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DEPLOYMENT AND FACT ANALYSIS OF THE IN2CARE® MOSQUITO TRAP, A NOVEL TOOL FOR CONTROLLING INVASIVE *AEDES* SPECIES

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ABSTRACT. During April–October 2019, the West Valley Mosquito and Vector Control District (Ontario, CA) deployed large numbers of In2Care® mosquito traps in a preliminary study to evaluate the trap's potential effectiveness at controlling invasive *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) in 6 cities of San Bernardino County, CA. The trap was used to attract ovipositing females, expose them to the juvenile hormone mimic pyriproxyfen and the entomopathogenic fungus *Beauveria bassiana*, and autodisseminate pyriproxyfen to other water sources prior to their death from fungal infection. The trap attracted *Ae. aegypti* and *Culex quinquefasciatus*, with the latter species predominating at much higher larval densities in the trap reservoirs. Field-collected larvae and pupae from the trap reservoirs showed complete adult emergence inhibition. Furthermore, the trap reservoirs retained high levels of residual larvicidal, pupicidal, and emergence inhibition activity after they were retrieved from the field, as indicated by laboratory bioassays against laboratory colony of *Cx. quinquefasciatus*. Results of this study support more detailed quantitative local evaluations on trap efficacy to measure the impact of the In2Care mosquito trap on wild invasive *Aedes* and *Culex* populations in future mosquito control efforts.

KEY WORDS *Aedes aegypti*, *Beauveria bassiana*, *Culex quinquefasciatus*, In2Care® mosquito trap, pyriproxyfen

INTRODUCTION

Mosquitoes and mosquito-borne diseases remain significant public health burdens, particularly in subtropical and tropical regions. Emerging and resurging mosquito species and the pathogens they transmit increase the challenges and complexities in integrated vector management (IVM) programs. The mosquitoes, *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse), are among the world's most invasive species (Lounibos and Kramer 2016). Their ongoing spread and establishment to regions outside their original tropical and subtropical territories has created a significant threat to public health and well-being across the world. In the USA, the potential for transmission of chikungunya, dengue, yellow fever, and Zika viruses has become more apparent following the geographical expansion of these invasive *Aedes*. Its initial discovery in central California was in 2013 (Porse et al. 2015, Metzger et al. 2017), and *Ae. aegypti* is now found in approximately 260 California cities (California Department of Public Health [CDPH] 2020), following multiple introductions from various geographic regions of North America over the past decade (Pless et al. 2017). A similar situation with less intensity also holds true for *Ae. albopictus*, with known infestations in 70 cities since 2011 (CDPH 2020). As of 2019, *Ae. aegypti* has expanded beyond its generally acknowledged northern limit (33°N latitude) in North America (Hahn et al. 2017) to the California city of Roseville (38°45′8″N, 121°17′17″W) (CDPH 2020).

Although the demand for innovative mosquito control tools has been on the rise, the availability of products that meet the standards of efficacy and safety remains limited because of strict regulations; high research, development, and registration cost; and a narrow market niche. Currently available products for mosquito control consist of just a few adulticides based on pyrethrins/pyrethroids and organophosphates, and larvicides formulated from microbials, insect growth regulators, and petroleum distillates. The development of resistance in species of concern, particularly to adulticides, has worsened the situation (Liu 2015, Su 2016). New active ingredients (AI) or optimized formulations of traditional AIs are strongly desired. Innovative control tools, such as bait stations or dissemination devices containing highly selective lethal agents with low effective concentrations and high stability under natural conditions (Lok et al. 1977), can reduce pesticide applications and minimize potential environmental and nontarget impacts as part of effective IVM programs targeting mosquitoes.

The In2Care® mosquito trap (In2Care BV, Wageningen, the Netherlands) was registered with the US EPA (EPA Reg. No. 91720-1) as a device to control *Ae. aegypti* and *Ae. albopictus*. Its innovative design targets both larval and adult mosquito life stages, respectively, by 2 bioactive pesticides, the juvenile hormone mimic pyriproxyfen (CAS No. 95737-68-1) (Farenhorst 2015, Unlu et al. 2020) and the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuil. (Hypocreale: Cordycipitaceae) (CAS No. 63428-82-0) (Blanford et al. 2011, Darbro et al. 2012). When combined with a source reduction program, the In2Care mosquito trap could be an integral component of a "push–pull" strategy to control container-breeding *Aedes* by attractive oviposition lures (pull) (Cook et al. 2007). Since inauguration, some limited field evaluations have been conducted (Farenhorst 2015), and promising results with variabilities have been reported (Snetselaar et al. 2014, Buckner et al. 2017, Salazar et al. 2019). Following the detections and population increase of *Ae. aegypti* since 2015 (Su et al. 2019a, Mullens et al. 2020), the West Valley Mosquito and Vector Control District (MVCD) in Ontario, CA conducted a study using the In2Care mosquito trap as one of its IVM tools in 6 cities of southwestern San Bernardino County, CA. Results of this investigation provide some insights for scaling up the use of this control device in the jurisdiction of the West Valley MVCD and in other areas infested with invasive *Aedes*.

MATERIALS AND METHODS

In2Care mosquito trap

The body of the In2Care mosquito trap is made of polyethylene, and consists of a lid, central tube, detachable interface, and reservoir filled with 3.5–4.5 liters of water infused with 2 yeast tablets as an oviposition attractant. A floating platform moves vertically along the central tube with the water level and holds a statically charged gauze strip coated with powder containing pyriproxyfen and *B. bassiana*. The gauze serves as a landing/resting site for adult mosquitoes that visit the trap, from which the 2 bioactives in the powder are transferred to mosquitoes. The bioactive mixture is provided in a separate, sealed aluminum refill sachet (In2Care® Mix) that also contains statically charged gauze strip and 2 odor tablets. In each sachet, there is a total 0.5 g of powder consisting of 74.03% of pyriproxyfen and 10.00% of *B. bassiana* strain GHA (not less than 4.5 $\times$ 10^9 viable spores/g). After egg deposition, mosquitoes exit the trap and disseminate pyriproxyfen to other breeding sources and die within 8–10 days after exposure to the entomopathogenic fungus. Traps are to be placed at a density of approximately 1 trap/400 m^2 and serviced every 4 wk by replacing the powder-treated gauze strip and refreshing the reservoir with water, bioactive powder, and yeast tablets (In2Care 2020).

In2Care mosquito trap deployment and servicing

The West Valley MVCD encompasses 544 km^2 in southwestern San Bernardino County, CA, and serves over 600,000 residents in the cities of Chino, Chino Hills, Montclair, Ontario, Rancho Cucamonga, Upland, and surrounding unincorporated county areas. The region experiences a Mediterranean type climate with hot, dry summers and rainfall occurring primarily in the winter (US Climate Data 2020). During recent years, the West Valley MVCD experienced an exponential increase of invasive *Ae. aegypti* and *Ae. albopictus* (Mullens et al. 2020).

To address the challenges of introduction and establishment of these species, the West Valley MVCD strategically deployed In2Care mosquito traps in stepped phases from April 26 to October 25 (calendar weeks 18–43), 2019, in *Aedes*-infested neighborhoods based on comprehensive mosquito surveillance data. Traps were placed in shaded or semishaded areas around homes and businesses in neighborhoods following new records of infestations of invasive *Aedes* collected in Biogents Sentinel (BGS) traps (Biogents AG, Regensburg, Germany). When setting up the traps, the In2Mix® sachet containing the biocides, odor tablets, and gauze was shaken well to maximize the attachment of biocides to the gauze. The remaining biocide powder was sprinkled into 3.8 liters of tap water inside the reservoir. During servicing, the reservoirs were emptied and rinsed with tap water, removing the remaining biocides, debris, and sediment. Each reservoir was refilled with the same amount of tap water and a new sachet was applied as described previously. Traps were replenished every 4 wk per product label instructions (In2Care 2020).

Profile of larval breeding in In2Care mosquito traps

Upon retrieval in early November 2019, traps were labeled by their placement and retrieval dates and locations. All larvae and pupae when present, and up to approximately 250 ml of water from each trap reservoir, were collected and placed together in a 480-ml Deli clear plastic container (First Street®, Los Angeles, CA) with a snap lid (cross cut made for ventilation) and brought back to the laboratory for processing. The trap reservoirs were transported to the District for further analysis (see below). The 3rd and 4th instar larvae were identified to species under a dissecting microscope (Nikon C-LEDS, Tokyo, Japan), while the 1st and 2nd instar larvae and pupae were counted only as identification of young larvae and pupae was not feasible.

Inhibition of adult emergence in collected larvae and pupae

Collection containers that held mainly late-stage larvae and pupae, and slight to moderate amounts of organic matter, were selected to observe adult emergence. To document the larval habitat profiles in these selected collections, 1st and 2nd instars of *Culex* and/or *Aedes* were identified visually by genus and counted; 2–3 from each genus were selected, killed with 95% ethanol, and identified to species under a dissecting microscope. The 3rd and 4th instars were estimated, and pupae were counted alive, if present. Adult emergence was checked daily and observations were concluded when no more surviving late instars or pupae were present in the

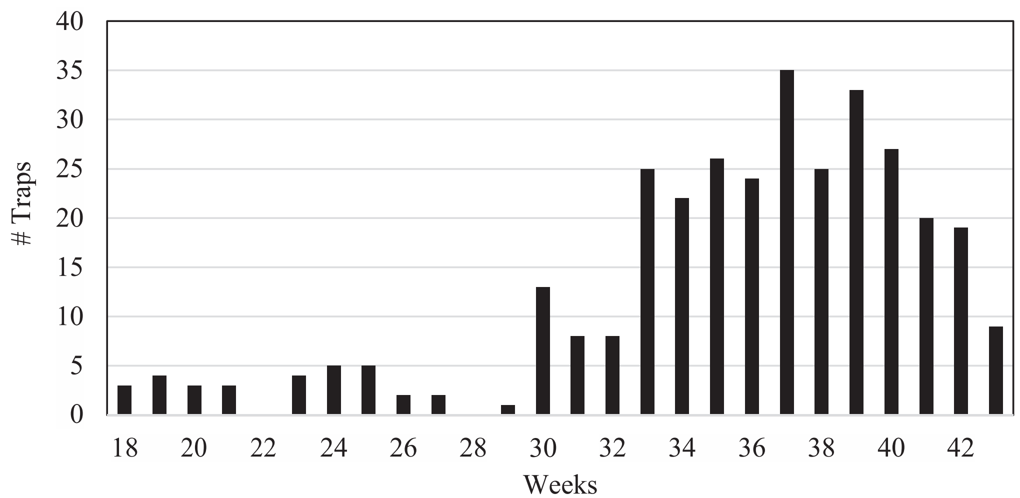

Fig. 1. Weekly deployment of In2Care® mosquito traps during April 26 to October 25, 2019.

containers. Free pupal exuviae, if present as criteria of successful adult emergence, were collected and counted.

Bioassays on residual activity of inhibition of adult emergence in In2Care mosquito traps

After traps were retrieved at the end of 2019 season, 2–3 reservoirs of the traps that were initially deployed and serviced ranging from 0 to 6 times since their deployment, were randomly selected for bioassay to determine the residual activity of adult emergence inhibition. Tap water was added to each reservoir in the amount of 3.8 liters. Two well-formed egg rafts that were oviposited overnight from a pyriproxyfen-susceptible laboratory colony of *Cx. quinquefasciatus* (West Valley MVCD internal data) were added to each reservoir. Rabbit pellets (2–3 g; Brookhurst Mill, Riverside, CA, 18% crude proteins) were placed in the water as organic enrichment to sustain larval growth. Egg hatch was verified on day 1 postintroduction, and larval growth, pupation, and mortality were checked twice daily. Viable pupae, when present, were collected and placed in 120-ml Styrofoam® cups, each with 100 ml of water from their respective reservoir. Each cup was covered by a plastic dome with a 2.5-cm-diameter window screen on the top for ventilation and to prevent emerging adults from escaping. Adult emergence was checked twice daily and free pupal exuviae were collected and counted. Successful emergence was defined as any adult that separated completely from the pupal exuvia. The percentage of inhibition of emergence (% IE) was calculated as % IE $= 100 \times$ (number of pupae – number exuviae)/number of pupae. Progressive mortality from 1st to 2nd and 3rd to 4th instars, intermediate form from larvae to pupae, and pupae was observed; photographs were taken when specimens were collected. Three new and clean trap reservoirs that were never used in the field, and did not supposedly have any activity of pyriproxyfen, were used as untreated controls in laboratory bioassays conducted in an insectary at 27.8–28.9°C, 40–60% relative humidity, and a photoperiod of 10 hr light and 14 h dark.

RESULTS

In2Care mosquito trap deployment and servicing

In total, 326 traps were deployed at different home and business addresses in 6 cities of the West Valley MVCD during April 26 to October 25, 2019. The largest number was in Chino (91; 27.91%), followed by Ontario (83; 25.46%), Montclair (66; 20.25%), Chino Hills (33; 10.12%), Upland (31; 9.51%), and Rancho Cucamonga (22; 6.75%). Minimum numbers of traps were deployed during weeks 18–29, followed by a gradual increase during weeks 30–36, a peak in week 35 (37 traps), and a general decline to week 43 (Fig. 1). Upon retrieval, 76 traps were still within the initial service interval (4 wk), 116 traps had been serviced once, 88 had been serviced twice, and the remaining traps (46) had been refilled 3–6 times.

Profile of larval breeding in In2Care mosquito traps

Of the 326 deployed In2Care mosquito traps, 105 traps (32.2%) contained immature mosquitoes, with a combined average of 37.4 larvae and pupae/trap. Based on identifiable larvae, 37 traps (11.3%) had *Ae. aegypti* (8.8 larvae/trap), 39 traps (12.0%) had *Cx. quinquefasciatus* (47.1 larvae/trap), and 24 traps (7.4%) had both species present, with averages of 5.0

Table 1. Presence of immature mosquitoes in In2Care® mosquito traps in 2019.[1]

	Aedes aegypti only	*Culex quinquefasciatus* only	Both species	Early larvae and/or pupae only	Total
No. positive traps	37	39	24	5	105
% positive ±SE	11.3 ± 1.8	12.0 ± 1.8	7.4 ± 1.4	1.5 ± 0.7	32.2 ± 2.6

[1] Three hundred twenty-six In2Care® traps were set and retrieved from April 26 to November 21, 2019. There were no other species present in the samples based on identifiable larvae.

Ae. aegypti and 25.0 *Cx. quinquefasciatus* larvae/trap, respectively. Five traps (1.5%) had early instars and/or pupae only, which could not be identified. Overall, early instars were present in 38 traps (17.5 larvae/trap), and pupae were detected in 40 traps (8.9 pupae/trap). Larvae from 3 collections with mixed *Culex* and *Aedes* were not counted because of heavy organic content and were excluded from larval density calculations (Tables 1 and 2).

Inhibition of adult emergence in collected larvae and pupae

Fourteen collections (Chino [7], Ontario [4], and Montclair [3]) with mainly late instars and/or pupae, and slight to moderate amounts of organic matter, were selected to observe adult emergence. From these collections, no adults emerged, resulting in 100% inhibition of adult emergence out of 79 and 7 late instars of *Ae. aegypti* and *Cx. quinquefasciatus*, respectively, and 160 pupae (Table 3). Mortality occurred in late instars, during transition from larvae to pupae, at intermediate forms between larvae and pupae, or at pupae. No incomplete adult emergence was noticed.

Bioassays on residual activity of inhibition of adult emergence in In2Care Mosquito Traps

Two rounds of bioassays were conducted with 16 In2Care mosquito trap reservoirs that were retrieved after various periods of deployment with 0–6 refills since initial placement in the field. In the 2 bioassays, respectively, 1–365 and 0–309 pupae were collected from all replicated reservoirs from each refill category (Table 4). Each bioassay demonstrated 100% inhibition of adult emergence in pupae from the reservoirs regardless of the numbers of refills and length of trap operation in the field (Table 4). The mortality in the untreated controls remained low, 5.3% and 1.3% out of 322 and 315 pupae, respectively, for each test series. Introduced egg rafts in each trap reservoir hatched normally, as indicated by open opercula and presence of live newly hatched larvae. However, progressive mortality of immature mosquitoes occurred at the following stages: early and late larvae, transitions from larvae to pupae ("puparvae"), or pupae (Fig. 2).

DISCUSSION

The West Valley MVCD explored the feasibility of the In2Care mosquito trap as a bait and dissemination station for controlling invasive *Aedes* in residential neighborhoods of 6 cities of the west valley area in southwestern San Bernardino County, CA. At this initial attempt, the trap density, however, was much lower than the label rate of 1 trap/400/m^2 because of limited resources. Although the agency has been undertaking many efforts to treat cryptic larval habitats, using available microbial and insect growth regulator larvicides, this trap's dual action offers an opportunity to change the control strategy from "delivery of pesticides to larval habitats by mosquito control technicians" to "an invitation to ovipositional females to lay their eggs in a lethal habitat and let the visiting females spread the pesticides before they are killed." It is application of pesticides, and controls labor costs. Labor is a major cost in large-scale larval control programs and could be substantially reduced if intervals for pesticide reapplications are reduced without jeopardizing control (Worrall and Fillinger 2011).

The yeast (odor) tablets induced fermentation, generating the desired ovipositional cues for both *Aedes* and *Culex* spp., presumably through biochemical and microbial activities, as evidenced by the

Table 2. Average number of immature mosquitoes in In2Care® mosquito traps in 2019.[1]

	Aedes aegypti only	*Culex quinquefasciatus* only	*Aedes aegypti*, *Culex quinquefasciatus*[2]	Early instars	Pupae	Total
No. positive traps	37	39	21	38	40	102
Avg. no./trap ± SE	8.8 ± 1.3	47.1 ± 7.2	5.0 ± 0.9 25.0 ± 5.3	17.5 ± 3.5	8.9 ± 1.3	37.4 ± 3.9

[1] Three hundred twenty-six In2Care® traps were set and retrieved from April 26 to November 21, 2019. There were no other species present in the samples based on identifiable larvae.
[2] Larvae from 3 collections with mixed *Culex* and *Aedes* and poor water quality were not counted and were excluded for larval density calculation.

Table 3. Inhibition of adult emergence (IE%) in 4th instars and pupae retrieved from In2Care® mosquito traps at end of the season in 2019.[1]

	Fourth instars		
	Aedes aegypti	*Culex quinquefasciatus*	Pupae
No. observed	79	7	160
% adults emerged	0	0	0

[1] Larvae and pupae for IE% observation were made from 14 traps in 3 cities. Mortality occurred at larvae, during transition from larvae to pupae, at intermediate forms between larvae and pupae, or at pupae. There was no incomplete adult emergence noticed.

presence of *Ae. aegypti* and/or *Cx. quinquefasciatus* larvae in 32.2% of the trap reservoirs. The trap-positive rate for *Ae. aegypti* alone (11.3%) was about the same for *Cx. quinquefasciatus* alone (12.0%), and some traps contained both species (7.4%). These results suggested that the fermented oviposition media did not repel *Ae. aegypti*, which is known to prefer water sources with light organic matter in which to lay eggs. Studies on oviposition attractants have indicated that a yeast-containing tablet was the most attractive odor lure for *Ae. aegypti* (Snetselaar et al. 2014).

The larval density for *Cx. quinquefasciatus* among the positive traps was considerably higher than that of *Ae. aegypti*. This difference held true for the traps that contained either 1 species only, or both species. These larval counts did not include the collections of early instars and pupae, as they could not be differentiated to species morphologically. This species-dependent larval density difference might be attributable to higher overall *Cx. quinquefasciatus* counts (averaging 28.1/trap night) compared to *Ae. aegypti* numbers (averaging 2.0/trap night) in the areas that were sampled, as shown in BGS surveillance traps in 2019 (West Valley MVCD internal surveillance data). It is also possible that the trap reservoirs could have become more attractive to *Cx. quinquefasciatus* at the end of each trap servicing cycle when water was accumulatively enriched by leaf litters, other organic matter, and microbial activity. Furthermore, *Ae. aegypti* employs skip-ovipositing behavior and lays its eggs in small quantities at multiple sites, which reduces the number of eggs laid at a single location compared to *Culex* females. Nevertheless, the organic content and attractiveness seemed not enough to attract *Cx. stigmatosoma* Dyar, the foul water mosquito, which prefers to lay eggs in water with high organic matter. This species is found in the same neighborhoods where the In2Care mosquito traps were placed (West Valley MVCD internal surveillance data).

The juvenile hormone mimic pyriproxyfen, an IRAC (Insect Resistance Action Committee) Group 7C pesticide, is considered a reduced risk and organophosphate alternative pesticide (US Environmental Protection Agency [US EPA] 2020). The amount of pyriproxyfen that was contained in each In2Mix® sachet was calculated to be 370.15 mg. Even though the concentration of the active ingredients in the reservoir water was unknown, and possibly varied for each trap, best efforts were made to measure the biological activity of pyriproxyfen upon trap retrieval at the end of the season. Complete inhibition of adult emergence was observed from larval and pupal collections from 14 traps, with most of the mortality occurring during larval stages and intermediate forms between larvae and pupae. The lack of incomplete adult emergence (i.e., the legs and/or wings still attached to the pupal exuviae) indicated the presence of overwhelmingly high concentrations of pyriproxyfen in water samples collected from the trap reservoirs. Residual pyriproxyfen in the samples could have been from the most recent biopesticide recharges, plus any amount from previous refills, as these 14 traps were in service for 52–167 days and refilled 2–6 times, with rinses at each service time. The relatively low percentage of mosquito-positive traps (32.2%) at the time of retrieval from the field in November 2019 could be due to the low oviposition activity in late season and the high cumulative pyriproxyfen concentrations that may have caused larval mortality.

The complete inhibition of adult emergence (100%) in samples described previously raised a critical question about whether some pyriproxyfen was sequestered by the trap reservoir, which is made of polyethylene. Pyriproxyfen and other juvenile hormone mimics, such as *s*-methoprene, tend to be retained by plastic and render long residual activity, as observed previously in field trials by the senior

Table 4. Residual activity of inhibition of adult emergence (% IE) in In2Care® mosquito traps upon retrieval from the field.

	First round				Second round		
Sachet refills	Trap in the field (days)	Pupae collected	Adults emerged	% IE ± SE	Pupae collected	Adults emerged	% IE ± SE
Unused reservoir	n/a	322	305	5.3 ± 1.2	315	311	1.3 ± 0.6
0	27–28	242	0	100	98	0	100
1	54–57	136	0	100	258	0	100
2	85	53	0	100	100	0	100
3	113	1	0	100	104	0	100
4	137	1	0	100	0	0	100
6	200	365	0	100	309	0	100

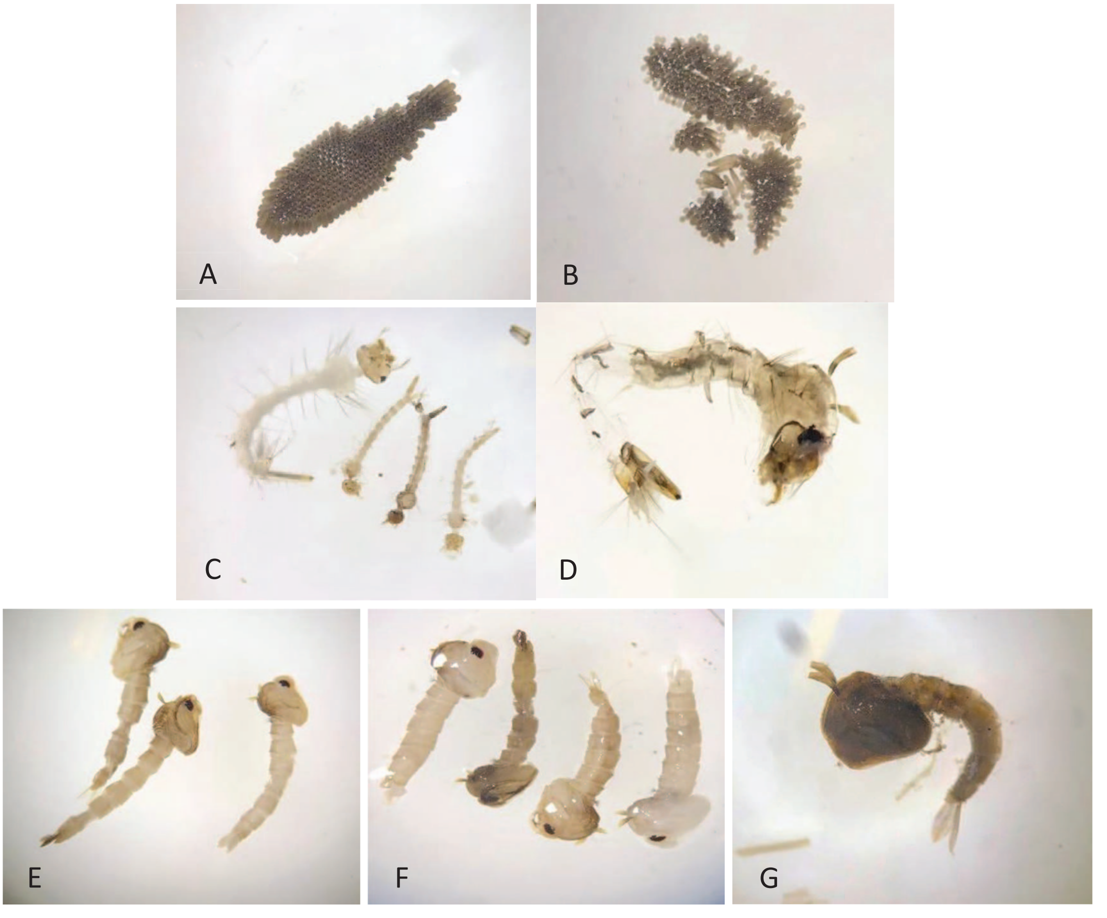

Fig. 2. Progressive mortality of immature stages of *Culex quinquefasciatus* during bioassay on residual activity from In2Care® mosquito trap reservoirs at the end of the season in 2019. (A) Egg raft introduced; (B) hatched eggs with open opercula; (C) mortality at larvae; (D) mortality at transition from larva to pupa (early puparvae); (E) mortality at early intermediate between larvae and pupae (puparvae); (F) mortality at late intermediate between larvae and pupae (late puparvae); (G) mortality at pupa.

author and others (Suman et al. 2013). In bioassay evaluations of the trap reservoirs, which were flooded in the laboratory using fresh tap water after they were retrieved from the field, 100% inhibition of adult emergence was achieved in 2 consecutive rounds of tests with negligible mortality in untreated control. Regardless of length in field and numbers of refills, progressive mortality was observed, ranging from newly hatched larvae to pupae. This result clearly indicated that a certain amount of pyriproxyfen was associated with, attached on, or even absorbed into, the trap reservoirs. These results, together with previously described 100% inhibition of adult emergence in field-collected larval and pupal samples from the water in trap reservoirs, implies the operational significance of the In2Care mosquito trap for mosquito control in the field. It is reasonable to assume that mosquito eggs laid into the trap reservoirs are unlikely to develop to adult stage when traps are serviced regularly, as documented in prior semifield studies (Snetselaar et al. 2014, Buckner et al. 2017).

The high and extended residual inhibition of adult emergence is related to the growth regulation profile of pyriproxyfen, the lethal concentration of which is the lowest ($IE_{90} < 10$ ppb) among the known insect growth regulators and microbial larvicides (Su and Cheng 2014; Su et al. 2018, 2019a, 2019b). Additionally, pyriproxyfen seems to have a low risk of resistance development (Schaefer and Mulligan 1991), even though some reports were documented earlier in whiteflies, houseflies, and recently, a separate field population of *Ae. aegypti* in southern California (Su et al. 2019a). Fortunately, once mosquitoes developed resistance to other larvicides, such as *Lysinibacillus sphaericus* (Myer and Neide) (Bacillales: Bacillaceae), spinosad, *s*-methoprene, or adulticide pyrethroids, they remained susceptible to pyriproxyfen (Su and Cheng 2014; Su 2016; Su et al. 2018, 2019a, 2019b). It is indeed worthwhile to emphasize the lack of 2-way high-level cross-resistance between pyriproxyfen and *s*-methoprene, as both belong to IRAC Group 7 and possess similar modes of action. The structural difference in both compounds could be among the reasons for a lack of significant cross resistance (Su 2016, Su et al. 2019a).

Also contained in each In2Mix sachet was 50 mg of the entomopathogenic fungus *B. bassiana* (no less than 2.25×10^8 viable spores). This fungus has drawn increasingly more attention recently because of its high lethal activity against multiple species (Blanford et al. 2011, Darbo et al. 2012). The slow lethal action of the fungus allows fungus-infected mosquitoes to spread pyriproxyfen for several days before death. The activity of *B. bassiana* spores, however, was unable to be determined in this current study, as it is intended for adulticiding.

The In2Care mosquito trap was used in this study as an investigative IVM measure to examine its potential to combat the spread of invasive *Aedes*, mainly *Ae. aegypti*, in impacted neighborhoods. Because of the presence of the oviposition lure, the In2Care mosquito trap acted as an egg sink (Buckner et al. 2017) by pulling in gravid *Ae. aegypti* and *Cx. quinquefasciatus*, then killing their offspring with pyriproxyfen. In addition, studies show that volatiles emitting from larvae are attractive to gravid dengue vectors (Wong et al. 2011) and may augment the attractiveness of the trap. Complete inhibition of adult emergence was observed in field-collected samples, as well as in laboratory bioassay tests on retrieved trap reservoirs. Mortality occurred in both larval instars and pupae. These results demonstrate that the In2Care mosquito trap is unlikely to foster adult mosquito production under monthly service cycles, by minimizing the risk of larval exposure to nonlethal doses. Sublethal doses of pyriproxyfen have been reported to reduce reproduction and vectorial capacity in mosquitoes (Vasuki 1999, Mbare et al. 2013) and are considered beneficial for disease risk reduction (Unlu et al. 2020), if the risk of resistance development is manageable (Schaefer and Mulligan 1991).

Although this study focused primarily on the impacts on immature mosquitoes inside the trap, other studies have demonstrated the In2Care mosquito trap's lethal effect on larval habitats in the vicinity of the trap (Snetselaar et al. 2014, Buckner et al. 2017, Smith and Thrall 2017). Large-scale deployment of the In2Care mosquito trap will be considered by the West Valley MVCD to establish quantitative evaluations on trap efficacy and actual impact on wild adult *Aedes* and *Culex* populations. Limited accessibility to individual properties can constrain optimal placement of autodissemination stations, a crucial component of effective levels of mosquito control (Caputo et al. 2012). However, even with frequent servicing and deployment limitations, the trap's relatively low cost makes it a promising addition to current vector control tools (Snetselaar et al. 2014). Based on earlier investigations and our promising results, the West Valley MVCD will continue to implement the use of the In2Care mosquito trap as a control strategy in its mosquito control operations.

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