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EFFICACY OF AUSTRALIAN QUARANTINE PROCEDURES AGAINST THE MOSQUITO *AEDES AEGYPTI*

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ABSTRACT. Methods employed by Australian quarantine officers to control *Aedes* mosquitoes in containers were tested against all stages of *Aedes aegypti*. Saltwater emersion killed all larvae but not pupae or eggs that were briefly exposed. Swimming pool chlorine, methyl bromide fumigation, and permethrin (2% active ingredient) spray provided 100% mortality of eggs, larvae, and pupae. Aerosol sprays incorporating synthetic pyrethrins are practical and also provide effective control of adults.

KEY WORDS *Aedes aegypti,* quarantine, mosquito control, synthetic pyrethrins, dengue

INTRODUCTION

Aedes albopictus (Skuse), a vector of dengue, is now established in the USA (Moore and Mitchell 1997) and parts of Europe (Mitchell 1995) after being introduced by infested cargo from Asia. Interceptions of *Ae. albopictus* larvae and adults have been made at Australian ports (Kay et al. 1990), leading to the establishment of mandatory fumigation of imported tires with methyl bromide (Laird et al. 1994). However, recent interceptions of *Ae. albopictus* in Australia indicate that individual wet containers, such as of tires and mining equipment, have replaced large shipments of tires as the primary source of imported *Ae. albopictus* (Foley et al. 1998; Ritchie et al., 2001a). These "traveling breeding sites" may contain recently emerged adult mosquitoes as well as aquatic stages; thus control methods must target all life stages.

The Australian Quarantine and Inspection Service (AQIS) is responsible for inspecting ports and imported goods for exotic insects including mosquitoes. If a suspected or active mosquito breeding site is encountered, control must be initiated immediately to kill aquatic stages and to prevent escape of adult mosquitoes. Control methods must be portable, inexpensive, rapid, and provide 100% kill of all life stages. Methods employed by AQIS officers include those designed to kill eggs on dry cargo (liquid chlorine and pyrethroid spray), aquatic stages within containers (chlorination with swimming pool chlorine, seawater, and pyrethroid spray), and adult mosquitoes within suspected cargo (pyrethroid spray and fumigation). Methyl bromide fumigation is frequently used to control insects in suspicious cargo, but is costly and requires tenting of the container, the set up of which is likely to flush adult mosquitoes. Use of synthetic pyrethroid aerosol sprays is a quick, convenient way to treat individual small to medium-sized containers that might harbor container-breeding mosquitoes. Aerosol spray offers quick knockdown of adult mosquitoes and should kill eggs and, potentially, larvae months after application (Ritchie et al., 2001b). The objective of these studies is to establish which of these measures could provide complete control against the target stages of *Ae. albopictus*. Because *Ae. albopictus* is an exotic mosquito in Australia, tests were conducted using *Aedes aegypti* (L.).

MATERIALS AND METHODS

Mosquitoes: Test mosquitoes were from a recently established colony (F_1–F_3) of *Ae. aegypti* collected in Cairns, Australia. Mosquitoes were reared at 28°C with adult mosquitoes maintained on honey water. Larvae exposed to treatments were provided with a pellet of fish food and incubated at 25°C. Strips of seed germination paper (paper U15, Robert Bryce & Co., Ltd., Blacktown, New South Wales, Australia) containing 20+ oviposited eggs were used to expose eggs.

Efficacy of chlorination against eggs, larvae, and pupae: Liquid formulations of sodium hypochlorite (bleach and swimming pool chlorine) are inexpensive and widely available. The AQIS uses 5–10% active ingredient (AI) sodium hypochlorite to treat flooded containers and to spray watermarks on machinery, tires, and other sources to kill *Aedes* eggs (William Crowe, AQIS, personal communication). Efficacy of chlorination was conducted using sodium hypochlorite and dry swimming pool pellets containing trichloroisocyanuric acid.

Effect of sodium hypochlorite against eggs was tested by submerging an egg strip in 100 ml of 1% or 5% sodium hypochlorite in a 200-ml plastic cup for 24 h. Egg strips placed in a dilute yeast–tap water solution served as a control, with 10 replicates. Egg mortality was assessed by examining eggs for hatching and the solution for viable larvae. To determine the efficacy of spraying watermarks with chlorine, 10 egg strips were dipped into a 1% and 5% solution for 1 sec, then allowed to dry for 24 h. Egg strips were then flooded and hatching percentage and larval survival determined as described above.

Larvae (4th instars) and pupae were exposed by placing 10 of each into a 250-ml plastic cup containing 200 ml of a chlorine–tap water solution. So-

Table 1. Percent (mean ± SD; n = 10) 24-h mortality of *Aedes aegypti* exposed to control measures used by Australian Quarantine and Inspection Service.

Treatment	Eggs: Dipped[1]	Eggs: Flooded	Larvae	Pupae	Adult females
Chlorination					
0.02% trichloroisocyanuric acid	ND[2]	ND	100	100	ND
1% trichloroisocyanuric acid	ND	ND	100	100	ND
1% sodium hypochlorite	100	100	100	100	ND
1% sodium hypochlorite + 10 g potting mix	ND	ND	100	100	ND
5% sodium hypochlorite	100	100	100	100	ND
Control	ND	3 ± 5	3 ± 5	2 ± 4	ND
Fumigation					
Methyl bromide (48 g/m³)	ND	100	100	100	100
Control	ND	5 ± 2	6 ± 8	3 ± 5	12 ± 11
Submergence in seawater					
50% seawater	ND	100	90 ± 11	2 ± 5	ND
100% seawater	5 ± 6	100	100	5 ± 4	ND
Control	6 ± 5	3 ± 3	1 ± 3	2 ± 4	ND
2% Permethrin spray					
1-sec spray	ND	100	100	100	100
3-sec spray	ND	100	100	100	100
Control	ND	6 ± 5	4 ± 3	2 ± 5	8 ± 6

[1] Paper strip with >20 *Ae. aegypti* eggs was dipped into treatment solution for 5 sec, allowed to dry for 24 h, then placed into freshwater hatching media.
[2] ND, not done.

lutions tested were 1% and 5% sodium hypochlorite (Savings Laundry Bleach, Grocery Holdings Pty. Ltd., Tooronga, Victoria, Australia), 0.02% and 1% trichloroisocyanuric acid (Homebrand Swimming Pool Tablets, Grocery Wholesalers Pty. Ltd., Yennora, New South Wales, Australia), and an untreated tap water control. Because organic matter can exhaust chlorine, potentially reducing efficacy, a similar trial was conducted using 200 ml of 1% sodium hypochlorite and 10 g of potting mix. Each trial was replicated 10 times and 1-h and 24-h mortalities were determined.

Efficacy of methyl bromide fumigation against eggs, larvae, pupae, and adult females: An egg strip was attached with a paper clip above the waterline to the inside of a 70-ml plastic cup containing 30 ml of water. Ten *Ae. aegypti* 4th-stage larvae and 10 pupae were placed in the water. This cup was placed inside a screened 750-ml plastic cup to which 10 adult females were added. Ten of the nested cups were placed inside a 1-m³ fumigation box and exposed to methyl bromide at 48 g/m³ for 24 h (standard rate). Egg strips were placed in a dilute yeast–tap water solution to induce hatching. Percent mortality for each stage was determined; similarly handled mosquitoes placed in an untreated fumigation box served as a control.

Efficacy of saltwater against eggs, larvae, and pupae: Because saltwater is readily available on wharves, it could be used as a quick, convenient method to kill mosquito pupae, larvae, and eggs inside containers. Eggs, larvae, and pupae of *Ae. aegypti* were placed in 200 ml of 50% and 100% seawater (1.7% and 3.4% NaCl, respectively). Handling procedures and assessment were similar to those described for chlorination. To test the effect of a brief emersion in saltwater on eggs, egg strips were dipped in 100% seawater for 5 sec, allowed to dry for 24 h, then flooded in a dilute yeast–freshwater solution to induce hatching.

Efficacy of permethrin aerosol spray against eggs, larvae, pupae, and adult females: Eggs, larvae, pupae, and adult females were placed in nested 250-ml/750-ml cups similar to that used to test methyl bromide. Each test container was sprayed with a 2% permethrin spray (Callington Aircraft Prespray Insecticide, Callington Haven Pty. Ltd., Rydalmere, New South Wales, Australia) for 1 or 3 sec duration; unsprayed cups served as a control. Mortality was assessed at 24 h although egg strips were then dried for 24 h before flooding.

RESULTS AND DISCUSSION

Efficacy of chlorine against eggs, larvae, and pupae

Both sources of chlorine (sodium hypochlorite and trichloroisocyanuric acid) caused 100% mortality of *Ae. aegypti* larvae and pupae (Table 1). Trichloroisocyanuric acid and 5% sodium hypochlorite had the fastest response, killing all larvae and pupae within 1 h (in fact, larvae were affected within minutes). The more dilute sodium hypochlorite (1%) resulted in a fast kill of larvae but pupae were somewhat more resistant, with only 30% morbid within 1 h; all subsequently died by 24 h. The addition of 10 g of potting mix did not affect the 24-h mortality, with all larvae and pupae dying. Control mortality of larvae and pupae was <10% in all cases.

Spraying with a chlorine solution is an effective method to kill *Aedes aegypti* eggs. Eggs (i.e., the chorion) cleared within 1 h of dipping in either 1% or 5% sodium hypochlorite. After a 24-h drying time, no eggs hatched when flooded with dilute yeast solution, whereas 93% of the control eggs hatched.

Clearly, trichloroisocyanuric acid and sodium hypochlorite, even at low concentrations, effectively kill all aquatic stages of mosquitoes. The addition of organic material may slow the effect somewhat, but does not reduce the overall effectiveness. The spraying of container watermarks with a sodium hypochlorite solution, if thorough, should kill all *Aedes* eggs. Given the inexpensiveness and availability of chlorine products, this is a practical and sure method to kill potential eggs, larvae, and pupae in water-bearing containers, even those with detritus.

Efficacy of methyl bromide fumigation against eggs, larvae, pupae, and adult females

Methyl bromide fumigation caused 100% mortality of all life stages of *Ae. aegypti* (Table 1). However, because methyl bromide is not soluble in water (methyl bromide Material Safety Data Sheet), submerged unhatched eggs could escape exposure to the fumigant and survive. Generally, this would not create a significant problem because most submerged eggs would hatch and all resulting larvae would be killed. Nonetheless, a small percentage of eggs might not hatch, raising the possibility of survival. A study should be conducted using unhatched submerged eggs.

Despite its overall effectiveness, it is difficult to imagine situations where methyl bromide fumigation would be practical to control adult mosquitoes. Adult mosquitoes would likely be flushed as the container was disturbed in preparation for fumigation.

Efficacy of saltwater against eggs, larvae, and pupae

Submersion in saltwater was an effective method to control eggs and larvae but not pupae of *Ae. aegypti* (Table 1). Complete control of eggs and larvae was obtained after 24 h in 100% seawater. Although 10% of the larvae exposed to 50% seawater survived for 1 h, they appeared sluggish and subsequently died within 24 h. Interestingly, most (> 90%) flooded eggs hatched but the 1st-stage larvae all died. Larval death, especially in 100% seawater, was apparently rapid because most larvae still had egg bursters and some failed to escape completely from the eggshell. However, dipping of eggs into 100% seawater for 5 sec did not kill eggs, with 95% hatching when flooded in freshwater 24 h after dipping, and with 99% of the hatched larvae surviving. Pupae were highly resistant to saltwater, with no significant control exhibited by either 50% or 100% seawater. In many replicates, adults successfully emerged.

Treatment of breeding containers with saltwater should be a last resort. Filling a container with seawater may kill larvae, but some eggs and pupae will survive. However, pupae from containers emptied into open seawater would likely perish from waves or predation.

Efficacy of permethrin aerosol spray against eggs, larvae, pupae, and adult females

Complete (100%) control of eggs, larvae, pupae, and adult *Ae. aegypti* was obtained using 1- and 3-sec exposures to the permethrin (2% AI) spray. Interestingly, most eggs (> 90%) hatched, although all larvae subsequently died, indicating that the permethrin remained active within the water. A similar result occurred with some pupae, where adults were killed as they emerged from the pupal skin. Adult female mosquitoes directly sprayed with permethrin were knocked down within ca. 10 sec and all subsequently died.

Use of permethrin spray is an excellent method to rapidly kill all stages of *Ae. aegypti.* Adults were rapidly knocked down (within 10 sec), larvae and pupae are killed, and even eggs that later flood yield larvae that perish. To ensure that egg progeny are killed, suspected oviposition sites should be sprayed to the point of runoff. Synthetic pyrethroid aerosols are widely available, inexpensive, and easy to apply. Furthermore, control can extend for several months (Ritchie et al. 2001b).

The studies identify practical methods that provide 100% control of *Ae. aegypti* in containers. Methyl bromide fumigation and permethrin spray both killed 100% of all stages of *Ae. aegypti.* The practicality and efficacy of the permethrin aerosol spray suggests that this should be introduced into AQIS procedures for inspecting cargo for mosquitoes. To prevent flushing adult mosquitoes, flooded containers suspected of containing mosquitoes should be sprayed with pyrethroid spray before inspection for larvae. Chlorine is also effective, but cannot be employed to kill adult mosquitoes. Flushing containers with saltwater should not be employed because eggs and pupae may not be necessarily killed. However, with the exception of fumigation, these methods would not be practical to treat large shipments of containers, such as tires, suspected of housing mosquitoes.

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