SURVIVAL OF OVERWINTERING *Aedes albopictus* EGGS UNDER NATURAL CONDITIONS IN NORTH-CENTRAL FLORIDA

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ABSTRACT. *Aedes albopictus* mosquitoes overwinter as eggs in north-central Florida. Knowledge of this species’ overwintering survival rate is of great interest to local mosquito control districts. In this study, field-collected *Ae. albopictus* eggs were exposed to natural conditions during winter 2016–17 in Gainesville, FL, to determine the overwintering survival rate. Individual strips of germination paper containing eggs of this species were collected from ovitraps in November 2016 then later clipped to the inner edge of empty flowerpots and placed in the Department of Public Works compound, City of Gainesville, in December. Egg strips that remained outdoors were later brought back to the laboratory and hatched in late March 2017. Significant differences were found among the 5 ambient environmental exposure methods (configurations) in terms of egg survival rate, egg collapsed rate, and egg unaccounted rate, whereas no significant difference was observed on the egg intact rate. Egg strips stored in flowerpots with the drainage hole sealed had the highest survival rate (18.3%), whereas eggs stored in an open covered area had the least survival rate (2.4%). The effect of different storage conditions on the survivorship of overwintering *Ae. albopictus* eggs in north-central Florida is discussed.

KEY WORDS *Aedes albopictus*, north-central Florida, overwintering, survival rate

INTRODUCTION

Since the 1st detection of *Aedes albopictus* (Skuse) in Jacksonville, North Florida (CDC 1986, Peacock et al. 1988), the Asian tiger mosquito has quickly spread to all 67 Florida counties, becoming one of the most abundant container-inhabiting mosquito species in many suburban and urban areas of the state. This rapid range expansion was likely facilitated by human-aided long-distance transport of artificial containers holding eggs and larvae (Lounibos 2002) as well as quick adaptation to North Florida climate. *Aedes albopictus* is not only an aggressive human-biter but also a capable vector of numerous arboviruses, including those responsible for dengue fever, chikungunya (Gratz 2004), and Zika (Grala et al. 2014).

One of the biological traits of *Ae. albopictus* populations is that they can overwinter as eggs in temperate regions (Wang 1962, Mori and Wada 1978, Mori et al. 1981). The strain introduced into North America was believed to originate from the temperate region of northern Asia and, as such, should possess characteristics such as diapause and cold hardiness (Hawley et al. 1987). In order to survive through the North American winter, eggs of this species undergo photoperiodic diapause, a crucial mechanism for overwintering survivorship (Tauber et al. 1986). Additional factors that may contribute to egg mortality have been reported, such as intrinsic (senescence) and environmental (desiccation, predation, and freezing). Therefore, the purpose of this study was to assess the overwinter survivorship of *Ae. albopictus* eggs in north-central Florida. Knowledge of survivorship of this species is important for mosquito districts as mosquito control programs may target the most vulnerable periods of this species by reducing the number of overwintering eggs. Mosquito control districts can also conduct outreach program to inform citizens about the important role they can play to prevent production of this species in containers around homes.

MATERIALS AND METHODS

*Aedes albopictus* eggs

All *Ae. albopictus* eggs used in this study were field-collected in the city of Gainesville, FL. Starting from May 2015, Gainesville Mosquito Control Services (GMCS) set out 42 ovitraps at 5 different locations as part of our routine ovitrap surveillance program for monitoring *Ae. albopictus* populations. Based on our egg hatching results in the laboratory, no *Ae. aegypti* (L.) mosquitoes have been recorded in the city for the last 3 years. Therefore, no *Ae. aegypti* eggs were involved in this study. Ovitraps used for this study consisted of 400-ml dark green plastic cemetery vases (Eaton Brothers Corp., Hamburg, NY) staked into the ground to reduce disturbance by lawn maintenance activities, wildlife, or harsh environmental conditions. The ovitraps were filled with 300 ml of oak leaf infusion and germination paper strip (12 cm × 25 cm) (Anchor Paper Co., Saint Paul, MN) was placed to cover the inside surface. The oak leaf infusion was formulated by adding 150 g of live oak (*Quercus virginiana* Miller) leaf litter to 15 liters of well water in a sealed container that was placed outside and allowed to ferment for 7 days. Ovitraps were collected and reset weekly. Three years’ prior ovitrap data by GMCS indicated that the 1st batch of *Ae. albopictus* eggs was normally...
collected from ovitraps in March, with the last batch collected in November. From those data, we found that egg abundance peaked in the middle of July with no egg hatch after early November, indicating that the eggs went into diapause around that time. Based on these data, all eggs used for the study were collected in late November. At that time, individual egg strips were examined under a stereoscopic microscope where intact eggs were counted, and damaged or collapsed eggs were removed with histological needles. After that, all egg strips were randomly assigned to the 5 different exposure methods.

Field study

The protocol used in Gimenez et al. (2015) study was adopted with revisions. In brief, individual egg strips collected from ovitraps were exposed to natural conditions from late November 2016 through the middle of March 2017. For this study, an individual egg strip was clipped to the inner edge of a 4.25-in. flowerpot with a single drainage hole at the bottom (Central Garden and Pet, Atlanta, GA). Five configurations of egg exposure were used: A, uncovered flowerpot; B, flowerpot covered with a fine mesh nylon screen (mesh size of 0.3 mm) and a flower saucer placed on top of screen to prevent access of macroscopic organisms and to prevent ambient rainfall from entering it; C, flowerpot covered with saucer only; D, flowerpot open but stored in an open covered area; and E, flowerpot open with the drainage hole sealed. The study started November 24, 2016. For each configuration of exposure, 15 pieces of egg strips were randomly picked up and assigned to 1 of 5 configurations, making 15 replicates (pots) for each exposure method. The number of eggs in each paper strip varied, ranging from 11 to 149. After that, all the flowerpots with egg strips were randomly placed outside on the ground next to the compound fence line in exposed areas (the oak tree branches from outside of the fence cast some shadow over the exposure areas), with the exception of one set of pots that were placed in an open covered area (vehicle parking bay). The testing site was located at the compound of Department of Public Works, City of Gainesville: 29°41.164′N, 82°19.503′W. Overwintered egg strips were brought back to the laboratory on March 15, 2017, when field eggs were observed to start hatching. Eggs were recorded at 3 different categories: intact (cigar-shaped with large central outer chorionic tubercles smoothly rounded), collapsed (uncompleted development of embryos or egg collapsed), and unaccounted (total number of eggs minus intact and collapsed eggs). Collapsed eggs were removed from the germination papers and discarded then immersed in 250 ml of dechlorinated water contained in plastic trays. Trays were then placed in a Panasonic Versatile Environmental Test Chamber (MLR-352H-PA; Panasonic Healthcare Corporation of North America, Aurora, IL) at a temperature of 28°C and photoperiods of 16 h light and 8 h dark. After 48 h, individual egg strips were removed from the water, air-dried, and the number of larvae were counted. The same procedure was repeated until no hatching was observed. After 4 hatchings, any remaining unhatched eggs were considered dead.

Meteorological data

Daily maximum/minimum temperature, relative humidity (%RH), and precipitation were obtained from the Weather Underground website available from https://www.wunderground.com/weather/us/fl/gainesville/KFLGAINEt64. This weather station is located at the City of Gainesville, Department of Public Works compound where the experiment was carried out.

Data analysis

Mean data subjected to statistical analyses consisted of the proportion of unaccounted eggs (eggs that could not be recovered after field exposure) in each pot, calculated as the difference between the total number of eggs initially exposed and the number of eggs recovered after exposure. The unaccounted egg rate was calculated by dividing the difference by the number of eggs initially exposed in that pot. Intact, survival, and collapsed rates were calculated by dividing the number of intact eggs (as defined in the field study), larvae, and collapsed eggs by the total number of eggs initially exposed, respectively. Intact egg survival rate was calculated by dividing the number of larvae by the number of intact eggs.

To determine the appropriate hypothesis test (parametric versus nonparametric), a preliminary goodness-of-fit (GOF) analysis was conducted to determine whether the data sets conformed to normal and homoscedastic behavior versus nonnormal and heteroscedastic behavior. Results of these GOF tests showed that these data sets conformed to nonnormal and heteroscedastic behavior (even after data transformation). Hence, a nonparametric 1-way Kruskal–Wallis test was applied to these data sets. Differences were considered significant at α = 0.05 Student–Newman–Keuls and Duncan multiple-range tests, as well as Scheffe multiple-contrast test (as identified from a post hoc optimization analysis) was conducted to determine differences between pairwise combinations of each factor and interaction contributing to the overall variability (sources of variance) at α = 0.05.

RESULTS

A total of 2,980 eggs were used in the study. Statistical analysis showed that there were no significant differences among the 5 groups in terms of the average number of eggs. At the end of
exposure, 1,712 eggs were recorded as collapsed, followed by the unaccounted (781) and intact (487).

Egg unaccounted (loss) was recorded for all the 5 exposure methods; statistically, there were significant differences among the different exposure methods \( (P < 0.0001) \) (Fig. 1). Configuration B, which was under protected conditions (fine nylon mesh and saucer), had the least unaccounted egg rate (2.7%); followed by D, open covered area (6.4%). Since A was uncovered, it had the highest unaccounted egg rate (39.9%).

Overall, 57.7% eggs were recorded as collapsed at the end of the exposure period. Collapsed rate (%) was significantly different among the 5 exposure methods \( (P = 0.0013) \) (Fig. 1). The highest collapsed rate was noted in the D (94.4%) and the lowest was in the A (53.4%).

There were no significant differences in terms of intact egg rate, among the 5 exposure methods \( (P = 0.0662) \) (Fig. 1). The highest intact rate was found in the B (22.9%) and the lowest was in the D (8.2%).

Overall, 191 (6.4%) larvae were hatched out from the total of 2,980 eggs exposed after 4 hatchings (Fig. 1). Again, there was a significant difference in the survival rate among the 5 exposure methods \( (P = 0.0112) \). Exposure method E had the highest survival rate (18.3%) and D had the lowest survival rate (2.4%).

Out of 487 intact eggs, 191 were hatched. Significant differences were found among the 5 exposure methods \( (P = 0.0132) \) (Fig. 1). The highest intact egg survival rate was recorded in the E (58.4%) and the lowest was recorded in the D (5.8%). The survival rates of A, B, and C were 32.7%, 14.9%, and 16.4%, respectively.

The cumulative hatched response showed that 90% of the eggs hatched within 2 immersions, with 77.5% (148 larvae/191 intact eggs) hatch after the 1st immersion, following by the 2nd with 1 immersion and 12.6% (24/191) hatch. No more hatching was observed after the 4th immersion.

Figure 2 shows the average, maximum–minimum temperature, and % RH for the months of December, January, February, and March. During the study period, the accumulated precipitation for the months of December, January, February, and March was 68, 36, 9.9, and 17 mm, respectively.

**DISCUSSION**

The overwintering survival of *Ae. albopictus* eggs under natural conditions during the winter months was significantly affected by exposure conditions. Results from this study clearly revealed that *Ae. albopictus* eggs survived better when exposed in outdoor containers that can collect rainwater (flow-er-puts with drainage hole sealed) than those in open covered area or protected (cover with saucers) conditions. Despite that the overwinter survivorship of *Ae. albopictus* eggs has been studied extensively in North America (Hawley et al. 1989, Hanson and Craig 1995a, Armbruster 2016), this appears to be the 1st study on the overwintering survival using actual field-collected *Ae. albopictus* eggs. Therefore, the results of this study reflect the real field survivorship of *Ae. albopictus* in north-central Florida.
The impacts of temperature, humidity, desiccation, origin of strains, and predation on the survival of *Ae. albopictus* have been studied extensively but not under different environmental exposure conditions. Hawley et al. (1989) stated that winter temperatures had direct effects on the survivorship of *Ae. albopictus* eggs. They found that 18% of eggs survived during the warmer winter (1986–87) but none survived the cold winter (1987–88). However, Hanson and Craig (1995b) believed that neither mean temperature nor absolute minimum temperature (a winter’s lowest temperature) accurately affected *Ae. albopictus* egg overwintering survivorship in the field. They believed that the duration of exposure to temperatures below a certain value had more influence on the survivorship of *Ae. albopictus* eggs. Later, Thomas et al. (2012) confirmed this assumption. The lower lethal temperature (the temperature that causes 50% mortality) for the North America strain is \(-12^\circ\text{C}\) (Hanson and Craig 1995b). However, ambient temperatures in Gainesville were never below \(-3.5^\circ\text{C}\) during our study; therefore, it may not have played a role in the reduction of overwintering survival of *Ae. albopictus* eggs in north-central Florida.

Desiccation is another critical factor contributing to the survival of *Ae. albopictus* eggs. Despite the fact that eggs can survive desiccation in a wide variety of indoor and outdoor artificial containers, survivability in low humidity appears to be dependent upon the developmental stage of embryos before they are exposed to dry conditions (Estrada-Franco and Craig 1995). A laboratory study by Gubler (1970) found that *Ae. albopictus* eggs were highly resistant to dry conditions if they were kept in humid conditions for 4 days before being exposed to dry conditions. In another laboratory experiment, Hien (1975) showed that eggs placed on wet cotton wool for 4 days, then exposed to 25–26°C air at 60–70% RH, were highly resistant to desiccation. Furthermore, maximal resistance to desiccation has been observed when eggs were exposed to humidity for at least 24 h, whereas partial or total mortality has been recorded when eggs <16 h old were dried (Hawley 1988). For our study, all initially field-collected eggs used in the exposure evaluations were preconditioned to the same field conditions including humidity for days to favor embryonation. Because all the collapsed eggs were removed before the study was started, we could correctly assume that they were embryonically fully developed. We noticed that the survival rate of eggs from the flowerpot with the drainage hole sealed was significantly higher than other exposure conditions. Rainwater had occasionally accumulated at the bottom of those pots. It is possible that the microhabitat humidity in those pots contributed substantially to the higher survival rate of the eggs. This result is also in agreement with our lab observations. Higher egg survival rate was observed when *Ae. albopictus* egg strips were kept in zipped plastic bags with wet cotton balls compared with ones without wet cotton balls.

The lowest number of eggs unaccounted were found to be from the protected conditions such as covered with fine nylon mesh and saucer (2.7%) and in the open covered area (6.4%). Egg loss was documented mainly by predation of other arthropods such as arachnids, psocids, orthopterans, isopods, and others. Gubler (1971) found that a natural population of *Ae. albopictus* from Calcutta, India, had >50% mortality during the 1st 24 h, primarily due to the predation by ants. A study conducted in New Orleans, LA, also examined the survivorship of *Ae. albopictus* eggs in tires (NOMCB 1989); predation

![Fig. 2. Daily maximum (Max) and minimum (Min) temperature, and % RH during the exposure period from December 1, 2016, to March 15, 2017.](http://meridian.allenpress.com/jamca/article-pdf/34/4/255/1816420/18-6775_1.pdf)
was considered the major cause of loss of *Ae. albopictus* eggs.

In the present study, 77.5% eggs hatched after the 1st inundation, followed by 12.6% after the 2nd inundation; thereafter, all the hatchings were minimal. This hatching pattern was similar to earlier observations (Gillett 1971, Hien 1975) when eggs went into a mass hatching followed by several additional small hatchings. *Aedes albopictus*, along with other species in the genus *Aedes*, has a hatching phenomenon known as instalment hatching (Gillett 1971). During instalment hatching, some eggs may hatch readily in response to the stimulus of inundation, while others may remain dormant for varying periods despite being submerged (Edgerly et al. 1993). The pattern of instalment hatching can be explained by different factors, including plasticity in response to environmental factors and genetic variation as a consequence of the adaptation to local climatic conditions or microhabitat conditions.

The results of this study provide a new insight into the effect of exposure conditions on egg survival in north-central Florida. Mosquito control programs should consider these findings and implement control measures such as the elimination of eggs during the cold and dry season when *Ae. albopictus* population remains mainly in the egg stage. Mosquito control programs can also reach out to homeowners to encourage storing containers indoors, rather than abandon them outside during the wintertime, as this would significantly reduce *Ae. albopictus* survival rate. Also, eliminating outdoor containers at the beginning of the warm and rainy season will reduce the rate. Also, eliminating outdoor containers at the beginning of the warm and rainy season will reduce the rate.

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