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Source: Florida Entomologist, 101(4) : 611-616

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.101.0423>

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Oviposition preference of rugose spiraling whitefly (Hemiptera: Aleyrodidae) on five host plant species

Siavash Taravati^{1,*}, Catharine Mannion², Cindy McKenzie³, and Lance Osborne⁴

Abstract

Rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae), is an invasive species that underwent a period of outbreak in southern Florida (Miami-Dade, Palm Beach, Broward, Martin, and Monroe counties) between 2010 and 2013. It infested numerous plant species in the landscape and nurseries of southern Florida and became a nuisance and economically damaging species in many urban areas. In order to study its oviposition preference, an experiment was conducted in a shadehouse using 5 known host plant species, namely coconut, *Cocos nucifera* L. (Arecaceae); gumbo limbo, *Bursera simaruba* (L.) Sarg. (Burceraceae); avocado, *Persea americana* Mill. (Lauraceae); black olive, *Bucida buceras* L. (Combretaceae) var. 'Shady Lady;' and giant white bird of paradise, *Strelitzia nicolai* Regel & Körn (Strelitziaceae). Gumbo limbo was the most preferred and giant white bird of paradise was the least preferred of the host plant species. This is consistent with survey data on frequency of plants serving on hosts provided by the Florida Department of Agriculture and Consumer Services—Division of Plant Industry. There was a significant difference between the number of eggs per spiral among host species with gumbo limbo having the highest number of eggs per spiral. No significant correlation was found between the leaf size and the number of eggs on each host species. A strong and significant correlation was found between the number of spirals and the number of eggs per plant species. Adult females deposited the first eggs on source plants, and later oviposited on test host plants, but always returned back to their eclosion site on the source plant. No statistically significant difference was found among the survival of rugose spiraling whitefly on different host plant species tested.

Key Words: Aleurodicinae; *Aleurodicus*; Florida

Resumen

La mosca blanca espiral rugosa (*Aleurodicus rugioperculatus* Martin; Hemiptera: Aleyrodidae) es una especie invasora que tuvo un brote de población en el sur de la Florida en los condados del Miami-Dade, Palm Beach, Broward, Martin y Monroe entre el 2010 y 2013. Se infesta muchas especies de plantas en el campo y en los viveros del sur de Florida y se convirtió en una especie molesta y económicamente perjudicial en muchas áreas urbanas. Para estudiar su preferencia de oviposición, se realizó un experimento en una casa sombreada utilizando cinco especies conocidas de plantas hospederas – coco, *Cocos nucifera* L. (Arecaceae); chaca, *Bursera simaruba* (L.) Sarg. (Burceraceae); aguacate, *Persea americana* Mill. (Lauraceae); olivo negro, *Bucida buceras* L. (Combretaceae); y ave de paraíso blanca gigante, *Strelitzia nicolai* Regel & Körn (Strelitziaceae). La chaca fue el más preferido y ave de paraíso blanca gigante fue la especie de planta hospedera menos preferida. Esto es consistente con los datos de la División de Industria de Plantas del Departamento de Agricultura y Servicios al Consumidor de Florida. Hubo una diferencia significativa entre el número de huevos por espiral entre las especies hospedadoras con el mayor número de huevos por espiral sobre la chaca. No se encontró una correlación significativa entre el área foliar y el número de huevos en cada especie hospedera. Se encontró una correlación fuerte y significativa entre el número de espirales y el número de huevos por especie de planta. Las hembras adultas pusieron los primeros huevos en las plantas fuente y más tarde se pusieron los huevos sobre las plantas objetivo pero siempre regresaron a su sitio de eclosión en la planta fuente. No se encontraron diferencias estadísticamente significativas entre la sobrevivencia de mosca blanca espiral rugosa en diferentes especies de plantas hospedera evaluadas.

Palabras Clave: Aleurodicinae; *Aleurodicus*; Florida

Rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae: Aleurodicinae), is an invasive species that first was reported in the USA in Florida, in 2009. This whitefly was originally described in 2004 in Belize (Martin 2004), and since its discovery has spread to 22 mostly coastal Florida counties (Francis et al. 2016). Not much is known about the biology of rugose spiraling whitefly but the management of this whitefly was heavily reliant on systemic insecticides (Taravati et al. 2013a). Nevertheless, several natural enemies have been identified on rugose spiraling whitefly infestations in southern Florida, including 3 parasitoids: *Encarsia noyesi* (Hayat) (Hyme-

noptera: Aphelinidae), *Encarsia guadeloupae* Viggiani (Hymenoptera: Aphelinidae), and *Aleuroctonus* spp. (Hymenoptera: Eulophidae), and 1 ladybird beetle, *Nephaspis oculata* (Blatchley) (Coleoptera: Coccinellidae) (Taravati et al. 2013b). When done properly, *E. guadeloupae* and *N. oculata* can easily be introduced and established to new areas containing whitefly-infested plants (Taravati et al. 2013a).

Rugose spiraling whitefly eggs usually are found in a waxy spiral on the abaxial side of the leaf. Similar to the spiraling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae) and giant whitefly, *Aleurodicus dugesii* Cockerell (Hemiptera: Aleyrodidae), rugose spiraling

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whitefly may have the potential to disperse to and become established in other regions of the US as well as in other countries. Rugose spiraling whitefly has already reached the southern Indian states of Kerala (Shanas et al. 2016) and Tamil Nadu, where they are likely to pose a threat to the coconut industry (Sundararaj & Krishnan 2017).

Most economically important whiteflies are known to be polyphagous. In contrast, many non-pest species are monophagous or oligophagous (Byrne & Bellows 1991). For example, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), an important pest worldwide, attacks more than 600 plant species (Gelman et al. 2005). Spiraling whitefly, *A. dispersus*, has a wide host range with at least 104 host plant species reported just from the northernmost parts of Queensland, Australia (Lambkin 1999). Similarly, more than 96 plant species have been reported as hosts for rugose spiraling whitefly, but it is not known how many of these species are true host plants (defined as a plant species on which the whitefly can develop from egg to adult) (Kumar et al. 2013, Francis et al. 2016). The most common whitefly-infested plants in the southern Florida landscape include gumbo limbo, *Bursera simaruba* (L.) Sarg. (Burseraceae); coconut, *Cocos nucifera* L. (Arecaceae); black olive, *Bucida buceras* L. (Combretaceae); avocado, *Persea americana* Mill. (Lauraceae); and *Calophyllum* spp. (Calophyllaceae) (Stocks & Hodges 2012).

Most members of Aleurodicinae are native to the Neotropics (Martin 2004) and only a few species, such as *A. dispersus*, have been able to disperse to different regions of the world and pose a problem (Waterhouse & Norris 1989). In Hawaii, *A. dispersus* was commonly found on many vegetable, ornamental, fruit, and shade trees crops, but some of the specific crops attacked include *Annona* spp. (Annonaceae); avocado; banana, *Musa* sp. (Musaceae); bird of paradise; breadfruit, *Artocarpus altilis* (Parkinson) Fosberg (Moraceae); citrus, *Citrus* spp. (Rutaceae); coconut, etc. (Kessing & Mau 1993). In Florida, *A. dispersus* prefers black olives over coconut; banana; mango, *Mangifera indica* L. (Anacardiaceae); seagrape, *Coccoloba uvifera* (L.) Crantz (Polygonaceae); grapefruit, *Citrus x paradisi* Macfad. (Rutaceae); and sweet orange, *Citrus x sinensis* (L.) Osbeck (Rutaceae), and its survival on black olive was significantly greater than on the other types of host plants (Cherry 1980).

Data on whitefly host preference may help researchers and regulatory agencies predict the host plant range more effectively and do more selected monitoring for this whitefly. In this paper, the results from oviposition preference and survival of whitefly on 5 known host plant species will be provided and discussed for the first time.

Materials and Methods

PRELIMINARY OBSERVATIONS

To evaluate the efficacy of releasing whiteflies into oviposition preference cages on cut leaves, whiteflies were collected from gumbo limbo trees from Miami-Dade and Monroe counties, Florida, by cutting tree branches containing infested leaves using an adjustable pole cutter and placing them in large plastic bags. These bags were transferred to the University of Florida, Tropical Research and Education Center, in Homestead, Florida, and placed in 6 field cages (1.83 × 1.83 × 1.83 m with 20 × 20 Mesh Lumite, BioQuip, Rancho Dominguez, California, USA) set up in open fields containing 5 individual plants from 5 different species. The 5 plant species were gumbo limbo, coconut, black olive, avocado, and giant white bird of paradise each 1.2 to 1.5 m (4–5 ft.) tall, all purchased from local nurseries around Homestead, Florida. Bags containing infested gumbo limbo branches and leaves were transferred to each field cage where they were gently opened, and placed

on the ground so that adult whiteflies could fly from the leaves and reach the host plants in the cage. Observations were made on the location of adult whiteflies and wax spirals.

An alternative experiment was conducted in a greenhouse at the University of Florida, Tropical Research and Education Center by placing small, 12 cm pots with live bird of paradise, and gumbo limbo plants infested with whitefly into cages containing clean plants of 1 or both species. Similar to the previous setting, observation was made on the location of adult whiteflies and newly deposited wax spirals.

HOST PREFERENCE

The host preference test was conducted in Nov 2013 using the 5 plant species mentioned above. These plants will be called ‘potential hosts’ hereafter. A total of 30 potential hosts were used in this experiment (6 from each plant species). Leaves on all plants were gently wiped with a damp paper towel to remove debris, pests, and mineral residues before placement into the cages and were irrigated using drip irrigation for 6 minutes per day in the morning.

Additional bird of paradise plant liners also were purchased and were repotted into 12 cm diam pots and kept in an air-conditioned greenhouse with temperatures ranging between 16 to 31 °C. These plants (“source plant” hereafter) were infested with whiteflies collected from gumbo limbo trees from Miami-Dade and Monroe counties, Florida. Infested gumbo limbo tree branches were cut using an adjustable pole cutter, placed into large plastic bags and transported to the laboratory where they were transferred to a glass-top cage (custom-made wood box, about 78 × 38 × 45 cm W × D × H). Source plants also were placed in the glass-top cage with infested gumbo limbo branches for oviposition by whitefly. After oviposition, these plants were transferred to an air-conditioned greenhouse for whitefly rearing for the oviposition preference experiment. All plants were fertilized with a slow release fertilizer (Suncote 16-9-12, Scotts Company LLC, Marysville, Ohio, USA). Plants were irrigated using drip irrigation set at once per d in the morning.

Six field cages (1.83 × 1.83 × 1.83 m with 20 × 20 Mesh Lumite, BioQuip, Rancho Dominguez, California, USA) were set up in late Oct 2013 and aligned in a north-south (Fig. 1) row inside a shadehouse at the University of Florida, Tropical Research and Education Center. On 6 Nov 2013, 1 whitefly-infested source plant was placed in the center of each cage to serve as the source of adult whiteflies in the experiment. Each source plant was surrounded by 5 target host plant species. The source plant was raised to 67 cm from the ground by placing it on a plastic platform made of a stack of 26.5 L pots. The source plant was tied to the platform for stability. All stems of the source plants were treated with Tanglefoot® Tangle Trap® insect trap coating (Scotts Company LLC, Marysville, Ohio, USA) in order to prevent ants from reaching whiteflies and interfering with the experiment. A randomized complete block design was used for this experiment. The order of the plant species around the source plant was randomly assigned in each cage. All the plants were placed in the cages in a way to equalize the distance between the source plant and the closest leaf on each potential host. To achieve this, the location and orientation of potential hosts and the source plant were carefully adjusted.

All the cages were monitored for oviposition activity after the introduction of the infested source plants. Leaves also were checked for the presence of predatory beetles and parasitoids that were quickly removed using an aspirator or by hand. Leaves were sampled from each target plant on 6, 10, 15, and 26 d after the infested source plant was introduced. On each sampling date, all the infested leaves were removed from potential hosts and taken to the laboratory to count the number of eggs and egg spirals. Eggs were cleaned of wax by blow-



Fig. 1. Photo showing the cage arrangement. Six cages ($1.83 \times 1.83 \times 1.83$ m with 20×20 Mesh Lumite) were constructed in a shadehouse in a north-south row. Each cage had 5 potential host plants and a source plant containing adult whiteflies.

ing a stream of air over them using a disposable plastic pipette, and the leaves were examined under a dissecting microscope to count the total number of eggs. The cumulative number of eggs for each target plant species was determined. The number of spirals per plant and the number of eggs in each spiral was recorded and calculated for both the source plant (bird of paradise) and the target host plants.

SUPPLEMENTAL FIELD DATA

To compare our data to field data in southern Florida, pest detection records were obtained from the Florida Department of Agriculture and Consumer Services—Division of Plant Industry (Ian Stocks, personal communication). These records were analyzed and summarized to find the total percent contribution of each host plant to the total number of samples sent to Division of Plant Industry for identification.

LEAF SIZE

The mean leaf surface area of each plant species was calculated by randomly choosing and collecting a subset (19–41, due to the limited number of leaves on bird of paradise) of infested leaves from each plant species. Each leaf was flattened on a cork board using insect pins, and a scientific ruler was placed close to the leaf to be used as a scale in digital

size calibration. Photos were taken of the leaves at a perpendicular angle to reduce any angular distortion. These photos were digitized using the program tpsDig v.2 (James Rohlf, Stony Brook University, Stony Brook, New York, USA). The leaf outline was digitized using the “background curve” tool in tpsDig and all curves were re-sampled to 50 points around the outline of the shape. The leaf area was calculated using the program Geometric Morphometrics Tool Package (Taravati 2009), and the result was imported into Microsoft Excel. Leaf area and oviposition data were used to investigate any correlations between them.

WHITEFLY SURVIVAL

Survival of the whiteflies on each target plant species was determined by counting 100 eggs on each host plant species using a magnifying hand lens. Leaves containing these selected 100 eggs were enclosed in a paint-strainer bag (Trimaco 3.78 L Elastic Top Strainer, Morrisville, North Carolina, USA) made of tight nylon mesh. The opening of the bag was wrapped snugly around the branch with a pipe cleaner wire tightly wrapped around the strainer and the branch to prevent natural enemies from contaminating the contents or adult whiteflies from escaping. The leaves were monitored for adult whitefly emergence. The number of emerged adults was counted, and the survival was calculated by dividing the number of adults by the number of eggs.

STATISTICS

The numbers of spirals, numbers of eggs, and data from the Florida Department of Agriculture and Consumer Services—Division of Plant Industry were analyzed using a chi-square test for equal proportions in the program R, ver. 3.3.1 (R Development Core Team 2008). Leaf area, eggs per spiral, and egg-to-adult survival were tested for normality using the Shapiro-Wilk test, and all were found to have non-normal distribution ($P < 0.05$). Thus, these data were analyzed using the non-parametric Kruskal-Wallis test. Pairwise (post-hoc) comparisons were conducted using the Mann-Whitney U test and Bonferroni correction in the statistical program PAST ver. 3.17 (Hammer et al. 2001).

Results

PRELIMINARY OBSERVATIONS

Observations on the oviposition behavior of whitefly revealed that when whiteflies were released on cut leaves and branches, adult females deposited wax and eggs indiscriminately on the closest leaves regardless of the plant species. Most of the eggs were deposited on bird of paradise. They also deposited many eggs on non-living objects, such as on the screen of the cage and plastic pots. Furthermore, a heavy aggregation of dead adult whiteflies was found on the top corner of cages toward the east. Most adult whiteflies died within 3 to 4 d after their release into cages. In contrast, adult whiteflies survived much longer, deposited fewer eggs per d, were rarely found dead on the corners of the cage, and very rarely deposited eggs on non-living objects when they were introduced into cages on live potted plants. Also, egg distribution was more even in the latter method. Based on these observations, whiteflies were introduced into cages on live potted plants instead of infested cut leaves in the oviposition preference test.

HOST PREFERENCE

There was a significant difference among the number of eggs deposited on different host plants ($\chi^2 = 8764.4$; $df = 4$; $P < 0.001$) with gumbo limbo having the most (16,956) and bird of paradise having the fewest (4,750) eggs (Table 1). The average cumulative number of eggs deposited over time for each host plant species is presented in Figure 2. The average cumulative number of eggs was greatest on gumbo limbo on all sampling dates.

The average number of eggs per spiral ranged from 22.5 to 31.2 when compared among the different plant hosts. There was a significant difference in the number of eggs per spiral deposited on different host plants ($\chi^2 = 99.42$; $df = 4$; $P < 0.001$), with gumbo limbo receiving the greatest number of eggs per spiral. Also, a strong and significant correlation was found between the number of spirals and the number of eggs per plant species ($r_s = 0.90$; $P < 0.05$).

In addition to the eggs deposited on the potential hosts, there was some oviposition on the source plants. Initially, some eggs were deposited on the source plant and then on the potential hosts. Oviposition continued on both the source and potential hosts. Among the potential hosts, the first egg spirals were seen on gumbo limbo and avocado. Adult females returned to the source plant after each egg laying on the target host plants. In other words, they flew back to the “emergence site” (the place they emerged as adults) after laying eggs on other parts of the source plant or potential hosts. The number of egg spirals continued to increase on both the source and potential hosts until the end of the study. On average, female whiteflies deposited $6.1 \pm 2.2\%$ of the total number of eggs on the source plant and the remainder on the potential hosts. At the end of the study, all of the potential hosts and source plants still had non-infested leaves or a considerable amount of clean area on their leaves available for oviposition.

SUPPLEMENTAL FIELD DATA

The analysis on data from the Division of Plant Industry revealed that out of 475 plant samples sent to them for verification of whitefly infestation, the highest number came from gumbo limbo (15%), followed by coconut (11%), *Calophyllum* spp. (Calophyllaceae) (11%), avocado (9%), black olive (5%), *Phoenix roebelenii* O'Brien (Aracaceae) (3%), *Mangifera indica* Bl. (Anacardiaceae) (3%), and bird of paradise (2%). There was a statistically significant difference among the number of reported infestations from the 5 host plants species used in our experiment mentioned above ($\chi^2 = 57.54$; $df = 4$; $P < 0.001$), with the trend largely consistent with our observations under controlled conditions.

LEAF SIZE

There was a significant difference in leaf size area among the 5 host plants ($\chi^2 = 101.7$; $df = 4$; $P < 0.001$) with bird of paradise having the largest leaf area and black olive having the smallest leaf area (Table 1). However, no significant correlation was found between the average leaf size and the total number of eggs deposited on each of the plant species ($r_s = -0.30$; $P = 0.51$).

WHITEFLY SURVIVAL

Egg-to-adult survival in our experiment ranged from 5 to 10% but there was not a statistically significant difference ($\chi^2 = 1.72$; $df = 4$; $P > 0.05$) in survival among the different host plants (Table 1).

Discussion

Preliminary observations showed that releasing whiteflies on cut leaves is not a suitable method for measuring oviposition preference, and may result in strongly skewed and inaccurate data. The principal

Table 1. Distribution of eggs and egg spirals recorded in the host preference test. Also provided are leaf area and the total number of eggs and egg spirals deposited on each plant species. Mean values are reported as mean \pm S.E.

	Gumbo Limbo	Avocado	Coconut	Black Olive	Giant White Bird of Paradise
Leaf area (cm ²)	24.4 \pm 4.8 a	58.6 \pm 8.5 b	39.3 \pm 5.5 b	3.3 \pm 0.6 c	1138.5 \pm 149.8 d
Total no. of spirals deposited	543	389	441	266	200
Total no. of eggs deposited	16,956	10,598	9,933	6,853	4,750
Eggs per spiral	31.2 \pm 1.3 a	27.2 \pm 1.3 b	22.5 \pm 1.1 c	25.8 \pm 1.5 b c	23.8 \pm 1.9 c
% Survival (Mean \pm SE)	9 \pm 5	5 \pm 2	10 \pm 4	5 \pm 2	7 \pm 2

Means \pm SE within a row followed by the same letter are not significantly different ($P > 0.05$).

reason for this could be because of the rapid deterioration of cut leaves and the resulting disturbance to adult whiteflies, forcing them to leave and find a new host. The aggregation of adult whiteflies on the eastern side of the cage is consistent with a previous study that showed a strong flight phototaxis, especially right after dawn, on the eastern side of the cages (Taravati et al. 2014). In the preliminary study using cut leaves, many adults died on the eastern corners of the cages, probably because of the disturbance caused by the destruction of the cut leaves and the fact that whiteflies were not able to return to their eclosion site to continue feeding.

Gumbo limbo was the most preferred, and bird of paradise was the least preferred host plant in our experiment. The preference for gumbo limbo is supported not only by the number of eggs deposited on this species, but also the fact that gumbo limbo was one of the 2 potential hosts that received the first series of eggs in our experiment. In addition, gumbo limbo had a significantly greater number of eggs per spiral among the host plant species. Gumbo limbo is one of the most commonly infested plants in the southern Florida landscape (Stocks & Hodges 2012). Our result is consistent with the Division of Plant Industry data, where gumbo limbo was the most-reported host plant and bird of paradise was the least-reported host plant species for whitefly among the 5 host plants used in our study. Based on the above-mentioned observations and the consistency of our data with that of the Division of Plant Industry, we believe that the result of our experiment is representative of the whitefly host plant preference in southern Florida fields. The lack of significant correlation between leaf size and the number of eggs deposited on leaves suggests that whitefly females do not simply prefer plant species

with larger leaves for oviposition when given the choices we provided in our experiment. Leaves of bird of paradise are about 20 times larger than avocado, and 380 times larger than black olive leaves on average, but despite these huge size differences, bird of paradise plants received the lowest number of eggs in our experiment. The plant species used in our host preference experiments had a very different spatial structure and leaf surface area. For example, bird of paradise has large, upright leaves, whereas black olive var. 'Shady Lady' has very small, mixed-angle leaves. The coconut plants received the lowest cumulative number of eggs in the beginning of the experiment, but later surpassed that of bird of paradise and black olive (Fig. 2). This could be an example of the influence of plant architecture, because coconuts in our experiment differed from the other plants in their spatial structure of the fronds, which are spread horizontally. Within the cage, a large number of coconut leaflets (on the fronds) were relatively far away from the source plant due to the horizontal spread. These leaflets did not receive many eggs in the early stages of the experiment, but started to receive more eggs as more and more leaves of other plant species that were closer to these leaflets were used for egg laying. As the experiment progressed, adult females probably had to increase their flight range in search of uninfested leaves within the cages, eventually encountering those coconut leaves further away. This hypothesis needs further investigation to be confirmed.

Survival from egg to adult was generally low on all plants, which may be partially explained by the fact that many of the whiteflies became infected with fungal pathogens. Our experiment site received a considerable amount of rain during the whitefly survival study period, which explains the prevalence of fungal pathogens and high mortality of whiteflies.

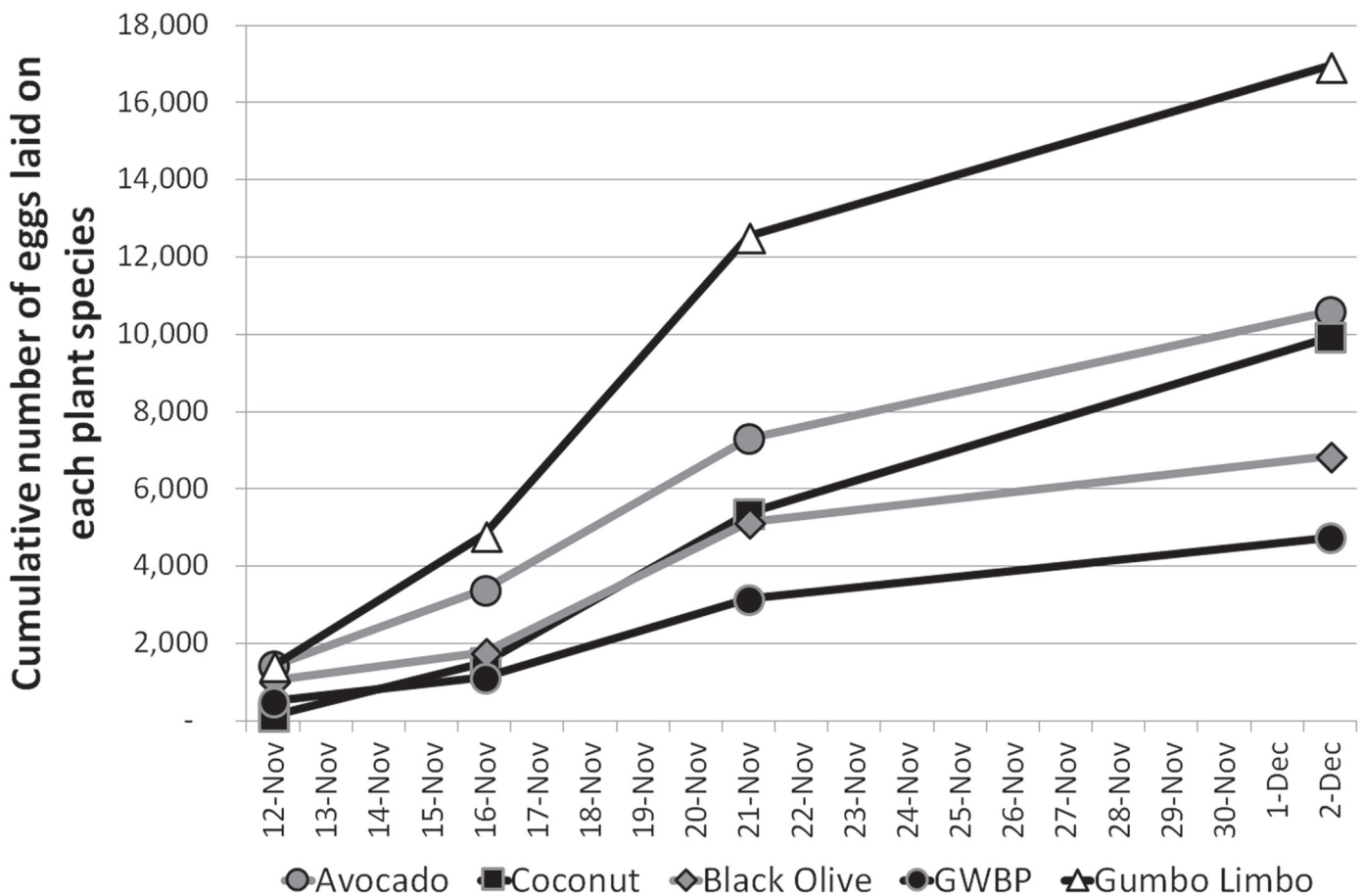


Fig. 2. The cumulative number of eggs deposited on different plants during the experiment. The average cumulative number of eggs was always greatest on gumbo limbo.

Observations during our experiment and the preparation period prior to experimentation suggest that the female whitefly deposits her first egg spirals close to the place where it emerged as an adult, regardless of the plant on which it developed. This is true provided that the host plant is not under stress or deteriorating. Observations that the adult female whiteflies return to their eclosion site after each egg laying effort is consistent with a previous study (Taravati & Mannion 2015), and suggests that whiteflies use one or more type of cues to locate their eclosion site.

Acknowledgments

We would like to thank Amanda Hodges (University of Florida, Entomology and Nematology Department, Gainesville, Florida) and Aaron Palmateer (University of Florida, Tropical Research and Education Center, Homestead, Florida) for their guidance as PhD committee members. Anthony Boughton (USDA, Laredo, Texas) made many suggestions regarding the experimental design, for which we are grateful. A special thanks to Ian Stocks (Florida Department of Agriculture and Consumer Services—Division of Plant Industry) for sharing the whitefly detection report database. Also, we are grateful to Holly Glenn and Rebecca Tannenbaum for assistance in data collection and experiment setup. A special thanks to James Colee (University of Florida, Department of Statistics, Gainesville, Florida) for his assistance in statistical data analysis.

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