

AEDES AEGYPTI OVIPOSITION DIFFERENCES AMONG ORNAMENTAL BROMELIADS WITH VARIABLE WATER LEVELS

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ABSTRACT

Aedes aegypti (L.) have recurrently been emphasized as a critical vector amidst the emergence, and re-emergence, of various anthroponoses. Bromeliads have been incriminated as an *Ae. aegypti* refuge. To investigate this, common ornamental bromeliads in the genera *Guzmania*, *Neoregelia*, and *Vriesea* were used in oviposition bioassays with *Ae. aegypti*. No choice assays were conducted with all three plant types alongside variations in water level, approximated as low, medium, or high based on a 25%, 50%, and 75% capacity. Gravid *Ae. aegypti* tended to deposit eggs in leaf axils of *Neoregelia* more than the central bowl, or tank. In contrast, *Guzmania* collected more eggs in the central bowl, and collected the most eggs when water levels were high. No other trends based on water level were apparent across the other types. *Vriesea* collected few eggs regardless of location or water level. *Neoregelia* collected more eggs overall, implicating this type of bromeliad is potentially suitable to *Ae. aegypti*. This was a preliminary investigation into the role of bromeliads for *Ae. aegypti* oviposition. However, even when mosquitoes were not allowed a choice, there were clear differences in egg deposition between bromeliad types. More information is needed that partitions the role of different bromeliads for the vectors of concern. Future operations and education should prioritize the bromeliads that have a clear connection with the target mosquitoes, particularly in light of controversy about the role bromeliads may play in mosquito-borne disease outbreaks.

Key Words: bromeliads, oviposition, mosquito, behavior

INTRODUCTION

Container-inhabiting mosquitoes, such as *Aedes aegypti* (L.), are peridomestic to human populations, proliferating in artificial containers such as trash, bird baths, open pipes, and buckets (Ngugi et al. 2017; Nordin et al. 2017). Source reduction programs are the main strategy for eliminating common artificial oviposition sites (Kittiyapong et al. 2008; Nordin et al. 2017). Unfortunately, the persistence of container-inhabiting mosquitoes is due in part because they also use natural containers (Nordin et al. 2017). Although container-inhabiting mosquitoes are highly productive in tires or plastic receptacles (Faraji and Unlu 2016; Unlu et al. 2016), the decline of Florida bromeliad specialists, such as *Wyeomyia vanduzeei* and *W. mitchellii* (O'Meara et al. 1995; Lounibos et al. 2003), and subsequent shifts in distribution in north Florida have allowed the en-

croachment of the aforementioned invasive *Aedes* species into bromeliads (O'Meara et al. 1995; Xue et al. 2018). Although artificial containers are still the primary concern for these *Aedes* species, the re-emergence of *Ae. aegypti* in the same areas where *Wyeomyia* have declined have increased the need to understand how container-mosquito oviposition ecology intersects with bromeliads.

Ornamental bromeliads have a wide variation in size and color, which leads them to being a common plant in both rural and urban environments throughout Florida, particularly southern Florida (Wilke et al. 2018). Their overlapping bowl-like axils collect water, providing an essential role in vegetative environments as a drinkable water source and also shelter for many insect and amphibious species, including some mosquitoes. Additionally, in urban settings ornamental bromeliads can be a coveted landscaping or decorative plant in both resi-

dential and public places, which can lead to difficulty in cultural control when home and business owners are not complicit in source reduction programs (Unlu et al. 2013; Wilke et al. 2018). Pesticide pressure among easy to diagnose harborages (Wilke et al. 2018) and skip-oviposition behavior (Colton et al. 2003) leads container-inhabiting mosquitoes to occupy otherwise atypical oviposition environments (Ramasamy et al. 2011; Chitolina et al. 2016).

The role that bromeliads play as a refuge when selective pressure (e.g., pesticide application) is high should be investigated to clarify whether *Ae. aegypti* is linked with particular bromeliad types. Key sites for container-inhabiting mosquitoes tend to require nutrient rich water and partial or indirect sunlight. Thus, bromeliads in the genus *Neoregelia* can become niches for *Ae. aegypti* because of large flowers that decompose in their water impoundments, possibly over-enriching the water to a degree that may exclude specialist competitors (J. H. Frank, pers. comm). In sampling throughout the jurisdiction of the Anastasia Mosquito Control District of St. Johns County, FL (AMCD), *Neoregelia* and *Guzmania* are commonly encountered bromeliad genera (Xue et al. 2018). *Vriesea* is less common, but is persistently available through local landscape providers. To take the first steps in investigating domestic mosquito preferences in bromeliads, we examined the oviposition of *Ae. aegypti* in *Neoregelia*, *Guzmania*, and *Vriesea* with three water levels.

MATERIALS AND METHODS

Aedes aegypti, 1952 Orlando strain, were acquired from the United States Department of Agriculture, Agricultural Research Service, Center for Medical and Veterinary Entomology. Mosquitoes were reared in the AMCD insectaries at 26.7°C, 80% RH, and 14:10 L:D photoperiod. Larvae were fed a 25 mg of a mixture of 1:1 yeast:liver powder bi-daily. Adult mosquitoes were kept on a diet of 10% sucrose solution. Once over seven days old, mosquitoes were blood fed and set

aside for 72 h to become gravid before use in bioassays.

Ten gravid mosquitoes were aspirated into a tented cage (BugDorm 1462W, Bioquip Products Inc., Rancho Dominguez, CA) that contained 10% sucrose solution and one of either a *Neoregelia*, *Guzmania*, or *Vriesea* bromeliad (Fig. 1). The total water-holding capacity across the bowl and axils for each plant type averaged 194 ml, 68 ml, and 64 ml for *Neoregelia*, *Guzmania*, and *Vriesea*, respectively. The bromeliads were coded as low (for ~25%), medium (for ~50%), or high (for ~75%) based on the qualitative water level maintained in the center and two prominent leaf axils. All water levels in each bromeliad were tested concurrently in no-choice assays where they did not have access to the other bromeliads or alternative breeding sites.

Once mosquitoes were added to the tents, they remained there for 3 days to allow sufficient opportunity to oviposit. Water levels were maintained at their respective assignments by manually adding reverse osmosis water to the bowl or axils both for the initial fill and daily during the bioassay. Upon concluding the bioassay, the central bowl and two prominent leaf axils were inspected for mosquito eggs. Larvae were then reared out from the eggs inside the bromeliad and cared for until adulthood using the same rearing conditions as the insectary. The assay was repeated three times, each time with new water. The difference in egg deposition between the bowl or leaf axils and low-, medium-, or high-water level was not distributed normally. Therefore, data were analyzed by bromeliad type using Kruskal-Wallis non-parametric ANOVA (Kruskal and Wallis 1952) and Steel-Dwass all pairs post-hoc test (Critchlow and Fligner 1991).

RESULTS AND DISCUSSION

There were no overarching trends in the number of eggs collected in the central bowl of the bromeliads when analyzed by the differences in water level (Fig. 2). For both *Neoregelia* and *Vriesea*, low- and high-water levels resulted in comparable amounts of eggs deposited, while medium-water lev-

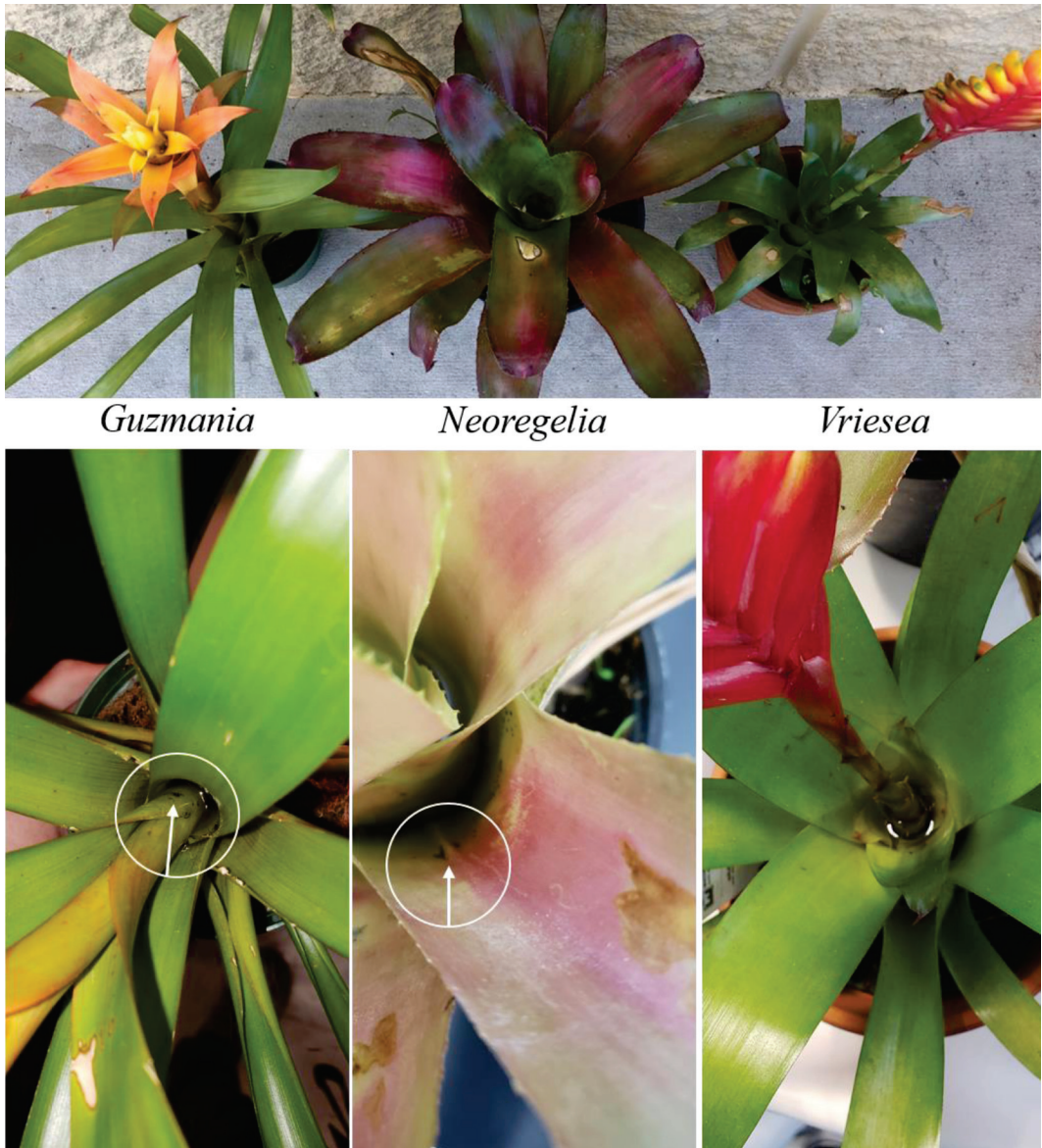


Figure 1. Common ornamental bromeliad types used in oviposition bioassays. Top: From left to right, *Guzmania*, *Neoregelia*, and *Vriesea*. Bottom: Post-bioassay images showing egg deposition within the center part (*Guzmania*), leaf axil (*Neoregelia*), or not at all (*Vriesea*).

els resulted in a visual but non-significant trend of fewer eggs than the other water levels. With *Guzmania*, low- and medium-water levels did not result in significantly different amounts of eggs deposited. The high-water level in *Guzmania* collected a comparable mean of eggs to *Neoregelia* (Fig. 2) with no statistical separation between the two plant types. When examining egg deposition by the central bowl of the plant or

the two prominent leaf axils, regardless of water level, there was a visual, but not statistical, trend for *Guzmania* to have more eggs deposited in the central bowl than in the leaf axils (Fig. 2). Similarly, *Neoregelia* tended to have more eggs deposited in leaf axils than the central bowl, but power was insufficient to statistically support the observation. Egg deposition in *Vriesea* did not favor either particular location.

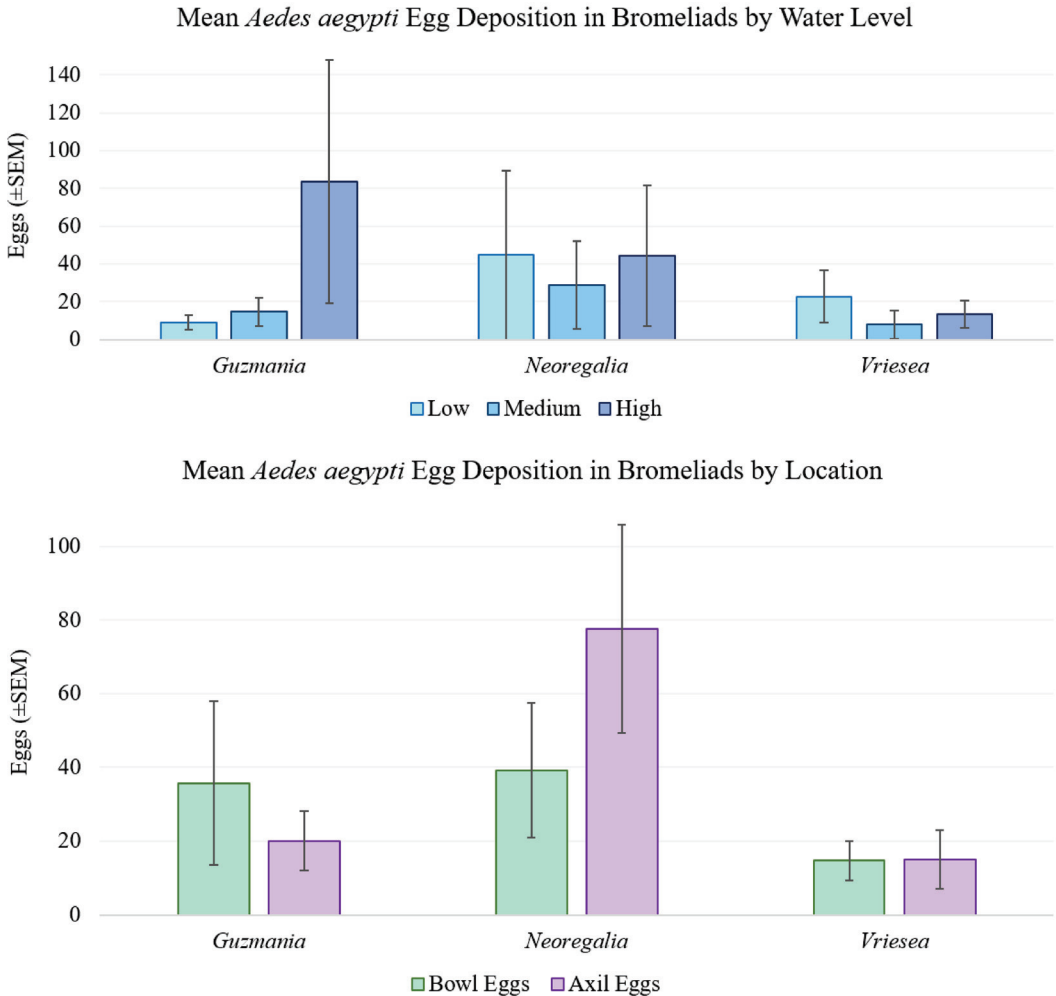


Figure 2. Trends in mean percent egg deposition by *Aedes aegypti* (L.) based on discriminating factors analyzed from non-choice tests, represented with standard error of the mean. Top: Deposition based on low- (~25%), medium- (~50%), or high-water (~75%) lines in the respective plant. Bottom: Deposition based on the central bowl or leaf axil in the respective plant.

Analyzing the total eggs per plant, regardless of water level or location, resulted in no statistical differences between *Guzmania*, *Neoregalia* or *Vriesea* with eggs totaling 321, 353, and 132, respectively. Though not significant between plants, it was observed that an average of 21%, 25%, and 41% of eggs in *Guzmania*, *Neoregalia*, and *Vriesea*, respectively, were floating atop the surface of the water rather than affixed directly to a leaf. Beyond that, there was an average of 68%, 20%, and 25% of assayed *Ae. aegypti* being found deceased in the impounded water within *Guzmania*, *Neoregalia*, and *Vriesea*, respectively. Post bioassay, larvae were

successfully reared to adulthood in all plants within 14 days without requiring any food input. There were no apparent differences among plants for the success of larval rearing, given the artificial conditions of the bioassay.

Although investigated through a series of no-choice assays, it was interesting to see that *Ae. aegypti* deposited eggs in all three types despite that bromeliads are considered inhospitable rearing environments for non-specialists (Lounibos et al. 2003; O'Meara et al. 2003; Mocellin et al. 2009; Lopez et al. 2011). Among the bromeliad types tested, it superficially appeared that

egg deposition was higher in *Neoregelia*. Other reports have tended not to specifically justify whether a certain genus of bromeliad is more prone to harboring invasive vector mosquitoes. If *Neoregalia*, or any one genus, were more prone to oviposition by *Ae. aegypti*, then source reduction could be more discriminatory about bromeliads that are considered harmful for public health. It is noteworthy that *Guzmania* harbored more eggs in the central portion than the leaf axils, but we attribute this to a difference in structure. The *Guzmania* used in these bioassays contained a central stalk composed of several interwoven leaf blades. Particularly with the finding that *Guzmania* contained more eggs at the high-water level, we believe that simply increased the surface area along the central stalk to which *Ae. aegypti* could adhere eggs (Fig. 1). *Vriesea* did not appear to be as suitable for oviposition, as even despite no preference, the fewest total eggs were deposited in either the central bowl or leaf axils. However, a caveat remains that the experiments were performed with colony mosquitoes. It is therefore possible that altering our experimental design to use mosquitoes reared from wild-collected eggs and increasing replication would have yielded different results. In addition, complementing experiments with sampling eggs from the same bromeliad species in the field may provide additional insight. Moreover, even though fewer eggs were found in *Vriesea* plants, they were still positive for the presence of *Ae. aegypti* eggs.

It is puzzling that such a high proportion of eggs were found on the surface of the water, as opposed to in available crevices. Historical study shows lower humidity to correlate with higher water oviposition (Chadee et al. 1995). However, we believe additional factors were more influential, such as texture. Smooth oviposition surfaces have been shown to encourage laying eggs on the water, or may lead to avoiding the oviposition site altogether (Madeira et al. 2002). *Aedes aegypti* have been reinforced as preferring rough, rugose (wrinkled) lining in oviposition sites

over smooth surfaces (Swan et al. 2018). We believe the leaves of certain bromeliads may lack sufficient texture for *Ae. aegypti* oviposition, which could also explain the post-bioassay mortality of adults that appeared to have fallen into the water. The lower mortality and water oviposition in *Neoregelia* assays may point to there being less of an obstacle through texture. But this may be better examined in future study through a combination of choice assays with artificial and natural containers, as well as possible substitution assays where a bromeliad leaf is used in lieu of standard substrates inside of an oviposition cup.

Bromeliad structure and prevalence in peridomestic landscapes is believed to contribute to vector risks (Wilke et al. 2018). However, historical study contradicts the importance that is attributed to bromeliads for *Ae. aegypti* (Frank and Curtis 1977; Frank et al. 1988; Mocellin et al. 2009). Therefore, we believe that such mixed findings are the result of bromeliad-specific differences that confound our understanding of bromeliads in the oviposition ecology of peridomestic vectors. Bromeliad utilization also may be a geographically linked phenomenon, as the plants may not be preferred but in some cases they may be the most abundant option in the landscape. Our current investigations are a preliminary attempt at understanding oviposition differences in natural containers, but show that, even in absence of top-down pressures, *Ae. aegypti* may not interact with all bromeliads equally. To resolve misconceptions, we propose that available source reduction education should begin prioritizing specific types of bromeliads that are liable to cause risks, which appear to be particular ornamental varieties (Wilke et al. 2018).

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