FIELD EVALUATION OF AUTOCIDAL GRAVID OVITRAPS AND IN2CARE TRAPS AGAINST *AEDES* MOSQUITOES IN SAINT AUGUSTINE, NORTHEASTERN FLORIDA

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ABSTRACT

Mosquito control programs are utilizing cost-effective long-term autocidal traps targeting the gravid population of container-inhabiting and other mosquito species, with the aim of reducing vector populations and disease transmission risk. In this field study we directly compared the efficacy of the Autocidal Gravid Ovitrap (AGO) and In2Care mosquito traps in St. Augustine, Florida. Total numbers of eggs (*Aedes aegypti* and *Ae. albopictus*) and adult mosquitoes were calculated at different weeks of trap deployment, pre-treatment (wk1-2), during-treatment (wk3-6), and post-treatment (wk7-8). There was a 72% reduction in both *Aedes* eggs in the two sites tested post-trap deployment, compared to pre-trap deployment. The mean numbers of eggs collected in the post-treatment, compared to pre-treatment showed that the In2Care traps had a higher reduction of mosquito oviposition (80%) than the AGO traps (23%). A total of 19 mosquito species included non container-inhabiting mosquitoes, *Aedes taeniorhynchus*, *Culex quinquefasciatus*, and *Cx. nigripalpus*, were collected by BG traps baited with BG lure and dry ice from the test sites. The species abundance varied between the two sites and collection weeks. The container-inhabiting mosquitoes, *Aedes aegypti* and *Ae. albopictus* were the major species. There was a significantly higher reduction in mosquito *Aedes aegypti* populations in the AGO (mean ± SE) (1.3 ± 1.7) and In2Care (4.9 ± 4.6) sites (X^2= 20.13, $P <$ 0.0001) post trap deployment, compared to pre-trap deployment. By week 8, the recovery rate of mosquito populations was highest in the In2Care trap site, followed by the AGO site. This result suggests that AGO traps were more effective than In2Care traps in reducing *Ae. aegypti* mosquito populations. For *Ae. albopictus*, the In2Care site had 100% reduction, and this was higher than the AGO site.

Key Words: *Aedes aegypti*, *Aedes albopictus*, Autocidal Gravid Ovitraps, In2care trap, Gravid mosquitoes

INTRODUCTION

Aedes aegypti (Linn.) and *Aedes albopictus* (Skuse) are highly specialized and selective domestic species that mostly oviposit in natural and man-made water containers associated with human dwellings and activities. Mosquito oviposition behavior (Bentley & Day 1989) has been a main target to develop novel approaches and tools for mosquito surveillance and monitoring vector population dynamics, and vector control (Reiter, 1983, Chadee and Corbet, 1987, Eiras et al. 2014). The first trap device used a combination of mechanical suction and organic plant-based infusion to collect eggs and attract gravid females (Reiter, 1983). Oviposition traps lined with polybutylene adhesive were successful to collect both *Ae. aegypti* and *Culex. quinquefasciatus* Say in Australia (Barbosa et al., 2010). This approach was further exploited and developed in attract-and-kill ovitraps and gravid traps, with the added advantage of attracting older mosquito cohorts that might be actively involved in disease transmission (Day, 2016).

The Autocidal Gravid Ovitrap (AGO) is a dual action surveillance and control tool that aims at capturing and killing gravid females of *Aedes* container-inhabiting mosquitoes (Barrera et al. 2014 a, b). The In2Care trap (In2Care) is a multi-purpose trap, containing both pyriproxyfen and the fungus *Beauveria bassiana*. Some field trials have been carried out to compare the efficacy of different trap types, such as gravid traps and AGOs under urban environmental conditions (Cilek et al. 2017) and AGOs and In2Cares (Buckner et al. 2017), where different levels of efficacies were observed (Su et al. 2020).

The AGO and In2Care traps have been preliminarily tested for control of *Aedes* mosquitoes in Saint Augustine, Florida (Autry et al. 2021). This is a continuation of direct comparison of the AGO and In2Care traps to determine their differential effectiveness against mosquitoes. Mosquito populations were monitored using host-seeking

Biogents-sentinel (BGs) traps (BioGents, Regensburg, Germany) and oviposition traps in both trap-treated sites. The expected outcomes of this study should inform mosquito abatement districts on the efficacy of the tested traps and the novel strategies for control of container-inhabiting mosquito vectors of diseases and nuisance species in urban areas.

MATERIALS AND METHODS

For this study, 100 AGOs and 100 In2Care traps were evaluated. Two sites were selected in downtown St. Augustine, Florida, based on their high abundance of *Ae. aegypti* and *Ae. albopictus* mosquitoes (Smith et al. 2018). All the traps were deployed over a one-day period, preceded by door-to-door interviews with residents of the households selected and providing educational brochures of the different traps being evaluated.

The selected sites were 18 acres (7.28 hectares) in size and 700 meters apart. Site 1 treated with AGOs and site 2 with In2Care traps. Site 1 had 91 houses, and Site 2 had 84 houses. Surveillance period (July 25-September 19, 2019) was 8-weeks and included pre-treatment for 2 wks, trap treatment for 4 wks and post-treatment (after trap removal) for 2 wks. Trap efficiency was carried out with nine ovitraps (1-L volume oviposition cups, ovicups) and six BGs traps. Three ovitraps and three BGs were deployed per site and remained throughout the whole 8-wk study period. The AGOs and In2Cares were used in the treatment period only. Ovitraps were fitted with seed germination papers and Cattail plant infusion water. The BGs traps were baited with BG-lure and dry ice.

The AGO trap was provided by SpringStar, USA. The trap consists of a 19-L black bucket with a fitted lid that houses a removable capture chamber. The capture chamber encloses a fitted sticky board and a small mesh screen on the bottom side of the capture chamber, which ensures the mosquitoes have no access to the water. Each AGO trap requires 8 L of water and no pheromones or pesticides are required. Holes were drilled at the 8-L mark to prevent excess water from rain or irrigation. The AGO traps were placed under trees, shrubs, and in the backyards to prevent damage or removal with 2-3 traps per household.

The In2Care trap, provided by Univar (Netherlands), is a small black bucket shaped like a planter pot. The trap lid has a 2.5 cm gap to the buckets rim that allows for mosquito entry but excludes debris and animals from the water inside the trap. Slots on the top of the trap drain excess water in the event of rainstorms and irrigation. This trap requires 3.5 L of clean tap water and a pre-supplied pesticide-treated gauze (includes the IGR, pyriproxyfen, the fungus *B. bassiana*, and Silicon Dioxide), which is placed onto a floating ring to keep the gauze upright. Two odor tablets supplied with the trap are added to the water to attract container-inhabiting mosquitoes. The In2Care traps were also placed under trees, shrubs, and in the backyards to prevent damage or removal with 2-3 traps per household.

The ovicups were black and could hold up to 750 mL of water and were purchased from Lowes, St. Augustine, FL. Each cup was filled with 500 mL of infusion water. To avoid overfill, a small hole was drilled above the water mark. Every week, the seed germination paper was collected, and new paper was placed with fresh infusion water.

A stock solution of infusion water was made from common Cattail plants (*Typha latifolia;* weighing around 1.36 kilograms; approximately 4-5 plants) collected from the field with green appearance. The Cattail plants were broken into smaller parts and placed in a large tank or dustbin and filled with water (up to 208.2 L mark) obtained from the retention pond onsite at Anastasia Mosquito Control District (AMCD), St. Augustine, FL. A stock solution of infusion water was prepared fresh at three-four days prior to putting the ovitraps in the field, to avoid over-fermentation and bacterial/mold growth. For effort and time effectiveness, infusion stocks were prepared for the whole experimental period and frozen and were thawed prior to field use.

Adult mosquitoes were collected from the BGs traps after 24 hr, while eggs were collected from ovicups weekly. The collected mosquitoes and egg papers were transferred to the AMCD lab for counting and identification of adult mosquito species.

All statistical analyses for AGO and In2Care trap data were analyzed using JMP statistical software. We explored the effects of AGOs and In2Care traps on *Ae. aegypti* and *Ae. albopictus* mosquito abundance and egg oviposition rates using a Shapiro-Wilk goodness-of-fit test along with a Kruskal-Wallis test, with significance levels set to 0.05. The data of non-targeted container-inhabiting species were not used and analyzed.

RESULTS

Mosquito species collected by BG traps. A total of 19 mosquito species were collected by BGs traps baited with BG lure and dry ice from the tested sites over the 8-wk period, with 18 and 17 species from Site 1 and Site 2, respectively (Table 1). The major species collected included target container-inhabiting mosquitoes *Aedes aegypti* and *Ae. albopictus*, and non container-inhabiting mosquitoes, *Ae. taeniorhynchus* Wied., *Cx. quinquefasciatus*

Table 1. Species of adult mosquitoes collected by BG sentinel traps baited with BG lure and dry ice from the AGO (site 1) and In2Care trap (site 2) on pre-treatment, during treatments, and post-treatments, St. Augustine, Florida, 2019.

Mosquito species	**AGO**	**In2Care**	**All**	**Species % of Total**
Aedes aegypti (Linn.)	**47**	**135**	**182**	**5.7**
Ae. albopictus (Skuse)	**758**	**96**	**854**	**26.8**
Ae. atlanticus Dyar & Knab	5	0	5	0.2
Ae. infirmatus Dyar & Knab	36	15	51	1.6
Ae. sollicitans (Walker)	0	4	4	0.1
Ae. taeniorhynchus (Wiedemann)	**55**	**646**	**701**	**22.0**
Anopheles Atropos Dyar & Knab	7	8	15	0.5
An. crucians Wiedemann	26	5	31	1.0
An. quadrimaculatus Say	11	2	13	0.4
Culex erraticus (Dyar & Knab)	30	1	31	1.0
Cx. nigripalpus Theobald	**263**	**143**	**406**	**12.8**
Cx. quinquefasciatus Say	**764**	**69**	**833**	**26.2**
Cx. restuans Theobald	2	1	3	0.1
Mansonia dyari Belkin, Heinemann & Page	2	4	6	0.2
Psorophora columbiae (Dyar & Knab)	11	5	16	0.5
Ps. ferox (von Humboldt)	4	2	6	0.2
Toxorhynchitus r. rutilus (Coquillett)	10	4	14	0.4
Uranotaenia sapphirine (Osten Sacken)	5	0	5	0.2
Wyomyia mitchelli (Theobald)	3	2	5	0.2
Total/Block	2039	1142	3181	100.0
(%) of total collected/Block	64.1	35.9	100.0	

Table 2. Number (mean ±SE) of target mosquitoes (eggs or adults) collected from different test sites, treated with AGO (Site 1) and In2Care (Site 2) traps on pre-treatment, treatment, and post-treatment, St. Augustine, FL, 2019. Different letters in column and row mean significant difference within the respective species.

Target	Traps deployed	Pre-treatment	Treatment	Post-treatment
Aedes aegypti & Ae. albopictus eggs	AGO	148.5±27.6 A	300.5±68.1 B	114.0±65.1 A
	In2Care	152.0±73.5 A	86.5±74.1 B	31.0±25.5 B
Aedes aegypti adults	AGO	1.0 ± 1.5 A	1.8 ± 2.0 B	0.83 ±1.2 A
	In2Care	3.8 ± 3.8 A	5.6 ± 5.3 B	4.7 ± 4.4 A
Aedes albopictus adults	AGO	19.2 ± 22.6 A	25.9 ± 21.4 A	17.7 ± 23.1 A
	In2Care	1.5 ± 2.8 A	4.5 ± 3.9 B	0.2 ± 0.4 A

Say, and *Cx. nigripalpus* Theobald, with variable abundance in different sites and collection weeks. *Aedes aegypti* and *Ae. albopictus* together represented 39.5% and 20.2% of total mosquitoes collected in Site 1 and Site 2, respectively.

Egg reduction of container-inhabiting mosquitoes. The total number of both *Aedes* mosquito eggs collected by ovitraps over the whole test period were the highest in the Site 1 (216±105 SE, n=1,727), followed by Site 2 (89±73, n=712). The mean egg numbers (eggs/week/trap ± SE) collected from post-treatment were significantly lower than those collected in pre-treatment and during the treatment (Table 2). Figure 1 shows that there is a general trend of reduction in eggs in the two tested sites based on the means in wk7 (1st week post-treatment) divided by the means in wk2 (2nd week pre-treatment); where the reduction rates were 59% and 88% in Site 1 and Site 2, respectively (Fig.1). Considering the whole period of post-treatment (mean of 2 weeks), compared to the pre-treatment (mean of 2 weeks), the reduction rates were 23% and 80% in Site 1 and Site 2, respectively. By comparing wk8 (2nd week post-treatment) to wk7, there were high recovery rates in mosquito populations, as indicated by increase in egg numbers by 130% and 300% in Site 1 and Site 2, respectively. The overall number of eggs collected in the two sites showed a 72% reduction in the post-treatment, compared to the pre-treatment period (Table 2 & Fig. 1).

Adult population reduction. Looking at the population dynamics of *Ae. aegypti* and *Ae. albopictus*, the two major dengue vectors targeted by the AGOs and In2Care traps, the results showed that in Site 1 and Site 2, the collected mean numbers of adult *Ae. aegypti* mosquitoes were 1.3 ±1.7 and 4.9 ±4.6, and *Ae. albopictus* were 22.2± 1.7 and 2.7± 3.6, respectively. In general, the two species *Ae. aegypti* and *Ae. albopictus* peaked at wk3 in the two treatment sites (Fig. 2 & 3). *Aedes aegypti* collections were higher in Site 2 than in Site 1 with mean (±SE) of 12.7±8.0 and 4.3±3.1 mosquitoes/trap, respectively. *Aedes albopictus* mean mosquito/trap (±SE) was highest in Site 1 (62.7±53.2) followed by Site 2 (14.0±12.1).

There was a reduction of 81.1% and 79.5% in *Ae. aegypti* mean numbers collected by BGs traps in wk7, compared to wk2 in both of Site 1 and Site 2, respectively (Fig. 2). By comparing mosquito mean numbers of wk8 to wk7, mosquito populations recovery rate was 353% in Site 2 higher than Site 1 with 85.7%. The mean numbers (i.e., mosquito/trap ±SE) in wk8 were 7.7±4.0 and 1.3±2.3, in Site 2 and Site 1, respectively (Fig. 2).

For *Ae. albopictus* collected by BGs, there was a general trend of population reduction by wk7, compared to wk2 in the two sites. The reduction rates were 100% in Site 2, compared to 72.8% in Site 1 (Fig. 3). By wk8, the mosquito

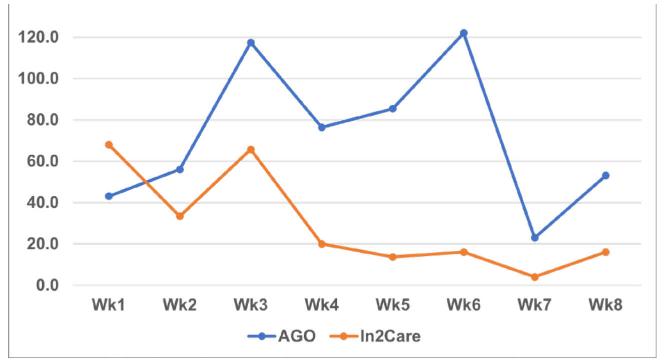

Fig. 1. Mean number of *Aedes aegypti* and *Aedes albopictus* mosquito eggs oviposited in ovitraps collected from different test sites, treated with AGO and (Site 1) and In2Care traps (Site 2) on pre-treatment, during treatment, and post-treatment.

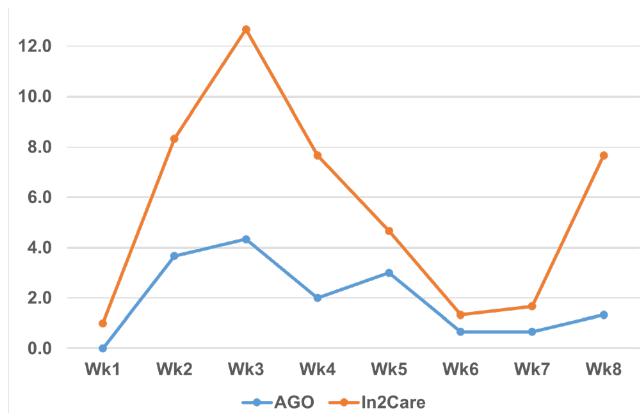

Fig. 2. Mean number of adult *Aedes aegypti* collected by BG sentinel traps from different sites, treated with AGO (Site 1) and In2Care (Site 2) traps on pre-treatment, during treatment, and post-treatment.

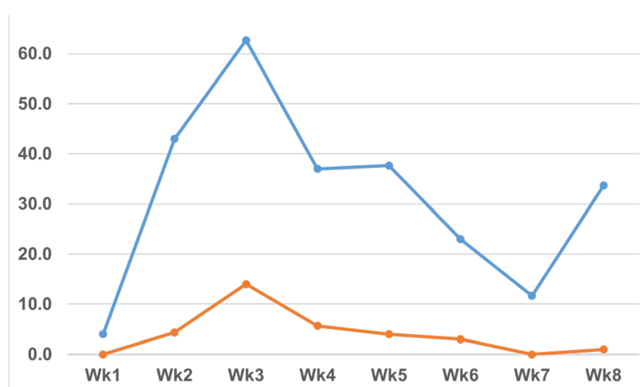

Fig. 3. Mean number of adult *Aedes albopictus* collected by BG sentinel traps from different test sites, treated with AGO (Site 1) and In2Care (Site 2) traps on pre-treatment, during treatment, and post-treatment.

population recovery rate was highest in Site 2 site followed by Site 1, with the means of 1.0±1.7 and 33.7±42.8 mosquito/trap in Site 2and Site 1, respectively.

For *Ae. aegypti*, in the treatment period, the mean mosquito numbers collected by BGs traps in Site 2 (5.6 ± 5.3 mosquito/trap) is 3-fold higher than in Site 1 (1.8 ± 2.0 mosquito/trap), but the difference is not significant (Table 2, Fig. 2). For *Ae. albopictus*, only in the treatment period, the mean mosquito numbers in Site 1 (25.9 ± 21.4 mosquito/trap) are significantly higher (X^2= 13.29, P = 0.0013) than Site 2 (4.5 ± 3.9 mosquito/trap) (Table 2 & Fig. 3).

DISCUSSION

In our study, we directly compared the field effectiveness of two new mosquito traps, the AGO and In2Care traps used as control tools, mainly against the major arboviral vectors and container-inhabiting, *Ae. aegypti* and *Ae. albopictus.*

Overall, the In2Care trap was the most effective in reducing mosquito populations for all container-inhabiting species collected at the end of the 4-wk trap deployment period, while the AGO was less effective. Looking at trap effectiveness on mosquito oviposition of *Ae. aegypti* and *Ae. albopictus*, via ovicups, the reduction rate observed in the In2Care site (88%) was higher than the AGO site (59%). However, in the post-deployment period, there was a remarkable increase in the total number of eggs in both In2Care and the AGO sites. This result shows a high recovery rate in the trap-treated sites, which could be considered an indication of the effectiveness of the In2Care and AGO traps. Ultimately, the In2Care traps had longer impact (i.e., after traps removal) on reducing the number of eggs laid by *Aedes* species than the AGOs (Fig. 1).

Due to the peculiar domestic container-inhabiting preference and oviposition behaviors of *Ae. aegypti* and *Ae. albopictus*, they have been the main species targeted for developing AGO and In2Care trapping strategies and tools, both for surveillance and control of these important arboviral vectors in different countries (Reiter, 1983, Ritchie et al. 2003,2009, Thavara et al. 2004, Gaugler et al. 2012, Barrera et al. 2014a,b, Buckner et al. 2017). In our study, the AGOs and In2Care traps had a significant impact on reducing adult *Ae. aegypti* populations, with the AGO traps being relatively more effective than the In2Care traps. Furthermore, after removal of traps, adult *Ae. aegypti* populations recovery rate in the AGO site was lower than in the In2Care site (Fig. 2). On the contrary, the In2Care trap were significantly more effective against *Ae. albopictus* adults than the AGO traps (Fig. 3). In Puerto Rico, AGO traps reduced *Ae. aegypti* populations by 60-80% with 85% area coverage (Barrera et al. 2014a,b). This reduction in vector population densities due to AGO deployment resulted in reduced transmission of Chikungunya virus (Barrera et al. 2016). Similarly, AGOs were effective in controlling gravid *Ae. aegypti* with good public acceptance in Australia (Mackay et al. 2013, Ritchie et al. 2009, Rapley et al. 2009).

The autodissemination stations (AS) showed variable efficacies against *Ae. aegypti* and *Ae. albopictus* in Florida based on mortality rates (29-45%) observed in sentinel ovicups, which measured the presence of competing natural oviposition sites and climatic conditions (Kartzinal et al. 2016). In addition, AS were able to transfer pyriproxyfen particles to most (85%) of cryptic ovicups and produced up to 41% mortality in *Ae. albopictus* pupal stages as well as a significant mortality in open ovicups. These mortality rates were compared to very low (0.3%) mortality in cryptic ovicups due to low-volume (LV)-*Bti* backpack sprayers use (Chandel et al. 2016). Similarly, in New Jersey, USA, Unlu et al. (2017) showed that pyriproxyfen-based AS were effective in reducing *Ae. albopictus* egg numbers and larval populations (collected by BGs traps, ovicups and sentinel cups), with significantly higher mortality in bioassays in trap-treated sites, compared to control sites. These studies are consistent with the partial efficacies of In2Care traps against *Ae. albopictus* in our study, which might be referred to the presence of cryptic or hidden larval sites, especially those created by the conditions of heavy rains such as from hurricane Dorian.

During this study, hurricane Dorian (August 29-September 5, 2019, i.e., wk6, the last week of trap deployment) caused heavy flooding, strong winds, abnormally high tides, and the destruction of environmental and artificial structures (roofs, trees, telephone poles, lawn décor, etc.) in both trap treatment sites. The intense wind and rain left debris in hard-to-reach areas as well as stacks of debris, which might have created new breeding sites and led to mosquito reinvasion into the treatment areas, especially for *Ae. aegypti* and *Ae. albopictus.* It is also possible that the intense wind and rain that came from multiple storms possibly flushed out the pyriproxyfen tainted containers in the In2Care traps resulting in pre-trap deployment-like conditions. The possibility for mosquito re-invasion into the treatment area is likely due to the surrounding housing and community structure in the treatment areas. However, the extent to which the homes and businesses surrounding both sites contributed to re-invasion is unknown.

A study using the In2Care trap showed that the combined use of IGR (pyriproxyfen) and entomopathogenic agents (the fungus *B. bassiana*) will ultimately target all mosquito life cycle immature and adult stages (Snetselaar et al. 2014). In the first semi-field efficacy trials of In2Care trap as autodissemination stations-based intervention in Florida, USA, traps were effective in attracting gravid females of both *Ae. aegypti* and *Ae. albopictus* with significant inhibition of adult emergence from the traps (Buckner et al., 2017). Pyriproxyfen particles were successfully autodisseminated to new oviposition sites, which in turn resulted in significant reduction in newly emerged mosquitoes. There was also a significant reduction in adult survivorship due to water sites contamination with fungal spores. In an 8-12-wk field trial using pyriproxyfen- autodissemination station, there was moderate (50%) pupal mortality in *Ae. albopictus* in peri-domestic habitats and 50% and 40% mortality in junkyard and tire piles, respectively. Site contamination with autodisseminated pyriproxyfen particles was 82.2%, with detection of pyriproxyfen particles in sentinel cups at a long distance (200 m) from ADS installment areas (Suman et al. 2018).

These field studies show the differential effects of the AGO traps and In2Care traps on different *Aedes* mosquito species. Based on the trap efficacy data on *Ae. aegypti* and *Ae. albopictus*, the AGOs and In2Care traps can be deployed for up to five weeks in the field, with extended post-treatment period (e.g., 4 wks from the last trap deployment week) to span a complete mosquito gonotrophic cycle and in different mosquito seasons. In addition, the mosquito population dynamics should be assessed for each individual species. For broad assessment of effectiveness, the sticky papers in the AGOs can be used periodically to identify the range of mosquito species collected. Egg and larval stage surveillance will be useful to evaluate the latent effect of mechanical killing of adults by AGO sticky papers or due to insecticidal efficacy of IGR- and fungus-autodisseminated particles on mosquito populations, especially in cryptic or hidden larval water habitats or containers. This is an important factor to measure the potential and cost-effectiveness of AGO and In2Care traps against *Ae. aegypti* and *Ae. albopictus.* When feasible, the public health gains from deployment of these dual surveillance and control tools can be assessed based on the outcomes reflected on reduced disease cases or incidence in the trap deployment areas. The present study adds more field-based information on the AGO and In2Care traps as novel, cost-effective toolset, which can be used by mosquito control districts for IVM. However, additional investigations of mass-trapping and population monitoring schemes are needed to enhance their effectiveness in the field.

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