Feeding and Development of Nephaspis oculata (Coleoptera: Coccinellidae) on Rugose Spiraling Whitefly (Hemiptera: Aleyrodidae)

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Feeding and development of *Nephaspis oculata* (Coleoptera: Coccinellidae) on rugose spiraling whitefly (Hemiptera: Aleurodidae)

**Siavash Taravati**, *, **Catharine Mannion**, **Lance Osborne**, and **Cindy McKenzie**

### Abstract

*Nephaspis oculata* (Blatchley) (Coleoptera: Coccinellidae) is a predatory lady beetle that has been studied for biological control of whiteflies. Here, we studied the feeding rate and development of this beetle on rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleurodidae), an invasive whitefly species that was first found in the United States in 2009. The objective of our study was to measure the feeding rate of *N. oculata* on rugose spiraling whitefly and compare the egg-to-adult development on eggs and nymphs. Our results showed that *N. oculata* is able to complete its development from egg to adult solely on rugose spiraling whitefly. At 26.7 °C, immature (1st to 4th instars combined) beetles consumed an average of 245.7 ± 14.4 rugose spiraling whitefly eggs prior to pupation. Adult beetles consumed 50.6 ± 1.8 eggs per day. There was no difference between the feeding rate of adult males and females or between immature males and females. Larvae searched for prey by sweeping the leaf surface in an arc-shaped area around them. Extra-oral digestion was observed during feeding, which involved withdrawing and regurgitating of fluids from and into the egg shell. It took 21.3 ± 0.7 d for the beetles to develop from egg to adult. The feeding rate of *N. oculata* on rugose spiraling whitefly was compared with that on *Bemisia tabaci* (Gennadius) (Hemiptera: Aleurodidae) at the same temperatures based on data from the literature. Adults of *N. oculata* consumed significantly lower number of eggs when fed on rugose spiraling whitefly compared with *B. tabaci*. However, rugose spiraling whitefly eggs are 3.7 times larger than *B. tabaci* eggs so the actual daily volume of rugose spiraling whitefly eggs consumed was 1.7 greater. Females of *N. oculata* produced significantly more adult progeny when fed on rugose spiraling whitefly eggs compared with adult progeny of *B. tabaci*. Therefore, rugose spiraling whitefly eggs could be considered a more suitable choice than nymphs for rearing *N. oculata*. Results of our study may help researchers to determine suitable beetle-to-whitefly release ratios and to improve rearing methods of this beetle.

### Key Words: biological control; lady beetle; Aleurodicinae; Florida

### Resumen

*Nephaspis oculata* (Blatchley) (Coleoptera: Coccinellidae) es un escarabajo maraquita depredador que ha sido estudiado para el control biológico de la mosca blanca. Aquí, se estudió la tasa de alimentación y desarrollo de este escarabajo sobre la mosca blanca espiral rugosa, *Aleurodicus rugioper- culatus* Martin (Hemiptera: Aleurodidae), una especie invasora de mosca blanca que se encontró por primera vez en los Estados Unidos en el 2009. El objetivo de nuestro estudio fue medir la tasa de alimentación de *N. oculata* sobre la mosca blanca espiral rugosa y comparar el desarrollo de huevo a adulto sobre los huevos y ninñas. Nuestros resultados mostraron que *N. oculata* es capaz de completar su desarrollo de huevo a adulto únicamente en la mosca blanca espiral rugosa. A los 26.7 °C, los escarabajos inmaduros (1º a 4º estadios combinados) consumen un promedio de 245.7 ± 14.4 huevos de mosca blanca espiral rugosa antes de la fase de pupa. Los escarabajos adultos consumen 50.6 ± 1.8 huevos por día. No hubo diferencia entre la velocidad de alimentación de los machos y hembras adultos o entre machos y hembras inmaduras. Las larvas buscan las presas mediante el barrido de la superficie de la hoja en un área en forma de arco alrededor de ellas. Se observó la digestión extraoral durante la alimentación, que implicaba la retirada y la regurgitación de líquidos desde y hacia la cáscara del huevo. Los escarabajos tomaron 21.3 ± 0.7 d para desarrollarse de huevo a adulto. Se comparó la tasa de alimentación de *N. oculata* sobre la mosca blanca espiral rugosa con la de *Bemisia tabaci* (Gennadius) (Hemiptera: Aleurodidae) a la misma temperatura en base a datos de la literatura. Los adultos de *N. oculata* consumieron un número de huevos significativamente menor cuando se alimentan de la mosca blanca espiral rugosa en comparación con *B. tabaci*. Sin embargo, los huevos de la mosca blanca rugosa espiral son 3.7 veces más grandes que los huevos de *B. tabaci* por lo que el volumen diario real de los huevos consumidos de la mosca blanca espiral rugosa fue de 1.7 mayor. Las hembras de *N. oculata* produjeron significativamente más progenies adultos cuando se alimentan de huevos de la mosca blanca espiral rugosa en comparación con las que se alimentaron sobre las ninñas de la mosca blanca espiral rugosa. Por lo tanto, se puede considerar que los huevos de la mosca blanca rugosa espiral es una opción más adecuada que las las ninñas de la mosca blanca espiral rugosa para la cría de *N. oculata*. Los resultados de nuestro estudio pueden ayudar a los investigadores a determinar el nivel de liberación adecuada de escarabajo-a-mosca blanca para mejorar los métodos de cría de este escarabajo.

### Palabras Clave: control biológico; mariquita; Aleurodicinae; Florida

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Rugose spiraling whitefly, *Aleurodicus rugioperculus* Martin (Hemiptera: Aleyrodidae), was first found in Florida in 2009, where it causes severe infestations of trees such as gumbo limbo (*Bursera simaruba* L.) Sarg.; Sapindales: Burseraceae), coconut (*Cocos nucifera* L.; Arecales: Arecaceae), black olive (*Buxus buxerus* L.; Myrtales: Commelinaceae), avocado (*Persea americana* Mill.; Laurales: Lauraceae), and *Calophyllum* species; Malpighiales: Calophyllaceae) (Stocks & Hodges 2012) and giant white bird of paradise (*Strelitzia nicolai* Regel & Körn.; Zingiberales: Strelitziaceae).

*Nephaspis oculata* (Blatchley) (Coleoptera: Coccinellidae) is a whitefly predator reported from the United States (Gordon 1985), Barbados (Peck 2009), and the Bahamas (Turnbow Jr & Thomas 2008). This beetle was first described in 1917 from Florida as *Synmus oculata* by Blatchley. Later, in 1952, Wingo described it as a new species, *N. nicola*, from Boone, Iowa, but the latter species is now considered as a synonym of *N. oculata* (Gordon 1985, 1997).

The biological control potential of this beetle has been studied against *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Liu et al. 1997; Liu & Stansly 1999, 2002) and spiraling whitefly, *Aleurodicus dispersus* Russel (Hemiptera: Aleyrodidae) (Kumashiro et al. 1983; Yoshida & Mau 1985). When fed on *B. tabaci* at 26.7 °C, adult beetles consumed 108 ± 15.6 whitefly eggs per day on average, and it took them 18.8 d on average to develop from egg to adult; males and females lived for 56.1 ± 9.4 and 67.5 ± 11.3 d on average, respectively (Liu et al. 1997). When fed on *A. dispersus* 4th instars, *N. oculata* completed egg-to-adult development within 26 ± 1 d on average at 23 to 26 °C (Yoshida & Mau 1985). It was found that *N. oculata* effectively controlled high population density of spiraling whitefly in Honolulu, Hawaii, whereas a parasitoid, *Encarsia hathiensis* Dozier (Hymenoptera: Aphelinidae), was more effective at low density of the same whitefly (Kumashiro et al. 1983).

*Nephaspis oculata* has been found on gumbo limbo trees infested with rugose spiraling whitefly in south Florida, where it feeds on rugose spiraling whitefly (Taravati et al. 2013a). This beetle has been collected from rugose spiraling whitefly infestations from 13 coastal counties in the south Florida region but probably has a much wider distribution in Florida (Francis et al. 2016).

*Nephaspis oculata* eggs are laid individually on the rugose spiraling whitefly-infested leaves and can be difficult to find because of small numbers and concealment in the wax flocculent material produced by rugose spiraling whitefly (Taravati et al. 2013a). Potential for establishment of *N. oculata* in rugose spiraling whitefly-infested landscape was first studied in Lee County on the west coast of Florida in 2011 where no beetles were found during initial sampling. After a single introduction of the beetle, it appeared to be successfully established, and beetle progeny were seen on both imidacloprid-treated and non-treated trees 6 mo after the insecticide application. Because of the natural association of *N. oculata* with rugose spiraling whitefly infestation in the landscape and the potential of combining biological and chemical control in the same area (Taravati et al. 2013b), it is important to quantify the predatory capacity of this beetle when feeding on rugose spiraling whitefly. In this paper, the feeding rate and development time of *N. oculata* on rugose spiraling whitefly eggs were studied to evaluate its ability in reducing rugose spiraling whitefly infestations. Also, the suitability of rugose spiraling whitefly eggs was compared with that of nymphs for rearing *N. oculata*.

**Materials and Methods**

Rugose spiraling whitefly and *N. oculata* adults were collected in Jun 2013 from gumbo limbo trees around Homestead, Florida. In-fested branch terminals containing whiteflies and beetles were cut using a pole cutter or a horticultural pruner and placed in plastic bags and transported to the laboratory. In the laboratory, these plastic bags were opened and placed in a glass-top wooden cage. Beetles were aspirated from the cage, and their sex was identified based on pronotum color, which is yellowish in males and dark in females (Gordon 1997). Rugose spiraling whitefly-infested leaves were collected from gumbo limbo trees at the University of Florida’s Tropical Research and Education Center (UF-TREC), Homestead, Florida. Male–female beetle pairs were placed into 60 × 15 mm polystyrene Petri dishes (Falcon® lined with a single layer of filter paper (Fischerbrand® qualitative grade PS) containing gumbo limbo leaf sections with rugose spiraling whitefly eggs.

Petri dishes each containing a pair of adult beetles were kept in an incubator set at 26.7 °C and checked daily for egg laying. Beetle eggs were distinguished from rugose spiraling whitefly eggs by their larger size (Fig. 1a) and different shape. Beetles eggs were gently transferred into a new Petri dish by cutting the leaf area around the egg using a pruner and using entomological forceps to remove the egg with minimum damage. There was 1 egg per Petri dish, and the filter paper in each Petri dish was kept wet by adding a few droplets of water as needed. Filter papers were replaced with new ones as needed. All Petri dishes were placed in large trays and kept in an incubator set at 26.7 °C. Twenty-three and 38 *N. oculata* eggs were collected and used for the 1st and 2nd repetitions of the experiment, respectively.

Beele eggs were evaluated daily for larval emergence and as soon as the larvae emerged, they were provided with rugose spiraling whitefly eggs from fresh gumbo limbo leaves collected at UF-TREC and taken to the laboratory to be provided to the beetles. To accurately count the whitefly eggs, the wax covering the eggs was partially removed from the leaves by blowing a stream of air over them with a disposable plastic pipette. Care was taken not to remove too much wax from the whitefly eggs as this could create a different situation to that which beetles encounter naturally in the landscape. Then, the whitefly eggs were counted and leaf sections containing the eggs were placed in Petri dishes with the beetle larvae.

Each day, the number of rugose spiraling whitefly eggs consumed since the previous day, the development stage and body length of *N. oculata* individuals, and any specific behavior of the beetle were recorded. Body length was measured from the anterior portion of the head capsule to the posterior portion of the uropod by using an ocular micrometer on a LEICA MZ 125 stereo-microscope. Larvae were measured when they were in a neutral position (i.e., not moving, neither stretched nor contracted). Both the body size and the presence of exuviae were used as criteria for identifying larval stages of the beetle. Exuviae were removed from the Petri dish as soon as seen to prevent confusion about the life stage on later days. Feeding rate of resulting adults was measured for 11 d after eclosion. Also, 20 adult beetles were collected from the field in the Homestead, Florida, area and randomly selected to be used in a similar feeding rate study for comparison with the feeding rate of laboratory-reared adult beetles. The experiment was repeated on 30 Jul 2013 using infested leaves of gumbo limbo trees at the UF-TREC, Homestead, Florida.

For comparing *N. oculata* egg consumption rate on *B. tabaci* and rugose spiraling whitefly eggs, the mean egg size of both whitefly species was calculated using an ocular micrometer on a LEICA MZ 125 stereomicroscope. Rugose spiraling whitefly eggs were obtained by placing clean giant white bird of paradise (*S. nicolai*) plants into 35.5 × 35.5 × 61.0 cm (14 × 14 × 24 inches) rearing and observational cages (BioQuip®, Compton, California) containing *S. nicolai* plants infested with adult whiteflies. After a few days, plants were removed from the cages, and leaf sections containing rugose spiraling whitefly eggs were selected randomly and cut from these plants for egg measurement.
Bemisia tabaci eggs were obtained from a colony reared on collard in the insectary and measured as above. Eggs \( n = 30 \) were assumed to have a perfect prolate spheroid shape (Vafaie et al. 2013) and the volume was calculated using the equation: \( V = \frac{4}{3}\pi \times a \times b^2 \), which is a formula for calculating the volume of a prolate spheroid where \( a \) is the major (longer) diameter of the ellipsoid and \( b \) is the minor diameter of the ellipsoid. The feeding rate data of \( N. oculata \) on rugose spiraling whitefly eggs were compared to the feeding rate data on \( B. tabaci \) eggs reared at the same temperature adopted from Liu et al. (1997).

Rearing tests were conducted in Jul–Aug 2014 in the UF-TREC insectary at 27 °C to compare the fertility of the beetle fed on rugose spiraling whitefly eggs versus nymphs. To do so, potted \( S. nicolai \) plants were infested with rugose spiraling whitefly eggs by placing them in whitefly colony cages for a few days to allow oviposition to occur on the leaves. The plants were removed from the whitefly colony cages and placed in new cages until the start of the experiment. Leaves containing 1- to 4-d-old eggs (2,000–3,000 eggs) and the ones containing 3rd to 4th instars (200–350 nymphs) were cut from the infested plants, moved to the insectary, and placed into water-filled flasks. The pedicel of the leaf was

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**Fig. 1.** Life stages of *Nephaspis oculata*. a) A single beetle's egg (arrow) laid adjacent to a row of rugose spiraling whitefly eggs. Rugose spiraling whitefly eggs become darker as they age as can be seen here. The wax on the eggs was removed in order to take this photo. b) Larva feeding on a rugose spiraling whitefly egg. c) An adult male feeding on a 4th instar of rugose spiraling whitefly. d) A hole (arrow) created by the feeding activity of a beetle. The hole looks similar to an exit-hole created by parasitoids but this one was created by *Nephaspis oculata* in the laboratory. Photo by Siavash Taravati.
Table 1 summarizes the development and feeding rate of *N. oculata* fed on rugose spiraling whitefly eggs. Daily feeding rate on rugose spiraling whitefly eggs increased with each subsequent larval stage. Adults reared from eggs in the laboratory consumed an average of 50.6 ± 1.24 (mean ± SE) eggs daily over an 11 d period after emergence. Adult beetles collected from the field consumed an average of 51.1 ± 1.8 eggs daily, which was not significantly different from the feeding rate of laboratory-reared adults (*W* = 569, *P* > 0.05).

The 4th instar had the longest development time and the 3rd instar had the shortest; however, the development time for all the instars except the 4th was between 2 to 3 d (Table 1). Immature (1st to 4th instars) beetles consumed 245.7 ± 14.4 rugose spiraling whitefly eggs on average. There was no significant difference in the total feeding rate of immature males (234 ± 13.8 eggs) and females (268 ± 21.2 eggs) (*W* = 6, *P* > 0.05) or adult males (595 ± 27.3 eggs) and females (581 ± 45 eggs) (*W* = 12, *P* > 0.05) during the 11 d study period. Egg-to-adult development took 21.3 ± 0.3 d on average.

Larvae searched for prey by sweeping an arc-shaped area around them and moved from point to point by securing themselves with their uropod. The 1st instars ate the fewest eggs and were unable to extract all the fluid contents of the eggs especially within 24 h after emergence. Consequently, consumed eggs had a depression on one side and the other side was intact. Beetle larvae and adults fed on eggs by piercing the egg chorion and withdrawing the fluids from within the egg. This was followed by regurgitation of fluids back into the egg shell. This process was repeated several times before the final withdrawal of the fluids. During feeding, the egg shell was seen shrinking and blowing up like a balloon due to the withdrawing and regurgitation of fluids.

Beetle eggs were 0.39 ± 0.01 mm long on average, and the egg-to-adult survival of beetles was 52% in the 1st experiment and 65% in the repeat test. Pupal stages were sessile and motionless except when disturbed by vibrations caused by moving Petri dishes or by poking. When disturbed, they waved back and forth by raising their anterior body segments from the leaf and swinging back to the surface while being anchored to the leaf by their uropod.

Table 2 compares data from rugose spiraling whitefly and *B. tabaci* egg measurements and their corresponding egg volume. Rugose spiraling whitefly eggs were 3.7 times larger (Table 2; Fig. 2) in volume than *B. tabaci* eggs on average, and there was a significant difference between the egg volumes of these 2 species. The estimated average daily volume of eggs consumed by *N. oculata* adults was also significantly different when feeding on rugose spiraling whitefly compared with data from Liu et al. (1997) on *B. tabaci*. The significance of the difference is indicated by a lack of overlap between the mean ± 95% C.I. (S.E.) of the 2 groups.

Females of *N. oculata* feeding on rugose spiraling whitefly eggs produced significantly more adult beetles in the next generation (F1) when compared with the females that fed on nymphs (*W* = 2.5, *P* < 0.05). Females that fed on eggs produced an average of 13.8 ± 3.2 adult beetles and those fed on nymphs produced 5.0 ± 1.3 adult beetles per female in the F1 generation over the average course of 23 d.

### Discussion

Results from the experiments showed that *N. oculata* is able to complete its development from egg to adult solely on rugose spiraling whitefly reared either on gumbo limbo or on *S. nicolai*. Our observa-
tion on the feeding behavior of *N. oculata* is similar to that of Yoshida & Mau (1985) in which beetles were seen withdrawing and regurgitating fluids and body contents from and to the eggs and nymphs of whiteflies. This behavior suggests an extra-oral digestion. In fact, many lady beetles show extra-oral digestion, and they extract and regurgitate fluids from and to the body of their host by piercing (Fig. 1b, c, and d) the cuticle of the hosts and withdrawing the pre-digested food (Hodek et al. 2012). This phenomenon is not limited to lady beetles but is very common in land-dwelling predatory arthropods with an estimation of 79% using extra-oral digestion (Cohen 1995). The movement and searching behavior of *N. oculata* in our study was similar to that described by Liu & Stansly (1999) in which the larvae swept their body over the leaf surface in an arc and used their uropod for securing themselves before moving to the next spot.

The finding that the feeding rate of field-collected *N. oculata* beetles was not significantly different from the feeding rate of laboratory-reared beetles on rugose spiraling whitefly eggs could be related to the fact that the lab laboratory-reared beetles were reared for only 1 generation, which may not be long enough to cause any major physiological or behavioral change in the insects.

We estimated that *N. oculata* adults consumed a greater volume of rugose spiraling whitefly eggs per day in our study compared with the volume reported by Liu et al. (1997) for *B. tabaci* eggs at comparable temperatures. However, the beetles in our study had a slower egg-to-adult development (21.3 d compared with 18.8 d). The effect of prey type on biological parameters of predatory Coccinellidae has been studied by various authors (Omkar & Srivastava 2003; Zhang et al. 2007). For example, Omkar & Srivastava (2003) found that *Coccinella septempunctata* L. develops fastest on *Lipaphis erysimi* (Keltenbach) and slowest on *Aphis nerii* Boyer de Fonscolombe among 6 aphid species (Hemiptera: Aphididae) used in the study. Similarly, the host plant can also influence the performance of predatory lady beetles. For example, *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae) showed highest predation on *B. tabaci* when fed whiteflies reared on cotton and had the lowest predation when fed whiteflies reared on *hibiscus* (Legaspi et al. 2006).

Although our experiment was conducted at the same temperature (26.7 °C) at which Liu et al.’s (1997) study was done, both the host plant and whitefly species were different between the 2 studies, which may explain the significant difference between the daily volume of eggs consumed and the development time of *N. oculata* on the 2 whitefly species. The faster development time of *N. oculata* in Liu et al. (1997) may be associated with higher nutritional value of *B. tabaci* (reared on collard) eggs compared with rugose spiraling whitefly eggs (reared on *S. nicolai*) in our study. Nevertheless, because these data are results of different experiments using potentially different strains of *N. oculata* from Florida, further studies might be needed to confirm this hypothesis. Although we estimated the standard error of Liu et al.’s (1997) data using error propagation, we could not run a statistical test because we did not have access to replicated data from that study. Hence, any statistical comparison using standard error range should be done with caution.

Our study showed a significantly higher reproduction of *N. oculata* when reared on rugose spiraling whitefly eggs compared with nymphs. We observed that adult beetles consumed eggs faster than 3rd and 4th instar beetle larvae. The 1st and 2nd instar beetle larvae are smaller in size than later stages and so have lower feeding capacity and are more affected by the size and hardness of their prey. A preference for eggs over nymphs of *B. tabaci* has been reported for *N. oculata* (Liu et al. 1997) and this is consistent with the result of our work mentioned above. In another study, it took 1st instars of *N. oculata* 22.3 and 194.8 min to consume the eggs and pupae of *B. tabaci* respectively; on the other hand, it took 4th instars of *N. oculata* 2.5 and 46.4 min to consume eggs and pupae of *B. tabaci*, respectively (Liu & Stansly 1999).

To conclude, our study quantified the feeding rate and development of *N. oculata* on rugose spiraling whitefly eggs and compared them with results from a similar study (Liu et al. 1997) on *B. tabaci*. Our study showed that rugose spiraling whitefly eggs are a more suitable choice than nymphs for rearing *N. oculata*. Because of the natural association of *N. oculata* with rugose spiraling whitefly infestations in the landscapes of south Florida, it may be useful to incorporate the results of this study into biological control strategies including the development of predator-to-pest release ratios. Female rugose spiraling whiteflies lay an average of 5.2 eggs per day when reared in groups on *S. nicolai* (Taravati & Mannion 2015). Considering the feeding rate of *N. oculata* adults (50.6 eggs per day) on rugose spiraling whitefly eggs, a single beetle can destroy all the eggs laid daily by 9.7 (50.6 ÷ 5.2) rugose spiraling whitefly females on average, which suggests an adult-beetle-to-adult-whitefly release ratio of 1:9.7.

The optimal beetle-to-whitefly release ratio on rugose spiraling whitefly infestations needs a separate study, but similar work on the same beetle species feeding on *B. tabaci* has shown the importance of release timing on the efficacy of biological control. It was shown that...
when released 1 d after B. tabaci introduction to a cage, N. oculata can efficiently control the whitefly population at the 1:4 and 1:20 beetle-to-whitefly ratios (Liu & Stansly 2005). However, when beetles were released 7 d after whitefly introduction, beetles were efficient only at the 1:4 ratio and not at the 1:20 release ratio (Liu & Stansly 2005).

We hope that our study will help researchers to identify the potential of this beetle for controlling whitefly infestations. This might become very important in dealing with new invasive whitefly species of the subfamily Aleurodicinae. Florida is very susceptible to invasion by the members of Aleurodicinae, which are native to the Caribbean Islands and parts of South America with similar climate and proximity to Florida. Although parasitoids such as Encarsia noyesi (Hayat) (Hymenoptera: Aphelinidae) are believed to be very effective in controlling rugose spiraling whitefly (Boughton et al. 2015), they are not effective against all species of whitefly. Consequently, having a predator such as N. oculata in the integrated pest management toolbox may help researchers and pest control professionals to control whitefly infestations with no or minimal insecticide applications whenever it is appropriate.

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