

**Manipulation of Female Parasitoid Age Enhances
Laboratory Culture of LYSIPHLEBUS TESTACEIPES
(hymenoptera: aphidiidae) Reared on TOXOPTERA
CITRICIDA (Homoptera: aphididae)**

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MANIPULATION OF FEMALE PARASITOID AGE ENHANCES LABORATORY CULTURE OF *LYSIPHLEBUS TESTACEIPES* (HYMENOPTERA: APHIDIIDAE) REARED ON *TOXOPTERA CITRICIDA* (HOMOPTERA: APHIDIDAE)

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ABSTRACT

Cultures of the endoparasitoid *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) on the brown citrus aphid, *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae), previously have been reported to be difficult to establish. In this study, *L. testaceipes* colonies were initiated from parasitized brown citrus aphids obtained from field-collected citrus foliage in Florida and successfully maintained for >25 generations in the laboratory. To enhance colony rearing methods, several aspects of the parasitoid's biology were examined. An evaluation of foraging by single or multiple females determined that the presence of multiple females did not influence mean progeny yield per female. However, the mean number of progeny produced by mature (25-49 and 49-73 h) *L. testaceipes* females was higher than that produced by younger (1-25 h) females over a 24-h period. In all three parasitoid age classes, each reared on second-, third- or fourth-instar aphid hosts, significantly more mummies containing *L. testaceipes* formed on a paper coffee filter covering the soil surface compared to the number of mummies formed on citrus foliage. Mummy formation off foliage has not been reported for this aphid-parasitoid complex in citrus. Mated females of *L. testaceipes* with access to honey and water and without access to aphids or honeydew lived longer than females that had access to aphid hosts and honeydew. These data provide novel findings on the biology of *L. testaceipes* when parasitizing the brown citrus aphid, particularly on mummification sites, and allowed us to develop a protocol for routine large-scale rearing of *L. testaceipes* on brown citrus aphids on citrus.

Key Words: *Lysiphlebus testaceipes*, *Toxoptera citricida*, laboratory cultures, host instar, citrus

RESUMEN

Las crías de el endoparasitoide *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) sobre el áfido de cítricos de color café, *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae), previamente han sido reportada difíciles de establecer. En este estudio, colonias de *L. testaceipes* fueron iniciadas de áfidos de cítricos de color café parasitados obtenidos del follaje de cítricos recolectado en el campo y exitosamente mantenidos por >25 generaciones en el laboratorio. Para mejorar los métodos de cría de la colonia, varios aspectos de la biología del parasitoide fueron examinados. Una evaluación del forraje de hembras individuales o hembras múltiples determinó que la presencia de hembras múltiples no tuvo influencia sobre el promedio de prole por hembra. Sin embargo, el número promedio de prole producido por hembras de *L. testaceipes* maduras (25-49 y 49-73 h) fue más alto de lo que fue producido por hembras más jóvenes (1-25 h) en un período de 24-h. En todas las tres clases de edad del parasitoide, cada cría en áfidos hospederos en el segundo-, tercero- o cuarto-estadio, significativamente más momias contenían *L. testaceipes* formado sobre un papel filtro de café que cubría la superficie del suelo comparada con el número formado sobre el follaje de cítricos. La formación de las momias no puestas sobre el follaje no ha sido reportado para este complejo de áfido-parasitoide. Hembras de *L. testaceipes* apareadas con acceso a miel y agua y sin acceso a los áfidos o a la miel del rocío vivieron más tiempo que las hembras con acceso a los áfidos hospederos o a la miel del rocío. Estos datos proveen descubrimientos nuevos sobre la biología de *L. testaceipes* en la parasitización del áfido de cítricos de color café, particularmente en los sitios de momificación, y nos permitieron desarrollar un protocolo para la cría rutinaria en gran escala de *L. testaceipes* sobre el áfido de cítricos de color café en los cítricos.

The brown citrus aphid, *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae), currently occurs on citrus throughout Florida where it is an efficient vector of citrus tristeza virus (CTV) (Costa & Grant 1951). In a classical biological

control program for the brown citrus aphid, the endoparasitoid *Lipolexis oregmae* (Gahan) (= *scutellaris* Mackauer, Miller et al. 2002) (Hymenoptera: Aphidiidae) was imported from Guam and released in citrus groves throughout Florida

(Hoy & Nuygen 2000). Another aphid parasitoid, *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphididae), already occurs in Florida and also has been recorded parasitizing brown citrus aphids (Michaud 1999; Yokomi & Tang 1996).

Existing accounts on the biology of *L. testaceipes* on brown citrus aphid are scarce; Carver (1984), Yokomi & Tang (1996), Michaud & Browning (1999) and Persad & Hoy (2003) have dealt specifically with this aphid-parasitoid complex. The biology of *L. testaceipes* on other aphid species is better known and include: Schuster & Starks (1975), Stary et al. 1988, Stadler & Volkl (1991), Volkl & Stadler (1991), Grasswitz & Paine (1992), Vansteenis (1994), Stechmann et al. (1996), Pike et al. (1997), Fernandes et al. (1997, 1998), Elliot et al. (1999), Rodrigues et al. (2001), Rodrigues & Bueno (2001), Gonzaales et al. (2002), Tang et al. (2002).

Previous studies were conducted to determine whether competition with *L. testaceipes* would affect establishment of *L. oregmae* in Florida (Persad & Hoy 2003). The intra- and interspecific interactions of both parasitoids on the brown citrus aphid were investigated in the laboratory and the data obtained suggest that *L. testaceipes* would not exclude *L. oregmae* during interspecific interactions and so may not affect its establishment in Florida. To conduct these competition studies, cultures of *L. oregmae* were maintained on brown citrus aphids on potted citrus in the laboratory using the method of Hill (2002), Walker (2002) and Hill & Hoy (2003). However, no protocol for rearing *L. testaceipes* on brown citrus aphids on citrus existed, so several attempts were made to initiate cultures from field-collected parasitoids.

Some researchers have reported that *L. testaceipes* is not easily cultured in the laboratory on brown citrus aphids. Carver (1984) was unsuccessful in rearing *L. testaceipes* on this host in the laboratory in Australia and considered oviposition by *L. testaceipes* in brown citrus aphids as an 'egg trap' because parasitism rates were high but adult emergence was low. Michaud & Browning (1999) failed to establish colonies of *L. testaceipes* on brown citrus aphid in Puerto Rico, even when parasitoids were used that were derived from brown citrus aphid populations exhibiting high rates of emergence of *L. testaceipes* adults.

This study describes the initiation and continued propagation of *L. testaceipes* colonies on brown citrus aphid in the laboratory for >25 generations after initial failures to establish thriving colonies. In an effort to understand the initial failures and to standardize a laboratory rearing system, several evaluations were conducted. To determine the nutrient requirements of adult *L. testaceipes*, survivorship of adult parasitoids with and without nutrients and the longevity of newly emerged females when allowed access to aphids and honeydew was evaluated. To resolve whether competing

L. testaceipes females affected progeny yield in cages, the mean number of progeny produced per female in colonies initiated from single females versus yield when the aphids were exposed to multiple (6) females, was evaluated. The relationships between female parasitoid age, mating status and aphid host stage on mummy location and progeny production also were investigated.

MATERIALS AND METHODS

Initiation of Laboratory Cultures of *L. testaceipes* on Brown Citrus Aphid on Potted Citrus

Brown citrus aphids were collected from young citrus foliage obtained from citrus groves in 15 counties in Florida between August and December 2001. Approximately 350 *L. testaceipes* adults were collected from field populations and used to initiate cultures. Care was taken to ensure that the collected aphids consisted solely of brown citrus aphids using the guidelines provided by Halbert & Brown (1996). Some of the leaves had mummified brown citrus aphids, indicating the presence of parasitoids. All collected foliage was held between crumpled sheets of absorbent paper in air-inflated plastic bags in the laboratory at 22-24°C, 55-65% RH and 16:8 h light:dark cycle. Condensation in each bag was wiped off twice daily. Under these conditions, citrus foliage could be maintained for 8 to 10 days, allowing parasitized aphids that were not yet mummified to be held sufficiently long to obtain adult *L. testaceipes*. This holding system allowed greater numbers of adult *L. testaceipes* to be collected than is obtained if only mummies are sampled.

Adult parasitoids that emerged were examined under the stereomicroscope. Apart from two species of hyperparasitoids, *L. testaceipes* was identified, using the guidelines of Evans & Stange (1997), as the only primary parasitoid emerging from field-collected brown citrus aphids. Emergence of *L. testaceipes* occurred mostly in the morning and adults were collected at 800, 1200 h daily by aspirator into 6 × 1.5 cm plastic vials. Groups of up to 20 parasitoids that emerged on the same day were held together in similar vials. Honey-saturated paper strips and moistened cotton was supplied within each vial and these were supplied whenever parasitoids were stored.

Mating pairs always were observed within 1 h of introducing parasitoids into the vials. Six presumably mated females were introduced into a 60 × 60 × 60 cm mesh (size 40/ mm²) cage which contained six potted citrus plants; each plant was infested with 200 to 250 brown citrus aphids of mixed instars. Water was provided on a moist cotton pad on the cage top and honey strips were attached to the upper corners of each cage. Adult *L. testaceipes* progeny emerging 9 to 10 d later were collected by aspirator and the cycle was re-

peated. In addition, parasitoid cultures were initiated with single *L. testaceipes* females in cages containing individual potted plants. Progeny from both culture systems were released into a 35 × 35 × 35 cm plexiglass cage to mix before re-introduction to cultures in an effort to preserve their genetic diversity.

The identity of *L. testaceipes* from our cultures was confirmed by Peter Stary, Institute of Entomology, Academy of Sciences, Czech Republic. Specimens of *L. testaceipes* were deposited at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, as voucher specimens 2002- 1742- 901. Cultures were routinely screened by extracting random samples upon emergence and observing these specimens under the dissecting microscope to ensure that only *L. testaceipes* were present in our rearing cages. All parasitoid adults used to start cultures were screened to confirm identity and establish sex ratios before introduction into cages with aphid-infested citrus plants.

The use of recently emerged female parasitoids (24 h or less) to initiate colonies resulted in similar numbers of progeny as that of the *L. testaceipes* parents and hence colonies did not increase in size. It was observed that when older (24- to 30-h-old) females were used, more progeny were obtained. To obtain mature females, all newly emerged *L. testaceipes* of both sexes (approx. ♂:♀ ratio of 1:1.5, and 25 to 30 individuals) were stored in 6 × 1.5 cm plastic vials for 24 to 30 h before allowing them access to aphids.

Inspections of mummies located on citrus foliage in these laboratory colonies revealed that most were still intact, with no emergence holes. Surprisingly, the numbers of mummies on foliage with exit holes were considerably lower than the numbers of adults obtained in the cages. Because *L. oregonae* was found to produce mummies on the soil surface when reared on brown citrus aphid (Hill 2002; Walker 2003; Hill & Hoy 2003) we examined the cages containing *L. testaceipes* and mummies containing *L. testaceipes* were noticed on the soil surface. To confirm that the excess *L. testaceipes* adults were coming from mummies on the soil surface, paper coffee filters were placed around the base of the potted plants. Parasitized aphids were observed walking or falling down to the base of the plant 5 to 6 d after adult *L. testaceipes* had been introduced into the cages and the mummies formed were sometimes firmly attached to the coffee filters. To quantify and confirm these results, and to develop a suitable rearing system for *L. testaceipes* on the brown citrus aphid, we conducted the following experiments.

Effect of Nutrients on Survival (days) of *L. testaceipes* Adults

Survival in days of *L. testaceipes* reared on brown citrus aphid was unknown, so adults were

held with or without nutrients and survivorship was determined. Mummies of brown citrus aphids containing *L. testaceipes* were collected from both citrus foliage and the coffee filter on the soil surface and placed individually in gelatin capsules (size 00). Emerging adult *L. testaceipes* were allowed to mate and were placed singly in 6 × 1.5 cm plastic vials, which contained a piece of fluted paper. Parasitoids were either offered no nutrients, water in moist cotton, pure honey on paper strips (0.75 × 2.5 cm) or both water and honey strips. Fifteen parasitoids of each sex were examined in each treatment.

To determine the effect of oviposition on longevity, 15 mated *L. testaceipes* females were housed in individual vials. These were allowed access to an excess of aphids (approx. 100 of mixed stages) on citrus foliage. The foliage was inserted into each vial and replaced every 12 h so that these female parasitoids had access to aphid honeydew, honey strips and water. Observations were made daily and a record of mortality kept. Comparisons of survival times of parasitoids that were not allowed to oviposit were made by ANOVA and LSD using Statview ver. 5.0 (SAS Institute 1999).

Effects of Using Young and Mature *L. testaceipes* Females on Total Parasitoid Progeny Production in Single vs Multiple (6) Parasitoid Culture Systems

Because Shekar (1956) had reported that *L. testaceipes* reared on *Aphis gossypii* Glover produced maximum progeny per day when mature (3 d), we compared total offspring produced by young and mature *L. testaceipes* reared on brown citrus aphid. Also, we compared total progeny produced per female in two culture systems to determine if competition/ interference during host seeking by multiple *L. testaceipes* females occurred.

Brown citrus aphid mummies containing *L. testaceipes* were collected from both coffee filters and citrus foliage from colony cages and stored individually in size 00 gelatin capsules in the laboratory. Ten female *L. testaceipes* were allowed to mate with one-d-old males upon emergence and a single female was introduced into each of 10 mesh (size 40/ mm²) (60 × 60 × 60 cm) cages within one h of emergence (young females). Mating occurred readily and generally lasted from 40 to 80 sec. Each cage contained six potted citrus plants each infested with 200 to 250 brown citrus aphids of mixed stages. Ten mated *L. testaceipes* females also were initially kept in vials for 24 h (mature females, 25-h-old) in the laboratory before introducing them individually into each of ten similarly prepared mesh cages. For evaluations of multiple females, mated young (1-h-old) *L. testaceipes* females were introduced into each of ten mesh cages in groups of six and this was repeated using mature (25-h-old) females. Both young and mature parasitoids were provided with

honey and water and were allowed to remain in the cage until death.

Mean total progeny obtained from cages in which single parasitoid females were introduced was compared to the mean produced by each female in a cage with 6 females and comparisons also were made between total progeny produced by young females and mature females in both culture systems (1 vs 6). These data were arcsine transformed and analyzed by ANOVA, using Statview ver. 5.0 (SAS Institute 1999) at the 5% significance level.

Effect of Age and Mating Status of *L. testaceipes* Females Over a 24-H Period on Mummy Location and Adult Parasitoid Emergence

To resolve the effects of female parasitoid age and mating status on mummy location and progeny, the following experiment was conducted. The trial was conducted for 24 h because the survivorship data indicated that ovipositing females only lived for an average of 1.4 d. Plastic wrap was placed around the base of a potted flushing citrus plant (24 cm tall) and taped around the base of the plant stem to form a barrier to aphids migrating down the stem toward the soil. A circular paper coffee filter was slit and placed on top of the wrap and secured in place with 3M® Scotch tape. Forty alate brown citrus aphids were placed using a damp sable hair-brush (size 000) onto young leaves of a potted citrus plant and left for 24 h. Alates were removed and the first-instar (L1) aphids present were allowed to molt to the third instar (L3); this stage was used to standardize possible variation in progeny production because of aphid size. Excess L3 aphids were removed to leave 100 L3 aphids on the plant, which was then covered with a plexiglass cylinder (13 cm diameter and 45 cm tall) with mesh tops and side windows. Plants prepared in this way did not require water for the duration of the experiments.

On emergence (usually between 900 and 1100 h), 10 female parasitoids were randomly collected and allowed to mate with one-day males (because younger males did not mate as readily) in 4 × 1 cm glass vials. After mating (<1 h), a single *L. testaceipes* female was introduced onto each of 10 plants. Each plant was pre-infested with 100 L3 *T. citricida* and the parasitoid was left on the plant for 24 h (1-25 h age class). Citrus infested with L3 brown citrus aphids yielded mummies which were located on both foliage and the coffee filter by day 6 after introducing *L. testaceipes*. Mummies were collected from both locations, labeled as to source and held individually in size 00 gelatin capsules until emergence.

Ten *L. testaceipes* females were held individually in vials for 24 h and allowed to mate (as described above); each was then introduced individually into cages containing citrus with L3

T. citricida and left for 24 h (25-49 h age class). Ten *L. testaceipes* females also were similarly treated and allowed to mate, but held for 48 h before introduction into cages containing plants for 24 h (49-73 h age class). All female *L. testaceipes* were introduced into the test cylinders between 1000 and 1200 h. Ten replicates of each of the three parasitoid age groups were evaluated for mummy location and total adult *L. testaceipes* progeny.

The experiment was repeated using virgin *L. testaceipes* females for each of the age classes (1-25, 25-49 and 49-73 h). Trials in which the parasitoid had died or could not be found after 24 h in all experiments were discarded. Mummies found on foliage and on the paper coffee filter (soil) were counted and transferred on the tip of a dampened hairbrush individually to gel capsules. The number of mummies and the percentage adult eclosion from both locations were recorded for each age group. Data were arc-sine transformed before analysis using the Students t-test (SAS Institute 1999).

Effect of *T. citricida* Host Instar on *L. testaceipes* Mummy Location and Percentage Adult Emergence

To resolve the effects of host instar on mummy location and adult emergence, we kept the age of females constant and tested all four instars of brown citrus aphid. Each of 10 potted citrus plants was infested with 100 L1, L2, L3 or L4 *T. citricida* by allowing the L1 to molt to the desired stage. A mated *L. testaceipes* female that was 24 to 30 h old was then introduced into each of 10 potted citrus plants containing each host instar and covered with a plexiglass cylinder. The plants were left in the laboratory for 24 h, after which the parasitoid was located and removed. Trials in which the parasitoid died or could not be found were not used in further analyses. The number of *L. testaceipes* mummies and adults emerging at each location (foliage versus soil surface) were recorded for each aphid stage tested and percentage adult emergence was determined. Data were analyzed as described in the preceding section.

RESULTS AND DISCUSSION

Effect of Nutrients on Survival (days) of *L. testaceipes* Adults

Adults of both sexes lived significantly ($P < 0.05$) longer when given both water and honey strips (Table 1). The data suggest that *L. testaceipes* needs both free water and an energy source for optimal survival. Mated females (data not shown and treated separately), when provided with water and honey and allowed constant access to aphids, lived a mean (\pm SD) of 1.4 (\pm 1.3) d ($N = 15$), which was comparatively shorter than mated females that were not allowed to oviposit (mean \pm SD of 3.7 (\pm 2.7) days ($N = 15$). Some fe-

TABLE 1. MEAN (±S.D.) SURVIVAL (DAYS) OF NEWLY EMERGED AND MATED *L. testaceipes* ADULTS.*

Treatment	Males	Females
No water or honey	1.0 ± 0.8 c ¹	1.2 ± 1.3 b
Water only	1.6 ± 1.1 b	1.8 ± 1.4 b
Honey only	0.9 ± 0.7 c	1.4 ± 0.5 b
Water and honey	2.8 ± 2.6 a	3.7 ± 2.7 a

*At 22-24°C, 55-65% RH and 16:8 h photoperiod. ¹Means followed by the same letters within a column are not significantly (P > 0.05) different by ANOVA and LSD.

males died while still attempting to oviposit and the urge for newly emerged adults to oviposit till death may have contributed to the failure of our initial colonies.

Effects of Using Young and Mature *L. testaceipes* Females on Total Parasitoid Progeny Production in Single vs Multiple (6) Parasitoid Culture Systems

Young females produced equal numbers (F = 0.02, df = 19, P = 0.88, n = 10, ANOVA) of total progeny whether introduced into cages as single females (Mean ± SD = 6.5 ± 3.6) or groups of six (4.7 ± 3.8). Total *L. testaceipes* progeny produced per mature female in which single (27.4 ± 12.8) or multiple (31.3 ± 14.7) females were introduced were not significantly different (F = 1.08, df = 19, P = 0.31, n = 10, ANOVA). These data suggest that competition/interference during host seeking by multiple *L. testaceipes* females may not have a significant effect on progeny yield when aphids are abundant.

However, younger *L. testaceipes* females (1-25 h after emergence) produced significantly fewer (6.5 ± 3.6) progeny compared to mature females (27.4 ± 12.8) (F = 53.8, df = 19, P = 0.0001, n = 10,

ANOVA) in single-female cultures. Likewise, in multiple-female cultures, young females also produced significantly fewer progeny (4.7 ± 3.8) per female when compared to mature females (31.3 ± 14.7) (F = 54, df = 19, P < 0.0001, n = 10, ANOVA). This deficit in total progeny production by younger females may have been a contributing factor to the low yields in our initial cultures when females were allowed access to aphids immediately upon emergence. It is common, when rearing short-lived aphid parasitoids, to introduce newly emerged females into cages as soon as possible in order to optimize their reproductive potential (Hill 2002; Walker 2002; Hill & Hoy 2003), but when rearing *L. testaceipes* on the brown citrus aphid this is counter productive. Weisser (1994) observed that older *Lysiphlebus cardui* Marshall (Hymenoptera: Aphidiidae) females produced significantly more progeny than younger females when reared on *Aphis fabae* Scopoli (Homoptera: Aphididae); he attributed this to increased patch residence time by older females.

Effect of Age and Mating Status of *L. testaceipes* Females Over a 24-H Period on Mummy Location and Adult Parasitoid Emergence

Significantly (P < 0.05) more mummies containing *L. testaceipes* were located on the paper coffee filter located on the soil surface and significantly (P < 0.05) more adults emerged from those mummies whether mated or unmated females were used (Table 2). Mated *L. testaceipes* females produced more adult progeny if exposed to hosts when they were 25-49 or 49-73 h old than the females in 1-25 h age class. Virgin and mated females produced the maximum number of progeny if they were in the 25-49 h age interval. Shekar (1956) observed maximum oviposition in *L. testaceipes* reared on *A. gossypii* on day 3, when para-

TABLE 2. NUMBER OF MUMMIES AND PERCENTAGE OF *L. testaceipes* ADULTS PRODUCED BY MATED AND VIRGIN FEMALES IN THREE AGE CLASSES* IN A 24 H PERIOD ON L3 BROWN CITRUS APHIDS.

	Mean ± S.D. number of mummies on			Mean ± S.D. percentage adults eclosing from		
	Foliage	Coffee filter	P value	Foliage	Coffee filter	P value
Mated						
1-25**	2.3 ± 1.8 b	19.4 ± 5.7 a ¹	<0.0001	20.5 ± 20.1 b	68.5 ± 18.8 a	0.0012
25-49	6.1 ± 2.5 b	37.6 ± 15.9 a	0.0001	3.3 ± 20.9 b	76.3 ± 11.3 a	<0.0001
49-73	6.9 ± 4.5 b	28.6 ± 14.7 a	0.0020	25.1 ± 22.8 b	73.3 ± 22.3 a	0.0056
Virgin						
1-25	1.1 ± 1.0 b	9.4 ± 3.6 a	0.0007	6.4 ± 5.2 b	55.5 ± 15.0 a	<0.0001
25-49	1.2 ± 1.1 b	19.8 ± 6.4 a	0.0002	8.1 ± 6.9 b	75.8 ± 17.4 a	<0.0001
49-73	2.5 ± 1.5 b	8.6 ± 4.3 a	0.0003	5.5 ± 6.8 b	59.0 ± 17.2 a	<0.0001

*When 100 L3 brown citrus aphids are exposed to parasitoids at 22-24°C, 55-65% RH and 16:8 h photoperiod.

**Three holding intervals (h) used after adult emergence to produce the three age classes.

¹Means ± S.D followed by the same letters in a row are not significantly different by Students t-test (SAS Institute 1999).

sitoids were allowed access to aphids for one-h periods on 3 consecutive days. This suggests that mature females of *L. testaceipes* also may produce more progeny when utilizing other aphid hosts.

Mated *L. testaceipes* females produced significantly more mummies and progeny than virgin females in all three age classes (Table 2, $F = 13.54$, $df = 59$, $P = 0.03$, $n = 10$ ANOVA). Although virgins of some parasitoid females may produce fewer progeny compared to mated females, in other parasitoid species the reverse may occur, or progeny yield may not differ (Godfray 1994). Michaud (1994) reported that virgin and mated females of *L. testaceipes* had similar parasitism rates on *Aphis fabae* Linneaus, while Shekar (1956) recorded that virgin females of *L. testaceipes* took from 2 to >30 times longer to begin oviposition in *Aphis gossypi* and had reduced fecundity. These combined reports suggest that oviposition behavior in *L. testaceipes* may be influenced by aphid species.

Effect of *T. citricida* Host Instar on *L. testaceipes*
Mummy Location, Percentage Adult Emergence

There was no significant ($P > 0.05$) difference in the number of mummies containing *L. testaceipes* located on foliage and on the coffee filter when first instar (L1) brown citrus aphids were parasitized by *L. testaceipes* (Table 3). However, significantly ($P < 0.05$) more mummies formed on the coffee filter than on foliage for all other *T. citricida* instars tested (L2, L3 and L4) (Table 3).

The percentage of adult *L. testaceipes* emerging from mummies located on the coffee filter was significantly ($P < 0.05$) higher than on the foliage for all instars of brown citrus aphids tested (Table 3). Mean percentage of *L. testaceipes* female progeny that emerged from L1 and L4 hosts on foliage was not significantly different to that observed on the coffee filter; however, significantly more females emerged from mummies of L2 and L3 aphid hosts on the coffee filter than on the foliage (Table 3). Generally, more female parasitoids than males are produced from larger aphid hosts (Godfray 1994) and our data are consistent with this because L4 hosts produced more females than males whether they formed on the foliage or on the coffee filter. However, the observation that mummies on foliage originating from L2 and L3 aphid hosts produce a male-biased sex ratio (66-70%) while mummies on the coffee filter from these same-sized hosts produce female-biased sex ratio (27-38% males) is interesting and needs further evaluation.

The mean number of mummies containing *L. testaceipes* that occurred on the foliage was not significantly different from that observed on the coffee filter when L1 hosts were parasitized (Table 3). However, L2, L3 and L4 hosts produced significantly more mummies on the coffee filter than on foliage (Table 3). Dissection of unclosed mum-

TABLE 3. MEAN NUMBER OF MUMMIES, MEAN PERCENTAGE *L. TESTACEIPES* ADULTS AND MEAN PERCENTAGE FEMALES PRODUCED BY MATURE *L. TESTACEIPES* FEMALES ON DIFFERENT INSTARS OF BROWN CITRUS APHID.

Host stage	Mean \pm S.D. number of mummies/♀			Mean \pm S.D. percentage adults/♀			Mean \pm S.D. percentage females/♀		
	Foliage	Coffee filter	P	Foliage	Coffee filter	P	Foliage	Coffee filter	P
L1	21.4 \pm 3.4 a	25.3 \pm 10.3 a ¹	0.2345	15.4 \pm 8.6 b	43.5 \pm 28.9 a	0.0258	36.4 \pm 8.9 a	35.4 \pm 8.8 a	0.3144
L2	13.5 \pm 5.3 b	38.1 \pm 14.7 a	0.0021	25.4 \pm 19.9 b	88.3 \pm 9.1 a	<0.0001	30.2 \pm 11.0 b	62.3 \pm 16.9 a	0.0012
L3	4.2 \pm 3.3 b	43.2 \pm 11.0 a	<0.0001	27.0 \pm 24.3 b	83.8 \pm 7.0 a	0.0002	44.4 \pm 14.9 b	72.9 \pm 18.2 a	0.0031
L4	3.1 \pm 2.3 b	29.8 \pm 6.4 a	<0.0001	17.9 \pm 21.1 b	94.7 \pm 4.9 a	0.0001	62.5 \pm 21.2 a	63.8 \pm 15.2 a	0.5124

mummies from citrus foliage in 0.8% saline under a dissecting microscope revealed many dead late-instar larvae, prepupae, or pupae of *L. testaceipes*. Stary (1989) termed this phenomenon 'incomplete parasitization'. In our study, mummies on the foliage produced higher rates of incomplete parasitization (73-85%) compared to that observed from mummies on the coffee filter (5-56%). Factors which cause more L1 hosts to produce mummies on foliage and mummies that exist on foliage to have greater rates of incomplete parasitism and a male-biased sex ratio are unknown.

Mummy location in *Lipolexis oregmae* also has been studied in the laboratory (Hill 2002; Walker 2002; Hill & Hoy; 2003). *Lipolexis oregmae* mummifies on (or in) the soil and mummy location is independent of brown citrus aphid instar. Mummification on (or in, because coffee filters would prevent movement of aphids into the soil) the soil, however, has not been described previously for *L. testaceipes* reared on brown citrus aphid on citrus in Florida. The mechanism controlling movement of parasitized aphids to areas where there is greater chance of predation or fungal infections is unknown (Godfray 1994). Chow and Mackauer (1999) reported that the percentage of mummification off the plant of the aphid *Acyrtosiphon pisum* (Harris) when parasitized by *Ephedrus californicus* (Baker) varied with aphid density. However, the location of brown citrus aphid mummies containing *L. testaceipes* does not appear to be dependent on aphid host density. When brown citrus aphids, in densities of 40 or 200, were parasitized by single *L. testaceipes* females in laboratory trials (data not shown) a mean (\pm SD) percentage of 60.2 (13.2) and 71.3 (17.4), respectively, were produced on the coffee filter ($P = 0.28$, $n = 10$, Students t-test).

Mummy location also was investigated in a citrus grove adjacent to the Department of Entomology and Nematology, University of Florida, Gainesville, in fall 2001 and spring and summer of 2002. Young citrus foliage was infested with 200-250 brown citrus aphids of mixed stages and were covered with mesh sleeves. When a single female *L. testaceipes* was allowed access to these aphids, mummies were formed off the foliage in significantly higher quantities than on the foliage. Potted citrus plants in mesh cages also were placed in the grove and produced similar results (Persad & Hoy, unpublished data). This suggests that movement of brown citrus aphids containing *L. testaceipes* to the soil is not restricted to laboratory colonies. Thus, these results indicate that analysis of parasitism by *L. testaceipes* of brown citrus aphid in the field in Florida should not be limited to examining the mummies occurring on foliage.

In field evaluations in Puerto Rico, Yokomi & Tang (1996) concluded *L. testaceipes* is an ineffective parasitoid of the brown citrus aphid because they observed an emergence rate of ca. 4.0% from

mummies located on field-collected citrus foliage. Michaud (1999) also commented on the low occurrence of emergence holes in mummies located on citrus terminals and observed that, despite the ubiquitous presence of *L. testaceipes*, rates of parasitism were generally too low to affect brown citrus aphid populations. Despite these negative evaluations of *L. testaceipes* as a parasitoid of the brown citrus aphid, our data suggest parasitism by *L. testaceipes* may be more extensive in citrus in Florida than previously recognized.

Rearing Protocol

The data in Tables 1 and 3 indicate that younger *L. testaceipes* females produce fewer progeny and often die shortly after being allowed constant exposure to aphids. In contrast, female parasitoids produced more progeny if their exposure to aphids was delayed for at least 24 h; it is unknown whether this effect is behavioral or physiological. This information is, however, crucial to the following guidelines for initiating and maintaining cultures of *L. testaceipes* on the brown citrus aphid:

Hold newly emerged adult parasitoids in vials with access to water and honey for 24 h before they are allowed access to aphids. Six potted citrus plants (prepared as described above), each infested with 200-250 brown citrus aphids of mixed instars, will yield 32 to 150 adult *L. testaceipes* (Mean \pm SD = 85 ± 61 , $n = 17$ culture cages) when 6 mated mature (24-30-h-old) females are allowed to parasitize their hosts until death. This protocol was used in summer 2002 to initiate 5 separate *L. testaceipes* cultures from field-collected citrus foliage infested with brown citrus aphid. Successful and expanding cultures resulted in all cases and populations increased within one generation using this protocol, indicating that no genetic selection of this parasitoid was needed to propagate it on brown citrus aphid.

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