COMPATIBILITY OF CYCLOPOID COPEPODS WITH MOSQUITO INSECTICIDES

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ABSTRACT. Larvivorous copepods (*Macrocyclops, Mesocyclops* and *Acanthocyclops*) were tested for their sensitivities to commonly used mosquito larvicides and adulticides. The cyclopoids were not harmed by *Bacillus thuringiensis* (H-14) (*B.t.i.*) or larviciding oil. Control of mosquito larvae in field trials was accelerated by applying *B.t.i.* at the same time cyclopoids were introduced to a breeding site. Among adulticides tested, the cyclopoids were least sensitive to permethrin. Field trials demonstrated that permethrin does not harm cyclopoids when applied at label specifications.

INTRODUCTION

Larvivorous copepods such as *Macrocyclops albidus, Mesocyclops longisetus* and *Mesocyclops aspericornis* are highly effective for controlling *Aedes* ssp. larvae in discarded tires (Marten 1990a, Rivière et al. 1987). They may also have considerable potential for controlling a variety of species of mosquito larvae in groundwater habitats (Marten 1990b). If cyclopoids are to be an operational part of mosquito control, it is highly desirable for them to survive and function alongside other mosquito control activities such as the use of larvicides and adulticides.

There is an additional reason that it would be desirable for cyclopoids to be compatible with larvicides. These tiny crustaceans prey on first instar mosquito larvae (Marten 1989). Even if they kill all new larvae that come along, the cyclopoids will not kill larger larvae already on site when they are introduced. Mosquito larvae can take many weeks to reach the adult stage if temperatures are low or their food supply is poor (Service 1985). As a consequence, some larval mosquito habitats can produce mosquitoes for many weeks after cyclopoid introduction (Marten 1990c).

If a compatible larvicide is applied at the same time as the cyclopoids, the larvicide can kill the existing mosquito larvae immediately, and the cyclopoids can kill all new larvae that appear. For this to work, it is necessary to use a larvicide that is harmless to cyclopoids at a concentration high enough to kill mosquito larvae.

We measured the sensitivity of larvivorous cyclopoids to commonly used mosquito larvicides and adulticides. For comparison, we measured the sensitivity of *Aedes albopictus* (Skuse) to the same larvicides that we tested with cyclopoids. We also evaluated cyclopoid survival during larviciding and adulticiding operations.

MATERIALS AND METHODS

The following larvivorous copepods were collected from natural populations in the New Orleans area: *Macrocyclops albidus* (Jurine) from a pond and discarded tires; *Mesocyclops longisetus* (Thiébaud) from a pond; *Mesocyclops ruttneri* Kiefer from a pond; *Acanthocyclops vernalis* (Fischer) from a pond and temporary pools. To supply animals for sensitivity tests and field trials, all species were established in laboratory cultures maintained according to procedures described by Suárez et al. (1992). All insecticides in the study were tested with *Macrocyclops albidus*. Permethrin was also tested with *Mesocyclops ruttneri* and *Acanthocyclops vernalis*.

Adult female *Ae. albopictus* were captured with an aspirator at a tire pile in New Orleans to provide larvae for sensitivity tests. Eggs were collected and hatched in the laboratory. Larvae were reared to the fourth instar under laboratory conditions.

Sensitivity tests: Twenty adult female cyclopoids were placed in disposable paper cups containing 200 ml of water with one of the following insecticides: *B.t.i.* H-14 (Vectobac®-12AS, 1200 ITU/mg); temephos (Abate®, 82.1% AI); malathion (Cythion®, 91% AI); permethrin (Permanone®, 10% AI); resmethrin (Scourge®, resmethrin 18% AI plus piperonyl butoxide 54% AI). Acetone was used as the solvent to achieve standard dilution series in water. There were 4 replicates at each concentration, plus a control with 0.1% acetone.

The same procedure was followed with methoprene (Altosid®, 5% AI), except early-instar cyclopoids (nauplii) were used instead of adults because methoprene is a juvenile hormone. Bircher and Ruber (1988) observed that cyclopoid nauplii were more sensitive to methoprene than adults.

The paper cups were held at 25°C, and surviving cyclopoids in each cup were counted at the end of 24 hours. Animals that moved abnormally or failed to show a normal escape reaction to a probe were considered moribund because we knew from experience they would soon die. The entire procedure was repeated 3 times for each insecticide.

The tests proceeded in 2 stages: 1) a broad range of 10 insecticide concentrations, and 2) a narrower range of 5 concentrations spanning the LD_5, LD_{50} and LD_{95} suggested by the first stage. The LD_5, LD_{50} and LD_{95} were estimated by fitting results from the second stage to a logistic curve using a probit analysis computer program. The LD_5 for cyclopoids was important because it indicated the concentration at which an insecticide started to have a negative impact on the cyclopoids.

Fourth instar *Ae. albopictus* larvae were subjected to sensitivity tests with the larvicides at the same time as the cyclopoids, following the same procedure. In the case of methoprene, first instar larvae were used for the test, and "surviving larvae" were counted as the number of adult mosquitoes that emerged within 14 days.

In addition to the 24-h sensitivity tests, *Macrocyclops albidus* and *Mesocyclops ruttneri* were placed in glass bowls containing 500 ml of water with a range of *B.t.i.*, methoprene and permethrin concentrations to assess the effect of long-term insecticide exposure. They were observed for 6 wk to see if they completed their life cycle in a normal fashion. There were protozoa in the containers to serve as food for the cyclopoids the entire time.

Enough Golden Bear larviciding oil to cover the surface of the water was applied to a laboratory culture dish (20 cm diam) containing each species of cyclopoid in the study. The oil was also applied to a ground pool in the field that contained *Acanthocyclops vernalis*, covering the entire surface of the water with oil. Cyclopoids in the dishes and pools were examined 1 day and 1 wk after applying the larviciding oil.

Field trials with B.t.i.: In June 1990, 50 *Macrocyclops albidus* were introduced to each of 10 tires in a pile of several thousand tires located at a clearing in a wooded area on the outskirts of New Orleans. At the same time, 10 tires were treated with *B.t.i.* (10,000 ITU/tire), and 10 additional tires were treated with both *B.t.i.* (10,000 ITU/tire) and 50 *Macrocyclops*. The *B.t.i.* was applied at the same time the *Macrocyclops* were introduced. Tires with different treatments were randomly interspersed among one another.

The numbers of *Macrocyclops*, mosquito larvae and mosquito pupae in each tire were counted 2 days, 10 days and 30 days after the initial treatment. On each occasion, 10 untreated tires that were interspersed among the treated tires were selected at random as controls to count the numbers of mosquito larvae and pupae.

Field trials with permethrin: In June 1992, *Mesocyclops longisetus* was introduced to every tire in 4 piles at an abandoned motel in New Orleans. The piles were 8–10 m in diameter, they contained about 200 tires, and the tires contained large numbers of *Ae. albopictus* larvae prior to treatment. In August, permethrin (Permanone, 10% EC) was sprayed over 2 piles from a hand-held Leco® ULV unit at a rate of 3.0 ml/sec while walking slowly across the upwind side of the pile. The duration of the spraying was 20 sec for one pile and 60 sec for the other. Fifteen tires were inspected immediately before spraying to verify that they contained *Mesocyclops longisetus*. The same tires were inspected 1 wk after spraying.

The other 2 tire piles were sprayed in early October. The same procedure was followed, except one pile was sprayed for 5 sec and the other for 10 sec, application rates that fall within Permanone label specifications (0.007 lb AI/acre). Ten sentinel cages (cardboard cylinders 5.1 cm diam × 8.9 cm long with plastic screen at each end), each containing 25 adult *Aedes aegypti* (Linn.), were placed at random locations on each of these piles immediately before spraying. Half the sentinels were on top of tires, and the other half were inside tires. The numbers of live and dead mosquitoes in the sentinel cages were counted 1 h after spraying. The same procedure was followed with control sentinel mosquitoes, except they were placed at a pile that was not sprayed.

RESULTS

Sensitivity tests of larvicides: Macrocyclops albidus was much more sensitive to temephos than the other larvicides that were tested. The LD_5, LD_{50} and LD_{95} for temephos with *Macrocyclops* (Table 1) were almost the same as the LD_5, LD_{50} and LD_{95} for temephos with *Ae. albopictus* (Table 2).

The LD_{50} for methoprene with *Macrocyclops* was 130× the LD_{50} for methoprene with *Ae. albopictus*. The LD_5 for methoprene with *Macrocyclops* was more than 20× the LD_{95} with *Ae. albopictus*, indicating that a dosage of methoprene sufficient to block *Ae. albopictus* pupation should kill few *Macrocyclops*. *Macrocyclops* was able to sustain a long-term population at methoprene concentrations up to 0.21 ppm, which is less than the LD_5 for *Macrocyclops* but 13× the LD_{95} for *Ae. albopictus*. *Macrocyclops* reproduction was impaired at higher concentrations.

The *B.t.i.* had no detectable toxic effect on any of the cyclopoids. They were harmed only when *B.t.i.* was so thick their movement was impaired. *Macrocyclops albidus* and *Mesocyclops ruttneri* completed their life cycles in a normal

Table 1. Sensitivity of *Macrocyclops albidus* to commonly used mosquito insecticides[a]

Insecticide	LD_5	LD_{50}	LD_{95}
Larvicides			
Temephos	0.0059 (0.0016–0.0085)	0.011 (0.006–0.013)	0.020 (0.016–0.037)
Methoprene	0.34 (0.28–0.38)	0.67 (0.63–0.73)	1.35 (1.16–1.72)
B.t.i.	3,323 (3,156–3,477)	5,375 (5,248–5,497)	8,692 (8,420–9,007)
Adulticides			
Resmethrin[b]	0.060 (0.055–0.063)	0.094 (0.091–0.097)	0.15 (0.14–0.16)
Permethrin	0.13 (0.10–0.16)	0.29 (0.26–0.31)	0.61 (0.53–0.78)
Malathion	0.02 (0.002–0.05)	0.76 (0.38–2.7)	ND[c]

[a] Concentration in parts per million (except for *B.t.i.*, which is ITU/ml). 95% confidence limits are in parentheses.
[b] Resmethrin mixed with piperonyl butoxide.
[c] Confidence limits unacceptably large.

Table 2. Sensitivity of *Aedes albopictus* larvae to commonly used mosquito larvicides[a]

Larvicide	LD_5	LD_{50}	LD_{95}
Temephos	0.0077 (0.0064–0.0088)	0.013 (0.012–0.014)	0.023 (0.021–0.027)
Methoprene	0.0017 (0.0011–0.0022)	0.0051 (0.0042–0.0060)	0.016 (0.013–0.020)
B.t.i.	0.058 (0.041–0.072)	0.12 (0.11–0.14)	0.27 (0.23–0.34)

[a] Concentration in parts per million (except for *B.t.i.*, which is ITU/ml). 95% confidence limits are in parentheses.

Table 3. Sensitivity of copepods to permethrin[a]

Species	LD_5	LD_{50}	LD_{95}
Macrocyclops albidus	0.13 (0.10–0.16)	0.29 (0.26–0.31)	0.61 (0.53–0.78)
Acanthocyclops vernalis	0.33 (0.24–0.41)	1.8 (1.6–2.0)	9.7 (8.2–12.0)
Mesocyclops ruttneri	0.49 (0.26–0.71)	1.9 (1.6–2.3)	7.7 (5.7–12.7)

[a] Concentration in parts per million (95% confidence limits in parentheses).

manner during 6 wk of observation at a *B.t.i.* concentration of 1,200 ITU/ml, nearly 5,000× the LD_{95} for *Ae. albopictus*. No deleterious effect on cyclopoids was observed in laboratory dishes or ground pools treated with larviciding oil.

Sensitivity tests of adulticides: Table 1 presents the LD, LD_{50} and LD_{95} for resmethrin, permethrin and malathion with *Macrocyclops albidus*. The relative sensitivity of *Macrocyclops* to field applications of these adulticides can be roughly compared by dividing the LD_{50} for each adulticide by its maximum label application rate. A larger ratio signifies less sensitivity. The ratios were: malathion = 0.76 ppm/0.23 lb/acre = 3.3 resmethrin = 0.094 ppm/0.007 lb/acre = 13.4 permethrin = 0.29 ppm/0.007 lb/acre = 41.4. *Macrocyclops* is least sensitive to permethrin by this measure.

Not all species of cyclopoids had the same sensitivity to the same larvicide. The LD_{50} of permethrin for *Acanthocyclops vernalis* and *Mesocyclops ruttneri* was 6× higher than the LD_{50} for *Macrocyclops albidus* (Table 3).

During the long-term exposure experiments with permethrin, some adult *Macrocyclops albidus* were able to survive and reproduce at a permethrin concentration of 0.38 ppm, equivalent to the LD_{75} for *Macrocyclops*. However, because *Macrocyclops* nauplii were able to survive at only half that concentration, *Macrocy-*

clops was able to complete its life cycle and sustain its population only when the permethrin concentration was less than 0.19 ppm, which corresponds to its LD_{20} for permethrin. *Mesocyclops ruttneri* was able to complete its life cycle at permethrin concentrations up to 0.57 ppm, which is the LD_8 for *Me. ruttneri.*

Field trials with B.t.i.: During the 30 days of observation, *Macrocyclops albidus* survived in all tires to which they were introduced (the *Macrocyclops* only treatment, as well as *Macrocyclops* combined with *B.t.i.*). There were substantial numbers of *Ae. albopictus* larvae in the control tires throughout the period of observation (Table 4).

More than 99% of the larvae in tires treated with *B.t.i.* were dead when the tires were examined 2 days after treatment, whether the tires contained *Macrocyclops* or not (Table 4). However, larvae were back within 10 days in tires treated only with *B.t.i.* (i.e., without *Macrocyclops*). By 30 days, the average number of larvae in tires treated only with *B.t.i.* was not significantly different from untreated controls (Table 4).

In tires treated only with *Macrocyclops*, there was a gradual reduction in *Ae. albopictus* larvae over a period of several weeks after the *Macrocyclops* introduction. The larvae eventually disappeared almost entirely from these tires, but it took more than a month in some.

Within 2 days after treatment, the larvae disappeared almost entirely from tires treated with both *B.t.i.* and *Macrocyclops.* Very few larvae were ever found in these tires during the entire period of observation (Table 4).

Field trials with permethrin: Nearly all sentinel mosquitoes were dead within an hour after the tire pile was sprayed for 5 sec (Table 5). All sentinel mosquitoes were killed at the pile sprayed for 10 seconds. No adverse effect was observed on *Mesocyclops longisetus* populations in the tires.

At the pile sprayed for 20 sec (several times the label rate), *Mesocyclops longisetus* was seriously reduced in 20% of the tires and unaffected in the rest of the tires. When water from the tires was taken to the laboratory to culture the *Mesocyclops*, the cyclopoids reproduced normally in water from tires whose *Mesocyclops* populations were unaffected by the spraying. The cyclopoids survived but failed to reproduce in water from tires where the population was reduced by spraying.

At the tire pile sprayed for 60 sec, the *Mesocyclops longisetus* populations disappeared from 65% of the inspected tires after spraying. When *Me. longisetus* was cultured in water from tires where it disappeared after spraying, all cyclopoids died in the water from some of the tires and they survived in the water from other tires. When they survived, reproduction varied from poor to normal depending on the tire.

Table 4. Number of *Aedes albopictus* larvae in tires treated with *Macrocyclops albidus* and *B.t.i.*[a]

Treatment	Time after treatment: 2 days	30 days
B.t.i. only	0.2 ± 0.2	34.1 ± 19.4
Macrocyclops only	30.4 ± 11.0	2.5 ± 2.1
B.t.i. + *Macrocyclops*	0.1 ± 0.1	0.1 ± 0.1
Controls	59.8 ± 21.4	41.0 ± 11.7

[a] Mean number per tire ± SE.

Table 5. Mortality of *Mesocyclops longisetus* populations and sentinel *Aedes aegypti* at tire piles sprayed with permethrin

Permethrin application: Duration[a]	Quantity[b]	*Mesocyclops* mortality[c]	*Ae. aegypti* mortality
0[d]	0	—	0%
5	15	0%	98%
10	30	0%	100%
20	60	20%	ND
60	180	65%	ND

[a] Number of seconds.

[b] Total quantity (ml) of Permanone (10% EC) sprayed at each tire pile.

[c] Percentage of tires in which the *Mesocyclops longisetus* population disappeared or was noticeably reduced.

[d] Control.

DISCUSSION

Bacillus thuringiensis var. *israelensis* and larviciding oil are clearly the most appropriate larvicides to use with cyclopoids. Rivière et al. (1987) noted the compatibility of *B.t.i.* with *Mesocyclops aspericornis.* The New Orleans Mosquito Control Board routinely applies *B.t.i.* when treating tires or groundwater habitats with cyclopoids. This procedure provides immediate and long-term control of larvae belonging to species for which cyclopoids are effective (Marten 1990b).

The relatively low sensitivity of cyclopoids to methoprene suggests that methoprene is probably compatible with cyclopoids as long as it is not applied above the label rate. Temephos is highly toxic to cyclopoids and completely incompatible. In fact, temephos is used as a control agent for cyclopoids in areas where they are alternate hosts for guinea worm (CDC/WHO 1989, Muller 1992).

Among the adulticides that were tested, permethrin is most compatible with cyclopoids. There should be no detrimental effect as long as permethrin is applied according to label specifications.

ACKNOWLEDGMENTS

Gerald Marten was supported by a National Research Council Senior Research Associateship with the Centers for Disease Control and Wenyan Che was supported by a Centers for Disease Control fellowship. Some of the research was supported by a contract with the Division of Vector-Borne Infectious Diseases, Centers for Disease Control. We thank Jerome Freier, Mieu Nguyen, Giai Ngo, Michael Carroll, Edward Freytag, Stephen Sackett, John Stennett and others on the staff of the New Orleans Mosquito Control Board for their contributions to this study. The cyclopoids were identified by Janet Reid (Smithsonian Institution).

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