Chartocerus sp. (Hymenoptera: Signiphoridae) and Pachyneuron crassiculme (Hymenoptera: Pteromalidae) are Obligate Hyperparasitoids of Diaphorencyrtus aligarhensis (Hymenoptera: Encyrtidae) and Possibly Tamarixia radiata (Hymenoptera: Eulophidae)

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CHARTOCERUS SP. (HYMENOPTERA: SIGNIPHORIDAE) AND PACHYNEURON CRASSICULME (HYMENOPTERA: PTEROMALIDAE) ARE OBLIGATE HYPERPARASITOIDS OF DIAPHORENCYRTUS ALIGARHENSIS (HYMENOPTERA: ENCYRTIDAE) AND POSSIBLY TAMARIXIA RADIATA (HYMENOPTERA: EULOPHIDAE)

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ABSTRACT

Two species of suspected hyperparasitoids, Chartocerus sp. and Pachyneuron crassiculme, emerged from parasitized Diaphorina citri nymphs collected in Punjab Pakistan over 15-22 Apr 2013. Exposure tests conducted in quarantine on D. citri nymphs parasitized by Tamarixia radiata and Diaphorencyrtus aligarhensis, as well as unparasitized D. citri nymphs, confirmed that Chartocerus sp. and P. crassiculme are hyperparasitoids. Both Chartocerus sp. and P. crassiculme successfully reproduced on D. aligarhensis, with one instance of P. crassiculme reproducing on T. radiata. There was no emergence from unparasitized D. citri.

Key Words: choice test, no-choice test, quarantine

MATERIALS AND METHODS

Parasitized D. citri host material returned from Punjab Pakistan to quarantine at UCR (15-22 April 2013) yielded previously collected T. radiata and D. aligarhensis, along with several species of known (Marietta leopardina Motschulsky [Hymenoptera: Aphelinidae], Aprostocetus (Aprostocetus) sp. [Hymenoptera: Eulophidae] [Hoddle et al. 2013]) or suspected (Chartocerus sp. [Hymenoptera: Signiphoridae], Pachyneuron crassiculme Waterston [Hymenoptera: Pteromalidae] and Psyllaphycus diaphorinae [Hymenoptera: Encyrtidae]) hyperparasitoids.

To confirm that Chartocerus sp. and P. crassiculme, both suspected hyperparasitoids, are not primary parasitoids of D. citri.

Asian citrus psyllid (ACP), Diaphorina citri Kuwayama (Hemiptera: Lividae), was discovered in California USA in 2008. D. citri vectors ‘Candidatus Liberibacter asiaticus’, a putative causative agent of huanglongbing (HLB), a lethal disease of citrus (Hoffman et al. 2013; Wang & Trivedi 2013). HLB was detected in California in Mar 2012 (Leavitt 2012). To mitigate the threat posed by D. citri-HLB to California’s citrus industry, a biological control program using Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) sourced from Pakistan was initiated (Hoddle 2012). Diaphorencyrtus aligarhensis (Shafee, Alam, and Agarwal) (Hymenoptera: Encyrtidae), a second parasitoid of D. citri also collected from Pakistan, is currently in quarantine at the University of California, Riverside (UCR). The purpose of this study was to confirm that Chartocerus sp. and P. crassiculme, both suspected hyperparasitoids, are not primary parasitoids of D. citri.

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were rotated through each of 4 treatment types between 26 Apr and 24 May, 2013 in quarantine at UCR. It was not possible to reliably sex live Chartocerus sp., so this species was exposed in groups (assumed to contain at least 1 female each) unless a pair was otherwise observed mating. Exposure treatments consisted of: (A) nymphs parasitized by T. radiata ($n=8$ replicates of 5-10 parasitized nymphs for Chartocerus sp. and 9 replicates of 5 parasitized nymphs for P. crassiculme), 5-9 days post-exposure to T. radiata; (B) nymphs parasitized by D. aligarhensis ($n=8$ replicates of 5-10 parasitized nymphs for Chartocerus sp. and 10 replicates of 5 for P. crassiculme), 10-14 days post-exposure to D. aligarhensis; (C) unparasitized third to fourth instar D. citri nymphs ($n=9$ replicates of 5-10 unparasitized nymphs for Chartocerus sp. and 10 replicates of 5 nymphs for P. crassiculme); and (D) each of the 3 previously listed host types (A, B, and C) presented simulta-
neously in a choice cage \((n = 9)\) replicates of 5-10 of each host type for *Chartocerus* sp. and 9 replicates of 5 of each host type for *P. crassiculme*.

Each replicate was comprised of host material for each treatment type exposed to a group of potential hyperparasitoids for 24 h each. Hosts were exposed sequentially in a different order for each replicate to prevent bias due to presentation order. Emergence rates of *T. radiata* \((n = 5)\) parasitized nymphs on each of 10 cuttings and *D. aligarhensis* \((n = 5)\) parasitized nymphs on each of 10 cuttings determined baseline mortality for primary parasitoids in the absence of hyperparasitoids. Unparasitized *D. citri* nymphs \((n = 5)\) provided data on nymph mortality in the absence of hyperparasitoids. Mummies of *T. radiata* and *D. aligarhensis* used in exposure experiments were sourced from colonies maintained in quarantine at UCR.

*Diaphorina citri* nymphs parasitized by either *T. radiata* or *D. aligarhensis* for no-choice treatments were presented on small *Citrus volkameriana* cuttings. *Citrus volkameriana* seedlings grown in 114 mL Cone-tainers™ (SC7 Stubby, 3.8 cm diameter, Stew & Sons Inc., Oregon) and infested with *D. citri* nymphs were used to expose unparasitized *D. citri* nymphs to *Chartocerus* sp. and *P. crassiculme*. Clear plastic vials (Thornton Plastic Co. 148 mL capacity, Salt Lake City, Utah) with three 12 mm diam ventilation holes covered with ultra-fine organza were inverted and placed over the top of the plant and fitted into the corresponding vial lid, which had a hole cut in the center to allow it to be fitted around the cone (Irvin et al. 2009).

Choice treatments were set up in 15 cm \(\times\) 15.3 cm \(\times\) 15.3 cm \((h \times w \times d)\) clear plastic boxes (S&W Plastics, Riverside, California) with a 30 cm sleeve sewn from no-see-um netting (Skeeta Mosquito & Other Insect Protection Products, Bradenton, Florida). Unparasitized *D. citri* nymphs in Containers and *T. radiata* and *D. aligarhensis*-parasitized nymphs on *C. volkameriana* cuttings in water were placed in the cage without ventilated vials on top to allow free access to all 3 host types simultaneously. After 24 h, each host type was enclosed with an inverted ventilated vial to contain all insects that emerged from each host type. All experiments were conducted in quarantine at UCR’s Insectary and Quarantine facility, at 27 °C, 50% RH, and 14:10 h L:D. Replicates were observed daily after initial exposure, and total numbers of each emerged species were recorded per treatment.

**RESULTS**

No-choice treatments resulted in *Chartocerus* sp. reproducing successfully only on *D. aligarhensis* (Table 1). Mean emergence time for *Char-

**Table 1. Emergence and Mortality Rates for *Chartocerus* sp. Exposed to UnParasitized Third and Fourth Instar *D. Citri* Nymphs, and Nymphs Parasitized By *T. radiata*, and *D. aligarhensis* in NO-CHOICE and CHOICE TREATMENTS.**

| Host         | Total % Host Emergence | % Host Parasitism | % Host Dead | % Host Missing | % Host Emergence | % Host Parasitism | % Host Dead | % Host Missing | % Host Emergence | % Host Parasitism | % Host Dead | % Host Missing |
|--------------|------------------------|-------------------|-------------|----------------|------------------|------------------|-------------|----------------|------------------|------------------|-------------|----------------|
| *D. citri*   |                        |                   |             |                |                  |                  |             |                |                  |                  |             |                |
| *T. radiata* | 65                     | 72.31%            | 0.00%       | 9.23%          | 18.46%           |                  |              | 5.97%          | 25.81%           |                  |              | 13.85%         |
| *D. aligarhensis* | 65 67                 | 67.16%            | 46.67%      | 16.67%         | 16.67%           |                  |              | 25.81%         | 33.33%           |                  |              | 29.23%         |

Percentage of *D. citri* adults that emerged from unparasitized nymphs.

Percentage of *T. radiata* adults that emerged from parasitized nymphs.

Percentage of *D. aligarhensis* adults that emerged from parasitized nymphs.

Percentage of hosts killed by parasitism. Actual number of hosts killed = 28. Actual number of *Chartocerus* sp. adults emerged = 33. Demonstrating 2 observed instances of superparasitism.
tocerus sp. offspring from D. aligarhensis was 18.36 days ± 2.34 (SE). Pachyneuron crassiculme produced progeny on D. aligarhensis and T. radiata in no-choice treatments, though parasitism was much higher on D. aligarhensis (Table 2). Mean emergence times for males and females were 12.83 days ± 2.48 (SE) and 11.33 days ± 2.05 (SE), respectively. Pachyneuron crassiculme had a single male emerge from T. radiata after 11 days. Emergence rates for control treatments of T. radiata, D. aligarhensis, and D. citri were 84%, 88%, and 88%, respectively (Table 3). Chartocerus sp. and P. crassiculme failed to reproduce on unparasitized D. citri nymphs.

Immature D. aligarhensis exposed to Chartocerus sp. in no-choice tests experienced 47% parasitism, 17% died from undetermined causes, 3% were unaccounted for, and 33% emerged as adult D. aligarhensis. In 20% of trials (i.e., 2 of 10 replicates) Chartocerus sp. exhibited superparasitism, with 11 adults emerging from 9 D. aligarhensis mummies in 1 replicate, and 6 adults emerging from 3 mummies in the second. In no-choice tests, immature T. radiata exposed to Chartocerus sp. exhibited 0% parasitism, 27% of mummies died from unknown causes, 6% disappeared, and 67% emerged as adult T. radiata. Unknown mortality may be attributable to superparasitism, host feeding, or a combination of both by P. crassiculme.

There was no successful parasitism of any host in choice tests for either Chartocerus sp. or P. crassiculme. However, elevated mortality rates were observed for T. radiata (26% when exposed to Chartocerus sp.; 28% for P. crassiculme) and D. aligarhensis (29%; 13%). In comparison, control mortality for T. radiata and D. aligarhensis were < 13% in the absence of these hyperparasitoids. When viewed collectively, data from exposure trials demonstrates that Chartocerus sp. and P. crassiculme are obligate hyperparasitoids within the D. citri-Yamaria-Diaphorencyrtus system. Immediately following the conclusion of trials, all Chartocerus sp. and P. crassiculme material was killed in quarantine and preserved in 95% ethanol. Voucher specimens were deposited in the Entomology Museum at UCR (Table 4).

Assuming Chartocerus sp. and P. crassiculme preferentially parasitize D. aligarhensis as these exposure trial data suggest, the frequency of Chartocerus sp. and P. crassiculme emergence in quarantine from material collected from Punjab Pakistan in April 2013 was significant in compar-
ison to *D. aligarhensis* emergence rates. *Chartocerus* sp. (237 individuals reared), *P. crassiculme* (181), and *D. aligarhensis* (743) represented 20%, 16%, and 64% of material reared, respectively, within this complex. A total of 292 *t. radiata* were reared from April 2013 collections. Exposure trials suggest that the lower numbers of *t. radiata* obtained from Pakistan in April 2013 were not likely due to hyperparasitism.

**Acknowledgments**

The authors would like to thank Roger Burks (UCR) for assistance with specimen mounting, photography, and identification. Serguei Triapitsyn (UCR) helped with specimen identification and deposition of voucher specimens. Anamaria Dal Molin (TAMU) identified *Chartocerus* sp. Funding for this project was provided, in part, by funds issued by the California Department of Food and Agriculture’s Specialty Crops Program and the California Citrus Research Board.

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**Table 3. Emergence Rates of Unparasitized Third and Fourth Instar *D. citri* Nymphs and Nymphs Parasitized by *T. radiata* and *D. aligarhensis* in Control Treatments Not Exposed to Hyperparasitoids.**

| Host            | Total No. Exposed | No. Adults Emerged | No. Dead Hosts | No. Missing Hosts |
|-----------------|-------------------|--------------------|----------------|------------------|
| *D. citri*      | 50                | 44\(^1\)           | 1              | 0                |
| *T. radiata*    | 50                | 42\(^2\)           | 6              | 2                |
| *D. aligarhensis* | 52              | 46\(^3\)           | 3              | 1                |

\(^1\)Total number of *D. citri* adults that matured from unparasitized nymphs.

\(^2\)Total number of *T. radiata* adults that emerged from parasitized nymphs.

\(^3\)Total number of *D. aligarhensis* adults that emerged from parasitized nymphs.

\(^4\)Total number of hosts found dead.

\(^5\)Total number of hosts unaccounted for at time of data collection.

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**Table 4. Specimen Accession Numbers for All Species Used in Exposure Trials and Deposited in the Entomology Museum at the University of California Riverside.**

| Species                 | Accession No.                                      |
|-------------------------|---------------------------------------------------|
| *D. citri*\(^1\)        | UCRC_ENT00334428                                  |
| *T. radiata*\(^2\)      | UCRC_ENT00334402-334418                           |
| *D. aligarhensis*\(^2\) | UCRC_ENT00334426-334427                           |
| *Chartocerus* sp.\(^2\) | UCRC_ENT00417173-00417182                         |
| *P. crassiculme*\(^2\)  | UCRC_ENT00417183-00417187                         |

\(^1\)Multiple individuals of Pakistani *D. citri* preserved in a single vial of 95% ethanol.

\(^2\)Point-mounted individuals.

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