Effect of Holding Diet on Egg Formation of Tamarixia radiata (Hymenoptera: Eulophidae), Parasitoid of Diaphorina citri (Hemiptera: Psylloidae)

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EFFECT OF HOLDING DIET ON EGG FORMATION OF *TAMARIXIA RADIATA* (HYMENOPTERA: EULOPHIDAE), PARASITOID OF *DIAPHORINA CITRI* (HEMIPTERA: PSYLLOIDAE)

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ABSTRACT

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae), is an arrhenotokous ectoparasitoid of *Diaphorina citri* Kuwayama (Hemiptera: Psylloidae), vector of huanglongbing (HLB) or citrus greening disease. Mass-rearing parasitoids for augmentative biological control has created the need to find storage conditions that will optimize egg load upon release. Food provided to females during the holding period may be foremost among factors that determine the number of eggs available for oviposition following storage. Pairs of newly emerged *T. radiata* were provided with 8 different diet treatments: water, honey, Nu-Lure® (a proteinaceous liquid made from 44% hydrolyzed corn gluten meal), host nymphs, honey+ Nu-Lure, honey+ host nymphs, Nu-Lure+ host nymphs, and honey+ Nu-Lure + host nymphs. Female wasps were dissected after 5, 10, 15, and 20 days and mature eggs counted. An average of 4.6 eggs was observed in ovaries of newly emerged females. Honey alone was sufficient for female survival, but egg resorption took place within 5 days after emergence. The combination of honey + Nu-Lure resulted in female survivorship similar to a diet of host nymphs, but egg formation was less than with nymphs provided. *Tamarixia radiata* formed more eggs feeding on mixed diets (Nu-Lure+ honey+ nymphs or Nu-Lure+ nymphs) compared to nymphs alone. However no artificial diet substituted for nymphal hemolymph.

Key Words: biological control, mass-rearing, storage, diet, oogenesis

RESUMEN

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) es un parasitoide arrenotoco de *Diaphorina citri* Kuwayama (Hemiptera: Psylloidae), vector del huanglongbing (HLB) o la enfermedad del greening. La cría masiva de parasitoides para ser utilizados en control biológico aumentativo ha conllevado a la búsqueda de condiciones de almacenamiento que optimicen la carga de huevos cuando van a ser liberados. La alimentación de las hembras previamente a su liberación es probablemente uno de los factores más determinantes en el número de huevos disponibles durante la ovoposición. Parejas recién emergidas de *T. radiata* fueron divididas en 8 tratamientos distintos: agua, miel, Nu-Lure® (un líquido proteico a base de 44% de gluten de maíz hidrolizado), ninfas del hospedero, miel+Nu-Lure, miel+ninfas, Nu-Lure+ninfas y miel+Nu-Lure+ninfas. Las hembras de los parasitoides se disectaron transcurridos 5, 10, 15 y 20 días y se contó el número de huevos maduros. En promedio se observaron 4.6 huevos en los ovarios de las hembras recién emergidas. La dieta basada sólo en miel fue suficiente para permitir la supervivencia de las hembras, pero se detectó reabsorción de huevos a los cinco días. La combinación de miel + Nu-Lure produjo una supervivencia similar a la de la dieta basada en ninfas del hospedero aunque la formación de huevos fue menor que cuando se le ofrecieron ninfas. *Tamarixia radiata* formó más huevos cuando se alimentó con dietas combinadas (miel+Nu-Lure+ninfas) comparada con una dieta basada en ninfas. Sin embargo, ninguna de las dietas artificiales es capaz fue sustituir completamente la hemolinfa de ninfas.

Palabras Clave: control biológico, cría masiva, almacenamiento, dieta, ovogénesis

Tamarixia radiata, is an arrhenotokous ectoparasitoid of the Asian citrus psyllid (ACP), *Diaphorina citri*, vector of citrus greening disease or huanglongbing (HLB). *Tamarixia radiata* was released in Réunion, Taiwan, and Guadeloupe (Aubert & Quilici 1984; Chien 1995), and was credited with reducing the populations of *D. citri* sufficiently in Réunion to mitigate the impact of HLB (Étienne & Aubert 1980; Chien & Chu 1996; Étienne et al. 2001).

Tamarixia radiata was first imported to Florida from Taiwan and Vietnam in 1998 and released in 1999-2001 (Hoy & Nguyen 2001). *Tamarixia radiata* is well established in citrus orchards throughout the state according to a survey conducted in 2006-07 (Qureshi et al. 2009). Incidence was highest in fall but low early in the growing season, presumably due to poor survivorship in the winter when new flushes necessary for development of host nymphs are scarce. This bottleneck in the life cycle of *T. radiata* has only been exacerbated by the now widespread practice of "dormant sprays" of broad-spectrum insecticides during the months preceding the spring flush (Qureshi & Stansly 2010). Thus, successful biological control using *T. radiata* would require augmentative releases to jump-start parasitoid populations early in the growing season. Mass-rearing of this parasitoid to obtain adequate numbers for release would be essential and entail temporary storage and stockpiling of the wasps. During the holding period, food provided to females may affect the number of eggs formed in ovaries, which would influence efficiency upon release.

Oogenesis is a nutrition-limited process, and nutrition is obtained during both the larval and adult stage. *Tamarixia radiata* has been characterized as synovigenic–autogenous, meaning that some eggs become mature in the newly emerged wasp without feeding, but that host- feeding is later required to mature more eggs (Chien et al. 1994a).

Insufficient nutrients always affect egg production (Wheeler 1996). Most synovigenic parasitoids, including *T. radiata*, can resorb eggs when hosts are absent or scarce (Chien et al. 1994a). Carbohydrates are the major energy source for most insects, but they are not the main nutrient that triggers egg formation in female insects. Egg resorption can occur in *T. radiata* at either 15 °C or 25 °C when only honey is provided, and egg resorption is positively related to the length of deprivation time (Chien et al. 1991, 1994a). Once suitable hosts are fed upon, new mature eggs can be produced in the ovary and oviposition can proceed normally (Chien et al. 1994a). Varley & Edwards (1957) reported that female *Nasonia vitripennis* (Walker) (Pteromalidae) fed on carbohydrates survived, but resorbed eggs, resulting in gradual decrease of eggs in the ovaries. Bownes & Blair (1986) found that oviposition and formation of vitellogenic oocytes in ovaries of *Drosophila melanogaster* (Meigen) were reduced significantly on sugar diets due to reduced fat body transcription of yolk protein and consequent low availability for oogenesis.

Host hemolymph is rich in protein and an important source of nutrients to many hymenopterous female parasitoids for increasing fecundity (Howard 1910; Rockwood 1917). Varley & Edwards (1957) observed that egg development by female *N. vitripennis* was initiated when or shortly after host hemolymph was consumed. Leius (1961) reported that female *Itoplectis conquisitor* (Say) (Hymenoptera: Ichneumonidae) fed on host body fluids laid more eggs than those fed on carbohydrates alone. Additionally, Leius (1961) found that pollen in diets significantly increased fecundity of *I. conquisitor* females, and females fed on a mixed diet (host body fluid and carbohydrates) deposited even more eggs. *Tamarixia radiata* females fed on a diet of honey and yeast extract significantly decreased host-feeding while maintaining or improving intrinsic rate of increase (0.2976 to 0.3014 per day) and net reproductive rate (140 to 187 female eggs per female), respectively (Chien et al. 1994b).

The objective of this experiment was to investigate the effects of food supplements on oogenesis with the ultimate goal of maintaining or increasing reproductive potential during storage, and presumably optimizing parasitism rate immediately upon release.

Materials and Methods

Colonies

Murraya paniculata (L.) Jack (Rutaceae) was used as a host plant to maintain a *D. citri* colony. Plants were grown in 3.9-L pots using peat potting soil containing 40% Canadian sphagnum (Fafard 4P Professional Growing Mix soil, Conrad Fafard Inc.) in a screen house (hoop style trussed, covered with insect screen mesh) under natural sunlight and passive ventilation. Plants were sprayed with 1% M-pede soap (Dow AgroSciences LLC) as needed to control insects and mites.

The *D. citri* colony was maintained in an air-conditioned rearing room at 28 ± 2 °C, 60-80% RH (Extech RH10 humidity and temperature datalogger, Grainger, Inc.), and 14:10 h L:D. Six *M. paniculata* plants, each with five to eight 4-cm long new sprouts, were placed in a wood frame cage (60 cm × 60 cm × 120 cm) covered on 3 sides with fine mesh for ventilation and the other sides with clear acrylic. Six hundred *D. citri* adults were released in each cage for 24 h to oviposit. All adult psyllids were then collected and removed. Plants were held for approximately 10 days (depending on temperature and humidity) until all nymphs reached the 4th instar.

Six *M. paniculata* plants with 4th instar nymphs were transferred into a clear acrylic cage (60 cm × 80 cm × 90 cm) with 3 sides of fine mesh in an air conditioned greenhouse maintained at 24-29 °C, 40-75% RH, natural sunlight. Fifty *T. radiata* females and 30 males were released in the cage for 48 h to parasitize hosts, and then these wasps were collected and removed. Plants were held for another 6 days until the parasitized hosts mummified. Young shoots with nymphs were excised and the nymphs were examined under a stereoscopic microscope. Small (2 cm) portions of shoots containing a parasitized nymph with the wasp close to the pupal stage were excised and placed individually in a glass tube (75 mm long × 12 mm diam, Fisher Scientific, Hampton, New Hampshire) in the air-conditioned rearing room. Tubes were checked frequently, and the wasps were removed immediately after emergence.

Diets

Each pair of newly emerged *T. radiata* was placed in a 50 mL centrifuge tube (Kendall Labware, Mansfield, Massachusetts), sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago, Illinois) and stored in a growth chamber (Percival Scientific, model I36LLC8, Perry, Iowa) at 17 C, 75~ 85% RH , and 14:10 h L:D. Wasps were provided with water alone, or 7 additional treatments with water plus: (1) honey, (2) Nu-Lure, (3) host nymphs, (4) honey + Nu-Lure, (5) honey + host nymphs, (6) Nu-Lure + host nymphs, and (7) honey + Nu-Lure + host nymphs. Nu-Lure® (Miller Chemical and Fertilizer, Hanover, Pennsylvania) is a proteinaceous dark brown liquid made from 44% hydrolyzed corn gluten meal, and 56% inert ingredients. Water, honey, and Nu-Lure were all provided on 8 cm long × 1.5 cm wide cellulose strips (Wypall-L30, Kimberly-Clark Professional). Host nymphs were provided on a freshly cut shoot with a mixed infestation of 2nd, 3rd, 4th instars. Water strips, Nu-Lure strips and host nymphs were changed every 24 h, and honey every 48 h to assure quality. Wasps in each treatment were held separately for 5, 10, 15, and 20 days. There were 10 replications of every treatment ´ day combination.

Egg Load

Tamarixia radiata females from each different treatment were dissected after 5, 10, 15, and 20 days as well as newly emerged *T. radiata* under a dissecting microscope (Olympus SZX 16, Olympus Corporation). Ovaries were photographed using an Olympus DP 21 camera, and the number of mature eggs was recorded.

Statistical Analysis

Data from the 8 treatments at each 5-day dissection intervals were compared using ANOVA with mean separation with Fisher's LSD (JMP, SAS Institute Inc. 2012).

Results

Newly emerged *T. radiata* females averaged 4.6 ± 0.65 eggs each. Significant treatment effects on egg load were seen at all 4 dissection intervals (for 5 days: $F_{7,48} = 11.1702$, $P < 0.0001$, for 10 days: $F_{5,35} = 12.72$, $P < 0.0001$, for 15 days: $F_{5,30} = 7.8989$, $P < 0.0001$, for 20 days: $F_{5,26} = 10.28$, $P < 0.0001$) for 5, 10, 15 and 20 days respectively. All of the wasps fed on just water, or water and Nu-Lure died within 10 days. Survivorship of females fed honey for 20 days was 40%, the same as wasps fed honey + Nu-Lure.

The addition of Nu-Lure increased the egg load associated with every diet more than honey. Significantly more eggs formed with honey + Nu-Lure compared to honey alone on all 4 dates, whereas no significant differences were seen between females provided with honey+ Nu-Lure compared to honey + nymphs until 15 days (Table 1). Nu-Lure + nymphs was significantly better than nymphs alone except at 10 days, whereas, nymphs + honey was not better than nymphs alone until 20 days. Nu-Lure + nymphs was better than honey + nymphs over all treatment periods, but nymphs + Nu-Lure + honey was never better than nymphs + Nu-Lure. Survivorship at day 20 from diets of nymphs, honey+ nymphs and Nu-Lure + nymphs were all 80%, whereas survivorship of the females fed on honey+ Nu-Lure+ nymphs was 90%.

Table 1. Number of eggs (Mean ± SE) per female by dietary treatment obtained by dissecting *Tamarixia radiata* females after 5, 10, 15, and 20 days on each diet at 17 C.

Treatment	5 days	10 days	15 days	20 days	Over all dates
Water[1]	1.83 ± 0.6 D	—	—	—	—
Nu-Lure[1]	2.14 ± 0.7 D	—	—	—	—
Honey	3.50 ± 0.4 C	3.67 ± 0.6 C	2.40 ± 0.4 D	1.00 ± 0.4 D	2.64 ± 0.24 E
Honey+ Nu-Lure	5.38 ± 0.7 B	5.30 ± 1.0 B	5.00 ± 0.7 C	4.60 ± 0.8 C	5.07 ± 0.07 D
Nymphs	5.50 ± 0.6 B	6.30 ± 0.8 AB	6.88 ± 0.7 BC	6.50 ± 0.8 B	6.30 ± 0.12 C
Honey+ Nymphs	6.00 ± 0.5 AB	7.00 ± 0.8 AB	7.70 ± 0.6 AB	8.00 ± 1.5 A	7.18 ± 0.18 B
Nu-Lure+ Nymphs	7.56 ± 0.6 A	8.00 ± 0.9 A	8.00 ± 0.9 A	8.30 ± 0.7 A	7.97 ± 0.06 A
Honey+ Nu-Lure+ Nymphs	7.20 ± 0.6 A	7.80 ± 0.6 A	8.00 ± 0.8 A	8.20 ± 0.8 A	7.80 ± 0.09 A

Means in the same column followed by the same letter are not significantly different (Tukey's Student's t-test HSD, $P < 0.05$).
[1]Wasps under these two treatments all died within 10 days.

Discussion

An average of 4.6 eggs were observed in the ovaries of newly emerged *T. radiata* females, despite no supplemental feeding, indicating that nutrition acquired during the larval stages was used to form the first clutch of eggs. Mating was not necessary for egg formation.

This study showed that honey alone was enough to keep wasp females alive, but egg resorption took place within 5 days after emergence in the absence of food. On the other hand, Nu-Lure alone with water did not support survival beyond 5 days. These results indicate that sugars provided by honey are necessary but not sufficient for survival, since nutrients from egg resorption were apparently also required. Addition of Nu-lure to the diet appeared to forestall egg resorbtion although it did not allow the number of eggs to increase. This result indicated that additional amino acids provided by Nu-Lure satisfied nutritional needs of the wasp but were insufficient for oogenesis. Access to nymphs allowed for a limited degree of oogenesis that was enhanced by the addition of honey, but even more so by the addition of Nu-Lure. This result indicated that while nutrients provided by host feeding were required for egg production, additional carbohydrates and especially amino acids were still beneficial. The fact that no additional benefit was conferred by the addition of honey to wasps provided with nymphs (Fig. 1) indicated that carbohydrates were not a limiting factor when hemolymph and an additional source of amino acids were provided.

Skelley & Hoy (2004) showed that *T. radiata* stored for up to 35 days at 17 °C with honey and yeast suffered less than 5% mortality. Chien et al. (1994a) reported that females stored for 20 days at 25 °C were able to lay a total of 156 eggs, compared with 98 eggs when stored at 15 °C. Thus, although less mortality was experienced at low temperatures, production suffered. Therefore, the ideal storage temperature should be determined according to specific objectives (establishment or augmentation) and conditions (host availability). Clearly, both honey and Nu-Lure or some facsimile are valuable supplements for wasps in storage at 17 °C. However, maximum benefit will be obtained if hosts can also be provided. Nu-Lure or other amino acid supplements could also enhance the efficiency of mass rearing. Studies are planned to evaluate benefits of providing carbohydrate and amino acid supplements to ovipositing *T. radiata* by assessing effects on fecundity and host feeding.

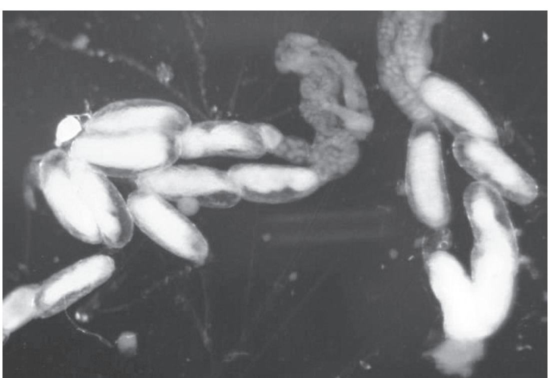

Fig. 1. Paired *Tamarxia radiata* ovaries of females that had been fed Nu-Lure + honey + *Diaphorina citri* nymphs for 5 days.

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