

SCIENTIFIC NOTE

SEMI-FIELD EVALUATION OF MODIFIED 00ZZZero® TRAPS WITH STICKY PAPER TO INCREASE THE COLLECTION EFFICACY OF GRAVID *Aedes aegypti*

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ABSTRACT. The 00ZZZero® trap (OZ) is a small black plastic container with permethrin and pyriproxyfen, designed and marketed to both attract and kill gravid *Aedes* mosquitoes (unmodified trap ([UMT] or modified trap [MT]). The OZ trap without the insecticide was modified with addition of a sticky paper, coated with an adhesive glue (MT) and evaluated for collection efficacy of released gravid mosquito, *Aedes aegypti* (L.) in outdoor screened enclosures in St. Augustine, in northeastern Florida. The mean numbers (\pm SE) of mosquitoes caught by MTs were 130.67 ± 23.95 (40.22 ± 5.78 mosquito/trap), compared with 2.33 ± 0.88 (0.79 ± 0.29 mosquito/trap) mosquitoes caught by UMTs. The MTs collected significantly more mosquitoes, 44.16% ($F = 4.495$, $P < 0.05$) of the released mosquitoes, than those captured in UMTs (0.83%). The number of immature mosquitoes (larvae and pupae) hatched from eggs laid in the MTs and UMTs were 79.3 ± 23.1 and 19.3 ± 6.8 , respectively. The mean numbers (\pm SE) of adults emerging in MTs and UMTs were 34.0 ± 22.3 and 14.0 ± 6.8 , respectively. These results show the potential of the sticky paper modified OZ traps to be used both to increase the efficacy of collection and as a sticky autocidal tool against gravid *Ae. aegypti*.

KEY WORDS *Aedes aegypti*, gravid trap, insecticide, oviposition, permethrin, pyriproxyfen

Aedes aegypti (L.) is one of the major mosquito vectors of arboviruses, the most important of which are yellow fever, dengue, chikungunya, and Zika (Smith et al. 2018). Autocidal gravid ovitraps (AGO) with sticky paper and other sticky ovitraps have been used for surveillance and control of *Aedes* mosquitoes in several studies (Ritchie et al. 2003, Chadee and Ritchie 2010, Mackay et al. 2013). Some sticky strips treated with a residual insecticide such as permethrin, have resulted in an overall suppression of mosquito populations (Williams et al. 2007, Bazin and Williams 2018). The 00ZZZero® (OZ) trap was designed to attract and kill gravid female *Aedes* mosquitoes and the immature stages that might emerge from any eggs laid in the trap water (Parker et al. 2017). The objective of this study was to determine whether a modification of the new trap, 00ZZZero® traps with a sticky paper (MTs) will increase the collection efficacy for gravid *Ae. aegypti*, compared with the unmodified traps (UMTs).

The OZ traps (00ZZZero, Gainesville, FL, <http://www.oozzero.com/trap.xhtml>) were provided by the Department of Entomology and Nematology, University of Florida, Gainesville, FL. The OZ trap is a small black plastic container with an internal material that is imbedded with an attractant to lure gravid

females to lay eggs, an adulticide (permethrin, AI 0.7%), and a larvicide (pyriproxyfen, AI 0.01%; Fig. 1A). In this study, the OZ traps without the insecticides were modified with the addition of sticky-paper sheets (20 × 10 cm; Atlantic Paste and Glue, Winchester, VA) fitted on the inside walls of the traps, referred to as modified traps (MTs). The MTs and the standard UMTs containing the insecticide sachets were evaluated for the collection efficacy of gravid *Ae. aegypti* in outdoor enclosures at St. Augustine, in northeastern Florida.

Female mosquitoes were used from a laboratory colony of *Ae. aegypti* 1952 Orlando strain, provided by the U.S. Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. This colony is reared at the insectary of Anastasia Mosquito Control District (AMCD) in St. Augustine, Florida. Blood-fed mosquitoes were allowed for 3 days to become ready to oviposit for experimental use, as described by Liu et al. (2019).

On the day of the experiment, the traps were filled with tap water (500 ml) and distributed on each side of the test screened enclosure (Fig. 1B). The screened enclosure dimensions were $12.19 \times 4.57 \times 6.1$ m with a centrally located 10.92-m² covered pavilion (arena). For each experiment, 3 traps (replicates) each were used for both MTs and UMTs, and the entire experiment was repeated 3 times. The trap positions in the enclosure were rotated at each experiment.

Gravid females (300 females per enclosure) were released into the test enclosure from the central arena and were collected after 48 h. In the laboratory, the sticky sheet of each trap was removed to enumerate the trapped mosquitoes. The water in each trap was

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Fig. 1. 00ZZZero traps (A) and the traps placed inside the screened enclosures (B) for the release of gravid *Aedes aegypti* females, Anastasia Mosquito Control District, St. Augustine, FL.

poured into clean 500-ml plastic cups. The eggs laid in each trap were collected on paper sheets, allowed to dry and mature for 2 days, and were then placed in clean water in small mosquito breeders (21 × 12-cm diam, BioQuip, Rancho Dominguez, CA) for larval hatching to adult emergence.

The mean differences in the numbers of collected gravid females between MTs and UMTs were calculated and analyzed by a Student's *t*-test. The numbers of immature mosquitoes and emerging adults in each breeder transferred from MTs and UMTs were analyzed, with the use of a 1-way ANOVA, with an alpha of 0.05 for all comparisons. All statistical analyses were performed with the use of SPSS version 18 (SPSS, Chicago, IL).

The results showed that of the total number of gravid mosquitoes released ($n = 900$), 44.33% ($n = 399$) were captured by all trap types. Of the total captured mosquitoes, ~98.2% ($n = 392$) were captured by the MTs; UMTs captured only ~1.8% ($n = 7$). The mean (\pm SE) of captured mosquitoes in MTs and UMTs were 130.67 ± 23.95 (86–168 mosquito/experiment) and 2.33 ± 0.88 (1–4 mosquito/experiment), respectively. The means (\pm SE) of captured mosquitoes per trap were 40.22 ± 5.78 and 0.79 ± 0.29 for the MTs and UMTs, respectively. The mean percentage of the mosquitoes captured from the released mosquitoes by the MTs was 44.16% (28.7–56%), compared to 0.83% (0.33–1.33%) captured by the UMTs. These data indicate that there is a significant increase in collection efficiency by MTs with the sticky paper, where it caught a significantly higher ($F = 4.495$, $P < 0.001$) number of gravid mosquitoes than the UMTs.

Preliminary biological observations on the extended effect of OZ traps on mosquito developmental stages from egg hatching, through adult emergence, showed no apparent abnormalities in morphological or behavioral characteristics of the mosquitoes emerged from MTs and UMTs. The mean (\pm SE) of immature mosquitoes (larvae and pupae) hatched from eggs laid in the MTs and UMTs were $79.3 \pm$

23.1 and 19.3 ± 6.8 , respectively. This indicates that MTs had significantly ($F = 16.97$, $P < 0.05$) higher number of immature mosquitoes than those collected by UMTs. The mean (\pm SE) of adults emerging from MTs and UMTs was 34.0 ± 22.3 and 14.0 ± 6.8 , respectively; the difference between the 2 was not significant. The male:female sex ratio was 1:1 in MTs and 0.4:1 in UMTs.

The original OZ trap contains a lure and 2 insecticides, an adulticide (permethrin) and a larvicide (pyriproxyfen). This trap design aims to both attract and kill gravid *Aedes* female mosquitoes seeking suitable water habitats for oviposition and probably kill any immature stages that might hatch from eggs laid in the trap. The present study showed an increased collection efficacy of gravid *Ae. aegypti* by modifying the original OZ trap with an adhesive glue coated on a sticky paper that is usually used in AGO traps. The MTs with a sticky paper and without the insecticide sachet enormously increased the collection efficacy of gravid mosquitoes (by >98%), compared with UMTs. The very low number of mosquitoes caught by the UMTs might be due to the mosquito escape from the trap or repellent effect of the insecticide treatment (permethrin) in the OZ traps.

This result also indicated that the insecticides (permethrin and pyriproxyfen) in UMTs might have latent effects that impaired (killed) larval developmental stages (late larval–pupal transformation (i.e., pyriproxyfen effect)). There was a 3–4-day lag time in egg hatching into 1st instars in UMTs than MTs. Even though the insecticides in the UMTs did not significantly affect adult emergence (pupal–adult transformation), compared to MTs with adhesive glue only, the male:female sex ratio was affected, with 0.4:1 in UMTs with apparent deviation of the 1:1 ratio in MTs. This needs more experimentation to quantify accurately the effect of the original OZ traps on the biological development and behavior of the captured mosquitoes that survive and lay eggs in the traps.

In summary, the results of our study on the sticky-paper MTs showed an increase in the collection efficacy of adult gravid mosquitoes similar to those reported by other workers about sticky ovitraps (Cheng et al. 1982, De Santos et al. 2012). Therefore, the sticky paper MTs could be used to improve the collection efficacy for gravid *Ae. aegypti* and add benefits to programs for surveillance and management of *Aedes* vector populations.

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