Mesocyclops aspericornis (Copepoda) and *Bacillus thuringiensis* var. *Israelensis* for the Biological Control of *Aedes* and *Culex* Vectors (Diptera: Culicidae) Breeding in Crab Holes, Tree Holes, and Artificial Containers

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J. Med. Entomol. 24: 425–430 (1987)

ABSTRACT In French Polynesia, the cyclopoid copepod *Mesocyclops aspericornis* was inoculated into burrows of the land crab *Cardisoma carnifex* and into tree holes, drums, wells, and tires. It successfully reduced larval populations of *Aedes polynesiensis* and/or *Aedes aegypti* by 91–99%. *Mesocyclops aspericornis* has persisted to date for 29 mo in crab holes and for 60 mo in some wells, tires, and tree holes. As *M. aspericornis* does not effectively reduce *Culex* populations, copepod suspensions can be mixed with *Bacillus thuringiensis* var. *israelensis*, without detriment to the copepod, to effectively kill larvae of both genera. Its oligophagic feeding pattern, ease of mass production or collection, and high fecundity within probable temperature and salinity ranges of 23–30°C and 0–4‰ make *M. aspericornis* a good candidate as a biological control agent.

KEY WORDS *Mesocyclops aspericornis, Cardisoma carnifex, Aedes polynesiensis, Aedes aegypti, Bacillus thuringiensis,* biological control

SERVICE (1983) reviewed progress in biological control and listed desirable characteristics of good candidates. He concluded that few of these had been developed sufficiently for practical large-scale use. *Bacillus thuringiensis* var. *israelensis* (BTI) and *Bacillus sphaericus* currently are considered the most promising of these candidates by Service and by the UNDP/World Bank/WHO Steering Committee (BCV) within the Special Programme for Research and Training in Tropical Diseases. We demonstrate herein that in French Polynesia the cyclopoid mosquito predator *Mesocyclops aspericornis* (Daday) can be used solely, or with other organisms, as a practical, cost-effective means of controlling larval *Aedes polynesiensis* Marks, especially in burrows of the terrestrial crab, *Cardisoma carnifex* (Herbst), tree holes, tires, wells, and in various other sites in which *Aedes aegypti* (L.) also breeds.

Although some copepod species are known to be predators of mosquito larvae (Bonnet & Mukaida 1957, Fryer 1957), it was not until 1976 that the accidental introduction of *Mesocyclops leuckuarti pilosa* (=*aspericornis*) into ovitraps from creek water prompted serious evaluation of the potential of this copepod as a biological control agent (Rivière & Thirel 1981). During a 14-mo observation period, ovitraps with copepods contained 85–91.6% fewer *Aedes* larvae than did ovitraps without copepods, but only slightly fewer *Culex quinquefasciatus* Say and *Toxorhynchites amboinensis* (Doleschall).

On Tahiti *M. aspericornis* occurs the year round mainly as part of freshwater benthos; it is found especially in aquaculture ponds but also in streams, wells, irrigation channels, and rarely in tree holes and terrestrial crab burrows. It also occurs in Lake Vaihiria (414 m altitude), the only lake on the island of Tahiti.

The *M. leuckarti* complex is cosmopolitan; Kiefer (1981) and Dussart et al. (1983) have revised the group. *M. aspericornis* is known to occur in French Polynesia, Australia, Hawaii, Indonesia, India, Singapore, the Mariana Islands, the Marshall Islands, the Philippines, Taiwan, and China, as well as in Neotropical areas such as the French Antilles, Venezuela, and Colombia (Kiefer 1981, Dussart et al. 1983). Laird (1956) possibly collected *M. aspericornis* (as *M. leuckarti*) from the Solomon Islands, Vanuatu, and Fiji. Recently in Hawaii and Colombia (Marten 1984, Suarez et al. 1984) *M. aspericornis* was used successfully to control *A. aegypti* breeding in artificial containers. Although *M. aspericornis* introductions are successful in most peridomestic situations, we believe that its greatest potential is for control of *A. polynesiensis* in sites for which no practical control strategies exist (e.g., terrestrial crab burrows and tree holes). This paper, therefore, reviews progress with *M. aspericornis* as a new tool for biological control and presents data on its usefulness for crab hole mosquito control.

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Materials and Methods

Natural Occurrence of *M. aspericornis*

Extensive surveys were carried out in various freshwater habitats, mainly in swamps and other large bodies of water, to establish the presence or absence of *M. aspericornis*. This was especially important in sites designated for studies of persistence.

Sampling of Immature Mosquitoes and Copepods

To determine the presence of mosquito immatures and copepods in breeding sites, the following methods were used. a) For ovitraps, tires, and drums, leaves and other debris were removed and the water poured into a white enamel tray. b) For tree holes and crab burrows, a siphon hose was inserted to the base and water pumped out into a 2-liter Erlenmeyer flask: after mud and sediment had settled, water was transferred into a white enamel tray for inspection. Residual sediments were returned to the laboratory in 70% alcohol for microscopic examination at 20×. c) Wells were sampled by drawing a 500-μm mesh net on an extension handle around the well margin for 2–3 min and inspecting the contents as above.

Percentage positive sites were calculated as number positive/number sampled × 100. To calculate numbers of mosquito immatures present, larvae and pupae were removed by pipette from the water samples and any additional ones found later in the sediments were added to these numbers. These counts were then averaged to give a mean score for each biotope.

Field Treatments

For each biotope the efficacy of treatment was based on comparison of the number of immatures collected from treated sites with numbers collected from similar untreated areas. Land crabs often dig numerous burrows in large taro pits, called *maite*, that were dug by early settlers. These pits may be 100 by 20 by 3 m and were dug just above the water table. At Kia Ora *maite* (Avatoru Island; Rangiroa Atoll) additional comparative adult biting collections were done before inoculation and 6 mo postinoculation of crab burrows to determine if adult populations of *A. polynesiensis* had been reduced. Two collectors aspirated mosquitoes at each site for 1 h. Daily scores were halved for each of four collections and adult densities calculated by averaging these scores.

Temperature (°C), salinity (‰), pH, dissolved oxygen content, and sometimes water volume (ml) were measured for a range of artificial containers, tree holes, and wells, and also for burrows of *Cardisoma carnifex*.

Crab Holes. From September 1983 on Avatoru Island, 2,432 burrows were treated with *M. aspericornis* as described below during different times of the year. Results of inoculation of crab holes at Kia Ora *maite* were examined in detail on five occasions over 29 mo for reduction of *A. polynesiensis*.

Other Biotopes. Efficacy and persistence of treatments were determined for *M. aspericornis* introduced into 236 tree holes in *Inocarpus fagifer* at the Papeari botanical gardens, Tahiti (treated October 1980); 127 drums (200 liters each) (10 at Paea, Tahiti, July 1981; 117 at Tiputa, Rangiroa Atoll, October 1981); 40 half tires at Paea (January 1979); 45 wells at Tiputa (October 1981) and 54 bottles (1 liter) used as ovitraps at Paea inoculated during October 1981.

Laboratory Tests

Using standard WHO testing procedures, the larval susceptibilities to BTI of species found in crab holes or in containers were determined.

Copepod Source

Mass rearing of *M. aspericornis* requires three distinct cultures: Culture 1 is the green alga *Chlorella,* (orcoccal) alga which constitutes the main diet of the five naupliar and young copepodid stages, and is also the diet of culture 2, consisting of mixed local zooplankton, *Rotifer longirostris* and large protozoans such as *Paramecium caudatum.* The zooplankton in culture 2 is used to feed copepodites stage III–V and adult *Mesocyclops* (Rivière et al. 1987) in culture 3. When mass rearing was terminated for reasons described below, some 5 × 10 adult copepods per month had been produced in 100-liter tanks at a cost of ca. US$200.

Baker's yeast or an infusion of dead leaves can be used as supplementary food for culture 2 above. Since copepods are often found in water containing dead leaves, an alternate method of rearing *Mesocyclops* solely on infusions of macerated mango and hibiscus leaves was compared with the above method; it was not as successful.

When it was discovered that unlimited quantities of *M. aspericornis* could be scooped from large-scale aquaculture ponds with 500-μm mesh nets, mass rearing was terminated. Nets were pulled through waters at the margins of the ponds; the contents were tipped into a plastic bucket and then poured into a portable cooler box through an 800-μm mesh sieve to remove debris, fish, shrimps, and other large organisms. Leaves were placed on the water surface within the cooler not only to prevent excessive agitation on the return trip to the laboratory but also as a means of reducing the numbers of ostracods, *Cypretta globulus,* in the concentrate; as ostracods adhere to leaves and twigs, they can be removed simply by replacing the vegetation.

For transport to other islands, suspensions were concentrated to 5–10 *M. aspericornis* per milliter and stored in 20-liter plastic jerry-cans. Under shaded conditions, the concentrate remained viable

Table 1. Comparison of numbers of *A. polynesiensis* breeding in crab holes treated with *M. aspericornis* or untreated at Kia-Ora *maite*, Avatoru

Zone	Months posttreatment					
	0	6	7	18	24	29
Treated						
No. crabholes sampled	30	22	20	28	9	29
% with *M. aspericornis*	0	100	100	100	100	89.6
% with *A. polynesiensis*	100	35	15	35.7	33.3	16.7
$\bar{x}$ no. larvae	865.3	1.6	0.6	9.9	0.3	3.6
$\bar{x}$ no. pupae	86	1.2	0.4	1.3	0.1	1.7
$\bar{x}$ no. adults	321	52	—[a]	—	—	—
Untreated						
No. crabholes sampled	20	24	20	38	11	60
% with *M. aspericornis*	0	0	0	0	0	0
% with *A. polynesiensis*	100	95.2	100	100	90.9	86.7
$\bar{x}$ no. larvae	—	175	160	351	212	138.2
$\bar{x}$ no. pupae	—	17.9	21.9	58.8	35.5	15.9
$\bar{x}$ no. adults	292	213	—	—	—	—
% reduction						
$\bar{x}$ no. larvae	—	99.1	99.6	97.2	99.8	97.4
$\bar{x}$ no. pupae	—	93.3	98.2	97.8	99.7	89.4
$\bar{x}$ no. adults	—	75.6	—	—	—	—

[a] —, not tested in Tables 1–3.

for at least 5 d, despite its putrid odor, but on arrival these stocks were usually transferred into vats (83 by 50 by 20 cm) and water added. For practical purposes, however, fresh stocks were flown to the atolls being treated whenever necessary.

More recently, it was discovered that the copepods can be transported on metal racks lined with moist adsorbent paper similar to the method used for transportation of fish eggs. Wet ice is placed at the base of the container to reduce the temperature.

Application Methods

For most small sites (e.g., crab holes, tree holes, and tires) ca. 40 *M. aspericornis* were inoculated into each site using a Solo 455 Hanjet (Solo Kleinmotoren Gmbh, Maichingen, Federal Republic of Germany). The diffuser and screen were removed from the nozzle cap at the end of a 70-cm-long wand. Nozzle diameter was rebored to 2 mm. Physical damage of the copepods by impaction was avoided by using low pressure.

Each solution was calibrated by spraying for certain time intervals into enamel pans. Because of the nonuniform distribution of copepods within the tank, the numbers inoculated varied by 2- to 3-fold.

Burrows of *C. carnifex* may be convoluted and as deep as 2 m but were seldom found when the water table was >1.25 m below the surface. Treatment following heavy rain facilitated inoculation, as water levels within the holes were raised (sometimes to the surface) and more easily reached with the wand. Treatment sometimes necessitated full extension of arm and wand down the burrow to ensure that the inoculum reached the water. During drier times, however, 500 ml of water was used to flush the inoculum to the base of the hole. One operator could treat 60–150 holes per hour, depending on conditions.

Because *A. polynesiensis* rarely occurs in salinities >4‰ and *C. carnifex* has been found in salinities of up to 52‰ on Moorea (Séchan & Rivière 1984), readings on selected holes within different zones obviated unnecessary treatment.

On some islets of Rangiroa Atoll, *C. roseni* Belkin and *C. quinquefasciatus* also breed in *Cardisoma* burrows and therefore BTI was also added to the tank. Data from preliminary field trials with BTI were not quantified, but mortality was recorded.

Results

Natural occurrence of *M. aspericornis*

In French Polynesia, surveys were conducted in four of the five archipelagos, and *M. aspericornis* was detected in permanent or semipermanent swamps, rivers, and ponds as well as in the sites mentioned: Society Island (Tahiti, in crab holes, buttresses of *I. fagifer;* Moorea, in crab holes; Huahine, in crab holes on the coast but not on the islets); Tuamotu Island (Rangiroa, in crab holes, a few wells at Tiputa, Takapoto); Marquesas Islands (Hiva-oa and Nuku-hiva, mixed with *Eucyclops serrulatus* (Fischer); Austral Island (Rurutu, also in taro swamps). *M. aspericornis* was not found on the following atolls: Scilly, Tetiaroa, Taiaro, Mataiva, Kauehi, Manihi, or the islets surrounding Bora Bora.

At Maeva on Huahine, where *M. aspericornis* occurs naturally, the mean biting rate of *A. polynesiensis* was 10 (n = 3 collections of 3 man-h) compared with 154 (n = 4) on the nearby islets where the copepod was absent.

Table 2. Comparative mean abundance of immature mosquitoes (% sites positive) from sites treated or not treated with *M. aspericornis*

Biotope	Species	Locality	*Aedes*		*Culex*	
			Treated	Not treated	Treated	Not treated
Crab holes	*A. polynesiensis* *C. quinquefasciatus* *C. roseni*	Avatoru	1.6 (35.3) 9.9 (35.0)	157.0 (95.0) 350.8 (100)	149.3 (47.0) 0.3 (3.6)	75.1 (57.1) 2.4 (5.6)
Ovitraps	*A. aegypti* *A. polynesiensis* *C. quinquefasciatus*	Paea	2.6 (21.3)	25.6 (88.3)	18.3 (45.5)	38.1 (52.2)
Tires	*A. aegypti* *C. quinquefasciatus*	Paea	1.3 (5.7)	32.3 (65.9)	20.1 (57.3)	11.5 (36.4)
Tree holes	*A. polynesiensis*	Papeari	1.1 (8.2)	12.4 (54.8)	—	—
Drums	*A. aegypti* *C. quinquefasciatus*	Paea	1.9 (5.0)	492.1 (78.9)	146.1 (78.9)	583.7 (50.0)

Field Treatments

Crab Holes. On Avatoru, 6 mo following inoculation, *M. aspericornis* was present in 54.6% of 2,432 crab holes, although the success rate varied from 0–100% in the seven different zones treated. *M. aspericornis* became well established in those zones with waters of temperatures from 28 to 29°C but not in those averaging 31.4°C; in salinities from 0.96–3.13‰ but not above 3.72‰. The pH of positive holes varied from 7.24 to 7.68 and dissolved oxygen from 3.8 to 12.3 g/liter.

At Kia Ora (Table 1), 26 of 29 holes sampled at the bottom of the *maite* contained *M. aspericornis* 29 mo postinoculation. Although 15–35.7% of crab holes with *M. aspericornis* still contained *A. polynesiensis*, larval and pupal numbers were reduced by 97.2–99.6% and 89.4–99.7%, respectively. When compared 6 mo posttreatment, biting rates of *A. polynesiensis* were 75.6% less in the *maite* treated with *M. aspericornis*.

Other Sites. *M. aspericornis* effectively reduced populations of *A. aegypti* and/or *A. polynesiensis* in oviposition bottles, tires, tree holes, and drums (as well as crab holes) but was ineffective against either *C. quinquefasciatus* or *C. roseni* larvae (Table 2). The copepods persisted particularly well in the tires at Paea over a 5-yr period (Table 3) and in shaded drums at Paea but not in those exposed to full sunlight at Tiputa. Water temperatures in exposed drums at Tiputa during January 1986 ranged from 29 to 38°C ($n = 73$), with a mean ±SEM of 32.2 ± 0.08°C. On Tahiti water temperatures in the half tires, drums, and tree holes during January 1986 were 28.5 ± 0.1°C ($n = 10$), 26.6 ± 0.3°C ($n = 8$), and 23.8 ± 0.3°C ($n = 26$), respectively. After 5 yr, only 17% of tree holes and 48% of wells contained *M. aspericornis*.

Laboratory Tests

In the laboratory, susceptibility tests with BTI (Bactimos, 6,000 IU/mg) indicated the following LC_{90}'s: against *A. polynesiensis*, 0.18 mg/liter; *A. aegypti*, 0.3 mg/liter; *C. quinquefasciatus*, 0.35 mg/liter; and *C. roseni*, 0.43 mg/liter. *M. aspericornis* showed no mortality at the highest dosage of 10 mg/liter.

Discussion

Previous studies (Rivière & Thirel 1981, Marten 1984, Suarez et al. 1984, Rivière 1985) and data from the present paper demonstrate that *M. aspericornis* meets the critera set for biological control agents and that it is effective against *Aedes* in various artifical containers, tree holes, and land crab burrows (Tables 1 and 2). Its compatibility with *Toxorhynchites* larvae (Rivière 1985) and with BTI (and probably also *B. sphaericus*) enhance its

Table 3. Persistence of *M. aspericornis* following inoculation into other biotopes of *A. polynesiensis* and *A. aegypti* in French Polynesia

Biotope	Locality	No. breeding sites	$\bar{x}$ temp (°C ± SE)[a]	% positive after months							
				1	2	6	12	18	24	48	60
Ovitraps	Paea	54	—	100	—	100	—	75	39	—	—
Tires	Paea	40	28.5 ± 0.1	100	98	96	84	80	65	—	100
Drums	Paea	10	26.6 ± 0.3	100	—	100	100[b]	—	—	10	—
Drums	Tiputa	117	32.2 ± 0.08	—	—	0	—	—	0	—	—
Tree holes	Papeari	236	23.8 ± 0.3	92	—	68	—	—	19	—	17
Wells	Tiputa	45	27.9 ± 0.3	—	—	35	—	—	—	—	48

[a] Readings taken during January 1986.
[b] Fish, *Poecilia reticulata*, were introduced into nine of these drums after 12 mo and consequently *M. aspericornis* disappeared.

prospects for integrated control. Conjoint use of BTI and *M. aspericornis* will kill both *Culex* and *Aedes* larvae and should provide some degree of persistence of control via colonization by *M. aspericornis*.

Gut analyses of various cyclopoid copepods indicate predation on other crustaceans, oligochaetes, protozoans, rotifers, and on young aquatic Diptera including mosquitoes and chironomids; green algae are also consumed (Fryer 1957, Rivière & Thirel 1981). Because *M. aspericornis* is benthic, it effectively preys on bottom-feeding *A. aegypti* and *A. polynesiensis* but does little to reduce populations of surface-dwelling *Culex*. Other copepods, however, are pelagic, and some have been recorded feeding on *Anopheles* (Hurlbut 1938, Lindberg 1949, Hintz 1951).

Although mass culture of *M. aspericornis* is inexpensive, the collection of naturally occurring copepods from aquaculture ponds is straightforward and costs nothing. In large freshwater ponds and swamps, *M. aspericornis* is part of a complex ecological community and, as one of the lower members of the food chain, probably exerts little or no effect on any mosquito larvae present. In more restricted biotopes, however, *M. aspericornis* becomes a more dominant member of the food chain. Elimination of *Aedes* spp. which feed on the bacterial film (i.e., colloids excreted by bacteria, captive green algae, and fungal mycelia), on protozoa and rotifers (and the eggs and cysts of both) and on the eggs of aquatic worms (Rivière 1985) makes available to *M. aspericornis* an abundant food source. The early nauplii of *M. aspericornis* feed on bacteria and algae; adults feed on algae and larger animals. Thus the ecological result of introduction of *M. aspericornis* in *Aedes* breeding sites is predation and food competition with target fauna.

To date, *M. aspericornis* has persisted in six different biotopes for 2–5 yr, reducing larval *Aedes* by 91–100% on average for least 6–12 mo. After the initial inoculation all of these breeding sites were left undisturbed except for periodic sampling (with partial replacement), and water levels were not replenished. At Paea the increased percentage of positive tires between 24 and 60 mo indicates recolonization, probably after rain had reflooded some dry tires. The gradual reduction in *M. aspericornis*-positive tree holes at Papeari suggests that on reflooding of dried tree holes, natural recolonization of these isolated sites was more difficult. Although resting stages and encystment occur in temperate *M. leuckarti* and other copepods (Fryer & Smyly 1954), our field data and preliminary laboratory studies suggest that *M. aspericornis* cannot resist desiccation. However, this requires investigation.

Nonpersistence in some other sites occurred for the following reasons: water stored in 200-liter drums in sunlit positions alongside houses at Tiputa often reached 37°C, limiting both *A. polynesiensis* and *M. aspericornis* but sometimes not *A. aegypti* populations; residents periodically emptied and scrubbed drums and water tanks; some wells were covered with materials that completely blocked sunlight necessary for algal photosynthesis.

The problem of controlling mosquitoes breeding in land crab burrows has never been addressed successfully. In West Africa (Bruce-Chwatt & Fitz-John 1951), Fiji (Burnett 1959), and Tahiti (Bonnet & Chapman 1958), crab holes have previously been treated with toxic chemicals that often resulted in the death of both mosquito larvae and the crabs themselves. As *Tupa* (*C. carnifex*) is part of French Polynesian mythology and part of the food chain, it is particularly important that any control attempts not be environmentally damaging.

Previous survey data (Klein & Rivière 1982) demonstrated the importance of land crab burrows in the production of *A. polynesiensis*. On Mataiva, these authors estimated a density of two burrows per square meter in a band 100 m wide for 28 km around the atoll; on Rangiroa during 1980–81 they estimated that the water in *C. carnifex* burrows produced 71–100% of adults biting, with each hole producing from 227 to 1,090 immature *A. polynesiensis*. Bonnet & Chapman (1958) listed tree holes, rock holes, and crab holes as the three most important breeding sites for *A. polynesiensis* on Tahiti.

Although the prospect of treating thousands of crab holes seems an impossible task, we believe that by 1) defining with a salinity/conductivity meter the zones that require treatment, 2) inoculating early in the wet season, and 3) using a modified back-pack sprayer, the task is well within the capabilities of local health teams and village communities; such treatment may be particularly useful for staff of tourist resorts. For example, treatment of most of the 2,432 crabholes on the island of Avatoru, <1 wk's work, possibly has reduced adult *A. polynesiensis* numbers by 75%, but more adult collections are required before the impact of this treatment can be fully assessed. Careful definition of the dispersal characteristics of *A. polynesiensis* could limit the treatment zones even further.

Gecardinid crabs occur throughout the world, and 140 species of mosquitoes have been associated with their burrows (Bright & Hogue 1972). As the distribution of *M. aspericornis* is widespread (Dussart et al. 1983), extension of these ongoing trials to other parts of the world should be simple and possibly of great practical value.

Acknowledgment

We thank R. Thirel, M. Faaruia, L. Colombani, A. Tetanui, M. Chebret, and J. Ferguson for assistance during these investigations and E. N. Marks and A. W. Sweeney for commenting on the manuscript. This work was supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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Received for publication 4 August 1986; accepted 12 November 1986.