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/m³ for 2 and 3 h and 48 g/m³ for 3 h, achieving complete control in 15, 115, and 255 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 0.17% AI) + petroleum oil (17% 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 (CH₃Br) 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/m³ 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/m³ for 2 and 3 h and 48 g/m³ 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-
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:

a. 16 ml/liter lambda-cyhalothrin (Demand 2.5% suspendable concentrate (SC); final concentration 0.04% AI),
b. 5 ml/liter bifenthrin (Talstar 8% SC; final concentration 0.04%),
c. AQIS Nursery Stock Dip (0.1% AI carbaryl + 0.1% AI malathion + 1% petroleum oil mixed in water at 35°C), or
d. 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/m³ 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 eclosed 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/m³ 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/m³ | 32 g/m³ | 48 g/m³ |
| 24 h posthatch | 5/5 | 5/5 | 4/5 | 0/5 |
| 96 h posthatch | 5/5 | 5/5 | 3/5 | 0/5 |

1 Number of egg strips positive for larvae/number of eggs strips exposed 24 h after inducement of 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/m³ 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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