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AN ADULTICIDAL STICKY OVITRAP FOR SAMPLING CONTAINER-BREEDING MOSQUITOES

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ABSTRACT. The efficacy of a standard ovitrap and an ovitrap featuring an internal wall covered by a polybutylene adhesive was compared in field studies in Cairns, Australia. The sticky ovitrap was as effective as the standard ovitrap in detecting *Aedes aegypti,* with 67.5% and 64% of traps positive for *Ae. aegypti,* respectively. Significantly higher numbers of *Ae. aegypti* were collected by traps set outside rather than inside premises. Sticky ovitraps also readily collected *Ochlerotatus notoscriptus* and, especially, *Culex quinquefasciatus.* With a 10× hand lens, mosquitoes of these species could readily be identified in traps set for 3 and 7 days. The sticky ovitraps were comparable in cost and as time efficient as standard ovitraps. The greatest advantage of the sticky ovitrap is the collection of adult female mosquitoes, negating the need to rear larvae for identification and providing a faster, more direct measure of the effectiveness of ovipositional attractants than egg counts. Finally, we demonstrated that sticky ovitraps, being adulticidal, have potential as a supplementary control measure, especially for quarantine programs designed to prevent the import and export of container-breeding vector mosquitoes at sea- and airports.

KEY WORDS Mosquito surveillance, *Aedes aegypti,* dengue, oviposition, ovitrap

INTRODUCTION

Ovitraps are widely employed to monitor populations of container-breeding mosquitoes such as *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Service 1993). A small, dark colored jar is filled with a hay infusion that is attractive to ovipositing mosquitoes. An artificial substrate, such as a wooden tongue depressor, a masonite or velour strip, or sturdy paper such as seed germination paper, is placed inside the jar. Ovipositing *Aedes* mosquitoes lay eggs directly upon the oviposition substrate. Ovitraps have also been used to monitor ports for exotic mosquitoes (Ritchie 2001), to study oviposition behavior (Allan and Kline 1995, Reiter et al. 1995), to measure the efficacy of control measures (Reiter and Gubler 1997), and to collect mosquitoes for research purposes such as mark–release–recapture (Reiter et al. 1995) and resistance studies (Canyon and Hii 1999). Autocidal ovitraps have been designed that kill larvae hatching within an ovitrap (Lok et al. 1977, Cheng et al. 1982), but these do not kill ovipositing mosquitoes.

Although effective in monitoring the presence of these mosquitoes, standard ovitraps do not indicate the actual numbers of ovipositing mosquitoes when egg numbers are >1. Apostol et al. (1994), with the use of DNA analysis to identify sibling eggs, estimated that female *Ae. aegypti* in Puerto Rico laid an average of 11 (range 1–58) eggs per ovitrap. Furthermore, because eggs are difficult to identify, ovitrap eggs are usually hatched and larvae reared to 4th instar for identification. This requires rearing facilities, which can be costly for quarantine surveillance operations, delaying identification for a week or more. Additionally, some species, such as members of the *Aedes* (*Stegomyia*) *scutellaris* (Walker) group, are difficult to distinguish as larvae and require rearing to adult stage, which induces further delays in identification.

Sticky traps provide a direct way to collect adult insects. Whereas sticky traps have been employed to collect adult mosquitoes (Service 1993), little has been done to employ the technique for container-breeding mosquitoes. Muir and Kay (1998) used a sticky "lure," consisting of a black cardboard "target" covered with a layer of polybutylene adhesive and containing a cardboard strip impregnated with a purported attractant, to collect *Ae. aegypti* in a mark–release–recapture study in Queensland, Australia. Kay et al. (2000) used adhesives to collect *Ae. aegypti* entering and exiting subterranean breeding sites, and a sticky ovitrap was used to collect released *Ae. aegypti* in a dispersal study in Mexico (Ordonez-Gonzalez et al. 2001). Although the latter reported that natural populations of *Ae. aegypti* and other mosquitoes were collected, they provided no quantitative data.

We, for the first time, report on studies that demonstrate the efficacy of an adulticidal sticky ovitrap to collect *Ae. aegypti* and other container-breeding mosquitoes in the field.

MATERIALS AND METHODS

Sticky ovitraps: A 1.2-liter black plastic "golf divot" bucket was used as the basic ovipositional container. Because the sides of the bucket were tapered, the adhesive strip was cut from a sheet of overhead transparency plastic into an arc 5.5 cm × 45 cm (top) and 36 cm (bottom) to fit snugly inside the top half of the bucket (Fig. 1). A thin layer of

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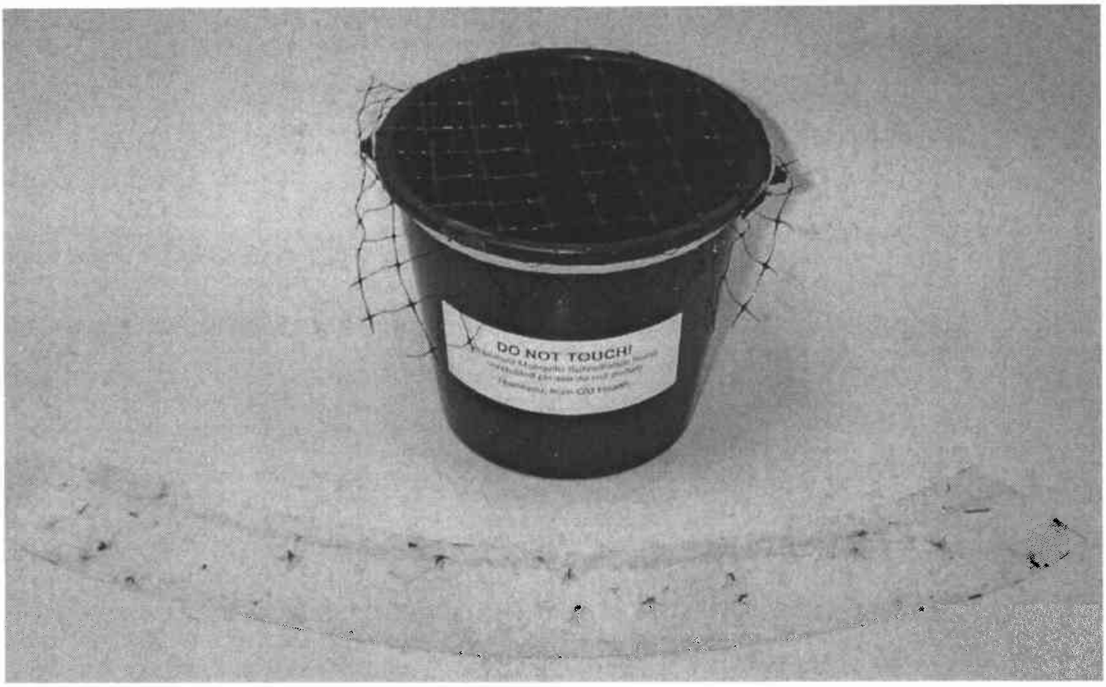

Fig. 1. Sticky ovitrap with divot bucket, plastic protective mesh, and overhead transparency plastic strip with adhesive. Note the *Ae. aegypti* on the glue strip.

polybutylene adhesive (Atlantic Paste and Glue Co., 170 53rd Street, Brooklyn, NY) was applied to one side of the plastic strip with a paint scraper. Thorough coverage was achieved by folding the sticky sides of the plastic strip together and then gently moving the plastic to smear the glue evenly. The strip was unfolded and placed into the bucket sticky side out. The buckets were filled with water until the water level reached the plastic strip.

The field trials were conducted in residential areas of Cairns in coastal northern Queensland, Australia, which has a history of dengue virus activity (Hanna et al. 2001). The sticky ovitraps were baited with 50% hay infusion (Reiter and Gubler 1997), supplemented with 2 0.2-g pellets of lucerne (alfalfa) to enhance the infusion for weekly trials (Ritchie 2001). A black plastic mesh (1.7 × 1.7-cm holes) was secured over the bucket with a rubber band to prevent animals from contacting the glue, and paired trappings with a conventional unscreened ovitrap indicated that the mesh did not significantly affect *Ae. aegypti* oviposition (P = 0.22). Because the glue loses tackiness when wet, traps were not set in the open to avoid rain.

Ability to identify mosquitoes captured by the sticky ovitrap: Sticky ovitraps were set in the field for 3 and 7 days in March–April 2002. Traps were returned to the laboratory where the sticky plastic strip was removed and placed onto "butchers" paper for inspection. Adult mosquitoes were identified in situ with a 10× hand lens and their position of attachment to the glue was recorded (e.g., ventral [bottom], lateral [side], dorsal [top], etc;). For each mosquito, the visibility of key morphological regions/features (body color, tarsi, proboscis, occiput, scutum, pleuron, and top, bottom, and side of abdomen) was assessed as + (clearly seen), ± (obscured but detectable), and − (undetectable).

Efficacy of sticky ovitrap to detect container-breeding mosquitoes: Initially, a study was conducted to determine if sticky ovitraps set outside collected significantly more *Ae. aegypti* than traps set inside. On May 28, 2002, 2 traps each were set inside and outside 15 residential premises for 5 days. Outside traps were placed under eaves, verandahs, and in open carports to protect them from rain. Indoor traps were placed against walls in a protected area of the house with no more than 1 trap in a room. The next week, the 10 premises with the largest collections of *Ae. aegypti* were sampled with 3 sticky ovitraps each inside and outside, respectively, for 5 days. The data for both trials were pooled and the mean numbes of *Ae. aegypti* were compared with an unpaired t-test. Additionally, female *Ae. aegypti* were graded as bloodfed, gravid, or neither by inspecting the abdomen with a hand lens.

A second study was conducted from June 18 to July 9, 2002, to examine the relative sensitivity of the sticky ovitrap and a standard ovitrap (identical except without sticky strip and with wooden tongue depressor for oviposition) to detect *Ae. aegypti*. *Ae-*

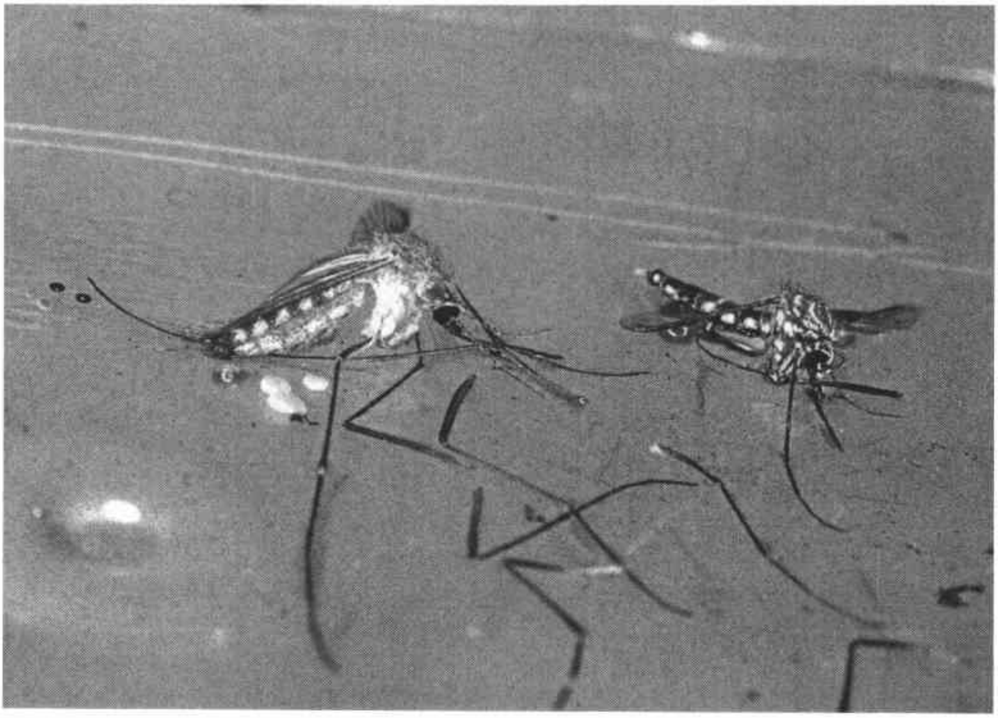

Fig. 2. Female *Culex quinquefasciatus* (left) and *Aedes aegypti* (right) captured by sticky ovitrap set for 3 days. The attachments are ventral and ventral/lateral, respectively.

des aegypti in paired premises at least 2 houses apart were sampled with either a sticky ovitrap or a standard ovitrap in 10 residential areas near Cairns. After 1 wk, new traps were set but the treatment positions were switched. This procedure was repeated, resulting in 4 trappings/premise over 4 wk and a total of 80 paired trappings. Paddles with eggs were returned to the laboratory, held for 2 days for conditioning, and flooded with a dilute yeast solution to induce hatching; larvae were reared to 4th instar for identification. Egg conditioning and hatching were repeated for those paddles where no eggs hatched upon first flooding. The frequencies of sticky and standard ovitraps detecting *Ae. aegypti* and *Ochlerotatus notoscriptus* (Skuse) were compared by a Mann–Whitney rank sum test. Furthermore, the mean numbers of *Ae. aegypti, Oc. notoscriptus,* and *Culex quinquefasciatus* Say collected by the sticky ovitrap were compared by the same test.

Efficacy of the sticky trap in a quarantine situation: Ovitraps and "sentinel" tires are commonly employed by quarantine workers to monitor ports for exotic and significant (e.g., *Ae. aegypti*) container-breeding mosquitoes (Ritchie 2001). We examined the efficacy of the sticky ovitrap at the Cairns International Airport where Australian Quarantine and Inspection Service (AQIS) monitors for *Ae. aegypti* in gardens outside the arrivals/departures terminal. AQIS routinely inspects the site for mosquito breeding and monitors for *Ae. aegypti* with 6 ovitraps baited with 0.5 g of lucerne (Ritchie 2001) and a "sentinel" tire treated with 1 pellet of *s*-methoprene; traps are serviced on a weekly schedule. From February 25, 2002, through May 9, 2002, 6 sticky ovitraps baited with 0.5 g of lucerne were set in the same area and monitored weekly. A cover cut from the bottom of a 10-liter plastic bucket was suspended on a wooden stake above the sticky ovitrap to protect it from rainfall.

Cost effectiveness of the sticky and standard ovitrap: During the sensitivity field trial, the cost of materials and the time (in minutes) were recorded for the setting of 20 sticky and standard ovitraps. Material costs included "start-up" costs, i.e., items needed to initiate the survey, and "running" costs for consumable items. Because the adhesive and the glue strips are not commercially available, running costs were calculated with glue transferred from commercial ratboards (Catchmaster 60 RB Ratboard, Atlantic Paste and Glue Co.) containing the same adhesive. The cardboard backing of the ratboards disintegrates when wet, making it unsuitable for ovitrapping. Instead, we transferred the glue from the ratboard to a plastic strip by pressing the 2 together firmly. A single ratboard can treat 4–5 plastic strips. In a field trial (30 paired trappings), sticky ovitraps made from ratboards caught slightly fewer female *Ae. aegypti* plus *Cx. quinquefasciatus* (mean = 4.9) than traps prepared from glue alone

Table 1. Point of attachment of mosquitoes (n = 82) trapped by a sticky ovitrap set for 3 days in the field.

Attachment point	Number	Percentage
Posterior	4	4.9
Ventral	26	31.7
Dorsal	1	1.2
Lateral	39	47.6
Posterior/lateral	9	11.0
Posterior/ventral	2	2.4
Leg	1	1.2

(mean = 6.9); the results were not significant (P = 0.438, Mann–Whitney rank sum test).

RESULTS

Identification of mosquitoes collected with the sticky ovitrap

Mosquitoes of the most common species captured by the sticky ovitrap (*Ae. aegypti, Cx. quinquefasciatus,* and *Oc. notoscriptus*) were readily identified with the hand lens. When captured by the glue, mosquitoes initially remained erect, attached to the glue by the legs and/or wings. Struggling mosquitoes often tore legs off, although these were located nearby and could still be used for identification (Fig. 2). After a day or so, the mosquito body became stuck, usually laterally or ventrally (Table 1), and various body parts became hidden from view and could not be used for identification (Fig. 2). However, about 85% of body features were clearly visible, with the head and abdominal characters most obscured (Table 2). After a few days, mosquito bodies mummified, preserving most characters. Sufficient characters were visible to identify 98% (n = 46) and 100% (n = 82) of trapped mosquitoes after 3 and 7 days of field exposure, respectively. If glue was applied too thickly, mosquitoes were more likely to become submerged and more difficult to identify. Also, rarely, a mosquito caught by its legs on the bottom of the glue strip would become submerged in the infusion, rendering it difficult to identify. Uncommon or small species, such as *Uranotaenia* spp., required a microscope for identification.

Table 3. Sensitivity of the sticky ovitrap and standard ovitrap to detect container-breeding mosquitoes in Cairns, Australia, from June 18 to July 9, 2002.

Mosquito	% positive trappings: Standard ovitrap (n = 78)	% positive trappings: Sticky ovitrap (n = 80)	P[1]
Aedes aegypti (female)	64.1%	67.5%	0.713
Ochlerotatus notoscriptus (female)	19.2%	7.5%	0.203

[1] A Mann-Whitney rank sum test was used to compare the frequencies of traps that were positive for a given mosquito species.

Collection of *Ae. aegypti* with sticky ovitraps set inside and outside premises

Significantly greater numbers of *Ae. aegypti* were collected in sticky ovitraps set outside rather then inside premises. For data pooled from both trials (n = 2 × 30), the mean (±SE) numbers of *Ae. aegypti* females captured in sticky ovitraps set inside versus outside were 0.28 ± 0.08 and 0.92 ± 0.16, respectively, a highly significant difference (t = 3.59; P = 0.0003). Indoors, bloodfed and gravid individuals represented 24% and 65%, respectively, of the female *Ae. aegypti* collected (n = 17), whereas outdoors they encompassed 8% and 71%, respectively (n = 55). For most of the gravid mosquitoes, some eggs were extruded from the abdomen onto the glue.

Sensitivity of the sticky and standard ovitraps

The sticky ovitrap was as sensitive as the standard ovitrap at detecting *Ae. aegypti* (Table 3). Sticky ovitraps were more effective at higher tem-

Table 2. Visibility of key morphological areas in mosquitoes captured by sticky ovitraps set for 3 or 7 days.

Score[1]	Body color	Scutum	Pleuron	Tarsi	Proboscis	Occiput	Abdomen (lateral aspects of tergites and sternites)	All characters
Traps set for 3 days (n = 82)								
+	97.6%	92.7%	92.6%	95.1%	98.8%	67.1%	48.8%	84.6%
±	0.0%	3.7%	3.7%	4.9%	1.2%	18.3%	34.1%	9.4%
−	2.4%	3.7%	3.7%	0.0%	0.0%	14.6%	17.1%	5.9%
Traps set for 7 days (n = 46)								
+	100.0%	95.7%	95.7%	95.7%	89.1%	69.6%	54.3%	85.7%
±	0.0%	4.3%	2.2%	2.2%	8.7%	17.4%	26.1%	8.7%
−	0.0%	0.0%	2.2%	2.2%	2.2%	13.0%	19.6%	5.6%

[1] Feature visibility scored as + (clearly seen), ± (somewhat obscured but detectable), and − (undetectable).

peratures; 72% and 95% of caged *Ae. aegypti* making contact with the glue were captured at 23°C and 30°C, respectively (S. Ritchie and P. Zborowski, unpublished data). Although there was no significant difference in the frequencies of sticky and standard ovitraps detecting *Oc. notoscriptus,* the low frequency of positive traps limits conclusions. During this trial, the sticky ovitraps collected 34 male and 511 female mosquitoes (Table 4), with female *Cx. quinquefasciatus* and *Ae. aegypti* dominating. In addition to the 6 species listed in Table 4, we have collected (rarely) female *Uranotaenia pygmaea* Theobald, *Culex sitiens* Wiedemann, *Culex hilli* Edwards, *Culex halifaxii* Theobald, and *Verrallina funerea* (Theobald) with the sticky ovitrap. Other dipterans, notably muscids, calliphorids, and psychodids, were commonly collected, along with, rarely, spiders and small lizards.

Efficacy of the sticky trap in a quarantine situation

The sticky ovitrap detected *Ae. aegypti* more frequently than did the combination of the standard ovitraps and the sentinel tire. The sticky ovitraps captured *Ae. aegypti* adult females on 4 of 9 wk of the trial, whereas the standard ovitraps and sentinel tire were positive for *Ae. aegypti* eggs and or larvae on only 0 and 2 occasions, respectively. Furthermore, the female *Ae. aegypti* collected by the sticky ovitraps were killed and posed no risk of entering the airport terminal.

Cost effectiveness of the sticky and standard ovitrap

The sticky ovitrap was comparable in cost to the standard ovitrap (Table 5), with start-up costs considerably lower because of the need for a microscope to identify larvae. Disregarding the microscope, start-up costs for the standard and sticky ovitraps were comparable (AUS$144 versus AUS$174, respectively), as were running costs. The labor (time) to conduct a survey with 20 traps was similar (Table 6). The preparation time for sticky ovitrapping would be reduced by the availability of a commercially available glue strip because 26% of the labor (93/358 min) was spent applying glue to and cleaning glue strips. Removal of this cost would reduce sticky ovitrapping labor to 265 min, 30% less than standard ovitrapping.

DISCUSSION

The sticky ovitrap was as sensitive as a standard ovitrap in detecting *Ae. aegypti.* It also successfully collected a range of container-breeding mosquitoes, suggesting that most species that enter the sticky ovitrap are captured.

The sticky ovitrap has several advantages over the standard ovitrap. Direct counts of ovipositing

Table 4. Mosquitoes collected by sticky ovitraps (n = 80) in Cairns, Australia, from June 18 to July 9, 2002.

Parameter	*Culex quinquefasciatus*		*Aedes aegypti*		*Ochlerotatus notoscriptus*		All mosquitoes[1]	
	Male	Female	Male	Female	Male	Female	Male	Female
% positive	15.0%	78.8%	13.8%	67.5%	0%	7.5%	26.3%	92.5%
Total	17	358	15	140	0	9	34	511
Mean ± SD[2]	0.21 ± 0.61	4.48 ± 5.04a	0.19 ± 0.51	1.75 ± 2.05b	0	0.11 ± 0.45c	0.43 ± 0.88	6.39 ± 5.42
Median	0	3	0	1	0	0	0	5
Maximum	4	22	2	12	0	3	4	22

[1] Other mosquitoes collected included female *Verallina carmenti* (1), *Culex annulirostris* (1), *Ochlerotatus palmarum* (1), and an unidentified *Culex*.
[2] Mean number of females in the same row followed by a different letter are significantly different by a Mann–Whitney rank sum test.

Table 5. Start-up costs and running costs (based upon 20 traps) for standard and sticky ovitrapping (in AUS$).

Standard ovitrap		Sticky ovitrap	
Start-up costs		Start-up costs	
Black buckets (box of 140)	$77.00	Black buckets (box of 140)	$77.00
Infusion bin, transport containers, and trays	$30.00	Infusion bin, transport containers and trays	$30.00
Dissecting microscope	$1,000.00	Hand lens	$26.00
Yeast	$2.95	Black mesh to cover trap	$2.90
Fish food	$2.85	OHP transparency plastic	$5.59
Lucerne pellets for infusion	$6.75	Paint scraper	$2.30
Hay	$6.00	Scissors	$2.10
Sandpaper	$3.60	Lucerne pellets for infusion	$6.75
Labels (pack of 100)	$2.53	Butcher's paper	$13.00
Paper clips (box of 50)	$1.05	Hay	$6.00
Total	$1,132.73	Labels (pack of 100)	$2.53
		Rubber bands (500)	$4.00
		Cleaners (baby oil, kerosene)	$4.00
		Total	$174.17
Running costs (for 20 traps)		Running costs (for 20 traps)	
Plastic cups	$5.00	Glue (1 $1.50 board makes 5 traps)	$6.00
Tongue depressors	$0.60	Latex gloves	$0.54
Total	$5.60	Total	$6.54

mosquitoes can be made, allowing useful population indices to be developed. Mosquitoes of the most important species are, for the most part, easily identified, although small and morphologically similar species may require microscopy. There is minimal delay in identification, unlike with standard ovitraps where larvae must be reared to 4th instar or adult for identification. The sticky ovitrap is cost and time efficient, particularly for identification procedures. Additionally, the ovipositing mosquitoes are killed and thus the trap is adulticidal.

Its ability to collect adult mosquitoes makes the sticky ovitrap an attractive tool for ecological and epidemiological research. The numerous studies on oviposition attractants that have employed ovitrap egg counts as a response variable could be improved by a direct count of gravid mosquitoes. Individual mosquitoes can be assayed for pathogens such as dengue virus (Bangs et al. 2001), tested for enzymes responsible for resistance (Rodriguez et al. 2001), and, if collected while still alive (24–48 h), dissected for age grading or aging (Desena et al. 1999). Sentinel ovitraps could be used to monitor vector populations and could be used to measure the efficacy of control programs.

Sticky ovitraps would be particularly useful for quarantine surveillance and the exclusion of exotic species from sea- and airports. Mosquitoes could be identified on the day of collection, improving the response time, and, in addition, any exotic spe-

Table 6. Labor, in minutes, to conduct a survey with 20 standard or sticky ovitraps.

Standard ovitrap		Sticky ovitrap	
Task	Time (min)	Task	Time (min)
Sanding tongue depressors	10	Making up glue strips	46
Measuring lucerne pellet dosage	38	Measuring lucerne pellet dosage	38
Producing and bottling infusion	10	Producing and bottling infusion	10
Setting traps	105	Setting traps	90
Picking up traps	90	Picking up traps	90
Counting eggs	30	Identifying mosquitoes	34
Flooding paddles with yeast solution	20	Cleaning glue strips	47
Rearing larvae to 4th instar	30	Data entry	3
Identifying 4th instar larvae (all or 10)	22	Total time (min)	358
Reflooding of unhatched eggs and subsequent identification	22	Total time (hour:min)	5:58
Data entry	2		
Total time (min)	379		
Total time (h:min)	6:19		

cies is killed. We demonstrated that an array of sticky ovitraps decreased the risk of exporting container-breeding vectors such as *Ae. aegypti* at international ports and suggest that their use at high-risk ports would improve the chance of capturing an imported gravid exotic mosquito before it escapes the quarantine area.

Control of container-breeding mosquitoes, especially *Ae. aegypti,* could be enhanced by sticky ovitraps. Ovitrap strips treated with residual insecticides such as deltamethrin were very effective at controlling *Ae. aegypti* in small cage trials (Zeichner and Perich 1999). Provided a sticky ovitrap collected a large proportion of visiting females, it could be an effective control agent to supplement dengue control programs. The results of our field trials, along with the laboratory studies indicating that 72%–95% of the *Ae. aegypti* contacting the glue were captured (higher numbers were collected at higher temperature), suggest that the adhesive used is a powerful killing agent when coupled with an effective attractant. Furthermore, infusion-baited sticky ovitraps are relatively selective and nontoxic, making them attractive to control resistant mosquitoes and to use in environmentally sensitive areas. Dengue control is enhanced by spraying insecticide inside premises (Reiter and Gubler 1997, Hanna et al. 2001, Perich et al. 2001), but this is not always practical or acceptable. The sticky ovitrap could be an attractive alternative for homeowners refusing insecticidal treatment. The use of host-seeking lures to capture bloodfeeding mosquitoes would improve the effectiveness of sticky traps.

In conclusion, the sticky ovitrap represents another successful application of adhesives to quantify the productivity of mosquito breeding sites. Although pupal surveys provide a direct estimate of the productivity of a breeding site and are especially useful for dengue research (Focks and Chadee 1997, Focks et al. 2000), many covert *Ae. aegypti* breeding sites are hard to access and difficult to sample for pupae with nets. Sticky traps have been employed to measure the mosquitoes produced by subterranean telecommunication pits (Kay et al. 2000), rainwater tanks, and sump pits (S. A. Ritchie, unpublished data) in north Queensland, Australia. A sticky ovitrap may prove useful for surveillance and to supplement control efforts of container-breeding mosquitoes in many areas. A commercially available sticky ovitrap would be an attractive proposition and could be appealing in the domestic market for control of *Ae. aegypti* and *Cx. quinquefasciatus.*

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