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Comparing the Fitness of Captive Bred *Anagyrus kamali* (Moursi) Wasps to Wild Populations

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COMPARING THE FITNESS OF CAPTIVE BRED *ANAGYRUS KAMALI* (MOURSI)
WASPS TO WILD POPULATIONS

By

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A capstone project submitted for
Graduation with University Honors

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APPROVED

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Abstract

Classical biological control is an effective method of reducing numbers of the invasive pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green). *Anagyrus kamali* (Moursi) is a parasitic wasp that has been used throughout the world for this type of control. Large scale insectaries breed millions of parasitoids for commercial release in agriculture, but little is known about the effect of long-term captive breeding on the fitness of *A. kamali*. This study evaluated the fitness of two populations of *A. kamali*, a wild type population collected in the U.S. and a captive bred population from Mexico. Average body size, an estimate of fitness, was found to be significantly different between the two populations. The Mexico population was consistently close to a constant average with a linear regression line slope of 0.002 from January to June, while the U.S. body size increased rapidly from February to April with a linear regression line slope of 0.157. Oviposition, another measure of fitness, generally was low in this study, but the U.S. oviposition rates were equal to, or numerically higher than, the Mexico population oviposition rates. In the first trial both populations had approximately 7% oviposition rate, but in the second trial the U.S. population oviposition rate was 13% compared to 1.7% for the Mexico population. These results challenge the conclusions of some previous studies as well as provide a basis for further research on the fitness of captive and wild populations of *A. kamali*.

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Introduction

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green) (Figure 1) first was described in India in 1908. Within four years in 1912, the first known invasion of *M. hirsutus* into another region was recorded in Egypt (Hall, 1926). From there it spread to Africa and Hawaii in 1956 and 1984, respectively, and in 1994 it was detected in the Caribbean on the island of Grenada (Hall, 1926; Williams, 1986; Michaud and Evans, 2000). Over the next several years it spread to the rest of the Eastern Caribbean, and in 1999 the first PHM population was discovered in North America in the Imperial and Mexicali Valleys of the United States and Mexico (Roltsch et al. 2006).



Figure 1. *Maconellicoccus hirsutus* egg sacs (A), 1st instar “crawlers” (B), 2nd instar female (C), 3rd instar female (D), immature male (E), mature male (F) (Chong et al. 2015).

Maconellicoccus hirsutus is known to infest over 330 different plant species, including many economically significant food crops, and species used for ornamental purposes (Meyerdirk et al. 2001, Chong et al. 2015). In the Coachella Valley (Riverside County, California), it readily infests carob tree (*Ceratonia siliqua*, L.) (Figure 2 G,H), hibiscus (*Hibiscus spp.*, L.) (Figure 2 C,D), mulberry (*Morus spp.*, L.) (Figure 2 A,B),

and silk oak (*Grevillea robusta*, Cunningham) (Figure 2 E,F). In addition to causing leaf and fruit damage, *M. hirsutus* has been found to transmit plant viruses, and their copious honeydew secretions encourage the growth of black sooty mold fungus (Eid, 2011).



Figure 2. Mulberry tree (A), PHM damage on mulberry branches (B), Hibiscus bush (C), PHM damage on hibiscus branches (D), Silk Oak (E), PHM damage on silk oak (F), Carob tree (G), PHM damage on carob tree (H).

The CABI International Institute of Biological Control imported the endoparasitoid wasp, *Anagryus kamali* (Moursi) (Figure 3), to the Caribbean in order to

implement a classical biological control program (Kairo et al., 2000). This program was shown to effectively reduce mealybug populations by up to 95% in the Imperial Valley of California in 2001-2004 (Roltsch et al. 2006).



Figure 3. Female *Anagyrus kamali* wasp under 20x magnification.

Due to the success of this program, and as *M. hirsutus* spread through the Eastern Caribbean to South and Central America, insectaries began to raise *A. kamali* for commercial release and many of these insectaries continue to rear this parasitoid. Very little is known about the effect of rearing many successive generations of *A. kamali* in captivity on their fitness and searching ability compared to wild populations. Traits that make *A. kamali* effective biological control agents in the field, such as searching ability over long distances, oviposition rate, and fecundity are not needed by captive *A. kamali* raised in small cages provided with an abundance of food and large densities of PHM for oviposition. Over time, parasitoids with traits that are more suited to life in a small cage can breed more prolifically in the cage than the wild type, eventually replacing them.

Loss of ability due to this natural selection in captive populations is a potential risk that could reduce the effectiveness of *A. kamali* in the field.

Sagarra et al. (2001) showed a positive correlation between body size and overall fitness of *A. kamali*. Because the fitness of *A. kamali* is paramount to its success as a biological control agent, the following questions were addressed; (1) is the body size of *A. kamali* significantly affected by long term captive breeding, and (2) is the oviposition rate significantly affected by long term captive breeding? These questions can be useful in expanding our understanding of how captive breeding impacts the overall fitness of *A. kamali*.

Materials and Methods

Rearing of Maconellicoccus hirsutus

Maconellicoccus hirsutus mealybugs were reared in a room maintained in 24 hour darkness at 26°C and 40% RH. The mealybugs were reared on sweet dumpling (*Cucurbita pepo* L. ‘Sweet Dumpling’ L.) and shishigatani squash (*Cucurbita moschata* Duchesne ‘Shishigatani’) fruit in the University of California Riverside’s (UCR) biosafety level 2 quarantine facility. Once the female mealybugs matured to adults, the squash were placed in a brood box with a pinpoint light directed at a manila folder placed in the box. Newly hatched 1st instar mealybugs called “crawlers” concentrated under the light for easy collection (Figure 4). The crawlers were collected with a soft paintbrush into a metal sieve and evenly distributed in a single layer onto a petri dish. A dissection microscope was used to search for mites (family Tetranychidae) among the PHM. Once the dish was confirmed to be free of mites, the PHM crawlers were transferred back to

the metal sieve using the soft paintbrush. This sieve then was used to evenly distribute PHM over fresh squash. These infested squash were kept in the rearing room until they were either used for the parasitoid colony or place in the brood box for collection of crawlers.



Figure 4. Brood box shown with pinpoint lights and aggregation of newly hatched crawlers.

Rearing of Anagyrus kamali

Anagyrus kamali were collected in the Imperial Valley in the fall of 2015 and used to start a colony. These wasps (designated U.S.) were reared in a 14:10 light:dark cycle at 26°C and 40% RH in the quarantine facility at UCR. When the hyperparasitoid *Chartocerus spp.*, a parasite whose host is *A. kamali*, was discovered in the rearing room, a monthly cage rotation was implemented to prevent its establishment in the colonies. Every weekday, fresh honey was streaked onto the top of the inside of each cage with an applicator made of a thin wooden dowel and a single cat whisker; *A. kamali* adults fed on this honey. Distilled water was added to small dishes filled with cotton to provide water

to the parasitoids. As needed, new squash with second instar mealybugs were taken from the PHM room and placed in each cage for wasps to infest. When PHM began to hatch from the older squash within the cages, those squash were removed in order to prevent the parasitoid cages from becoming overrun with crawlers. For the Mexico population, *A. kamali* adults were reared at the Laboratorio Regional de Reproducción de Agentes de Control Biológico, Valle de Banderas, Nayarit. CP. 63730, Mexico. These parasitoids have been reared for 10 years, and with a generation time estimated at 30 days, this is equivalent to some 150 generations. Insects from Mexico were sent to UCR to start a colony that was used for the oviposition study. This colony was kept in the quarantine facility in a room separate from the U.S. colony to prevent accidental colony blending.

Body Measurements

Sagarra et al. (2001) determined that body size of *A. kamali* adults was positively correlated to overall fitness in several categories including longevity, mating preference, fecundity, reproductive longevity, progeny emergence, and sex ratio. In addition, body size was positively correlated to tibia length (Sagarra et al. 2001). To confirm the relationship between body size and tibia length, 10 individuals were collected from 6 different shipments that arrived from Mexico between October 2015 and February 2016. Parasitoids were placed into small glass vials filled with 70% EtOH until the measurements were made. To measure the body size and tibia lengths, each insect was placed in a petri dish and covered with ethanol and the measurements were made with a micrometer etched into one of the lenses of a dissecting microscope; all measurements were made at 20x magnification. The tibia of *A. kamali* is defined as the fourth segment of the leg, counting from the body of the parasitoid, and it is considered analogous to the

tibia of the human leg. The body length was measured from the vertex of the head between the eyes to the tip of the abdomen, and tibia length was measured from its connection to the femur (third segment of the leg) to its connection with the basitarsus (fifth segment of the leg).

Body lengths of female parasitoids reared in Mexico were compared to U.S. parasitoids. For this study, adult parasitoids from Mexico were collected from 6 different shipments sent from December 2015 to June 2016. Ten adults from each shipment were placed into small glass vials filled with 70% ethanol (EtOH) and kept until the measurements could be made. Parasitoids from California were collected from quarantine cages at 6 different times between February 2016 and April 2016. 10 females were collected each time and placed in a vial of 70% EtOH for transport out of quarantine. All measurements were made at 20X magnification as described above.

Oviposition

Two different methods of providing leaf material as food for PHM were tested. In the first method, cotton was wrapped around either hibiscus or citrus leaf stems, then placed in a small glass vial with the stem submerged in water and the leaf sticking out the top as shown in Figure 5.



Figure 5. First method of providing leaf material. Leaves are wrapped in cotton at the stem and placed in glass vial filled with water.

Ten second instar mealybug nymphs were removed from infested squash with a paintbrush, and placed on each leaf. These infested leaves were observed for 10 days to see how well the PHM settled on the leaves. While the hibiscus leaves were highly preferred to the citrus leaves, neither retained a satisfactory number of mealybugs after 24 hours. Thus a second method was developed. Arenas were constructed out of two petri dishes, the smaller one 3.8 cm in diameter with a hole drilled in the bottom (Figure 6 B). A small strip of cotton was threaded through the hole in the bottom of the smaller dish through which water would wick from the larger dish to the smaller dish. The wick was held in place by a circle of cotton that covered the bottom of the smaller dish. A circle of hibiscus leaf was cut to fit the petri dish (Figure 6 A,B) and it was placed on top of the cotton. The inside edge of the dish was lined with cotton, completely encircling the leaf (Figure 6 C). This smaller dish was placed inside a larger 6.4 cm diameter dish (Figure 6

C). The larger dish was supplied with a small amount of water to keep the smaller dish and leaf hydrated, and a lid with a mesh hole was placed on top of the small dish to keep the wasps from escaping (Figure 6 D).

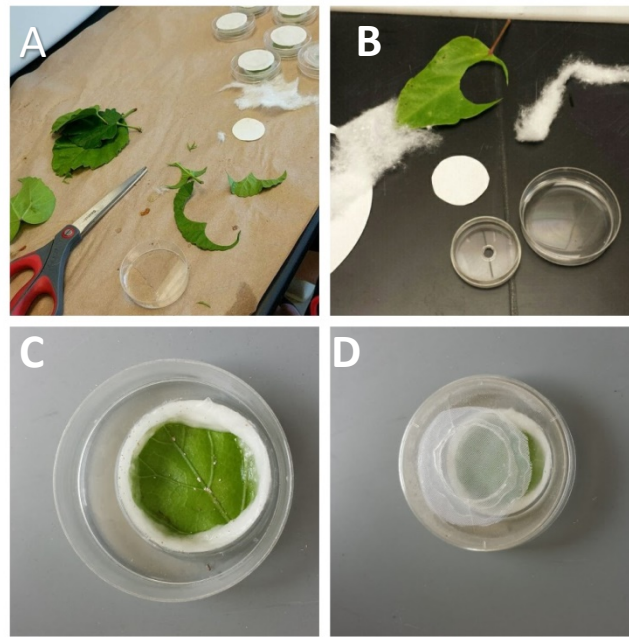


Figure 6. Assembly of arenas used to study parasitism of pink hibiscus mealybug. (A) foreground shows scissors and cut leaves, background contains completed arenas; (B) Cotton, leaf, circle mold for cutting leaves and cotton, and two petri dishes used in assembly, (C) Completed arena with lid off, (D), Completed arena with lid on.

15 second instar mealybug nymphs were individually transferred from squash to each arena using a small paintbrush. In the parasitoid room, 10-20 male and female wasps were separated from the colony into a smaller jar for 24 hours preceding the experiment to ensure they were at least 24 hours old and mated. The arenas with PHM were transported from the PHM room to the wasp room. Parasitoids in the smaller, separate jar were cooled with ice, then females were placed individually into the arenas using a small paintbrush. After 24 hours, wasps and mealybugs in each arena were collected into small labelled glass vials containing 70% EtOH. Mealybugs were observed under a microscope to search for egg pedicels which indicated that the egg had been parasitized. (Figure 7).

The number of pedicels per mealybug in each arena was recorded, and the average parasitization for Mexico parasitoids and U.S. parasitoids was determined.

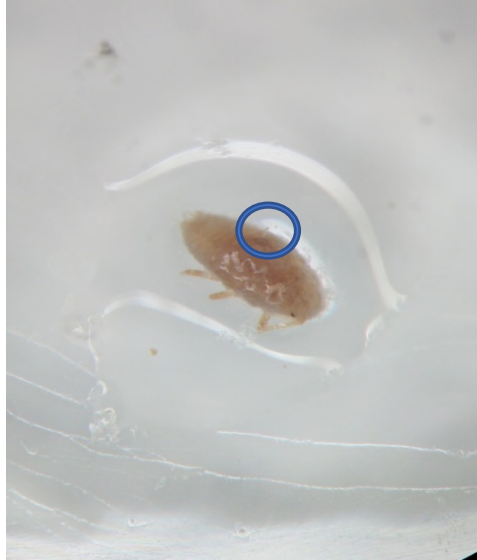


Figure 7. Circle indicates an egg pedicel placed in the body of a mealybug by a parasitoid.

Statistical Analyses

The relationships between body length and tibia length was determined using linear regression (Excel 2013, Microsoft Corp.). In addition, linear regression was used to regress time on *A. kamali*. The body length versus tibia length experiment had 1 treatment with 6 replicates over time, while body length had 2 treatments (Mexico and U.S.) with 6 replicates over time.

The relationship between place of origin (Mexico and U.S.) and oviposition was determined with a one-way Analysis of Variance (PROC ANOVA, SAS Institute, Cary, NC. v.9.4) for each trial (17 Feb. and 25 Feb). For these analyses, each parasitoid was treated as a replicate so there were 20 replicates in the first trial and 10 replicates in the

2nd trial. The percentage data were square root transformed to satisfy the conditions of ANOVA.

Results

Body Measurements

The R^2 value for the regression of body size on tibial length for *A. kamali* was 0.04537, indicating a very low relationship between the two variables (Figure 8). Because of this result, only body size was used to compare the Mexico population to the parasitoids from the U.S. The average body length of U.S. *A. kamali* for 6 trials ranged between 62.3 μm and 76.6 μm with a length versus time regression line R^2 value of 0.81664 (Figure 9). Average body length of Mexico *A. kamali* for 6 trials ranged between 68.2 μm and 70.8 μm with a length versus time regression line R^2 value of 0.01872 (Figure 9). The average body length of U.S. *A. kamali* increased rapidly over time compared to Mexico *A. kamali*, with a slope of 0.157. The slope of Mexico *A. kamali* over time was 0.0023 (Figure 9).

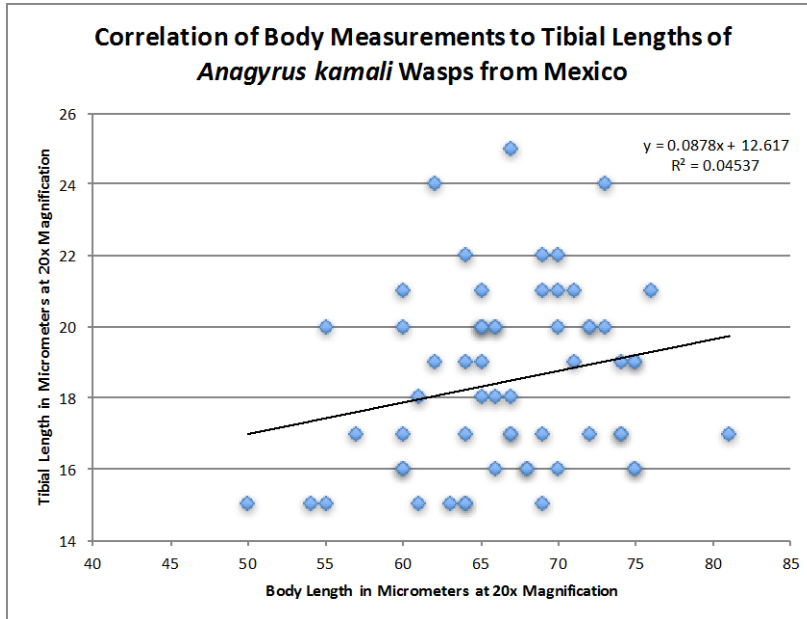


Figure 8. Linear regression of body measurements on tibial lengths of *A. kamali* wasps from Mexico. Data points were taken from 10 wasps each on 6 collection dates.

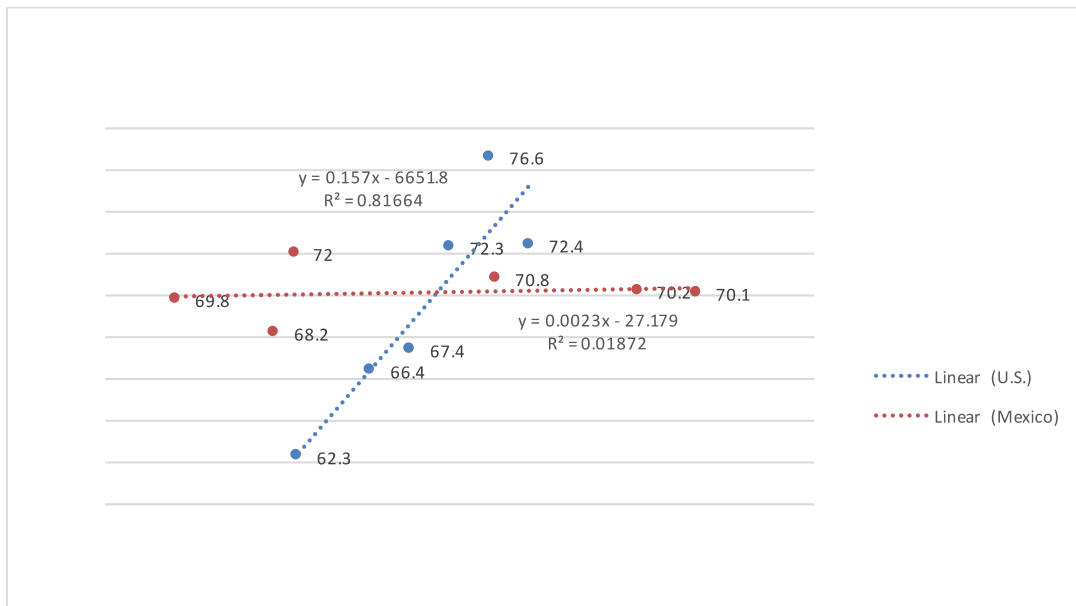


Figure 9. Comparison of body sizes of *A. kamali* from the U.S. and Mexico. Each point represents the average measurement from 10 wasps collected on 6 dates.

Oviposition

In the February 17 trial, the oviposition rate for the U.S. population was 7.3% and for the Mexico *A. kamali* it was 6.8%. These values were not statistically different ($P > 0.9663$). For the February 27 trial, there was a larger difference in the oviposition rates between the two populations, with the U.S. population having a rate of 13.0% while the rate for Mexico was 1.7% (Figure 10). Even with this difference, there was a substantial amount of experimental error in this study and so many of the parasitoids failed to lay any eggs. Thus there was no significant difference between the average oviposition rates of the two populations ($P > 0.1163$).

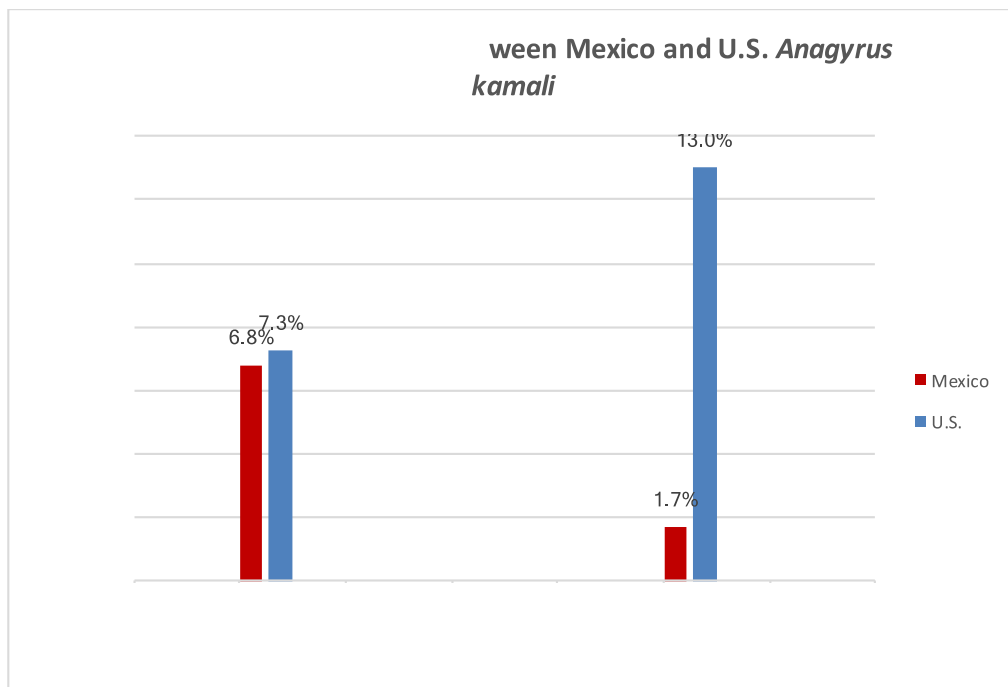


Figure 10. Comparison of oviposition rates between U.S. and Mexico *A. kamali*. Data were taken from one trial of 20 arenas for each country (17 Feb), and one trial of 10 arenas for each country (25 Feb)

Discussion:

Body size has been positively correlated to overall fitness in *A. kamali* (Sagarra et al. 2001). In the present study, there was a rapid increase in size of U.S. *A. kamali* over

time which may indicate that *A. kamali* has a higher level of fitness overall as the months get warmer. Our first Mexico measurements were made in November and the first U.S. measurements were made in January, both times when winter temperatures were prevalent. As the months got warmer the average parasitoid size increased, albeit at a much lower rate in the Mexico parasitoids. While all parasitoids used in this study were reared in a quarantine facility at constant temperatures, the U.S. population was a much newer colony and therefore most likely closer to a wild type population. The increase of body size may be a seasonal part of *A. kamali*'s development, perhaps in order to survive colder temperatures with a smaller body size. The large difference in size between the U.S. and Mexico parasitoids both in January and in April suggests that populations closer to wild types have a higher level of fitness in spring and summer months and a lower level of fitness in winter months. The relatively steady body size of the Mexico *A. kamali* suggests their fitness is more stable over time, possibly a result of their being in captivity for at least 150 generations. A year-long study of the body measurements of U.S. *A. kamali* compared to Mexico would help to determine how body size fluctuates throughout the year. If the fitness of *A. kamali* is significantly affected by colder temperatures, they may not be a good candidate for releases in northern parts of the U.S. if or when PHM reaches these areas. Cold hardiness of *A. kamali* is currently unknown but should be tested before continuing to expand releases into North America (Chong et al., 2015).

Oviposition rates of U.S. and Mexico *A. kamali* were statistically similar in these studies. The two populations were nearly identical in the February 17 trial but the U.S. population laid substantially more eggs than the Mexico population in the February 27

trial. The U.S. population, which had been in colony for just 15 generations at the time these studies were conducted, had an equal or greater rate of oviposition compared to the Mexico population, suggesting a greater fitness. What is interesting to note is these data were collected in February, a month in which the body size of U.S. *A. kamali* was smaller than the Mexico *A. kamali*. This casts some doubt on the correlation between body size and fitness shown by Sagarra et al. (2001). While the overall oviposition rate of U.S. parasitoids was higher than Mexico, both rates were much lower than what has been cited in other studies. Sagarra et al. (2000) studied the effect of host density, temperature, and photoperiod on the fitness of *A. kamali*. When a host density of 10 PHM was offered to individual parasitoids, 95-100% of the mealybugs were parasitized. Similarly, Sagarra et al. (2002) in their work on the mating impact of *A. kamali*, determined that 23.5% of the PHM were parasitized when the parasitoids were given 6 hours to oviposit into 20 PHM. This number is higher than our highest recorded oviposition rate, despite only allowing the parasitoids one quarter of the time to oviposit. Finally, Roltsch et al. (2006) conducted field surveys finding a 99% decrease in PHM populations four years following the release and establishment of *A. kamali* and another PHM parasitoid, *Gyranusoidea indica* Shafee, Alam & Agarwal. While this last study was not conducted in vitro at the other studies, it does indicate a high oviposition rate for *A. kamali* in the field.

In the present study, the low oviposition rate may have been due to the methodology used. The age of parasitoids used was not standardized beyond validating they were at least 24 hours old, and parasitoids were not observed to ensure that the females had been mated. In Sagarra et al. (2001), body size was an indicator of parasitoid quality in male and female *A. kamali*, and parasitoid age was standardized by

placing a single *A. kamali* mummy (a PHM with a larval stage *A. kamali* inside) into gelatin capsules so the emergence of each wasp could be recorded. Should the present experiment be repeated, we should standardize the parasitoid age and insure mating, both of which could improve oviposition percentages. The optimum age for oviposition is known to be after the first 24 hours to 5 days after emergence (Sagarra et al. 2001). In addition, more replicates over time would show if the difference in oviposition rate between the Mexico and U.S. population shown in the present study is maintained. Furthermore, it would be relatively straightforward to take body size measurements on the same insects used in the ovipositional study to confirm or refute the Sagarra et al. (2001) study correlating body size with overall fitness.

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