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SHORT-TERM STORAGE OF ADULT *TAMARIXIA RADIATA* (HYMENOPTERA: EULOPHIDAE) PRIOR TO FIELD RELEASES FOR BIOLOGICAL CONTROL OF ASIAN CITRUS PSYLLID

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Tamarixia radiata (Waterson) (Hymenoptera: Eulophidae) is a solitary ectoparasitoid of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). The psyllid is an important citrus pest because it vectors pathogens responsible for a serious disease of citrus known as huanglongbing (Bové 2006; Gottwald 2010). Regarded as one of the psyllid's most important natural enemies (Hall et al. 2013), *T. radiata* can be mass-reared on psyllid colonies maintained on potted *Citrus* or other host plants such as *Murraya exotica* L. (Skelley & Hoy 2004). As new adult parasitoids emerge, they can be collected and field-released to establish the parasitoid in new areas or to augment existing parasitoid populations. However, sometimes it may not be possible to immediately release adults, in which case it would be advantageous to know how best to store them until release.

The objectives of experiments presented here were to investigate survival of adult *T. radiata* stored under different food, light and temperature conditions. Adults were obtained from a USDA-ARS colony of the parasitoid established during 2011. This colony is maintained using psyllids reared on alemow, *Citrus macrophylla* Wester, following the basic procedures presented by Skelley & Hoy (2004). Four storage experiments were conducted. In each experiment, adult *T. radiata* (< 24 h old) were aspirated into clear-styrene, 9-dram plastic vials (26 mm diam × 67 mm tall) with snap-on lids (#8909, BioQuip Products, Gardena, California) using a BioQuip aspirator (#1135A) operated manually. A 1.5 × 5 cm piece of tissue paper was saturated with pure honey, blotted with paper toweling to remove all excess honey, and then placed against the inside wall of a vial before aspirating adults, after which the lid was snapped into place anchoring one end of the honey strip.

In one experiment, adult survival was compared in vials with or without honey. Fourteen vials, each with 20 adults, were prepared for the experiment, 7 with honey and 7 without. The vials were held at 25 °C with a 14 h light photo phase and checked daily to determine number of live adults. All adult *T. radiata* held without honey were dead after 5 days of storage while all adults held with honey were still alive. These survival results agreed with findings by Chu & Chien (1991) and Chien et al. (1994).

In a second experiment, survival was compared among parasitoids held in vials either in the dark or under a 14 h light photo phase. Three vials each containing 20 parasitoids and honey were subjected to each light treatment, one set of vials at 20 °C and a second set at 25 °C. Adult survival was relatively good whether they were held in the dark or not, although survival tended to be better among adults provided with light on a number of observation dates (Fig. 1). After 14 days of storage, there were no significant differences in adult survival with respect to temperature ($F_{1,11}$ = 0.28, P = 0.61) or replication ($F_{2,11}$ = 1.12, P = 0.38), but survival was significantly reduced under the dark treatment (93%) as compared to the light treatment (98%) ($F_{1,11}$ = 7.00, P = 0.03).

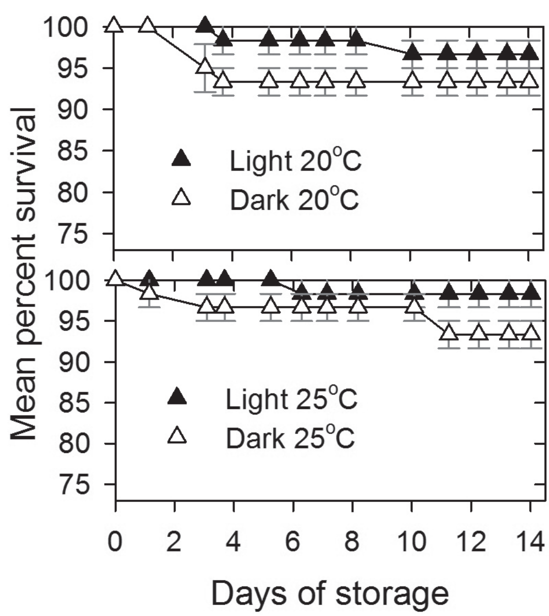

Fig. 1. Storing adult *Tamarixia radiata* at 20 or 25 °C with honey in darkness or under a 14 h light photo phase.

In a third experiment, newly-emerged adult *T. radiata* were stored with honey in vials at 4 different temperatures: 15, 20, 25 or 30 °C (all under a 14 h light photo phase). Four vials of 30 adults were studied at each temperature, and the experiment was repeated twice. Parasitoid survival was good at 20, 25 and 30 °C but poorer at 15 °C (Fig. 2). On day 14, mean percent survival of adults stored at 15 °C (72%) was significantly less than that of adults stored at 20 to 30 °C (95 to 97% survival) ($F_{3,15}$ = 18.2, P = <0.0001). Chien & Chu (1993) reported a higher survival rate of adults at 15 °C than we observed in our study. A major difference between the 2 studies was the time allowed for parasitoids to feed – Chien & Chu (1993) moved stored parasitoids from 15 to 25°C every other day and allowed them to feed for an hour, while we moved the parasitoids from 15 °C to ambient laboratory temperature conditions just long enough to determine if the adults were alive. Thus, survival rates at 15 °C in our study might have been greater had we allowed the adults more time to feed at a temperature above 15 °C.

Based on the 3 experiments, newly-emerged adult *T. radiata* can be stored at 20 to 30 °C in vials containing honey with relatively good survival rates for up to 14 days. Of interest is whether parasitoids stored for some period of time exert the same parasitism rates as newly-emerged parasitoids. This we investigated using procedures similar to those described by Chen et al. (2013). Six *C. macrophylla* plants each with at least 3 new flush shoots (~3 cm in length) were placed in a large cage and 600 adult psyllids were released into the cage for a 24 h oviposition period. The adults were then removed and the plants were held for 9 days (when most nymphs had reached the 4th instar of development) in a rearing room at 25 °C, 60% RH, 14 h light photo phase. A small brush was then used to remove nymphs until approximately 120 nymphs remained on each plant. Each plant was then placed individually into a clear acrylic cylindrical cage (12.5 cm diam × 43 cm tall) after which 3 *T. radiata* females and 2 males were released into each cage. Three caged plants were randomly selected to receive newly-emerged parasitoids and the other 3 received parasitoids that had been stored for 14 days at 25 °C with honey under a 14 h photo phase. Subsequently beginning 7 days later, each caged plant was inspected daily to count and remove adult *T. radiata* and psyllids. Each day we counted and sexed parasitoid progeny and calculated parasitism rates based on the 120 original nymphs. Means of 91 and 75% parasitism rates were observed for parasitoids that were fresh and stored for 14 days, respectively; these means were significantly different (t = 3.9, P = 0.02, df = 4). Emergence of new *T. radiata* adults peaked at 13 days and was completed within 20 days after parental parasitoids were introduced onto nymph-infested plants regardless of whether the parents had been stored or not. However, there was a general delay in the emergence of adult progeny from parental parasitoids that had been stored compared to those from parents that had not been stored: 42 or 65% progeny adults emerged after the 13-day peak when parental parasitoids were fresh or stored, respectively. Means of 79 and 70% of the progeny parasitoids obtained from fresh and stored parents, respectively, were female; these percentages were not significantly different (t = 1.1, P = 0.3, df = 4).

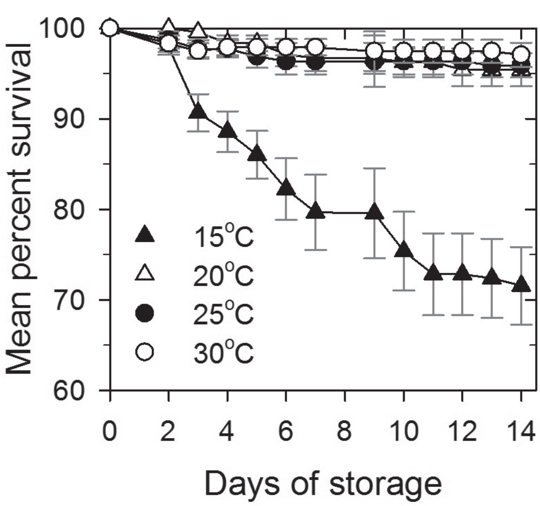

Fig. 2. Storing adult *Tamarixia radiata* with honey under a 14 h photo phase at 15, 20, 25 or 30 °C.

Our results generally support published observations on survival rates of stored adult *T. radiata*. Chien et al. (1993) reported that storing adults at 25 °C with honey provided 10 to 20 days of good survival, although these authors did not discuss light conditions. Chien et al. (1994) reported equal survival rates of females and males held at 25 °C with honey. Chen et al. (2013) reported good survival (~97%) of adult *T. radiata* over a 14-day period when stored at 25 °C (60% RH, 14 h photo phase) with pure honey as a food source, which closely agrees with our findings. Skelley & Hoy (2004) reported less than 5% mortality over 30 days when adults were held at 17 °C and provided every other day with a mixture of honey and yeast extract. Our results differed from those of Chien et al. (1993) in that they reported better survival of parasitoids stored with honey at 20 °C than at 25 or 30 °C. While it may be necessary to store adult parasitoids for some period of time before releasing them, our study showed that parasitoids stored at 25 °C for 14 days exerted lower parasitism rates than freshly emerged parasitoids.

Summary

Tamarixia radiata is regarded as one of the Asian citrus psyllid's most important natural enemies, and is thus currently being mass-reared and released by a number of laboratories in North America. It may not always be possible to immediately release newly-emerged adults, in which case it would be advantageous to store the parasitoids under optimal conditions until release. We found that newly-emerged adult *T. radiata* can be stored at 20 to 30 °C (14 h light photo phase) in vials containing honey with greater than 95% survival for up to 14 days. However, parasitoids stored for 14 days with honey at 25 °C parasitized fewer psyllid nymphs than newly emerged parasitoids. Thus, adult parasitoids can be stored, but they should be released as soon as possible for maximal parasitism.

Key Words: diet, ectoparasitoid, honey, mass rear, parasitism rate, survival, temperature

Resumen

Tamarixia radiata es considerado como uno de los principales enemigos naturales del psílido asiático de los cítricos, y es así que actualmente se cría en masa y liberado por un número de laboratorios en Norteamérica. No siempre es posible liberar inmediatamente los adultos recién emergidos, en cuyo caso sería ventajoso el almacenar los parasitoides en condiciones óptimas hasta su liberación. Encontramos que se puede almacenar de 20 a 30°C (fotoperíodo de luz de 14 horas) los adultos de T. radiata recién emergidos en viales que contienen miel con una sobrevivencia de mas del 95% por 14 días. Sin embargo, los parasitoides almacenados durante 14 días con la miel a 25° C parasitaban menos niinfas de psílidos que los parasitoides recién emergidos. Por lo tanto, se puede almacenar los parasitoides adultos, pero deben ser liberados tan pronto como sea posible para un parasitismo máximo.

Palabras Clave: dieta, ectoparasitoide, miel, criá en masa, tasa de parasitismo, sobrevivencia, temperatura

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