

## EFFICACY OF METHYL BROMIDE FUMIGATION AND INSECTICIDAL DIPS AGAINST *Aedes* EGGS ON LUCKY BAMBOO

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**ABSTRACT.** Importation of lucky bamboo (*Dracaena* sp.) stalk clusters has led to the accidental importation of *Aedes albopictus* in some countries. Current methods of fumigation using methyl bromide are harmful to live plants and thus are not acceptable. We exposed *Aedes aegypti* eggs to methyl bromide at rates of 32 g/m<sup>3</sup> for 2 and 3 h and 48 g/m<sup>3</sup> for 3 h, achieving complete control in 0/5, 1/5, and 5/5 replicates, respectively. Submersion for 1 min in aqueous solutions of 0.04% active ingredient (AI) lambda-cyhalothrin, 0.04% AI bifenthrin and carbaryl + malathion (both 0.1% AI) + petroleum oil (1% AI) provided 100% control of *Ae. aegypti* eggs laid on *Dracaena* stalk clusters. These methods, apparently harmless to plants, would have application in preventing the importation of exotic *Aedes* eggs on lucky bamboo shipments.

**KEY WORDS** *Aedes albopictus*, quarantine, lucky bamboo, synthetic pyrethrin, *Aedes aegypti*

### INTRODUCTION

*Aedes albopictus* (Skuse), a known vector of dengue, has colonized and rapidly expanded its range in several geographic areas (Moore 1999, Urbanelli et al. 2000). Previous introductions have been via eggs laid on imported used tires (Moore 1999) and active breeding sites on bulk equipment, such as mining equipment, imported from countries where *Ae. albopictus* is endemic (Ritchie et al. 2001). The Australian Quarantine and Inspection Service (AQIS) minimizes introduction of *Ae. albopictus* by requiring that imported tires be fumigated with methyl bromide (CH<sub>3</sub>Br) and that high-risk cargo (e.g., tires, construction equipment) be inspected, fumigated, or treated with insecticide or bleach (Ritchie 2001). Nonetheless, *Ae. albopictus* has been intercepted several times in Australia, although quick response by vector-control organizations has helped prevent its establishment (Ritchie et al. 2001).

Recent importations of *Dracaena* sp., lucky bamboo, cuttings for ornamental use have led to the accidental introduction of *Ae. albopictus* in California (Madon et al. 2002, Linthicum et al. 2003). Cuttings of bamboo stalks are often arranged in sequential rings around a piece of polyvinyl chloride (PVC) pipe (Fig. 1) or tied in bundles and then arranged upon arrival. The bamboo bundles are set in shallow water within a plastic box, where, if exposed to *Aedes* mosquitoes, oviposition on the stalks can occur. Current importations are largely from southern China, where *Ae. albopictus* is endemic.

A strategy to destroy all *Aedes* eggs on imported lucky bamboo bundles is urgently needed. At the standard AQIS rate of 32 g/m<sup>3</sup> for 24 h, methyl bromide results in 100% mortality of *Aedes aegypti* L. eggs (Ritchie 2001). However, it is harmful to

live plants (Bill Crowe, AQIS, personal communication). As an alternative, AQIS dips live plants into a mixture of 0.1% active ingredient (AI) carbaryl + 0.1% AI malathion + 1% petroleum oil mixed in water at 35°C for 1 min. We conducted laboratory trials to see if shorter methyl bromide fumigation times, along with insecticidal dips, would kill *Ae. aegypti* (*Ae. albopictus* does not occur in Australia, thus *Ae. aegypti* was used as a proxy) eggs on bundles of *Dracaena sanderiana*.

### MATERIALS AND METHODS

#### Methyl bromide fumigation

Fumigation with methyl bromide took place within an AQIS-approved fumigation tent at rates of 32 g/m<sup>3</sup> for 2 and 3 h and 48 g/m<sup>3</sup> for 3 h. Strips of cardboard upon which *Ae. aegypti* had oviposited ca. 2–4 wk previously (50–100 eggs/strip) were exposed in open 70-ml plastic containers within the fumigation tent (Ritchie 2001). While these eggs were potentially more exposed to methyl bromide than eggs laid on bamboo clusters, the ability of methyl bromide to rapidly and deeply penetrate sorptive material such as plants is documented (Monro 1969, Gerozisis and Hadlington 1999) and suggests that the fumigant should readily reach eggs laid on bamboo clusters. Thus, the exposure of eggs on cardboard to methyl bromide was considered to be an adequate simulation. Five replicates, including an untreated control, were conducted. A day after exposure, egg strips were flooded in a dilute yeast solution and live larvae were counted after 24 h (Ritchie 2001).

#### Insecticidal dips

To simulate a small lucky bamboo cluster, 2 sequential rings of 3- and 5-cm stalks (diameter of 0.5–1.2 cm, leaves removed) of *D. sanderiana* were arranged around a 10-cm-long piece of 2-cm-diameter PVC pipe and held together with a rubber band. Each bamboo bundle was placed in a 1.2-

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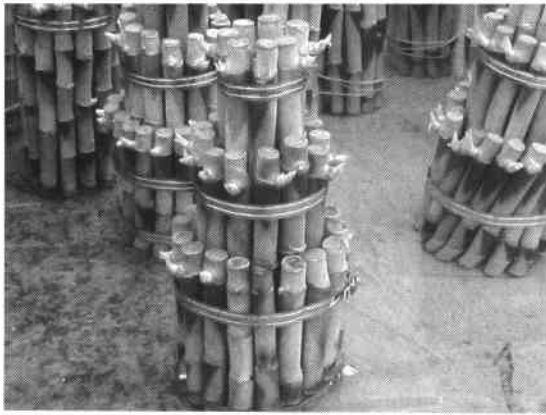


Fig. 1. Lucky bamboo stalk bundles.

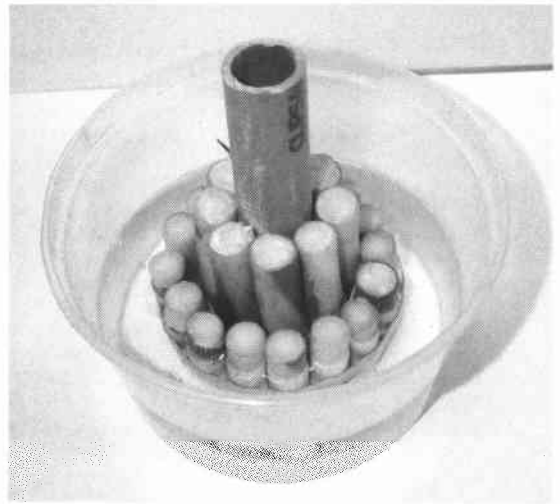


Fig. 2. Bundle of *Dracaena sanderiana* used in the dipping trials.

liter plastic container with 3-cm-deep water (Fig. 2) that was then placed into a 9-liter bucket. The bucket was covered with fine cloth screening and 10 gravid *Ae. aegypti* were placed into the bucket. After 5–7 days, mosquitoes were removed, the water was poured from the container, and the lucky bamboo bundle was air dried for 24 h. The bundle was then dipped by submerging it for 1 min into a tray containing 10 liters of the following solutions mixed in tapwater:

- 16 ml/liter lambda-cyhalothrin (Demand 2.5% suspendable concentrate (SC); final concentration 0.04% AI),
- 5 ml/liter bifenthrin (Talstar 8% SC; final concentration 0.04%),
- AQIS Nursery Stock Dip (0.1% AI carbaryl + 0.1% AI malathion + 1% petroleum oil mixed in water at 35°C), or
- An untreated control consisting of tap water.

Five replicates were conducted for each solution. After dipping, the bamboo bundles were placed onto a piece of paper towel to remove excess solution, then transferred to a clean plastic container. After 24 h, each container was filled with 500 ml of a dilute yeast solution to stimulate egg hatch. The dead and live larvae were counted at 24 and 96 h posthatch. Each bamboo bundle was air dried for 1 wk, then resubmerged in dilute yeast solution to hatch eggs that may not have hatched upon initial flooding. Again, larval counts were made after 24 h.

## RESULTS AND DISCUSSION

### Methyl bromide fumigation

Methyl bromide fumigation achieved 100% control only at the highest rate (Table 1). Microscopic examination of the egg strips indicated that, after exposure to 32 g/m<sup>3</sup> methyl bromide, most of the eggs hatched but larval eclosion was not successful. Thus, this dose of methyl bromide did not directly

kill the embryos inside the eggs. The large numbers of dead larvae that did not successfully eclose indicates that methyl bromide affected the larvae within the egg, preventing successful eclosion, or left a toxic residue (bromide) on the egg chorion that subsequently killed hatching larvae. The results using the higher rate (48 g/m<sup>3</sup> for 3 h) suggest the methyl bromide did penetrate the chorion; most of the eggs failed to hatch, indicating that the embryos were affected. Larvae that hatched from trials with the lower exposures successfully pupated, suggesting no overt sublethal affects.

### Insecticidal dips

The 3 dipping solutions provided 100% control of *Ae. aegypti* eggs on the *D. sanderiana* stalk bundles (Table 2). No larvae were observed after the 2nd flooding (1 wk postexposure), except in one of the controls where 8 larvae were recorded 24 h posthatch. These data indicate that the dipping solutions should provide 100% control of *Aedes* eggs laid on lucky bamboo bundles and provide control for subsequent hatchings upon later flooding.

These methods could be employed by quarantine workers (or exporters) to treat imported shipments of lucky bamboo stalk bundles originating from

Table 1. Proportion of *Aedes aegypti* egg strips (50–100 eggs/strip) hatching after exposure to methyl bromide.

	Control	32 g/m <sup>3</sup> for 2 h	32 g/m <sup>3</sup> for 3 h	48 g/m <sup>3</sup> for 3 h
24 h posthatch	5/5 <sup>1</sup>	5/5	4/5	0/5
96 h posthatch	5/5	5/5	3/5	0/5

<sup>1</sup> Number of egg strips positive for larvae/number of eggs strips exposed 24 h after inducement of hatching.

Table 2. Efficacy of a 1-min insecticidal dip against *Aedes aegypti* eggs laid on *Dracaena sanderiana* stalk bundles ( $n = 5$ ); bundles submerged in hatching media 24 h after dipping.

Dip	Larvae alive	Larvae dead
AQIS nursery stock dip <sup>1</sup>	0 <sup>2</sup>	30.2 ± 11.8 <sup>2</sup>
Bifenthrin (80 SC 5 ml/liter)	0	57.6 ± 24.5
Lambda-cyhalothrin (25 SC @ 16 ml/liter)	0	93.6 ± 83.3
Control	80.0 ± 18.7	0

<sup>1</sup> 0.1% AI carbaryl + 0.1% AI malathion + 1% petroleum oil mixed in water at 35°C; AQIS, Australian Quarantine and Inspection Service; SC, suspendable concentrate.

<sup>2</sup> Mean number of larvae ± SD 24 h after hatching.

countries with *Ae. aegypti* or, potentially, *Ae. albopictus*. The dips do not harm plants and lucky bamboo clusters exhibited normal growth 3 wk after a 3-h exposure to 48 g/m<sup>3</sup> methyl bromide (Bill Crowe, AQIS, unpublished data). However, bamboo stalks with poorly developed roots or that have been stressed by cold temperatures or prolonged darkness (e.g., seafreight consignments are shipped 2–3 wk in dark boxes) often die after the 3-h methyl bromide fumigation (Bill Crowe and Keryl Jacobi, AQIS, personal communication). Use of these strategies should reduce the risk of importation of *Aedes* from lucky bamboo shipments and some have been used operationally by AQIS to treat imported lucky bamboo.

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