al. (2011) indicated that longevity was a function of biotic and abiotic conditions experienced by insects, as well as handling and nutrition.

Mean consumption capacity of C. valida Larva 2 on D. citri 1-2 nymphs during the first 6 hours of exposure was greater than that reported by Cortez-Mondaca et al. (2011) for C. valida (84). Mean consumption rate of Nymphs 3-4 by Larva 2 was similar to that reported by Ail-Catzim et al. (2012) with C. carnea preying on large nymphs of Bactericera cockerelli (Sulc.). C. valida Larva 2 preying on D. citri nymphs 3-4, consumed more on the 1st day of exposure. This coincided with the finding of Maia et al. (2004), with C. externa Larvae 2 and 3 on nymphs of Rhopalosiphum maidis (Fitch) under similar conditions.

In general, predator consumption capacity depended on the size of the predator and prey (Chesson 1989). C. valida Larva 3 was evaluated using immature D. citri of known age, showing greater consumption by Larvae 1 and 2. This result agreed with those of Alcantra et al. (2008) and Salamanca-Bastidas et al. (2010) with C. cubana and C. externa. Most consumption by Larva 3 could be attributed to size, ability to search for prey, and storage of energy for the pupal stage (Auad et al. 2001, Alcantra et al. 2008, Ail-Catzim et al. 2012). Less
consumption of Nymph 5 by predatory larvae was attributed to larger nymph size and greater quantity of secreted wax that hinders predatory potential, all of which was consistent with findings by Goncalves-Gervásio and Santa-Cecília (2001).

According to Rashid et al. (2012), feeding rate increased with predator larval age, the situation observed in this study for mean consumption capacity of C. valida Larvae 1, 2, and 3 on 3-4 nymphs of D. citri in a greenhouse. It also confirmed the findings of Auad et al. (2003) with C. externa fed nymphs of Uroleucon ambrosiae (Thomas).

Mean consumption capacity of C. valida Larvae 1 and 2 on D. citri nymphs 3-4 was greater under controlled conditions, compared to the assay in the greenhouse. Similar results were obtained by Su et al. (2004) with Larvae 1-2 of Chrysopa phyllochroma (Wesmael) fed Aphis gossypii, as well as those obtained by Auad et al. (2003) with C. externa Larvae 1-3 fed U. ambrosiae nymphs.

According to Cortez-Mondaca et al. (2008), predatory capacity, dominance in the agroecosystem, resistance to environmental conditions, feasibility of mass reproduction, and the life cycle must be considered when choosing a biological control agent against D. citri immatures. The larval life cycle of C. valida in this study (18.5 days) was a major feeding period compared to species of the genus Chrysoperla. This could be considered an advantage in the field because C. valida might spend more time consuming pests (Albuquerque et al. 2001) and is a local resource already adapted to the environment. C. valida should be evaluated in the field to revise predator-prey interactions in the natural environment.

Acknowledgment

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