Journal of the American Mosquito Control Association, 17(3):206–208, 2001

SCIENTIFIC NOTE

EFFECT OF SOME ANIMAL FEEDS AND OVIPOSITION SUBSTRATES ON *AEDES* OVIPOSITION IN OVITRAPS IN CAIRNS, AUSTRALIA

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ABSTRACT. Animal feed pellets containing lupin seed or alfalfa were added to ovitraps set in Cairns, Australia. Although they collected fewer *Aedes* eggs than Centers for Disease Control enhanced ovitraps, they did outperform tap water alone. A wooden tongue depressor collected comparable number of *Aedes* eggs as a Masonite board and seed germination paper.

KEY WORDS *Aedes aegypti*, ovitrap, oviposition attractants, Australia

Ovitraps (ovipositional traps) are commonly used to monitor container-breeding *Aedes* mosquitoes (Service 1993). In Australia, ovitraps containing tap water and a Masonite® (a dark-brown, compressed-wood hardboard) oviposition substrate are employed in *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) surveillance programs. Reiter et al. (1991) documented that ovitraps enhanced with hay infusion were positive more frequently, with a higher number of eggs, than ovitraps with tap water. The Centers for Disease Control (CDC) enhanced ovitrap (Reiter et al. 1991) employs 2 jars containing 100% and 10% hay infusion and uses seed germination paper as an oviposition substrate. Because the paper covers the entire inner surface of the jar, all eggs laid are recovered, eliminating the risk that eggs laid on the inner surface of the ovitrap will produce mosquitoes.

However, hay infusions may be inconvenient to produce and transport to the field. To eliminate these problems, infusions can be grown in situ by adding animal feed to ovitrap water. Haber and Moore (1973) and Freier and Francy (1991) employed rabbit pellets to create an ovipositional infusion. Quarantine officers in Cairns, Australia, create an infusion in the trap by adding a small amount of hay to ovitrap water. To standardize this approach, plant-based animal feeds such as guinea pig chow could be used to create an infusion in an ovitrap. Furthermore, Masonite boards are dark, requiring microscopy to detect eggs. The objective of this study was to test ovitraps employing animal feeds and lighter-shaded oviposition substrates in Cairns, Australia.

Ovitraps consisted of a 750-ml plastic container with the exterior painted black. A Latin square design (Cochran and Cox 1957) was used to compare treatments. Trial A compared ovitraps containing 400 ml of tap water, two ovitraps containing 100% and 10% hay infusion (CDC enhanced ovitrap; Reiter et al. 1991), tap water supplemented with 0.5 g of lupin-based animal chow, or tap water supplemented with 1.0 g of lupin-based animal chow. The animal chow consisted of compressed seeds of the legume *Lupinus* sp. A wooden tongue depressor, roughened with sandpaper, was used as an oviposition substrate. Traps were reset weekly and treatments were switched so that each treatment was set at each location once. All paddles were examined with a dissecting microscope and mosquito eggs (hatched and unhatched) were counted. Paddles with mosquito eggs were incubated at 25°C for 1 wk before hatching of eggs and subsequent identification of larvae. Four replicate trials, encompassing 16 trappings/treatment, were conducted. The data were pooled and a Kruskal–Wallis analysis of variance on ranks was performed with Sigmastat 2.0 (SPSS Inc., 444 North Michigan Avenue, Chicago, IL). A Tukey's multiple-range test was used to separate means.

Because the lupin pellets often created a film on the water surface that killed larvae, trial B compared ovitraps containing an alfalfa (*Medicago sativa* L.)-based animal feed that, because it is composed of compressed leaves and stems rather than fat-rich seeds, should reduce putrefaction. Treatments were ovitraps containing 400 ml of tap water, tap water with 2 (0.25 g) lupin pellet, or tap water with 1 (0.5 g) pellet of the alfalfa-based animal feed. The data from 8 replicates (total of 24 trappings/treatment) were pooled and analyzed as before.

A 3 × 3 Latin square design was used to test 3 oviposition substrates (trial C). Ovitraps were baited with 400 ml tapwater plus 0.5 g of lupin and provided with the following oviposition substrates: Masonite paddle (15 × 2 cm), wooden tongue depressor (15 × 2 cm) roughened with course sandpaper, or seed germination paper (Paper U15, Robert Bryce & Co., Ltd., 32-34 Tattersall Road, Blacktown, New South Wales, Australia). Paddles were secured with a large paper clip, whereas a 28

Table 1. *Aedes* oviposition in ovitraps with different attractants set for 1 wk in Cairns, Australia.

	Trial A[1] (n = 16)				Trial B[2] (n = 24)		
	CDC enhanced ovitrap (10% and 100% hay infusion)	Tap water + 0.5 g lupin	Tap water + 1 g lupin	Tap water	Tap water + 0.25 g lupin	Tap water + 0.50 g alfalfa	Tap water
% positive	100	81	63	75	75	83	67
Mean[3] ± SD no. eggs	129A ± 170	81AB ± 90	57AB ± 80	19B ± 16	26A ± 45	27A ± 36	11A ± 18
Median no. eggs	54	43	10	26	3	9.5	2

[1] Conducted between Oct. 23 and Nov. 13, 1998.
[2] Conducted between April 23 and May 5, 1999.
[3] Means followed by a different letter are significantly different ($P < 0.05$) by Tukey's multiple-range test.

× 10-cm piece of the paper was used to line the inside of the ovitrap.

Addition of animal feed to ovitraps generally increased the number of eggs laid, but not significantly (Table 1). The CDC ovitrap was always positive for eggs, with the other methods less frequently so. In trial A, only the CDC enhanced ovitrap, employing 2 ovitraps baited with 10% and 100% hay infusion, had a significantly higher mean number of eggs than did an ovitrap baited with tap water. However, the CDC ovitrap did not have significantly more eggs than the traps containing lupin pellets and the median number of eggs was comparable to that of ovitraps baited with 0.5 g of lupin. Of the 40 positive egg batches identified by rearing larvae, 95, 23, and 3% contained eggs of *Ae. aegypti, Ochlerotatus notoscriptus* (Skuse), and *Ochlerotatus palmarum* Edwards, respectively. However, lupin pellets often caused putrefication of water, killing larvae.

In trial B, both lupin pellets (0.25 g or 2 pellets) and alfalfa pellets (0.5 g or 1 pellet) enhanced oviposition, but not significantly (trial B, Table 1). *Aedes aegypti* and *Oc. notoscriptus* were found in 100 and 13% of egg batches identified (n = 31), respectively. Both treatments did not create a surface film on water; no dead larvae were observed in 21 ovitraps with larvae.

Oviposition rates were comparable for the 3 oviposition substrates (Table 2), with *Ae. aegypti* and *Oc. notoscriptus* comprising 95 and 10% of the egg batches identified (n = 42), respectively. However, some methods had practical advantages. The seed germination paper, because it covered the interior of the ovitrap, prevented unwanted oviposition. Conversely, off-target oviposition was common in ovitraps with narrow oviposition substrates. Off-target oviposition was examined by wiping the interior of the trap (6 each containing a Masonite paddle or tongue depressor) with a moist paper towel. Respective means of 14.5 and 13.2 eggs were collected, accounting for 54 and 13% of all eggs laid in the ovitraps containing wooden and Masonite paddles. However, most of the eggs on the paper had hatched in response to excessive water absorption by the paper. Seed germination paper used by the CDC (Reiter et al. 1991) is superior (#76 seed germination paper, Extra Heavy Weight, Anchor Paper Co., Box 65648, St. Paul, MN) and should be considered. Finally, paper and the tongue depressor provided a light background upon which eggs could be detected without magnification. The dark Masonite paddles required microscopic examination to detect eggs.

Table 2. *Aedes* oviposition in ovitraps (n = 18) employing different oviposition substrates and set for 1 wk in Cairns, Australia (trial C, conducted Jan. 1 and 28, 1999).

	Mansonite paddle	Seed germination paper	Wooden tongue depressor
% positive	89	78	72
Mean[1] ± SD no. eggs	65A ± 123	93A ± 102	73A ± 132
Median no. eggs	29	60	16

[1] Means followed by the same letter are not significantly different ($P > 0.05$) by Tukey's multiple-range test.

These trials indicate that lupin- or alfalfa-based animal pellets can be employed to enhance oviposition by aedine mosquitoes. Because animal feeds can create a surface film, they should be used in small amounts (<0.5 g/ovitrap). Ovitrap paddles made of light-shaded wooden tongue depressors allow assessment of positive paddles by the naked eye and are a preferable oviposition substrate to dark Masonite. Although supplementing ovitraps with animal feeds is practical and effective, the method is not a replacement for the CDC enhanced ovitrap. The latter is set for 24 h and, utilizing a ready-made infusion, provides an immediate measurement of oviposition rate. The traps described create an infusion over the week and thus measure oviposition over a different time and conditions.

I thank Brian Montgomery and Ross Spark for reviewing the manuscript and the Australian Quarantine Inspection Service for supporting the research.

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