SHORT COMMUNICATION

Evaluation of Insecticide Impregnated Baits for Control of Mosquito Larvae in Land Crab Burrows on French Polynesian Atolls

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ABSTRACT Land crab burrows are larval mosquito habitats of major significance in the Pacific region. They are constituted by a sinuous tunnel leading to a chamber in contact with the water table, where mosquito larvae proliferate. Controlling larvae in these sites is difficult, because the configuration of burrows prevents the use of standard techniques. An experiment was carried out in French Polynesia to control *Aedes polynesiensis* Marks and *Culex* spp. breeding in burrows of the land crab *Cardisoma carnifex* (Herbst). The technique was based on the crab's behavior, which involves the crab carrying food into its burrow. It was shown that appetizing baits impregnated with an insecticide were carried by crabs into the flooded chamber of their burrows. A field treatment of burrows was carried out by sowing insecticide impregnated baits on the ground. The treatment coverage was almost perfect and the easy implementation of the technique enabled large areas to be treated in a short time. The bait was developed by compacting various flours, which easily incorporate a large variety of insecticide formulations. Although the baits can be easily stocked, a reliable insecticide is still to be found. The results indicate that our technique could be a method of choice for treating crab burrows.

KEY WORDS *Aedes polynesiensis, Culex* spp., *Cardisoma carnifex,* mosquito control, French Polynesia, land crab

IN FRENCH POLYNESIA, *Aedes polynesiensis* Marks is the main vector of *Wuchereria bancofti* (Cobbold), the causative agent of Bancroftian filariasis (Rosen 1955), and is also a vector for dengue viruses (Maguire et al. 1971). It breeds prolifically in various artificial and natural biotopes (Jachowski 1954). The main larval habitat of the mosquitoes is the burrow of the gecarcinid land crab *Cardisoma carnifex* (Herbst) on atolls outside villages (Lardeux et al. 1992). Land crab burrows have long been recognized as mosquito larval habitats in French Polynesia (Bonnet and Chapman 1958). The crab digs (burrows) down to the water table, where mosquito larvae can develop in the small flooded chamber. It was estimated that throughout the world, 141 mosquito species, including 10 species that vector diseases to humans, are ecologically dependent on these burrows (Bright and Hogue 1972). On atolls in French Polynesia, *A. polynesiensis* densities can be up to 1,000 immature per burrows (Rivière et al. 1987). *Culex quinquefasciatus* Say, *Cx. roseni* Belkin, *Cx. annulirostris* Skuse, and *Cx. sitiens* Wiedeman are also among the most numerous mosquito species in burrows (Rivière 1988). *Cardisoma carnifex* is found on most atolls in the Pacific region and in the low areas near the sea or lagoons of high islands, where burrows are significant mosquito larval habitats (Laird 1988). The control of mosquitoes in these sites has never been addressed successfully (Bruce-Chwatt and Fitz-John 1951; Bonnet and Chapman 1958; Burnett 1959; Rivière and Thirel 1981; Laird et al. 1985, pp. 395–428; Gardner et al. 1986; Rivière et al. 1987; Lardeux et al. 1990) with two obstacles contributing to failures: (1) the lack of insecticide efficacy and (2) the practical difficulties associated with treating the burrows.

Several insecticides have been tested without much success in large scale field experiments. These include insect growth regulators (Laird et al. 1985), bacterial toxins such as *Bacillus thuringiensis* variety *israelensis* de Barjac (Rivière et al. 1987), and biological agents such as the copepod *Mesocyclops aspericornis* Daday (Rivière et al. 1987, Lardeux et al. 1992) or the entomopathogenic fungus *Tolypocladium cylindrosporum* Gams (Gardner et al. 1986). However, even if a good control candidate were found, introducing it down into the crab chamber is not straightforward. Techniques to correctly treat the burrows must take into account its configuration, and in particular, the sinuous tunnel (up to 1.5 m long) that leads to the chamber. Usually, treatment is based on the inoculation of each individual burrow with the control agent by means of manually operated equipment such as backpack sprayers (Rivière et al. 1987) or by inserting a hose down into the chamber and flushing it with control agent (Lardeux et al. 1992). These techniques were criticized because crab holes could be left behind and more specifically because flooded chambers

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at the end of burrows may be inoculated incorrectly. Moreover, all these inoculating techniques were person/h expensive.

Cardisoma carnifex were observed carrying food into their burrow. Our hypothesis was that if appetizing insecticide baits were presented to the crabs, they would transport it down into the water table.

The research was carried out on Rangiroa atoll and evaluated the mosquito control efficiency of land crabs that self-treated their burrows with insecticide impregnated baits. As part of this evaluation, we determined what percentage of burrows were visited each day by crabs on a delineated area, whether crabs carry items from the ground down into the burrow chamber, and, if they did, whether this behavior can be used to inoculate burrows with an insecticide bait. In addition, physicochemical characteristics of burrows were recorded to give basic ecological information on these not well-known mosquito habitats.

Materials and Methods

Study Area. The study was carried out in French Polynesia, on Tereia Island (15° 05″ S, 147° 57″ W), which is part of Rangiroa atoll. Rangiroa atoll belongs to the Tuamotu Archipelago, and is ≈80 by ≈40 km in size and lies ≈300 km NE of Tahiti. Islets of this atoll are typical of many others in the Tuamotu Group, some of which have been described in greater detail by Rivière (1979). Tereia island is low (<3 m above sea level) and ≈500 by 500 m in size. It is composed of accumulated coralline-shell materials and decomposed organic matter. The ocean side of the isle is composed of a higher zone of coarse coralline material and impenetrable scrub unsuitable for *C. carnifex*. The part of the isle facing the lagoon is lower (<1 m above sea level) and marked by an extensive coconut plantation. The degradation products of these trees and other vegetation provide suitable habitats for burrows of the terrestrial crab. The experimental zone for treatment was delineated in this coconut plantation and consisted of ≈3,600 m^2 low area densely populated with *C. carnifex* (mean number of burrows, >1 per m^2).

Burrows Characteristics. The presence of mosquito larvae was monitored by inserting a hose down into the base of the burrows. Any water present was pumped with a boat hand pump into a 2-liter flask. Burrows <4 cm in diameter were not sampled. When mosquito immatures were present, they were identified to species and poured back into the burrow chambers with the water. Basic physicochemical characteristics were recorded for each burrow. In addition to the length, diameter, and water volume of each tunnel, a portable probe (YSI model 30 Handheld, Yellow Springs, Yellow Springs, OH) was used to measure pH, temperature, salinity, and dissolved oxygen.

Crab Behavior and Principles of Control. The percentage of burrows visited by crabs each day was estimated by obstructing the burrow entrances with a grill made from wood sticks. The number of opened entrances was then scored the following day. The experiment was replicated three times on three successive days, with 185 burrows observed in total. Burrows in each experimental area were also checked for the presence of water.

A special appetizing bait was developed for the crabs. The bait was a mixture of fish flour (60%), wheat flour (20%), gluten (20%), and red dye (Rhodamine). The dye colored the burrow's water red, thus facilitating the location of the baits after inoculation. The components were thoroughly mixed, slightly moistened, and pressed through a grid to produce small (≈3 cm long and ≈1 cm in diameter) granules. The bait was dried at 60°C for 24 h. The result was a bait with an appearance like dehydrated mice food. Two experiments were carried out on two (400 and 350 m^2) areas. Each area harbored 65 and 50 burrows, respectively. The experiments involved "sowing" the baits on the soil surface at a density of two to three granules per burrow and then scoring the percentage of burrows that had Rhodamine colored water at 24 h.

Insecticide Baits and Field Trial. Once the feasibility of using insecticide treated baits was demonstrated, a field trial was done using the same type of baits as above, except that Rhodamine dye was substituted with Reldan 40 EC (400 g [AI] per liter chlorpyrifos-methyl). Chlorpyrifos-methyl is an organophosphate compound that kills insects by contact, ingestion, or vapor action. The operational dosage for mosquito larvae is 0.1 ppm or 0.1 mg/liter. Twenty kilograms of baits was manufactured, which produced ≈20,000 granules. The baits were impregnated with 25 ml of Reldan 40 EC so that each granule contained ≈0.5 mg of chlorpyrifos-methyl. As such, one granule may control immature mosquitoes in burrows containing five liters of water, which is largely in the range of volumes found.

The field trial was carried out on the 3,600 m^2 area described above. Burrows were counted, measured, and checked for the presence of water and mosquito larvae. Wet and mosquito positive burrows were individually identified with a colored peg. The treatment involved sowing the baits on the ground at a density of ≈3 per burrow. The operation was carried out by one person and lasted only a few minutes. Previously mosquito-positive burrows, as well as some negative flooded ones, were checked >48 h after treatment for the presence of larvae.

The percentage of burrows still positive for mosquito larvae after treatment was compared with the pretreatment situation using a chi-square test.

Results and Discussion

Burrows Characteristics. Flooded burrows were ≈24 cm longer than dry ones and ≈4 cm larger (Table 1). In general terms, ≈37.1% of flooded burrows were positive for either *A. polynesiensis* or *Culex* spp. Flooded burrows exhibited a large variation in water volume, from mud up to >25 liters, but with a mean of ≈3 liters. *Aedes polynesiensis* was found in burrows where mean salinity was 7.8 ppt, whereas *Culex* spp. occupied burrows with a higher mean salinity level of

Table 1. Physicochemical characteristics of land crab *Cardisoma carnifex* burrows on Tereia island (Rangiroa atoll)

Characteristic	*n*	Mean	SD	Min.	Max.
Length of flooded burrows, cm	714	70	22	10	130
Length of dry burrows, cm	942	46	20		
Diameter of flooded burrows, cm	714	9.1	5.2	3.0	25
Diameter of dry burrows, cm	942	5.3	3.6		
Volume, liter	714	2.74	4.19	0.05	>25
pH	139	7.35	0.34	6.37	8.52
O^2 (mg/liter)	44	3.92	1.52	0.4	6.6
Temp, °C	652	27.00	1.0	24.90	34.3
Salinity (parts per thousand)	651	9.48	6.75	0.70	26.2
Conductance (micro-Siemens)	652	15.89	10.62	1.46	47.5

13.5 ppt. As a rule, in association with the high level of organic matter in burrows, the quantity of dissolved oxygen was low, with a mean level of 52% of saturation.

Crab Behavior and Principles of Control. After 24 h, >99.4% of burrows were visited by crabs with only one dry burrow out of 185 not visited. The treatment with Rhodamine impregnated baits in two small areas showed that only one burrow out of 23 flooded ones (4.3%) and eight out of 65 (12.3%), respectively, were incorrectly treated. Hence, the contained water was not colored red. On average, 90% of flooded burrows were treated in <24 h by the crabs, indicating that a treatment involving the crabs themselves as vector of insecticide could be carried out.

Insecticide Baits and Field Trial. The treated area consisted of ≈3650 crab holes distributed over ≈3,600 m^2. Among them, 1,656 had a diameter larger enough to be sampled, 148 (8.9%) were flooded, and 55 (37.1% of flooded burrows) harbored mosquito larvae. Of the mosquito positive burrows, 10 (6.8%) contained *Ae. polynesiensis* and 45 (30.4%) held *Culex* spp. Two days after treatment, 48 of the 55 mosquito positive burrows were resampled and 41 (86%) were found negative. Only seven (14%) were found positive, but each had only one pupae per burrow (and no larvae). This was far less than the tenth of larvae sampled before treatment. The reduction of the percentage of positive burrows was statistically significant (χ^2 = 21.72, $P <$ 0.0001). Sixteen formerly negative burrows were resampled and none were found positive. Three burrows harbored eggs of *Culex* spp. The eggs were maintained in the water pumped from the burrows, and all larvae were killed soon after hatching. This is to be from the effect of insecticide. The treatment was successful, because no larvae were found, and only a few surviving pupae escaped the effect of insecticide.

It has been noted that treating thousands of crab holes seemed to be an impossible task (Rivière et al. 1987, Lardeux et al. 1992). The method developed in this study is easy and fast, enabling effective treatment of all burrows over a large area in a very short time. Because the bait developed is dry, it is well suited for stocking, yet still rapidly dissolves in water, releasing the control agent.

Controlling land crab burrows has been a real challenge. Early works advocated the destruction or reclamation of crab habitats, or the destruction of the crabs by various techniques (Dalziel 1920, Scharff and Tweedie 1942, Wanson 1935, Bruce-Chwatt and Fitz-John 1951). More recently, less destructive and more environmentally acceptable control techniques have been evaluated, which involve the use of larval mosquito predators (Rivière et al. 1987, Lardeux et al. 1992; Gardner et al. 1986). However, all field experiments failed because of poor control efficacy and inadequate inoculation of the predator into burrows. The technique developed here solves the inoculation problem. However, research is still needed to develop an efficient insecticide. A product with high efficacy and selectivity for *Aedes* spp. and *Culex* spp. larvae; that is active in briny waters with high organic contents; with residual activity; with low risk of resistance; that can be incorporated in crab baits; and that does not produce acute or chronic toxic effects in the nontarget crabs. Products such as sustained release s-methoprene and *Bacillus sphaericus* Neide formulations warrant investigation.

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