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# SCIENTIFIC NOTE

## GRASS INFUSIONS IN AUTOCIDAL GRAVID OVITRAPS TO LURE *AEDES ALBOPICTUS*

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ABSTRACT. *Aedes albopictus*is a vector of several pathogens of significant public health concern. In this situation, gravid traps have become a common surveillance tool for *Aedes* spp., which commonly use hay infusions as an attractant. Diverse grass infusions have been assessed to enhance the attraction to this vector mosquito. However, these studies have focused on the oviposition effect, and the attraction potential to gravid *Ae. albopictus* females has not been evaluated yet. Here we report the attractiveness of infusions of 4 different botanical species (*Cenchrus purpureus*, *Cyanodon dactylon*, *Megathyrus maximus*, *Pennisetum ciliare*) as baits in sticky ovitraps and autocidal gravid ovitraps (AGOs) under laboratory, semifield, and field conditions. In the laboratory, *Cynodon dactylon* showed attractiveness, whereas in semifield conditions, both *C. dactylon* and *Megathyrsus maximus* were similarly attractive for gravid *Ae. albopictus*. None of the infusions conducted with AGOs were able to lure *Ae. albopictus* and other species of mosquitoes in a 14-wk field experiment. Our results demonstrate the feasibility of finding more attractive infusions for *Ae. albopictus* females to improve the efficacy of AGO traps, but further testing of infusions in AGOs in field settings is needed.

**KEY WORDS** *Aedes albopictus*, attractant, bait, grass infusion, ovitraps

*Aedes albopictus* (Skuse) is a known vector of dengue, chikungunya, Zika, and other pathogens (Parry et al. 2021). There is a lack of effective vaccines to control these pathogens, therefore most current strategies focus on surveillance and vector control approaches to diminish the arboviral disease impact on human settlements, especially in the Americas. Among novel advances in vector surveillance and control tools, autocidal gravid ovitraps (AGOs) (Mackay et al. 2013) using hay infusions of *Cynodon nlemfuensis* Vanderyst have been effective in attracting gravid *Ae. aegypti* (L.) females in Puerto Rico (Barrera et al. 2019). Nonetheless, the use of hay infusions to attract and control the other important dengue vector, *Ae. albopictus* (Skuse), has been negligible. In these situations, to lure *Ae. albopictus* females, attempts in laboratory settings with limited success have been conducted including organic infusions of *Cynodon dactylon* (L.) and *Quercus* spp. (Zhang and Lei 2008, Dixson et al. 2020). Here we report a Mexican study to improve the efficacy of AGOs traps to collect *Ae. albopictus* matched with 4 different grass infusions under lab conditions. Additionally, we also tested these infusions under semifield and field conditions as attractants for *Ae. albopictus* gravid females.

*Aedes albopictus* were generated from a colony originally collected from Tapachula, Chiapas, Mexico, bred in the insectary facilities of the Centro de Biotecnología Genómica from Instituto Politécnico Nacional in Reynosa, Mexico (García-Munguía et al. 2011). Males and females were allowed to mate in 50 × 50 × 50 cm cages with 10% sucrose ad libitum; females 4–6 days old were blood fed on an immobilized rabbit for 40 min, and 3 days before the experiments unfed females were removed from the cage, leaving gravid females to be used for the bioassays.

For infusions using *Megathyrsus maximus* (Jacq.), *C. dactylon*, *Cenchrus purpureus* (Schumach), and *Pennisetum ciliare* (L.), dehydrated green leaves collected from an experimental station in Las Huastecas, Tamaulipas, Mexico (22°34′2.93″N, 98°9′53.64″W) were prepared as reported by Reiter et al. (1991). Then a 10% infusion concentration in unchlorinated water was prepared for use in the bioassays. For each bioassay, 4 replicates of twenty 7-to-9-day-old gravid females each were tested in mosquito cages (50 × 50 × 50 cm), containing 2 black cups with 100 ml of either unchlorinated water as control or grass infusion placed diagonally in the corner and lined up with a strip of AGO replacement glue board (4 × 21.5 cm). The position of each cup was switched between replicates; after 24 h, the cups were removed from the cage and the captured females in each cup counted.

Identical infusions were assayed to lure *Ae. albopictus* released in a 3.35 m long × 2.74 m wide × 1.98 m high greenhouse during August–November 2022 in Reynosa, Tamaulipas, Mexico (26°04′09.2″N, 98°18′46.8″W). The greenhouse, which was empty, received natural light

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and maintained an average temperature of 26.66 ± 3.59°C, with relative humidity 69.79% ± 9.51%.

Four replicates using a group of 100 blood-fed females for each of the 4 grass infusions and controls were assayed. Each replicate had 2 AGOs containing either 10 liters of unchlorinated water (control) or grass infusion placed diagonally 4.3 m apart from opposing greenhouse corners. The position of AGOs was switched between replicates to account for location bias. *Aedes albopictus* females were released in each replicate between 0800 h and 0830 h at the center of the greenhouse, approximately 2.15 m away from AGOs. After 48 h, the AGO traps were retrieved, and the mosquitoes captured on the glue boards were counted for 4 replicates.

Field experimental studies were conducted from March 29 to July 7, 2023, in the residential neighborhood of Pedro José Méndez (26°01′04.0″N, 98°16′29.3″W) in Reynosa. The site was selected due to the highly observed populations of *Ae. albopictus* during monitoring studies in the spring, summer, and fall of 2021 and 2022.

We selected *C. dactylon* and *M. maximus* as the most effective infusions for *Ae. albopictus* under lab and semifield conditions. Experimental design included individual or combinations of infusions in a 50:50 mixture as follows: 1) *C. dactylon*, 2) *M. maximus*, 3) hay, 4) tap water, 5) *C. dactylon* + *M. maximus*, 6) *M. maximus* + hay, and 7) C. *dactylon* + hay. Positive control was an infusion of *Medicago sativa*.

Under field conditions, 14 trap positions were selected in the study area, with collection sites located at least 30 m apart. Two replicates were conducted concurrently each week by setting 7 at the beginning of the week and 7 more later in the week. Trap positions were reset every 7 days. Sticky glue boards were removed, and mosquitoes were counted and identified visually to gender and species level. To evaluate the attractiveness of the infusions by trap positions, traps and baits were rotated between each site. The grass infusions were replaced and evaluated after 7 wk, following the study of Santana et al. (2006).

Data obtained from lab and semifield conditions were tested for normality (Shapiro-Wilk test) and equality of variance (Bartlett's test). One-way ANOVA followed by post hoc Student-Newman-Keuls (SNK) analysis was conducted to compare the mean (+SE) of gravid *Ae. albopictus* females caught on glue board in ovitraps or AGOs across replicate/infusion treatment and control groups.

For the field experiment, the difference in the number of captured mosquitoes across infusions and controls was compared using a Generalized Linear Mixed Model (GLMM) with the GLIMMIX procedure, with a negative binomial distribution to account for nonnormality and overdispersed data. All statistical analyses were performed in SAS OnDemand for Academics (SAS 2021) and $\alpha = 0.05$ for statistical differences.

Under lab conditions, there was a significantly higher number ($F_{(1,\ 6)} = 15.47$, $P = 0.0077$) of *Ae. albopictus* counted in the cups containing *C. dactylon* (mean of 11.75 ± 0.62 SE) compared to the control (8.25 ± 0.62 SE). However, no significant differences were observed between the other infusions: *M. maximus* (mean of 11.0 ± 1.08 SE; $F_{(1,\ 6)} = 1.71$, $P = 0.2383$), *C. purpureus* (mean of 11.50 ± 1.19 SE; $F_{(1,\ 6)} = 3.18$, $P = 0.1250$), *P. ciliare* (mean of 11.0 ± 0.70 SE; $F_{(1,\ 6)} = 4.00$, $P = 0.0924$), and controls (9.0 ± 1.08, 8.5 ± 1.19, 9 ± 0.70 SE, respectively; Fig. 1A).

Under semifield conditions, both *C. dactylon* ($F_{(1,\ 6)} = 460.15$, $P < 0.0001$) and *M. maximus* ($F_{(1,\ 6)} = 248.47$, $P < 0.0001$) infusions lured *Ae. albopictus* gravid females, showing a higher number (mean of 74.25 ± 2.05 SE and 70.25 ± 2.01 SE, respectively) of attracted mosquitoes than that of controls (20 ± 1.47 SE and 26.25 ± 1.93 SE, respectively). No variation between the infusions of *C. purpureus* (mean of 44 ± 2.94 SE; $F_{(1,\ 6)} = 2.25$, $P = 0.1841$), *P. ciliare* (mean of 48.25 ± 2.56 SE; $F_{(1,\ 6)} = 0.40$, $P = 0.5525$), and controls (50.75 ± 3.40 SE and 45.75 ± 3.03, respectively) was noted (Fig. 1B).

In the field experiment, when assessing the attractant effect of organic infusions with AGOs, a total of 308 *Ae. Aegypti*, 181 *Culex quinquefasciatus* Say, and 22 *Ae. Albopictus* were collected over 14 wk (Table 1). None of the 6 infusions, whether tested individually or in a mixture, resulted in a significantly higher collection of female mosquitoes than that of the control water. Furthermore, there was no significant difference in the number of either *Ae. Aegypti* females (GLIMMIX: $F_{6,187} = 0.84$, $P = 0.5405$), *Ae. Albopictus* (GLIMMIX: $F_{6,187} = 1.05$, $P = 0.3965$), or *Culex quinquefasciatus* (GLIMMIX: $F_{6,187} = 0.84$, $P = 0.5437$) or the total of mosquitoes (GLIMMIX: $F_{6,187} = 0.59$, $P = 0.7341$) captured among all the infusions tested.

Diverse grass infusions have been assessed against vector mosquitoes, including *Ae. aegypti* and *Ae. albopictus* under both laboratory and field conditions (Dormont et al. 2021). These studies have primarily focused on evaluating the oviposition response of these species to grass infusions as an indicator of their attractiveness (Santana et al. 2006). However, some authors have suggested egg count as an indirect indicator for measuring attraction of female *Aedes* spp. because of their skip-oviposition behavior (Mulatier et al. 2022). Instead, we employed sticky-screen glue boards from ovitraps and AGOs as a direct indicator of attraction of *Ae. albopictus* gravid females to the infusions.

Our results from the lab and semifield bioassays demonstrate the relative attraction of *Ae. albopictus* females to *C. dactylon* and *M. maximus* infusions, with more females caught in traps baited with these infusions than in control traps. These findings support previous results indicating *Ae. albopictus* females being attracted to infusions of *C. dactylon* (Zhang and Lei 2008), and *M. maximus* (Santana et al. 2006, Santos et al. 2010), in lab bioassays. However, none of these infusions lured *Ae. albopictus* females when evaluated under field conditions in earlier studies (Burkett-Cadena and Mullen 2007, Brisco et al. 2023).

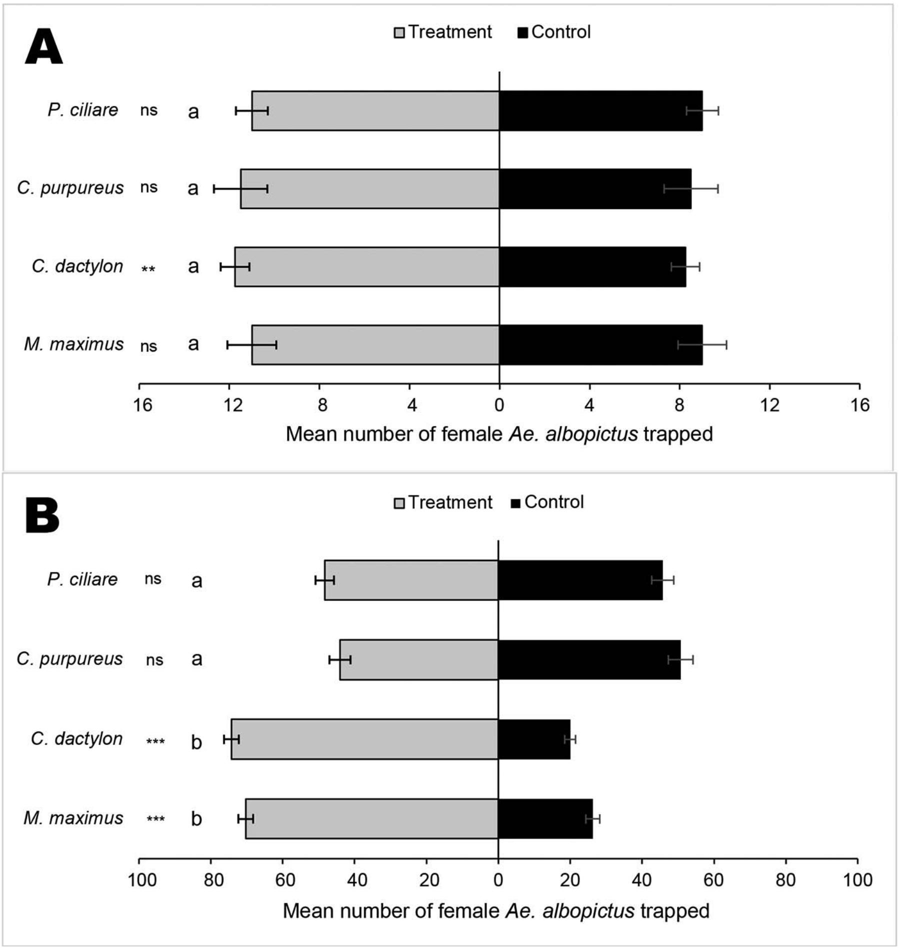

Fig. 1. Mean number (±SE) of *Ae. albopictus* gravid females caught in traps using grass infusions and tap water as control. (A) Under lab conditions, 20 gravid females were released in each of the 4 replicates. (B) Under semifield conditions, 100 gravid females were released in each of the 4 replicates. Statistical significance is denoted by asterisks (*$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$), and different letters (a, b) indicate treatments with statistical difference based on a post hoc Student-Newman-Keuls analysis.

Some limitations in this study should be mentioned. First, the low mosquito densities can be attributed to abiotic factors, particularly the low precipitation in Tamaulipas documented in 2023 when the field study was conducted. Without enough precipitation, mosquitoes lack proper habitats for laying eggs, and their aquatic larvae struggle to thrive and grow because of the competence between larvae and pupae at higher population densities (Morin et al. 2015). Additionally, in the summer, temperatures can reach 40–42°C, and temperatures

Table 1. Average number of mosquitoes trapped at AGOs baited with organic infusions under field conditions in Reynosa, Tamaulipas, Mexico.

| Infusion | *N* | *Aedes aegypti* (mean ± SE)[1] | *Ae. albopictus* (mean ± SE)[1] | *Culex quinquefasciatus* (mean ± SE)[1] |
|---|---|---|---|---|
| *C. dactylon* | 28 | 1.17 ± 0.40 | 0.35 ± 0.25 | 0.78 ± 0.20 |
| *M. maximus* | 28 | 2.17 ± 0.39 | 0.07 ± 0.04 | 0.78 ± 0.18 |
| Hay | 28 | 1.75 ± 1.29 | 0.14 ± 0.08 | 0.85 ± 0.23 |
| Tap water | 28 | 1.53 ± 0.32 | 0 | 0.78 ± 0.20 |
| *C. dactylon* + *M. maximus* | 28 | 1.53 ± 0.44 | 0.14 ± 0.08 | 1.00 ± 0.23 |
| *C. dactylon* + hay | 28 | 1.14 ± 0.30 | 0.03 ± 0.03 | 0.71 ± 0.16 |
| *M. maximus* + hay | 28 | 1.67 ± 0.30 | 0.03 ± 0.03 | 1.03 ± 0.21 |

[1] SE, standard error of the mean. None of the means shown here differ significantly from any other ($P > 0.05$).

above 35°C lead to a decline in *Aedes* mosquito populations (Jia et al. 2019). Therefore, our study was limited to only 14 wk, from March to the beginning of July, preceding the onset of the period of high summer temperatures.

Further field studies are needed at different seasons, localities, and collection sites to confirm the efficacy of these grass infusions to lure *Ae. albopictus* using AGOs, aiding public health agencies in surveillance and control efforts for this dengue vector, with the ultimate goal of developing an AGO for *Ae. albopictus*.

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