# ELIMINATION OF *AEDES ALBOPICTUS* FROM TIRE PILES BY INTRODUCING *MACROCYCLOPS ALBIDUS* (COPEPODA, CYCLOPIDAE)

GERALD G. MARTEN[1]

*Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, CO 80522 and New Orleans Mosquito Control Board, New Orleans, LA 70126*

ABSTRACT. The copepod *Macrocyclops albidus* is an unusually promising new form of biological control for mosquito larvae. When introduced to two isolated tire piles, *M. albidus* eliminated all *Aedes albopictus* larvae from both piles within 2 months. Adult *Ae. albopictus* around the tire piles disappeared within another month. Complete suppression of *Ae. albopictus* larvae was still in effect in all treated tires a year later.

## INTRODUCTION

The Centers for Disease Control and the New Orleans Mosquito Control Board recently collaborated in field trials to evaluate the ability of 6 species of cyclopoid copepods to control *Aedes* larvae in tires (Marten 1989). *Macrocyclops albidus* (Jurine) was the most effective predator. Once established in tires, *Macrocyclops* always killed all first instar larvae of *Aedes albopictus* (Skuse), *Ae. aegypti* (Linn.) and *Ae. triseriatus* (Say) (Marten 1990a).

A small percentage of discarded tires in New Orleans contain natural populations of *Macrocyclops* that appear to have been in the tires for years (Marten 1989). Using *Macrocyclops* for biological control of mosquito larvae is a matter of introducing *Macrocyclops* into tires that do not already have them naturally. *Macrocyclops* were introduced to experimental tire piles to assess their impact on both mosquito larvae in the tires and the population of adult mosquitoes around the tires.

## MATERIALS AND METHODS

In June 1989, three tire piles infested with *Ae. albopictus* were established in a wooded area of New Orleans (Big Oak Island), which was virtually free of this mosquito previously. Each pile consisted of 100 naturally infested tires brought from another area. Only tires without a natural population of *Macrocyclops* or other larvivorous cyclopoids were used. The tires were stacked against each other, all touching the ground and arranged so that the water inside could be removed for examination without moving the tires. No maintenance was provided. The only source of water for the tires was rainfall. Some of the tires dried out from time to time.

The tire piles were 500 m from one another in a triangular arrangement. They were more than 500 m from other *Ae. albopictus* breeding areas. *Aedes albopictus* populations at each pile were isolated by a paucity of discarded containers and tree holes in the surrounding woods to serve as breeding sites. *Aedes albopictus* does not normally disperse more than a few hundred meters (Hawley 1988). The very low populations of *Ae. albopictus* in the surrounding woods during May–November 1989 were verified by means of oviposition traps.

Each pile had approximately 15,000 immature *Ae. albopictus* at the end of July, when 10 adult female *Macrocyclops albidus* were introduced into every tire in 2 of the piles (designated "treated" piles). The *Macrocyclops* came from a laboratory colony initiated 3 months earlier from a natural population in a tire. No *Macrocyclops* were introduced to the third pile, which served as a control.

Immature and adult mosquitoes were monitored at each tire pile from June through November. A random sample of 10 tires at each pile was examined every 2 weeks for larvae and pupae. All water and detritus were removed from each tire with a plastic cup and examined in a large shallow glass dish with intense illumination from the top, bottom and sides. Larvae and pupae were removed from the sample, identified and counted live with a stereomicroscope, and returned with water and detritus to the tire. Standard errors of estimates of the number of immature *Ae. albopictus* in each tire pile varied from 17 to 43% of the estimate. Every tire in the *Macrocyclops*-treated piles was examined on every sampling occasion during September–November, when there were few or no *Ae. albopictus* larvae in those piles; the standard errors of those samples were zero.

*Macrocyclops* in the treated piles were monitored by counting copepodids and adults in the same samples that were taken for mosquito larvae and pupae. Standard errors of estimates of

[1] Current address: New Orleans Mosquito Control Board, 6601 South Shore Harbor Drive, New Orleans, LA 70126.

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the number of *Macrocyclops* in each tire pile varied from 16 to 30% of the estimate.

The adult mosquito population was assessed at weekly intervals, using 2 different measures. The first was landing rate on human bait. Two persons stood simultaneously at different parts of the same tire pile, using dry ice as an attractant and collecting mosquitoes with hand-held aspirators. Each person collected for a total of 10 min in two 5-min sessions at different parts of the pile. Landing rates were always conducted in immediate succession at all piles between 0900 and 1100 h the same day. The same people measured landing rates for the entire study.

Numerous species of adult mosquitoes were collected: *Aedes atlanticus* Dyar and Knab, *Ae. sollicitans* (Walker), *Ae. triseriatus*, *Ae. vexans* (Meigen), *Anopheles crucians* Wied., *Coquillettidia perturbans* Dyar, *Culex nigripalpus* Theobald, *Cx. salinarius* Coq. and *Psorophora ferox* (von Humboldt). Only *Ae. albopictus* were counted. Standard errors of *Ae. albopictus* landing rates ranged from 11 to 55% of the estimate. The relatively high standard errors were associated with very low landing rates.

The second measure of the adult population was oviposition. The ovitraps were black plastic cups containing water in which rabbit food pellets had previously been incubated. Eggs were collected on red velour paper strips (10 × 2.5 cm) clipped to the inside of each cup and immersed halfway in the water. Ten ovitraps were set among the tires at each pile. Ovistrips were collected weekly.

*Aedes triseriatus* eggs appeared on the ovistrips occasionally, but only *Ae. albopictus* eggs were counted. Standard errors of *Ae. albopictus* egg counts ranged from 14 to 29% of the average.

*Macrocyclops* were killed in most of the tires by a severe freeze in December 1989, when the temperature stayed below −6°C for 3 consecutive days. Water in the tires froze solid.

*Macrocyclops* were reintroduced to all tires in the 2 treated piles in March 1990, at which time immature and adult *Ae. albopictus* were observed at the piles. In June 1990, all tires at the treated piles were examined for *Macrocyclops* and mosquito larvae and pupae; a sample of 10 tires at the control pile was examined for the same. Landing rates, based on 4 persons collecting for a total of 60 man-minutes at each pile, were measured at the same time. Ten oviposition traps were operated for a week at each pile.

## RESULTS

It took several weeks after introduction for the *Macrocyclops* to build up their numbers in the treated tires (Fig. 1A). By one month, *Ma-*

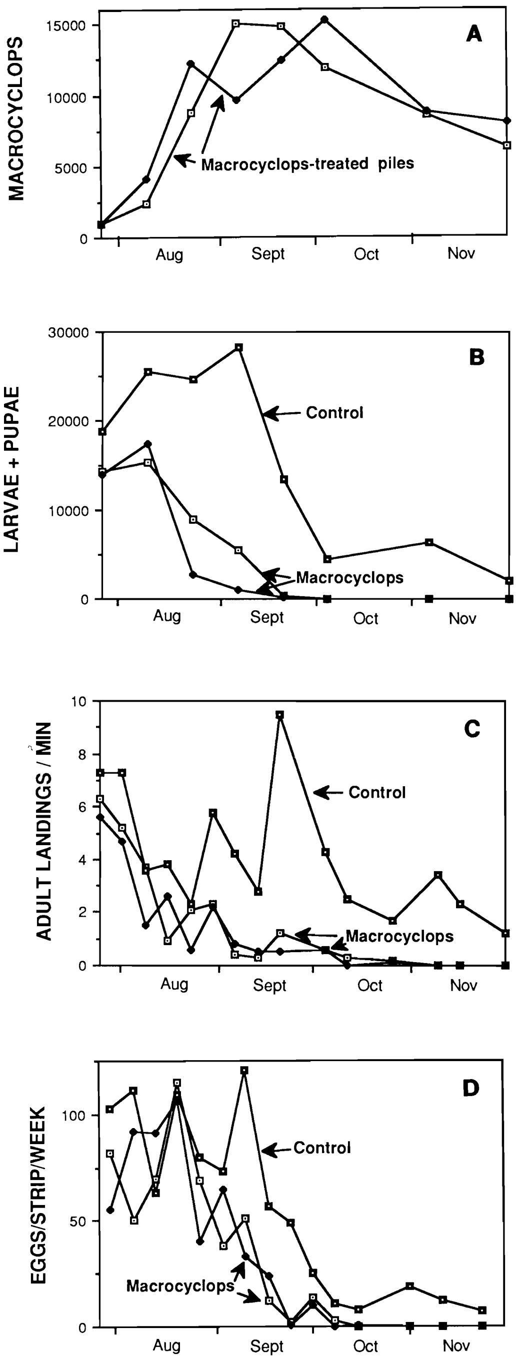


Fig. 1. *Aedes albopictus* populations at tire piles after introducing *Macrocyclops albidus:* A, number of *Macrocyclops* in the 2 tire piles to which *Macrocyclops* were introduced; B, number of *Ae. albopictus* larvae and pupae in each tire pile; C, landing rate of adult *Ae. albopictus* on human bait at each tire pile; D, oviposition of *Ae. albopictus* in ovitraps at each tire pile. The time axis of the graphs starts on the date that *Macrocyclops* were introduced into the tires.

*crocyclops* numbers averaged more than 100/tire in each of the treated piles, but 5–10 tires in each pile contained fewer than 20 individuals. A month later each treated pile contained at least 50 *Macrocyclops*, and most tires had more than 100. *Macrocyclops* were present in all treated tires until December.

Small numbers of *Ae. triseriatus* larvae (averaging less than 1 larva/tire) were observed in the control pile throughout the study. Similar numbers were found in the treated piles during June–September, but no *Ae. triseriatus* larvae were observed in the treated piles during October–November. Large numbers of *Cx. salinarius* larvae were present in all piles, particularly during October–November, with no difference between treated and control piles. Larvae of *Toxorhynchites rutilus* (Coq.) were sometimes found in tires at both treated and control piles. Most tires with *Tx. rutilus* in the control pile contained substantial numbers of *Ae. albopictus* larvae and pupae.

The average number of *Ae. albopictus* larvae and pupae in the control pile was more than 200/tire until the middle of September (Fig. 1B). The number of *Ae. albopictus* larvae and pupae in the treated piles started to decline in the middle of August, 2 weeks after *Macrocyclops* introduction. Larvae and pupae were nearly gone from the treated piles by the middle of September, 8 weeks after *Macrocyclops* introduction. No *Ae. albopictus* larvae or pupae were found in the treated tire piles during October and November. The control pile averaged 43 ± 9.8 (SE) immature *Ae. albopictus* per tire during October and November.

The landing rates of most species of mosquitoes were substantial at all 3 tire piles throughout the study. In contrast, *Ae. albopictus* landing rates and oviposition at the 2 treated piles started to decline about 1 week after the decline of larvae and pupae, i.e., 3 weeks after *Macrocyclops* introduction (Fig. 1, C and D). Landing rates and oviposition at the treated piles were close to zero by the 11th week after *Macrocyclops* introduction. *Aedes albopictus* landing rates and oviposition at the control pile declined during October, but the decline started after *Ae. albopictus* populations at the treated piles were already close to zero.

No adult *Ae. albopictus* were collected at the treated piles during November (Fig. 1C), nor were any *Ae. albopictus* eggs collected by ovitraps at the treated piles during that month (Fig. 1D). Adult landings of *Ae. albopictus* at the control pile averaged 2.3 per min during November, a total of 138 ± 12 landings during that period. Oviposition at the control pile averaged 13 eggs/ovistrip/week during November, a total of 650 ± 78 eggs.

*Macrocyclops* were present in all tires in the treated piles when they were examined in June 1990. No *Ae. albopictus* larvae or pupae were found in either of the treated piles (Table 1). However, very small numbers of adult *Ae. albopictus* were collected at both treated piles in June, and small numbers of *Ae. albopictus* eggs were collected in ovitraps at the treated piles. *Aedes albopictus* larvae, pupae, adult landings and oviposition at the control pile were normal for that time of year.

## DISCUSSION

In summary, *Macrocyclops* eliminated all *Ae. albopictus* larvae from the treated tire piles within 2 months. It took an additional month for the adult mosquitoes to disappear. *Aedes albopictus* larvae did not reappear in the tires the following summer, but adults reappeared in small numbers.

It is possible that a natural decline due to autumn temperatures and diapause contributed to the disappearance of adult *Ae. albopictus* from the treated piles during October–November. However, diapause was incomplete that year; *Ae. albopictus* populations remained substantial at the control pile and elsewhere in New Orleans until December.

Adult populations of *Ae. albopictus* at treated and control piles were drastically different by November; a null hypothesis that the treated piles and control pile had the same number of mosquitoes can definitely be rejected. Assuming

Table 1. *Aedes albopictus* at the 3 experimental tire piles in June 1990.[a]

| Tire piles | Larvae + pupae[b] | Adult landings[c] | Oviposition[d] |
|---|---|---|---|
| *Macrocyclops*-treated[a] | 0 | 0.05 ± 0.03 | 9 ± 3 |
| *Macrocyclops*-treated[a] | 0 | 0.03 ± 0.02 | 7 ± 3 |
| Control pile[e] | 142 ± 31 | 2.20 ± 0.21 | 118 ± 13 |

[a] Three months after reintroducing *Macrocyclops* to the 2 treated tire piles.
[b] Average number of immature *Aedes albopictus* per tire ± SE.
[c] Average landings per minute ± SE.
[d] Average number of eggs per ovistrip in 1 week ± SE.
[e] No larvivorous copepods in control tire pile.

that adult landings followed a Poisson distribution, if the expected number of landings at a treated pile was actually the 138 landings observed at the control pile during November, the probability of no observed landings at a treated pile would be $P[0] = e^{-(\text{expected value})} = e^{-138} = 10^{-60}$. For oviposition, assuming that a mosquito lays an average of 10 eggs each time it deposits eggs at a container, the 650 eggs at the control pile represent approximately 65 independent sets of eggs on the ovistrips. The probability of no observed eggs at a treated pile would be $P[0] = e^{-65} = 10^{-29}$. The hypotheses that each treated pile was the same as the control pile can therefore be rejected at an extremely high level of significance: $P = 10^{-60}$ for adult landings; $P = 10^{-29}$ for oviposition.

We do not know whether there was still a small population of adult *Ae. albopictus* at the treated piles during November, but if there were any adults, their numbers were extremely small. Assuming a Poisson distribution, the fact that no landings or eggs were observed at either treated pile during November gives a 99% level of confidence that the total expected number of landings or egg sets at each treated pile during November was less than 1.15—much less than the 138 adult landings and 65 eggs sets observed at the control pile during the same period.

Because there were no *Ae. albopictus* larvae in the treated tires in June 1990, it is unlikely that the small numbers of adult *Ae. albopictus* around the treated piles at that time came from the piles themselves. The experimental introduction of *Ae. albopictus* appears to have established a small population in the surrounding woods. This interpretation is supported by the observation of low levels of oviposition and adult landings in the woods between the tire piles in June 1990.

The results of this study suggest that *Macrocyclops albidus* should be considered seriously as a biological control agent for operational use in tires. The prospects for an inexpensive and convenient supply are promising (Rivière et al. 1987, Marten 1990b). Techniques for mass production, storage and transport are currently under development at the New Orleans Mosquito Control Board.

Methods for applying *Macrocyclops* to large tire piles remain to be worked out in detail. *Macrocyclops* can be squirted into tires with a hand-held, pressure-pumped larvicide sprayer. Broadcast spraying is also a possibility; *Macrocyclops* survive when sprayed from a backpack air-blast sprayer. Because *Macrocyclops* survive without water as long as they are damp, it may be possible to apply them with a granular carrier similar to ones that have proved effective for delivering larvicides to tire piles (Novak et al. 1990).

A point of concern is the time that it took *Macrocyclops* to eliminate all larvae from the treated tires during July–August 1989. The delay was due in part to the time it took for *Macrocyclops* to build up their numbers in the tires. This part of the delay could be eliminated by introducing more *Macrocyclops* at the beginning (e.g., 100 individuals/tire).

A second and equally important source of delay arises because cyclopoid copepods are not large enough to kill third and fourth instar mosquito larvae. Any large larvae that are in a tire when *Macrocyclops* is introduced will escape predation and may eventually become adult mosquitoes. If a tire is crowded with larvae, the food supply is reduced, larval development is slow, and it can take more than a month for some of the larvae to emerge as adults.

The second delay could be eliminated by applying a larvicide at the same time *Macrocyclops* is introduced. The larvicide kills all larvae, mosquito production is terminated, and the *Macrocyclops* continue the treatment after the larvicide loses its potency. Several commonly used larvicides (e.g., diesel oil, *B.t.i.*, and methoprene) are compatible with *Macrocyclops* (W. Y. Che and G. G. Marten, unpublished data).

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