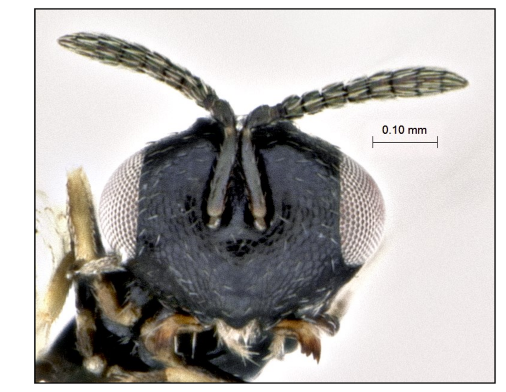
Hyper Alert: Confirming two Pakistani wasps are hyperparasitoids of valuable ACP biological control agents

Allison Bistline-East[1] and Mark S. Hoddle[1,2]

[1] Department of Entomology, University of California, Riverside

[2] Center for Invasive Species Research, University of California, Riverside



Abstract

Exposure trials were conducted in quarantine to determine if *Chartocerus* sp. (Hymenoptera: Signiphoridae) and *Pachyneuron crassiculme* (Hymenoptera: Pteromalidae) collected from Punjab, Pakistan are primary parasitoids of Asian citrus psyllid (ACP), *Diaphorina citri*, or hyperparasitoids capable of utilizing parasitized ACP. Each replicate consisted of a group of potential hyperparasitoids being serially exposed to four treatments in randomized order for 24 hours each. Unparasitized ACP nymphs and mummies of each of two primary ACP parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) comprised three no-choice treatments, while a single choice treatment presented all three hosts simultaneously. No-choice exposures resulted in a distinct preference of both *Chartocerus* sp. and *P. crassiculme* for reproduction on *D. aligarhensis*, with only one recorded instance of reproduction on *T. radiata*. There was no reproduction on unparasitized ACP nymphs. This lack of successful reproduction on unparasitized ACP and utilization of *D. aligarhensis* larvae as hosts indicate that both *Chartocerus* sp. and *P. crassiculme* are obligate hyperparasitoids. Further research into endemic California species closely related to these two hyperparasitoids is warranted to determine if potential biotic challenges exist which would hinder establishment of *D. aligarhensis* in California as a biological control agent against ACP.

Introduction

Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an invasive pest of citrus that was first detected in Southern California in 2008. ACP is capable of vectoring the bacterium *Candidatus* Liberibacter asiaticus, which causes huanglongbing (HLB), a lethal disease of citrus which is highly difficult to treat (especially in a commercial orchard environment). It was detected in California in Mar 2012 (Hoddle & Hoddle, 2013). In 2012, a classical biological control program was initiated using natural enemies of ACP. Aside from the target species (*Tamarixia radiata*, *Diaphorencyrtus aligarhensis*), several other parasitoid species were collected. The identification of *Chartocerus* sp. (**Fig. 1**A. male, B. female) and *P. crassiculme* (**Fig. 2**A. male, B. female) as hyperparasitoids gives insight into the dynamics of the host/parasite system in ACP's natural home range (Bistline-East & Hoddle, 2014). This information can also guide further study examining closely-related species in Southern California to evaluate host preference for introduced biological control agents to determine if these native hyperparasitoids pose any challenges to the efficacy of *T. radiata* or *D. aligarhensis*.

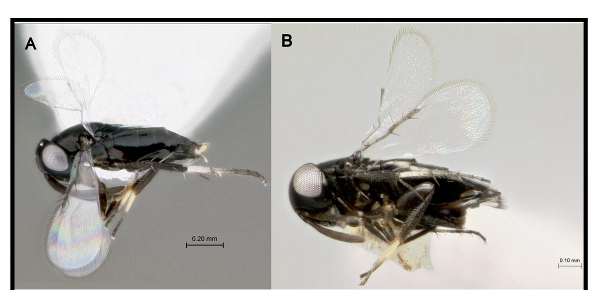


Figure 1. *Chartocerus* sp. male (**A**); female (**B**)

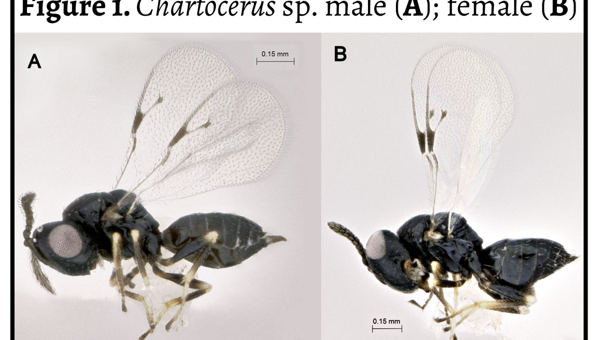


Figure 2. *Pachyneuron crassiculme* male (**A**); female (**B**)

Materials & Methods

Specimen Collection

A total of 6 trips over 3 years were made to Punjab, Pakistan (part of ACP's home range) to collect natural enemies. From the most recent expedition (15-22 Apr 2013), parasitized ACP host material harvested from citrus orchards were imported into quarantine at UCR under USDA-APHIS permit No.P526P-11-00103. In addition to the two target species (*T. radiata* and *D. aligarhensis*), several species of known (as detailed in Hoddle et al., 2013) or suspected (*Chartocerus* sp. and *P. crassiculme*) hyperparasitoids emerged from collected host material.

Experimental Setup

Exposure trials were set up with 10 replicates for each potential hyperparasitoid species, with each replicate using groups of 4-7 *Chartocerus* sp. and mated pairs of 1 ♂ and 1 ♀ *P. crassiculme* that emerged from material collected in Pakistan. It was not possible to reliably sex live *Chartocerus* sp., so this species was exposed in groups (assumed to contain at least 1 ♀ each) unless a pair was otherwise observed mating. Subjects of each replicate were rotated sequentially through each of 3 treatment types between 26 Apr and 24 May, 2013, in quarantine at UCR. Replicates were comprised of 3 no-choice treatments defined by potential host species: (A) *T. radiata* mummies, 5-9 days post-oviposition; (B) *D. aligarhensis* mummies, 10-14 days oviposition; (C) unparasitized third to fourth instar ACP nymphs; as well as 1 choice treatment: (D) all 3 potential hosts presented simultaneously (**Fig. 3A-D**). Potential hyperparasitoids were exposed to each treatment type for 24 hours before being moved to subsequent hosts. Hosts were presented sequentially in a different order for each replicate to prevent bias. *T. radiata* and *D. aligarhensis* mummies were presented on *Citrus volkameriana* cuttings in water (**Fig. 4A**). Small *C. volkameriana* seedlings infested with ACP nymphs were used to expose unparasitized ACP nymphs to hyperparasitoids (**Fig. 4B**). Choice treatments presented *T. radiata*, *D. aligarhensis*, and unparasitized ACP simultaneously within a clear acrylic sleeve cage (**Fig. 4C**). After 24hr, each host type was isolated with an inverted ventilated vial so that any hyperparasitoids that emerged would be accurately recorded for the associated host species. Emergence rates of *T. radiata*, *D. aligarhensis*, and ACP were measured in the absence of hyperparasitoids to establish baseline mortality for each host. Host material used in exposure trials were sourced from colonies maintained at UCR. All experiments were conducted in quarantine at UCR at 27 °C, 50% RH, and 14:10 h L:D. Replicates were observed daily after final exposure rotation, and the total number of each emerged species was recorded per treatment.

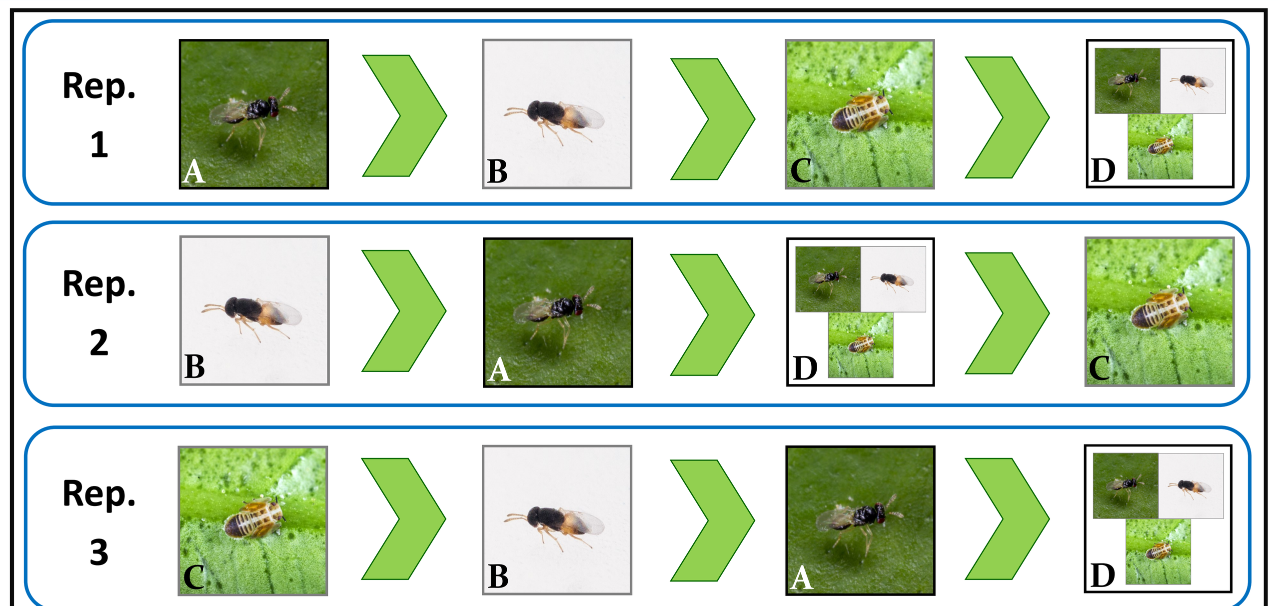


Figure 3. Each replicate consisted of hyperparasitoids rotated serially through 4 different treatment types: *T. radiata* (**A**) mummies, *D. aligarhensis* (**B**) mummies, unparasitized ACP nymphs (**C**), and a choice cage (**D**) containing A-C. Hosts were exposed for 24hr each, and each replicate presented host species in a different order.

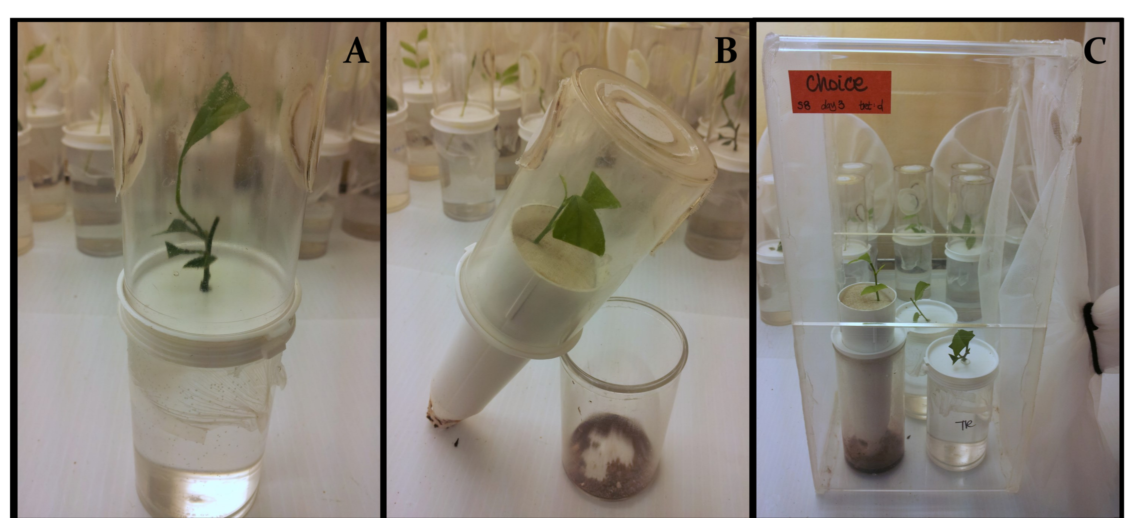


Figure 4. No-choice treatments were set up using cuttings in water (*T. radiata, D. aligarhensis* mummies) (**A**) and unparasitized third and fourth instar ACP nymphs on live *Citrus volkameriana* seedlings in 114mL Cone-tainers (**B**). Choice treatments containing all 3 potential hosts were set up in large acrylic cages with fine mesh sleeves to allow hyperparasitoids free access to all hosts simultaneously (**C**).

Acknowledgements

Our greatest thanks to Michael Lewis and Roger Burks for providing photographs; Serguei Triapitsyn for identification of hyperparasitoid species; and Christina Hoddle for advising on experimental design. Funding was provided by the California Department of Food and Agriculture Specialty Crops Program and the California Citrus Research Board.

Results Discussion

Results from exposure trials indicated that both *Chartocerus* sp. and *P. crassiculme* are hyperparasitoids, with successful reproduction observed on *T. radiata* and *D. aligarhensis* mummies, but none on unparasitized ACP. In no-choice treatments, *Chartocerus* sp. parasitized 47% of *D. aligarhensis* mummies, with 2 instances of successful superparasitism (**Fig. 5**). *Chartocerus* sp. did not parasitize *T. radiata*. *P. crassiculme* parasitized 28% of *D. aligarhensis* mummies, and had one recorded instance of parasitism on *T. radiata* (2% of mummies), with 1 ♂ offspring emerging after 11 days, in no-choice treatments (**Fig. 6**). Mean emergence times for *Chartocerus* sp. and *P. crassiculme* offspring on *D. aligarhensis* were 18.36 days ± 2.34 (SE) and 12.83 days ± 2.48 (SE) (♂) and 11.33 days ± 2.05 (SE) (♀), respectively. Unparasitized ACP nymphs did not experience parasitism by either hyperparasitoid. Neither *Chartocerus* sp. nor *P. crassiculme* showed any parasitism in choice cages on any host type.

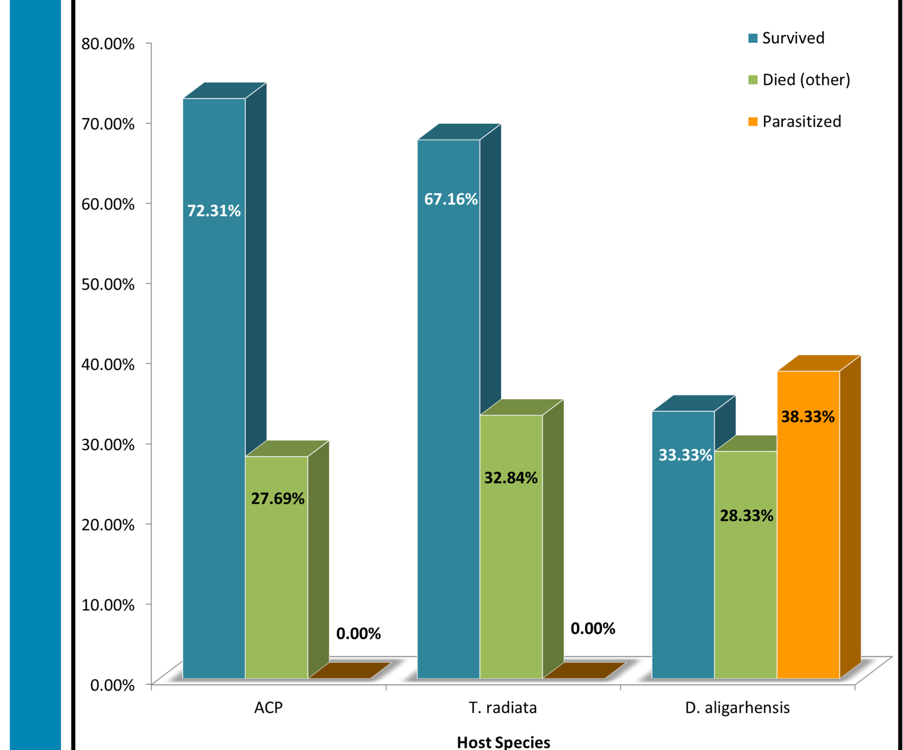


Figure 5. Host fate in no-choice treatments when exposed to *Chartocerus* sp. "Died (other)" = dead + unaccounted for hosts.

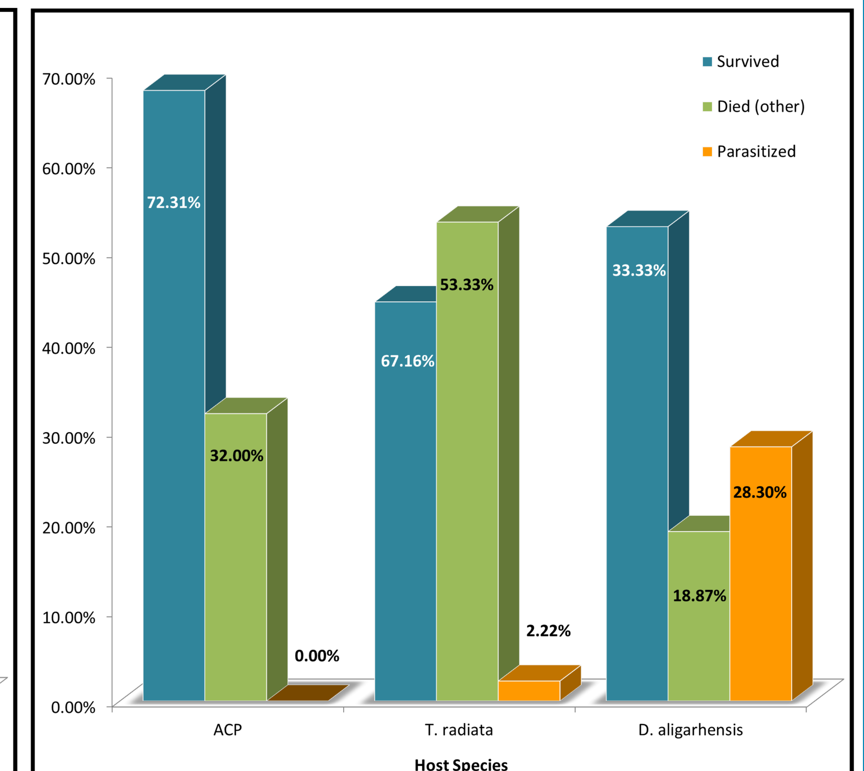


Figure 6. Host fate in no-choice treatments when exposed to *P. crassiculme*. "Died (other)" = dead + unaccounted for hosts.

All potential host species exposed to either species of hyperparasitoid also displayed elevated levels of mortality likely due to host feeding, superparasitism, or a combination of the two (not including parasitism). Across both no-choice and choice treatments, ACP experienced "Other" mortality rates of 23% and 29%; *T. radiata* experienced 33% and 41%; and *D. aligarhensis* experienced 38% and 16% when exposed to *Chartocerus* sp. and *P. crassiculme*, respectively (as compared to 2%, 16%, and 8% (respectively) in the controls). Chi-square analyses comparing mortality rates in *T. radiata* and *D. aligarhensis* exposure trials to control showed this elevated mortality to be statistically significant in *T. radiata* under exposure to both *Chartocerus* sp. (χ^2= 4.43, *df*=1, P<0.05) and *P. crassiculme* (χ^2=5.43, *df*=1, P<0.05). Mortality rates were significantly greater in *D. aligarhensis* when exposed to *Chartocerus* sp. excluding death by parasitism (χ^2=10.60, *df*=1, P<0.05), but *D. aligarhensis* exposed to *P. crassiculme* showed significantly elevated mortality only when death by parasitism was included (χ^2=9.23, *df*=1, P<0.05). Fisher's Exact Test comparing mortality rates of ACP also resulted in significantly higher mortality in exposure versus control treatments (P<0.05, with 95% confidence interval for both hyperparasitoid species).

Literature Cited

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