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Potential Community-Based Control by Use of Plastic Film to Block *Aedes aegypti* (L.) Egg Adhesion

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Abstract. Monitoring and control programs for yellow fever mosquito, *Aedes aegypti* (L.), usually do not focus on the egg as a potential target for control. The egg is the most numerous life stage but is invisible to conventional inspection by a sticky pad that attaches it. This laboratory study evaluated the potential ovicidal effect of five commonly used plastics. Plastic liners in oviposition containers were exposed to gravid female mosquitoes in an insectary. The percentage of eggs that hatched was recorded. The plastic liners altered the places where eggs were laid, i.e., 27.0% were glued onto the plastic film, 70.0% remained floating, and 3.0% were submerged. Vinyl blocked most egg adhesion, with a mean of 7.05 ± 10.1 eggs, compared to 170.7 ± 68.6 eggs for the check. Pooled numbers of glued, floating, and submerged eggs showed fewest eggs hatched on vinyl or low-density polyethylene, resulting in the death of 94.7% of the embryos. Plastics waterproofing property might be blocking the hyaluronic acid, the component of the sticky substance of mosquito eggs. Results demonstrated the potential use of plastic strips as an ovicide. Plastics should be studied further for use in community-based programs to control dengue.

Introduction

Dengue and yellow fever are two of the most important viral diseases transmitted by arthropods (Gould and Solomon 2008). The yellow fever mosquito, *Aedes aegypti* (L.), is the main vector of dengue in Mexico (García-Rejón et al. 2011). According to the World Health Organization, application of organophosphate granules (Temephos 1%) in domestic breeding sites is recommended to control *Ae. aegypti* larvae. Elimination of adults sometimes relies on using truck-mounted ultra-low volume sprayers to disperse insecticide mist to permeate indoor spaces and kill resting mosquitoes. The methods are costly, have low efficacy, select for insecticide-resistant populations of mosquitoes, and negatively impact non-target species (WHO 2009). The egg stage of *Ae. aegypti* is often ignored as a target for control.

During oviposition, female *Ae. aegypti* select moist areas above the water line to lay eggs. The accessory gland of the mosquito female secretes a sticky

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substance to glue the egg onto a surface. Embryogenesis begins shortly after (Clements 1996). The sticky substance contains several chemical compounds, with hyaluronic acid the major constituent. This acid is hydrophilic and absorbs water before hardening (Christophers 1960, Padmaja and Rajulu 1981). Desiccation of the embryo begins shortly after the serosa cuticle is synthesized (Lazzaro-Rezende et al. 2008). The serosa cuticle develops during embryogenesis and becomes the third layer of the egg shell, just beneath the endochorion (Beckel 1958, Clements 1996). Failure of egg adhesion after oviposition can prevent cuticle formation. As a consequence, the mosquito embryo life cycle and survival are disrupted and population growth reduced.

Physical properties of some widely used plastics such as cellophane (vinyl and polyethylene) may prevent oviposition, either repelling or preventing the "stickiness" of eggs (Alvarado-Moreno 2011). We hypothesized that placing plastic strips onto preferred egg-laying areas such as 200-liter drums and cement tanks or "pilas" could control *Ae. aegypti* in dengue fever-endemic countries. This ovicidal control strategy could be used in a community-based program because of the availability and low cost of plastic supermarket and trash bags.

The behavioral response of *Ae. aegypti* and efficiency of different plastic films for blocking adhesion of mosquito eggs were evaluated under laboratory conditions. The number of eggs that hatched but were unable to adhere to a container wall also was assessed.

Materials and Methods

Properties of Plastic Films. Commercially available vinyl, high- and low-density polyethylene, cellophane, and polyvinyl chloride plastics were evaluated. A description of the properties of each plastic follows.

Vinyl is a clear, colorless plastic often described as having a sweet fruit aroma (Design Institute for Physical Properties 2003). It is waterproof and resistant to warm temperatures and chemical solvents. Annual worldwide production is estimated at 500 billion tons of the polymer.

High- and low-density polyethylene plastics are insoluble in water and permeable to organic vapors and oxygen (Plastics Pipe Institute 1993). With both polymers, there is an increase in temperature. They are not especially sensitive to moisture absorption. A paraffinic structure makes them stable and inert.

Cellophane is a natural polymer derived from cellulose (Tibbets 1931). It is thin, transparent, and flexible. Low permeability to air, grease, and bacteria makes it useful as a wrapping material.

Polyvinyl chloride is a thermoplastic, odorless, tasteless, and harmless plastic (Mingwang et al. 2004). It is resistant to most chemical solvents, lightweight, and non-flammable. It does not degrade or dissolve in water and is recyclable. It is stable and inert and used extensively where hygiene is a priority.

***Aedes aegypti* Colony.** *Ae. aegypti* mosquitoes (F_1) collected from Monterrey city area and continuously reared in a laboratory were used. Larvae were fed fish food (trademark of the local fish store), and adults that emerged were fed 10% sucrose solution. Blood meals from anesthetized rats (Gadelha and Toda 1985) were provided in 15-minute intervals on two consecutive days to sugar-starved (24-hour old) female mosquitoes.

Experiments with Plastic Film. Cohorts of 10 gravid, 6-7 day old *Ae. aegypti* females were released into five 30-cm^3 cages. Before release, five 100-ml oviposition containers with 85 ml of dechlorinated water and a 6 x 15 cm plastic strip just above the water line were placed in each cage. Vinyl, high- and low-density polyethylene, cellophane, and polyvinyl chloride plastics were individually tested. A matched set of five cages lined with filter paper (Whatman no. 4) were used as checks. Cages were protected from light and maintained at 28°C and 67.5% relative humidity. Egg laying generally began immediately after the test was set up. In all cases including the check, oviposition was permitted for 24 hours. Experiments using five-cage replications per plastic material were done four times between February and June 2010. The number of individual replications testing the anti-adhesion properties of each plastic material totaled 125, with an additional 125 replications for the matched filter-paper check. In a preliminary study, Alvarado-Moreno et al. (2012) noticed a change in the ovipositing behavior of female *Ae. aegypti* after plastic film was used as an egg substrate. Some batches of eggs were glued, most were floating on the water surface, and a few were submerged. All were deposited in the same oviposition container in the cages. Considering these findings, eggs were counted in three categories: glued to the plastic liner, floating on the water surface, or submerged below the water surface. A dissecting microscope at 30x magnification was used while directly counting eggs in the oviposition container.

Assessing the Number of Eggs that Hatched. To determine the potential inhibitory effect of the five plastics on incubation and hatching of first-instar larvae, glued, floating, and submerged *Ae. aegypti* eggs were separated and incubated for 48 hours to promote embryonation (Clements 1996). Afterward, counted eggs were placed into individual 500-ml polystyrene containers filled with dechlorinated water. Eggs that hatched and 1^{st}-instar larvae that survived were counted during a 3-day period. Temperature and relative humidity were maintained at 26°C and 70%, respectively.

To determine the effectiveness of each of the five tested plastics for blocking egg adhesion, data from the five experiments were pooled (n = 125). Means and standard deviations and percentages for egg adhesion were computed for the kinds of plastic and egg categories (glued, floating, and submerged). Analysis of variance (ANOVA) and paired student *t*-tests were used to compare means among plastics and egg categories. Data were transformed ($1 + \log_{10}$) before statistical analysis (Zar 1999). SPSS software (2008) was used to compute means to compare the effect of egg-hatching inhibition of each plastic material.

Results

Blocking Egg Adhesion. Change in egg location was recorded for each kind of plastic. Only 27.0% of eggs were glued onto the oviposition liners, whereas 70.0% were floating on the water surface and 3.0% were submerged (Table 1). Analysis of the number of glued eggs by plastic material showed that vinyl most disrupted egg adhesion, with a mean of only 7.1 eggs, and high-density polyethylene, with a mean of 73.4 eggs, was least efficient (Table 2). Vinyl prevented almost 96% of eggs compared to the check. Vinyl blocked adhesion of significantly more eggs than did the other plastics ($F = 169.5$, df = 624, $P < 0.01$). Fewer eggs also were glued onto low-density polyethylene (18.6 ± 15.9) and polyvinyl chloride (21.1 ± 20.1). As expected, most floating eggs, 159.4 ± 124.5, were found with vinyl. An unexpectedly large mean of 142.7 ± 101.1 eggs was found with low-density polyethylene. A paired *t*-test comparison showed no

Table 1. Percentages of *Ae. aegypti* Eggs Glued, Floating, and Submerged after Exposure to Five Plastic Films under Insectary Conditions

Plastic film	N	Glued		Floating		Submerged	
		Eggs laid	%	Eggs laid	%	Eggs laid	%
VIN	125	881	4.1	19,922	93.0	629	2.9
HDPE	125	9,172	75.6	2,837	23.4	127	1.0
LDPE	125	2,328	11.4	17,842	87.1	306	1.5
CLP	125	2,596	38.8	3,671	54.8	431	6.4
PVC	125	2,640	53.8	1,377	28.1	885	18.1
Check	125	64,240	100.0	-	-	-	-

VIN = vinyl, HDPE = high-density polyethylene, LDPE = low-density polyethylene, CLP = cellophane, PVC = polyvinyl chloride

Table 2. Mean Number (±SD) of *Ae. aegy*pti Eggs Laid after 24 Hours On 125 Oviposition Containers Lined with Five Plastic Materials under Insectary Conditions

Plastic film	Glued	Floating	Submerged
VIN	7.1 ± 10.1	159.4 ± 124.5	5.0 ± 11.2
HDPE	73.4 ± 34.1	22.7 ± 14.5	1.0 ± 2.4
LDPE	18.6 ± 15.9	142.7 ± 101.7	2.4 ± 4.9
CLP	20.8 ± 23.6	29.4 ± 31.1	3.4 ± 4.7
PVC	21.1 ± 20.1	11.0 ± 12.8	7.1 ± 8.2
Check	170.7 ± 68.6	---	---

VIN = vinyl, HDPE = high-density polyethylene, LDPE = low-density polyethylene, CLP = cellophane, PVC = polyvinyl chloride

statistical differences between vinyl and low-density polyethylene (t = 1.202, df = 124, P = 0.232). The mean number of submerged eggs differed statistically (F = 14.1, df = 624, P < 0.01) among the five plastic materials. Fewest submerged eggs were found with high-density polyethylene (1.0 ± 2.4), low-density polyethylene (2.5 ± 4.9), and vinyl (5.0 ± 11.2).

Assessing Egg Hatching Rate. Inhibition of oogenesis in the embryos of *Ae. aegypti* was evaluated by assessing the failure of eggs to hatch on each of the five plastic films. Vinyl most inhibited hatching, with a mean of 1.0 ± 2.5, while low-density polyethylene was the least efficient at stopping eclosion, with a mean of 33.8 ± 27.4 (Table 3). Only 17.7 and 11.4% of 1st-instar larvae survived on vinyl and low-density polyethylene compared to 98.0% of the check group. However, paired mean comparisons showed vinyl and low-density polyethylene resulted in similar hatching rates for attached eggs (t = -3.490, df = 124, P = 0.01).

The mean hatching rates for the plastic films differed statistically (F = 106.6, df = 624, P < 0.01). Mean numbers of floating eggs on each of the five plastic films differed statistically (F = 6.8, df = 624, P < 0.01). For floating eggs, viability was inhibited more with polyvinyl chloride, with a mean of 4.2 ± 7.8 eggs, compared to cellophane where an increase in eclosion was observed, with a mean of 10.9 ± 15.6. However, these low means resulted in greater survival of 1st instars (43.9 and 42.4%, respectively). Vinyl had a high ovicidal effect, with a mean of 7.4 ± 9.3 eggs, representing 5.3% larval survival.

Table 3. Means (± SD) and Percentages of Survival for 1st-instar *Ae. aegypti* Larvae Calculated from Hatching Rates after Exposing Commonly Used Plastics as Oviposition Substrates under Insectary Conditions

	Pooled eggs		Glued eggs		Floating eggs		Submerged eggs	
Plastic film	Mean	% surviving	Mean	% surviving	Mean	% surviving	Mean	% surviving
VIN	8.6 ± 9.7	5.7	1.0 ± 2.5	17.7	7.4 ± 9.3	5.3	0.1 ± 0.5	2.2
HDPE	41.5 ± 30.1	42.2	33.8 ± 27.4	45.3	7.6 ± 7.7	33.8	0.02 ± 0.9	1.6
LDPE	9.3 ± 10.5	5.4	2.5 ± 3.8	11.4	6.6 ± 9.3	19.5	0.3 ± 0.7	11.1
CLP	18.0 ± 24.8	38.3	6.8 ± 9.8	38.2	10.9 ± 15.6	42.4	0.2 ± 0.9	4.9
PVC	15.7 ± 20.5	44.1	10.0 ± 13.4	49.5	4.2 ± 7.8	43.9	1.7 ± 2.9	28.3
Check	169.1 ± 68.2	98.0	169.1 ± 68.2	98.0	---	---	---	---

VIN = vinyl, HDPE = high-density polyethylene, LDPE = low-density polyethylene, CLP = cellophane, PVC = polyvinyl chloride

Most submerged eggs also failed to hatch with vinyl and high-density polyethylene, 0.1 ± 0.5 and 0.02 ± 0.9 eggs, respectively (Table 3). These corresponded to 2.2 and 1.6% surviving 1st-instar larvae on vinyl and high-density polyethylene, respectively. The numbers of submerged eggs that hatched differed significantly (F = 30.6, df = 624, P < 0.01) among the five kinds of plastic. Comparisons showed vinyl-low-density polyethylene, vinyl-cellophane, and low-density polyethylene-cellophane resulted in similar hatching rates of submerged eggs (t = -1.064, df = 124, P = 0.289; t = -0.495, df = 124, P = 0.621; and t = 0.366, df = 124, P = 0.715, respectively).

Analysis of hatching of the pooled eggs (glued, floating, and submerged) by the kind of plastic showed vinyl had the least, 8.6 ± 9.7, while high-density polyethylene had the most, 41.5 ± 30.1. Numbers of pooled eggs that hatched differed significantly (F = 52.2, df = 624, P < 0.01) on the five kinds of plastic.

We calculated the pooled ovicidal effect of each kind of plastic by combining mortality and failure to hatch. Mortality rates were calculated by subtracting the percentage of eggs that hatched. Low-density polyethylene resulted in death of most embryos, 88.6%, and high-density polyethylene least, 45.3% (Fig. 1). Vinyl resulted in death of most floating eggs, 94.7%, while polyvinyl chloride was least effective at 56.1%. For submerged eggs, high-density polyethylene was most effective, 96.7% mortality, followed by vinyl and cellophane at 95.4 and 95.0%, respectively. Pooled mortality of glued, floating, and submerged eggs for each plastic showed vinyl was the most effective ovicide, resulting in 94.3% mortality.

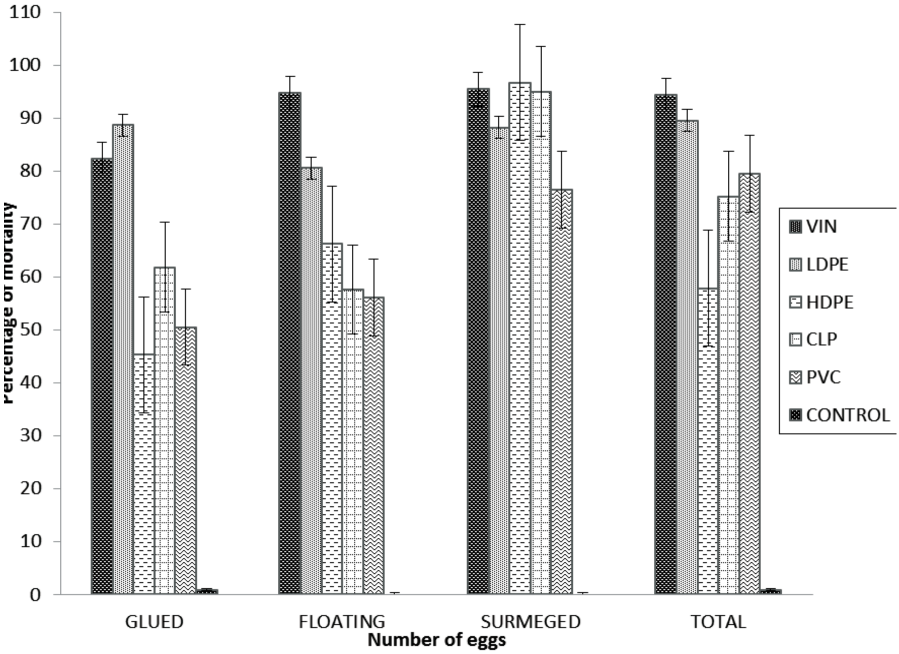

Fig. 1. Mortality of glued, floating, and submerged *Ae. aegypti* eggs that failed to hatch when plastic liners were used as oviposition substrates.

Discussion

Overall, each plastic with different chemical structure showed three abilities to inhibit oviposition of gravid *Ae. aegypti* females: deterrence of oviposition, blocking egg adhesion, and failure of hatching with consequent embryo death. Lack of adhesion can be understood considering opposite hydrophilic properties of hyaluronic acid versus hydrophobic or water-proof properties of plastic materials (Gibbs 1988). As documented by Padmaja and Rajulu (1981), the acid absorbs water and hardens. Hyaluronic acid is known to absorb water 600 times its molecular weight (Kablik et al. 2009). Therefore, some variation in blocking the egg adhesion substance by the five plastics might be explained by different amounts of hydrophobicity exhibited by the chemical composition. With this scenario, vinyl showed the strongest water-proofing, resulting in less than 4.1% of eggs glued per surface, and high-density polyethylene resulted in most eggs glued to the surface (almost 50%) compared with those on the filter paper, 75.6 and 100.0%, respectively (Table 1). Most eggs during all experiments were floating. After explanation of the hydrophobic properties of the plastic inhibiting egg adhesion, it is reasonable to expect that ovipositing female mosquitoes were deterred from the sides of the container yet continued laying eggs on the water surface. We contend the smoothness of the plastic deterred females. The tarsal claws of the female had difficulty grabbing the plastic film during oviposition. If we accept this, we conclude the plastic acted as a deterrent. It is understandable that combined action of both water-proofing and deterrence would support the fewest glued (7.4) and most floating eggs (159.4) observed with vinyl.

Smoothness of a plastic is the result of lack of porosity and the tridimensional shape of the major polymers. These results suggested that in the future, technology could be used to manufacture anti-mosquito plastics with molecular compounds that eliminate porosity and inhibit mosquito tarsi from attaching to the substrate before resting, biting, and ovipositing. This nanocloth concept was used for anti-dust mite mattresses increasing in popularity in developing countries (Buczylko et al. 2008). On the other hand, few submerged eggs were found in any treatment, ranging from 1.0-7.1 (Table 2).

Fewest eggs laid by individual *Ae. aegypti* females (7.4%) were submerged with all plastics. This group started sinking within 24 hours after being oviposited, as a result of being heavier than floating eggs. Although embryonic development or the outer chorion layers were measured in this study, it is likely that water penetrated throughout the incipient egg layers, thus increasing the egg weight and resulting in immersion. It is documented that the exochorion is made of two sheets that trap a film of air between them and function as a plastron in the respiratory system of insect eggs (Hinton 1970, Clements 1996). Clearly, the egg plastron was allowed to develop in the floating eggs and absent from those submerged.

Failure to hatch was recorded with all plastic types and glued, floating, and submerged eggs. Eggs glued onto vinyl and low-density polyethylene resulted in least hatching of 1st-instar larvae (Table 3). Survival of immature *Ae. aegypti* larvae was as low as 5.7 and 5.4%, respectively, for the plastic types. Hatching of floating eggs was least with vinyl and low-density polyethylene, both with 9.3% survival.

Submerged eggs also showed the least eclosion and survival, i.e., 1.6% for high-density polyethylene. We hypothesized that several factors are involved in embryonic mortality in each egg category. The sticky pad of hyaluronic acid that wraps the egg is needed to provide stable physico-chemical conditions and promote embryo development. The hours after oviposition are critical for maturation of the serosa cuticle that protects the egg from desiccation (Lazzaro-Rezende et al. 2008). Hydrophobic plastics block hyaluronic acid from absorbing enough water to wrap the egg, resulting in damage to the early stages of embryogenesis. We postulated that the few embryos that survive acquired enough water in hyaluronic acid to survive and hatch. Alternate mechanisms resulting in damage to embryogenesis would include decreased oxygen intake as a result of poorly developed aeropyles (Jones 1968, Mohinder 2001), as well as lack of a water-proofing wax layer that is part of the serosa cuticle (Beckel 1958, Christophers 1960, Clements 1996).

Although none of the plastics tested produced 100% embryonic mortality under insectary conditions, both vinyl and low-density polyethylene resulted in ≥90% ovicidal activity after pooling data on eggs that were glued, floating, and submerged (Fig. 1). Both plastics, e.g., grocery and trash bags, are commonly found in the home. Use of vinyl and low-density polyethylene strips at breeding sites could reduce abundance of *Ae. aegypti*. This method could be a simple, feasible, and affordable control option for community-based programs against dengue fever.

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