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TOOLS FOR EVALUATING *LIPOLEXIS OREGMAE* (HYMENOPTERA: APHIDIIDAE) IN THE FIELD: EFFECTS OF HOST APHID AND HOST PLANT ON MUMMY LOCATION AND COLOR PLUS IMPROVED METHODS FOR OBTAINING ADULTS

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ABSTRACT

Lipolexis oregmae Gahan was introduced into Florida in a classical biological control program directed against the brown citrus aphid, *Toxoptera citricida* (Kirkaldy), on citrus. Prior to evaluating distribution, host range, and potential nontarget effects of *L. oregmae* in Florida, we evaluated the role of other potential host aphids and host plants on mummy production and location. Under laboratory conditions, this parasitoid produced the most progeny on the target pest, the brown citrus aphid on citrus. This parasitoid, unlike the majority of aphidiids, did not produce mummies on any of the host plants tested when reared in black citrus aphid *T. aurantii* (Boyer de Fonscolombe) on grapefruit, spirea aphid *Aphis spiraecola* Patch on grapefruit and pittosporum, cowpea aphid *A. craccivora* Koch on grapefruit and cowpeas, or melon aphid *A. gossypii* Glover on grapefruit and cucumber. Thus, sampling for *L. oregmae* mummies of these host aphids and host plants must involve holding foliage in the laboratory until mummies are produced. This parasitoid requires high relative humidity to produce adults because no adults emerged when mummies were held in gelatin capsules, but high rates of emergence were observed when mummies were held on 1.5% agar plates. In addition, we compared the color of 6 aphid hosts and the color of mummies produced by *L. oregmae* when reared in them to determine if color of mummies could be used to identify *L. oregmae*. Mummy color varied between aphid hosts and tested host plants, and is not a useful tool for identifying *L. oregmae* for nontarget effects.

Key Words: classical biological control, monitoring, mummy color, mummy location, parasitoid

RESUMEN

Lipolexis oregmae Gahan fue introducida en Florida por medio de un programa de control biológico clásico dirigido contra el áfido pardo de los cítricos, *Toxoptera citricida* (Kirkaldy), sobre *Citrus*. Prioritario a la evaluación de la distribución, el rango de los hospederos y los efectos potenciales de *L. oregmae* sobre los organismos que no son el enfoque de control en la Florida, nosotros evaluamos el papel de otras especies de áfidos y plantas hospederas potenciales sobre la producción y ubicación de las momias. Bajo condiciones de laboratorio, este parasitoide produjó el mayor número de progenies sobre la especie de plaga enfocada, el áfido pardo de los cítricos sobre *Citrus*. Este parasitoide, no como la mayoría de los afidíidos (Hymenoptera), no produjó momias sobre cualquiera de las plantas hospederas probadas cuando fue criado sobre el áfido negro de los cítricos, (*T. aurantii* (Boyer de Fonscolombe)) en toronja, el áfido spirea (*Aphis spiraecola* Patch) en toronja y pittosporum, el áfido del caupí (*A. craccivora* Koch) en toronja y caupí o el áfido del melón (*A. gossypii* Glover) en toronja y pepino. Así, el muestreo para momias de *L. oregmae* de estas especies de áfidos hospederos y plantas hospederas tiene que incluir el mantenimiento de follaje en el laboratorio hasta que se produzcan las momias. Este parasitoide requiere una alta humedad relativa para producir los adultos por que ningún adulto emergió cuando las momias fueron mantenidas en cápsulas de gelatina, pero una tasa alta de emergencia de adultos fue observada cuando se mantuvo las momias en platos con agar al 1.5%. Además de esto, nosotros comparamos el color de 6 especies de áfidos hospederos, así como el color de las momias producidos por *L. oregmae* cuando fue criado sobre ellos para determinar si se puede usar el color de las momias para la identificación de *L. oregmae*. El color de las momias varia entre las especies de áfidos hospederos y las plantas hospederas probadas, por lo que no es una herramienta útil para la identificación de *L. oregmae* o para la evaluación de su efecto sobre los organismos que no son el enfoque del control.

Lipolexis oregmae Gahan (Hymenoptera: Aphidiidae) was introduced in a classical biological control program into Florida as *Lipolexis scutellaris* (Mackauer) (Hymenoptera: Aphidiidae) in 1999 to control the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphi-

didae) on citrus (Hoy & Nguyen 2000a, 2000b; Persad et al. 2007). *Lipolexis oregmae* was obtained from Guam and is designated as the Guam biotype because parasitoid populations from different geographic areas often have unique host ranges and other biological attributes (Stary 1988). Furthermore, host aphids from different populations may be considered biotypes with different effects on their parasitoids. For example, Oliver et al. (2003) found that populations of aphids may have different facultative endosymbionts that can confer resistance to parasitoid wasps while other populations lack such secondary endosymbionts. Thus, it is difficult to predict parasitoid-host aphid interactions when introducing a parasitoid from one geographic area into a new environment with aphid hosts that could be genetically distinct. In Guam, *L. oregmae* was reported to attack brown citrus aphids, spirea aphids (*Aphis spiraecola* Patch) (Homoptera: Aphididae), melon aphids (*Aphis gossypii* Glover) (Homoptera: Aphididae), and cowpea aphids (*Aphis craccivora* Koch) (Homoptera: Aphididae) (Miller et al. 2002), and we expected these aphids to also serve as hosts in Florida.

As with other members of the Aphidiidae, *L. oregmae* pupates inside its aphid host in a 'mummy'. Aphidiid mummies most often are found on the leaves and stems of plants, but may occur on or in the soil (Mackauer & Kambampati 1988; Chow & Mackauer 1999). Host aphid, host plant, and parasitoid species play a significant role in determining the location of mummies and these factors could affect the color of mummified aphids, which could be used to recognize parasitoid species (Stary 1970).

Mummy location can affect the accuracy of detecting parasitoids in a given geographical area (Mackauer & Kambampati 1988) and can affect the assessment of the host range of parasitoids (Stary 1988). Evaluation of the impact of hyperparasitoids (Stary 1988) and predation on parasitoids (Hill & Hoy 2003; Persad & Hoy 2004) can be affected by mummy location. During laboratory rearing of the Guam biotype of *L. oregmae* on brown citrus aphids, mummies were found off the citrus plants and on coffee filter papers, which covered the soil surface of the potted citrus trees (Hill & Hoy 2003; Walker & Hoy 2003; Persad & Hoy 2004). The location of *L. oregmae* mummies produced when reared in other aphids has not been reported, but this information is needed to design appropriate sampling methods to evaluate the establishment of *L. oregmae*, its host range, and nontarget effects on other aphid species, if any.

Persad et al. (2007) reported that *L. oregmae* is established and widely distributed throughout all citrus-growing regions in Florida. Persad et al. (2004) used a high fidelity polymerase chain reaction (PCR) assay on aphids collected from citrus, weeds, and vegetables in Florida and detected *L. oregmae* in field-collected brown citrus aphids, black citrus aphids, spirea aphids, and melon aphids from citrus, and cowpea aphids from eggplants. The high-fidelity PCR assay provides a sensitive qualitative assessment (presence or absence) of aphids parasitized by *L. oregmae*, but a quantitative assessment of the proportion of aphids parasitized by *L. oregmae* would require testing each aphid individually, which is time consuming and costly. Furthermore, this method does not provide any adults with which to confirm species identity by morphological traits. Collection of mummified aphids from foliage is commonly done in faunistic studies, but this method will be inappropriate if mummies are produced off the host plant (Mackauer & Kambampati 1988).

Several methods have been developed to obtain adults from mummies for species confirmation. Mackauer & Kambampati (1988) suggested that parasitoid adults could be obtained from mummified aphids obtained from foliage by holding them in clear gelatin capsules or cotton-stoppered glass vials held under controlled temperatures and relative humidity conditions. This method has not been successful with *L. oregmae* (Hill & Hoy unpublished), but Persad & Hoy (2003) successfully obtained emergence of *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) adults from brown citrus aphids collected from the field by placing citrus foliage infested with aphids between crumpled sheets of absorbent paper in plastic bags. This method originally was used by Smith & Hoy (1995) to obtain adults of citrus leafminer (*Phyllocnistis citrella* Stainton) (Lepidoptera: Gracillariidae) from citrus leaves with pupal chambers because these insects require a high relative humidity to emerge successfully. Zappalá & Hoy (2004) placed pupae of the parasitoid *Ageniaspis citricola* Logvinvskaya (Hymenoptera: Encyrtidae) dissected from pupal chambers on 1.5% agar plates to obtain adults because pupae of this parasitoid also require high relative humidity to emerge. The agar plate method has not been evaluated as a method to obtain adults of *L. oregmae* from mummies but, if effective, would allow analysis of percentage emergence and provide adults for taxonomic confirmation.

The color of the mummies has been used as a method for identifying some aphid parasitoids (Stary 1970), but the host aphid, host plant, and parasitoid species could affect the color of mummies produced. Kavallieratos & Lykouressis (2004) reported the color of *A. gossypii* mummies was useful in identifying the genus and species of aphidiine parasitoids, and we investigated whether mummy color could be used to identify *L. oregmae*.

The objectives of this study were to develop methods that would enable us to conduct field sampling of aphid hosts on different host plants

in a manner that would allow us to hold the samples in order to obtain mummies and adult parasitoids. We compared the location of *L. oregmae* mummies produced in 6 representative aphid hosts and on 5 host plants to determine whether mummy location was consistent. We evaluated a method to hold parasitized aphids in order to obtain mummies and adult parasitoids, and compared the color of *L. oregmae* mummies produced in the different aphid species tested when reared on different host plants to determine if color varied among host aphids and host plants. These methods could be used in future field studies to evaluate geographical distribution, host range, and potential nontarget effects.

Materials and Methods

Source and Rearing of Aphid Species

Aphids were collected from citrus (*Citrus sinensis* Pers.), cucumbers (*Cucumis sativus* L.), scarlet milkweed (*Asclepias curassavica* L.), and cowpeas [*Vigna unguiculata* (L.)] in Alachua County, Florida between Apr and Jun 2005. Healthy adult females were hand transferred to clean host plants to obtain pure colonies. Aphid species were confirmed with identification keys by Blackman & Eastop (2000) and Halbert & Brown (1996) and confirmed by Keith S. Pike. Colonies of *T. citricida*, *T. aurantii,* and *A. spiraecola* were reared on potted grapefruit (*Citrus* × *paradisi* Macfad.), whereas *A. gossypii* and *A. craccivora* were reared on potted cucumber (*Cucumis sativus* L. var. Cucumber-Poinsett 76) and cowpea [*Vigna unguiculata* (L.) var. Cream 12 Peas] plants, respectively. *Aphis nerii* Boyer de Fonscolombe was reared on scarlet milkweed. Colonies were maintained in PVC cages (61 × 61 × 61 cm) covered with mesh sleeves (BioQuip Products, Gardena, CA) at 22-25°C, 60-70% RH under an 18:6 (L:D) photoperiod.

Rearing of *L. oregmae*

Lipolexis oregmae was reared on potted citrus trees infested with mixed stages of *T. citricida* (Walker & Hoy 2003). Seven citrus plants infested with approximately 500-700 aphids of mixed stages per plant were transferred to wood-framed mesh parasitoid cages (87 × 48 × 50 cm) twice a week (Walker & Hoy 2003). Parasitoid males and females were aspirated into a 50-mL conical centrifuge tube (USA Scientific, Ocala, FL) and fed honey and water for 6-12 h prior to release. At least 50 *L. oregmae* females and ~20-30 males were released per cage. Each colony cage was provided with a honey strip and 2 water-soaked cotton pads at the time of parasitoid release and at the time of adult emergence of the next generation because adults require free water to drink and a high relative humidity. The colony was maintained at 25 ± 1°C, 60-70% RH under an 18:6 (L:D) photoperiod.

Host Plant Rearing

Grapefruit trees (35-40 cm tall) grown in 11.4-liter pots were pruned and soil in the pots was soaked with 250 mL of bleach (sodium hypochlorite 6.15% a.i., Ultra Clorox Germicidal Bleach, Clorox Professional Products Company, Oakland, CA) solution diluted (5 mL of bleach/100 mL water) to reduce fungal growth that could kill aphid mummies (Walker & Hoy 2003). After 20 min of treatment with bleach, pots were drenched with water. Plants were fertilized (Peter's 20-20-20; United Industries, St Louis, MO) and sprayed with 0.26% (20 mL/7.57 L) of horticulture grade oil (Dyne-Amic, Setre Chemical Company, Memphis, TN) to reduce insect contamination. Plants were transferred to a greenhouse and kept there for approximately 2 weeks to allow them to produce tender new shoots (=flush) prior to being infested with aphids. *Pittosporum tobira* (Thunb.) W. T. Aiton plants purchased from Lowe's (Gainesville, FL) were pruned, fertilized and sprayed with 0.26% of horticultural oil. Pruned plants were maintained in a mesh cage at 22-25°C, 60-70% RH under an 18:6 (L:D) photoperiod in the greenhouse until new growth was produced.

Cowpeas were raised from seeds in 3.8-L pots in a greenhouse. Once the plants produced 3 to 4 true leaves, 10 plants were transplanted to the center of another pot prior to being infested with aphids. Cucumber plants were raised from seeds in 3.8-L pots. Six seeds per pot were sown and thinned to 4 plants each when seeds emerged. Milkweed plants potted in 3.8-L pots were purchased (Lowe's, Gainesville, FL). Cowpeas, cucumbers, and milkweeds were not treated with chlorox prior to being infested with aphids.

Assessment of *L. oregmae* Mummy Location When Reared on Different Aphids and Host Plants

Once the grapefruit trees had produced flush 1-2 cm in length, the soil surface was soaked for a second time with the bleach solution for 20 min and then flushed with water. The pots were then filled to the top rim with potting mix (Jungle Growth, Professional Grower Mix, Piedmont Pacific Inc., Statham, GA). The pot surface then was lined with plastic wrap (Borden Packaging & Industrial Products, North Andover, MA) and covered with 32 cm diameter filter paper (Whatman Ltd., U.K.) and taped to the sides of the pot with Duck tape (Henkel Consumer Adhesives, Avon, OH). The holes on the bottom of pots were sealed with tape. Trees were placed into the colonies of aphids for infestation. After 2 d, the plants were removed from the colonies and examined for a

desired density of approximately 150-200 mixed stages of immature aphids per plant. Infested plants were placed into wood-framed mesh parasitoid cages in which the bottom was lined with a plastic sheet so that mummies could be seen, counted, and removed. The Pittosporum plants were treated similarly.

Cowpea, melon, and milkweed plants were allowed to establish for 3-4 d, the soil surface was soaked with the bleach solution for 20 min and then flooded with water. The pots were then treated as described for the grapefruit trees until being infested with aphids. After 2 d, plants were removed from the aphid colonies and examined for a desired density of approximately 150-200 mixed stages of immature aphids. Then infested plants were placed in PVC cages covered with mesh sleeves and the bottom of each cage was lined with a plastic sheet.

One female and 2 males of *L. oregmae* less than 24 h old were collected in a plastic vial and kept for 2 h to ensure mating. A honey strip was placed inside the vial and a water-soaked cotton pad was placed on the top of the vial covered with mesh to provide food and water for the parasitoids. The parasitoids were released after 2 h into the wood-framed mesh parasitoid cages (citrus and Pittosporum), or the PVC cages (milkweeds, cowpeas, cucumbers), which were monitored daily for the production of progeny (mummies). For all experiments, but one, 5 replicates were evaluated. In the case of cowpeas, 15 replicates were observed. In the case of grapefruit, milkweed, and Pittosporum, 1 plant per pot was used, whereas 10 plants per pot were used for cowpeas and 4 plants per pot for cucumber to yield the desired total of ca. 150-200 aphid hosts per cage. The number and location of the progeny mummies (on plant leaves, on stems, or on the bottom of the cage) was recorded 8, 10, and 12 d after parasitoids were released into the cage. The number of mummies produced on these different host aphids and plants was analyzed by ANOVA (SAS) and means were separated by Fisher's protected least significant difference (LSD) test, 5% level.

Aphid and Mummy Colors When Reared on Different Host Plants

The color of aphid mummies produced by *L. oregmae* on different host aphids and host plants was determined by matching mummy color to color chips in the Munsell book of color (Anonymous 1942). Five mummies were randomly selected from each host aphid and host plant combination. Individual mummies were matched to the color chips in daylight outdoors at noon under a 10× dissecting microscope following the instructions provided. For the Munsell charts, data were recorded as HV/C, where 'H' is hue name, 'V' is the value in the vertical column on the left of the chart, and 'C' is chroma in the horizontal line at the base of the chart. Similar procedures were followed to determine the color of wingless adult aphids from the colonies in order to compare aphid and mummy color. After obtaining the color values from the Munsell charts, the color designations were compared to the ISCC-NBS color name charts (Anonymous 1964) and the name of the colors observed was recorded.

Handling of *L. oregmae* Mummies for Adult Emergence

A single citrus shoot infested with brown citrus aphids was collected from cages of the laboratory colony of *L. oregmae* 4 d after release of adult parasitoids. The number of aphids per shoot was estimated and shoot length recorded for each of the 6 replicates. Each shoot was then placed in a plastic bag (60 × 30 cm) inflated with air along with 2 moist paper towels at 24 ± 1°C, 50-60% RH under an 18:6 (L:D) photoperiod. Mummies were removed from the bags after 6 and 10 d and counted; on each date, half were plated on 1.5% agar (non nutrient) plates (Difco Agar granulated, Becton, Becton, Dickinson & Company, Sparks, MD) and the rest were kept individually in gelatin capsules (No. 3, Eli Lilly and Company, Indianapolis, IN) within a 50-mL vial for 8 d to evaluate adult emergence. The number of mummies counted after 6 and 10 d were summed and the proportion of adults that emerged on both dates was calculated. The percentage parasitism of the aphids on the citrus shoots was calculated based on the number of mummies divided by the greater value (300) of the estimated number of aphids per shoot (range: 140-300) at the beginning of the experiment, and then average percentage parasitism was obtained for the 6 replicates.

Results and Discussion

Assessment of *L. oregmae* Mummy Location When Reared on Different Aphids and Host Plants

Host aphid and host plant did not affect the location of mummies of *L. oregmae* (Table 1). All mummies of *L. oregmae* produced in all host-aphid and host-plant combinations were found on the cage bottom or beneath the pots and sticking to the tape used for covering the holes on the pot bottoms. No mummies were found on the foliage and/or stems. Hill & Hoy (2003) obtained similar results with mummies of *L. oregmae* reared in brown citrus aphids on citrus and reported 98.6-99.2% of the mummies were on/in the soil surface. Thus, based on this sample of diverse aphids and host plants, it appears that *L. oregmae* only produces mummies off the host plant.

In these no-choice tests, when *L. oregmae* was reared in 5 different host aphids reared only on grapefruit, a significant difference ($F = 98.29$, $df =$

TABLE 1. MEAN NUMBER OF MUMMIES COLLECTED FROM LEAVES, STEMS, AND CAGE BOTTOM WHEN *L. OREGMAE* WAS REARED ON DIFFERENT HOST APHIDS AND HOST PLANTS.

Host aphid	Host plant	Mean (±SD) no. of *L. oregmae* mummies collected from		
		Leaves	Stems	Cage bottom
T. citricida	Grapefruit	0	0	188.2 ± 16.4
T. aurantii	Grapefruit	0	0	132.8 ± 14.4
A. spireacola	Grapefruit	0	0	144.0 ± 11.4
A. gossypii	Grapefruit	0	0	58.2 ± 9.8
A. craccivora	Grapefruit	0	0	54.2 ± 16.2
A. spiraecola	Pittosporum	0	0	51.0 ± 8.5
A. gossypii	Cucumber	0	0	58.6 ± 10.5
A. craccivora	Cowpea	0	0	0.7 ± 1.7
A. nerii	Milkweed	0	0	48.6 ± 17.6

4, $P < 0.0001$) was observed in the mean number of mummies produced (Table 1). *Lipolexis oregmae* produced more mummies (mean = 188.2) in brown citrus aphid hosts followed by spirea aphid (144.0), black citrus aphid (132.8), melon aphid (58.2), and cowpea aphid (54.2) (Table 1). The higher rate of reproduction on brown citrus aphids on citrus is significant because this pest was the target of the classical biological control introduction.

When *L. oregmae* was reared in spirea aphid on grapefruit, a mean of 144.0 progeny were produced, which was significantly ($F = 217.86$, $df = 1$, $P = 0.0001$) higher than when *L. oregmae* was reared in spirea aphid on Pittosporum (51.0), suggesting that host plant may affect parasitism by *L. oregmae*. No significant difference ($F = 0.09$, $df = 1$, $P = 0.7816$) was observed in the mean number of mummies produced by *L. oregmae* when reared in melon aphids on grapefruit (58.2) or on cucumber (58.6). The mean number of mummies produced in cowpea aphids on grapefruit (54.2) was higher ($F = 55.91$, $df = 1$, $P = 0.0017$) than in cowpea aphids on cowpeas (0.7), which suggests that cowpeas affected oviposition or developmental success by the parasitoid. Despite the fact that we increased the number of replicates over a period of several months, *L. oregmae* mummy production remained low on cowpeas, indicating that this host plant and aphid combination would be unlikely to yield significant numbers of *L. oregmae* in a field survey.

Lipolexis oregmae produced an average of 48 mummies in the oleander aphid *A. nerii* (Boyer de Fonscolombe) on milkweed, which appears to be a new host record for this parasitoid (Table 1). This suggests that the host range of *L. oregmae* may be broader than previously recorded and that evaluation of nontarget effects in Florida should involve sampling diverse host plants and aphid species.

Because the number of aphid hosts provided (150-200) to the females was similar among the aphid-host plant combinations, these laboratory data indicate that *L. oregmae* performs better on brown citrus aphids on citrus, the target of the classical biological control program, than on other aphid hosts on citrus or on other host plants. In general, *L. oregmae* appears well adapted to aphids on citrus (Table 1).

Aphid and Mummy Colors When Reared on Different Host Plants

The color of the individual wingless adult aphids reared varied when reared on the same host plant under our laboratory conditions, but greater variability was observed when the aphids were reared on different host plants (Table 2). Brown citrus aphids, which probably develop only on citrus, were "Reddish yellow red" to "Yellow red" by the Munsell values and "Brownish black-Black" by the ISCC-NBS color chart, and black citrus aphids appeared similar to the brown citrus aphids when using the Munsell and ISCC-NBS color values (Table 2). Halbert & Brown (1996) described brown citrus aphids as shiny black and black citrus aphids as matte black in color. According to Blackman & Eastop (2000), the color of brown citrus aphids and black citrus aphids vary from very dark brown to black and reddish brown to brown black, respectively. Thus, different people may use different terminology to describe the same colors; for example, cowpea aphids reared on grapefruit are described in the Munsell Book of Color Values as "Greenish yellow to Yellow green yellow" while the ISCC-NBS color chart calls the same colors "Light olive gray to Olive black" (Table 2). However, by using either color chart, it is possible to provide consistent color designations, even though the color terminology used may not be consistent. These color evaluations were conducted between 1200 and 1400 h in sunlight and with a dissecting microscope, as recommended by the Munsell Book of Color Values, because color evaluations should be conducted with standardized light conditions.

TABLE 2. RANGE OF COLORS OF APHIDS WHEN REARED ON DIFFERENT HOST PLANTS AS DEFINED BY MUNSELL COLOR VALUES AND THE ISCC-NBS COLOR CHART.

Host plant	Brown citrus aphid	Black citrus aphid	Cowpea aphid	Melon aphid	Spirea aphid	Oleander aphid
Grapefruit						
MBCV	Reddish yellow red (2.5YR3/2) to Yellow red (5YR2/2)	Reddish yellow red (2.5YR2/2) to Yellow red (5YR2/2)	Greenish yellow (7.5Y6/2) to Yellow green yellow (10Y4/2)	Yellow (5Y7/6) to Greenish yellow (7.5Y6/6)	Yellow green yellow (10Y7/8) to Yellow green yellow (10Y7/6)	n. a.
ISCC-NBS	65 Brownish black to 267 Black	267 Black only	112 Light olive gray to 114 Olive black	87 Medium yellow to 107 Medium olive	100 Deep greenish yellow to 103 Dark greenish yellow	n. a.
Cowpea						
MBCV	n. a.	n. a.	Greenish yellow (7.5Y4/2) to Yellow green yellow (10Y4/2)	n. a.	n. a.	n. a.
ISCC-NBS	n. a.	n. a.	111 Dark gray olive to 114 Olive black	n. a.	n. a.	n. a.
Cucumber						
MBCV	n. a.	n. a.	n. a.	Yellow (5Y6/6) to Greenish yellow (7.5Y6/6)	n. a.	n. a.
ISCC-NBS	n. a.	n. a.	n. a.	106 Light olive to 107 Medium olive	n. a.	n. a.
Pittosporum						
MBCV	n. a.	n. a.	n. a.	n. a.	Greenish yellow (7.5Y8/10) to Yellow green yellow (10Y7/6)	n. a.
ISCC-NBS	n. a.	n. a.	n. a.	n. a.	97 Very greenish yellow to 103 Dark greenish yellow	n. a.
Milkweed						
MBCV	n. a.	n. a.	n. a.	n. a.	n. a.	Reddish yellow (2.5Y8/12) to Reddish yellow (2.5Y8/10)
ISCC-NBS	n. a.	n. a.	n. a.	n. a.	n. a.	82 Very yellow to 83 Brilliant yellow

MBCV: Munsell Book of Color Values.
ISCC-NBS: ISCC-NBS Color Name Charts.
n.a.: This aphid does not grow on this host plant or was not reared on it.

The color of cowpea aphids also varied when reared on grapefruit and cowpeas (Table 2). Halbert & Brown (1996) and Blackman & Eastop (2000) described adult wingless cowpea aphids as shiny black in color. Melon aphids differed only slightly in color when reared on grapefruit and cucumber. Spirea aphids varied in color when reared on grapefruit or on Pittosporum. The oleander aphid could not be reared on citrus, but was a strong yellow when reared on milkweed (Table 2).

The color of mummies produced by *L. oregmae* when reared in different host aphids reared on the same or different host plants also varied (Table 3). *Lipolexis oregmae*, when reared in different host aphids but on the same host plant, produced mummies ranging from light gray yellowish brown to dark gray yellowish brown, medium brown to dark yellow brown, and grayish brown to dark grayish brown.

The color of mummies on same host aphid, but a different host plant, also varied (Table 3). In the case of oleander aphids reared on milkweed, the mummies produced were strikingly different from all other host aphid and plant combinations. The color of the mummies produced when *L. oregmae* was reared in spirea aphids on Pittosporum was reddish brown compared to grayish brown when mummies were produced in cowpea aphids, black citrus aphids, and brown citrus aphids.

These differences in the color of mummies produced by *L. oregmae* in different host aphids reared on the same or different host plants make it difficult to use color as a method for identifying *L. oregmae* mummies, especially if sampling among an array of host plant and aphid species. However, all mummies appeared similar in texture and size, even though they differed in color. *Lipolexis oregmae* mummies, at least when reared in these aphid hosts, are not shiny and appear somewhat 'fluffy' because silk strands protrude from the broken surface of the aphid exoskeleton. Typically, there are multiple breaks in the aphid exoskeleton surrounding the parasitoid with the parasitoid cocoon silk extruding. We have seen no evidence that *L. oregmae* glues the aphid exoskeleton to the substrate, unlike the behavior of many aphidiid species (Stary 1970).

Handling of *L. oregmae* Mummies for Adult Emergence

When *L. oregmae* was reared under laboratory conditions, the individual shoots had 140-300 brown citrus aphids per shoot, averaging 193.3 (SD = 54.3). The number of mummies produced per shoot ranged from 116 to 223, averaging 153 (38.6). Percentage parasitism ranged from 68.2 to 88.7, averaging 79.7% (7.5). A large proportion of the mummies recovered from the bags were found between the folds of the paper towels and were attached only very loosely to the rough paper by the silky strands protruding from the mummy. This behavior suggests that the parasitized aphids move to darker, more humid and secure areas before becoming mummified in the field.

Mummies (n = 202) of *L. oregmae* kept in gelatin capsules did not produce any adults. This was unexpected because many aphidiid mummies readily produce adults in gelatin capsules. We suspect that the failure to obtain adults was due to lower-than-optimal relative humidity conditions but other factors may influence emergence of adults from mummies. Walker & Hoy (2003) only achieved a mean percentage emergence (SE) of 44.5% (1.6) of *L. oregmae* adults from 100 second-instar brown citrus aphids reared on potted citrus plants enclosed in plastic cylinders under laboratory conditions, which suggests that mortality of *L. oregmae* mummies could have occurred due to fungal pathogens as well as to insufficiently high relative humidity on the surface of the soil.

For the 353 mummies that were placed on the 1.5% agar plates, the mean percentage emergence (SD) of adults was 95.7% (5.3). These mummies were 1-6 d old when placed on the agar and were held for 8 d. Most of the adults emerged within 4-6 d after being placed on the agar. Due to the high relative humidity in the petri dish, some fungal growth was observed growing on the mummies and on the nonnutrient agar after several days, but this apparently did not prevent emergence of the parasitoid adults.

The high rate of emergence of adults from mummies on the agar plates allowed us to ship *L. oregmae* mummies to Jamaica via express delivery service (in boxes that did not contain ice) during Oct and Dec 2005, where they were to be reared for a classical biological control program. D. Clarke-Harris (personal communication) kindly recorded the number of adults that emerged from these 2 shipments, which experienced unknown temperature conditions. In the first, 80.9% of the 110 mummies produced adults, while in the second shipment 109 adults were produced from 450 mummies (24.2%). Because adults of *L. oregmae* are short lived and difficult to ship, this agar plate method appears to be an effective method for shipping this species, as well as a useful method for obtaining adults for taxonomic confirmation.

Conclusions

These results indicate that sampling of aphids on host plants other than citrus should be conducted to determine the host range of *L. oregmae* in Florida. It is likely that *L. oregmae* utilizes aphids on other plants, including vegetables, ornamentals, and weeds during periods when aphids are rare in citrus. Monitoring will require potentially parasitized aphids to be held until

TABLE 3. RANGE OF COLOR OF *L. OREGMAE* MUMMIES WHEN REARED IN DIFFERENT APHID AND HOST-PLANT COMBINATIONS AS DEFINED BY MUNSELL COLOR VALUES AND THE ISCC-NBS COLOR CHART.

Host plant	Brown citrus aphid	Black citrus aphid	Cowpea aphid	Melon aphid	Spirea aphid	Oleander aphid
Grapefruit						
MBCV	Yellowish yellow red (7.5YR4/4) to Yellow red yellow (10YR3/4)	Yellow red yellow (10YR6/2) to Reddish yellow (2.5Y5/2)	Yellow red (5YR4/4) to Yellowish yellow red (7.5YR3/2)	Yellow red (5YR5/4) to Yellowish yellow red (7.5YR4/4)	Yellowish yellow red (7.5YR6/4) to Yellow red yellow (10YR4/4)	n. a.
ISCC-NBS	78 Dark yellow brown to 81 Dark grayish yellow brown	80 Grayish yellow brown to 64 Brownish gray	61 Grayish brown to 62 Dark grayish brown	58 Medium brown to 78 Dark yellow brown	79 Light gray yellowish brown to 58 Medium brown	n. a.
Cowpea						
MBCV	n. a.	n. a.	Yellowish yellow red (7.5YR4/4) to Yellow red yellow (10YR4/2)	n. a.	n. a.	n. a.
ISCC-NBS	n. a.	n. a.	78 Dark yellow brown to 61 Grayish brown	n. a.	n. a.	n. a.
Cucumber						
MBCV	n. a.	n. a.	n. a.	Yellowish yellow red (7.5YR5/4) to Yellowish yellow red (7.5YR4/4)	n. a.	n. a.
ISCC-NBS	n. a.	n. a.	n. a.	75 Deep yellow brown to 78 Dark yellow brown	n. a.	n. a.
Pittosporum						
MBCV	n. a.	n. a.	n. a.	n. a.	Red yellow red (10R4/6) to Reddish yellow red (2.5YR3/6)	n. a.
ISCC-NBS	n. a.	n. a.	n. a.	n. a.	43 Medium reddish brown to 42 Reddish brown	n. a.
Milkweed						
MBCV	n. a.	n. a.	n. a.	n. a.	n. a.	Yellow red (5YR4/8) to Yellowish yellow red (7.5YR4/4)
ISCC-NBS	n. a.	n. a.	n. a.	n. a.	n. a.	55 Slightly brown to 78 Dark yellow brown

MBCV: Munsell Book of Color Values.
ISCC-NBS: ISCC-NBS Color Name Charts.
n.a.: This aphid does not grow on this host plant or was not reared on it.

mummies are produced because *L. oregmae* mummifies off all host plants tested. The improved mummy holding method will allow *L. oregmae* adults to be obtained in a consistent and inexpensive manner for field studies designed to evaluate host range, seasonality, and nontarget effects of this introduced natural enemy.

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