

INFECTION OF A FIELD POPULATION OF *Aedes cantator* WITH A POLYMORPHIC MICROSPORIDIUM, *Amblyospora connecticus* VIA RELEASE OF THE INTERMEDIATE COPEPOD HOST, *Acanthocyclops vernalis*

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ABSTRACT. The microsporidium *Amblyospora connecticus* was successfully introduced into a larval field population of *Aedes cantator* via the release of the infected intermediate copepod host, *Acanthocyclops vernalis*. The tests were conducted in 3 steel drums that were centrally placed within a salt marsh pool that supports breeding populations of both hosts. A total of 2.7×10^4 2nd-instar *Ae. cantator* larvae were exposed to spore doses ranging from 2.0×10^3 to 2.8×10^4 spores/larva through the release of more than 2,000 live infected copepods. The majority of infections were acquired by 2nd- and 3rd-instar larvae during the first 3 weeks of exposure, and maximum infection rates ranging from 16 to 24% were obtained by the time of pupation.

INTRODUCTION

Amblyospora connecticus Andreadis is a naturally occurring microsporidian parasite of the brown saltmarsh mosquito, *Aedes cantator* (Coquillett). It has a complex and highly specialized life cycle that includes obligatory development in an intermediate copepod host, *Acanthocyclops vernalis* (Fischer). It is transmitted vertically and horizontally and produces 3 different spore types, including 2 in the mosquito and one in the copepod (Andreadis 1983a, 1985, 1988b).

A generalized scheme of the life cycle is shown in Fig. 1. *Amblyospora connecticus* is transovarially transmitted by adult female *Ae. cantator* that are infected with "binucleate spores." Infections appear to delay egg hatch and only rarely do infected eggs hatch during the summer. These individuals (mostly females) usually develop a benign infection that leads to ovarian infection and transovarial transmission once again. Most infected eggs do not hatch until the fall. These larvae (both sexes) develop patent infections within their fat bodies and die during the 4th stadium. This results in the formation and release of large numbers of "meiospores" that are orally infectious to an intermediate copepod host, *A. vernalis* (females only). The microsporidium invades ovarian tissue and overwinters in the copepod host. Infected copepods usually succumb to the parasite in the spring and "haploid spores" formed in these hosts are subsequently released into the water. These spores are ingested by early instar mosquito larvae which hatch at this time. Larvae develop benign infections and emerge as unapparently infected adults. Female mosquitoes thus infected eventually go on to transmit the microsporidium transovarially to complete the cycle (Andreadis 1983a, 1983b, 1985, 1986, 1988a, 1988b).

Although there has been significant progress in our understanding of the basic biology and ecology of various *Amblyospora* spp. in mosquitoes (Sweeney et al. 1985, 1988, 1989; Becnel 1986), no attempts have been made to infect mosquitoes outside of the laboratory in the form of a field release. The present study describes the first attempt to infect a field population of mosquitoes with a polymorphic microsporidium via the release of infected copepods and specifically determine whether infections can be induced in larvae under field conditions.

MATERIALS AND METHODS

Test Site: The study was conducted in a semi-permanent, brackish water pool (approximately 8.5×5.5 m) located in a coastal salt marsh in Guilford, Connecticut. This site habitually supports a large larval population of *Ae. cantator* in the spring and all stages of *A. vernalis*, which are both generally free of infection from *Amblyospora connecticus*.

The experiments were conducted in 6 galvanized steel drums (3 treatment and 3 control) (1.2 m high \times 50.8 cm diam) that were centrally placed within the pool (Fig. 2). These drums were recessed (ca. 20–30 cm) into the bottom of the pool before larval hatch in early March and subsequently seeded with resident 1st- and 2nd-instar *Ae. cantator* larvae 1 week prior to the release of infected copepods (April 5).

Source of Inoculum: The *Amblyospora connecticus*-infected *A. vernalis* copepods used for release were obtained from two sources. One group (treatment 1) was experimentally infected in the laboratory, and the other (treatments 2 and 3) was naturally infected from the field.

The laboratory-infected copepods used for

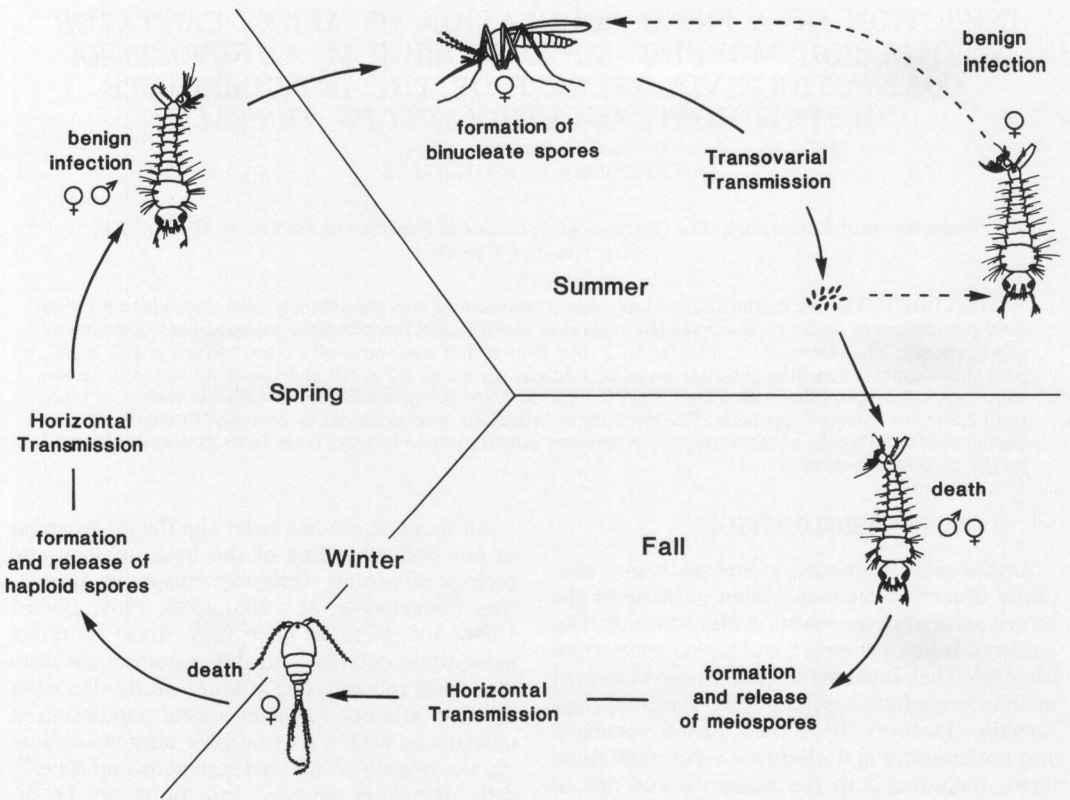


Fig. 1. Generalized scheme of the life cycle of *Amblyospora connecticus* in *Aedes cantator* and *Acanthocyclops vernalis* (Adapted from Andreadis 1983a, 1983b, 1985, 1986, 1988a, 1988b) (See text for details).



Fig. 2. Test release site in Guilford, Connecticut, showing the placement of the treatment and control steel drums in the salt marsh pool.

in 3.5 liter glass jars containing water from the breeding site. On March 1, copepod densities were determined and infectious meiospores, which had been harvested from field-collected *Ae. cantator* larvae obtained the previous fall, were added to the jars at a rate of 1.3×10^4 spores/ml or 2.5×10^4 spores/female copepod. Copepods were maintained as described above for 4 weeks. At the end of the exposure period, copepod densities were estimated once again, and the prevalence of infection in adult females was determined from microscopic examination (40 \times) of whole wet mounts ($n = 25$). Spore densities were also determined from hemacytometer counts ($n = 5$ copepods), and these data were collectively used to calculate the total quantity of spores (no. female copepods/jar (3150) \times % female copepods infected (0.533) \times mean no. spores/female copepod (1.6×10^5) = 2.7×10^8 spores) available for release on April 5.

Field-infected copepods were used in treatments 2 and 3. These individuals were obtained in March from 2 salt marsh pools in Guilford and Milford, Connecticut, with natural infection rates of 26.4 and 15.2% in the adult female copepod populations. They were maintained un-

treatment 1 were originally collected from 3 salt marsh pools in Guilford and Milford, Connecticut during February 1988. These individuals were held at 15 $^{\circ}$ C under a 16:8 LD photoperiod

der identical conditions as laboratory infected copepods until their release on the same day. The estimated quantity of spores released in treatment 2 was 2.3×10^7 (941 copepods \times $0.152 \times 1.6 \times 10^5$ spores/copepod) and treatment 3 was 4.1×10^7 (980 copepods \times $0.264 \times 1.6 \times 10^5$ spores/copepod).

Assay Procedures: Resident populations of adult female *A. vernalis* and larval *Ae. cantator* that were developing within the pool (outside of the drums) were monitored for infection with *Amblyospora connecticus* both before and after the release. This began in February and provided a baseline infection rate for both hosts throughout the experiment. Weekly samples of female copepods and larval mosquitoes were collected from the pool and transported to the laboratory. Representative samples of both hosts (n = 25) were smeared on slides, stained with a Giemsa solution and microscopically (100X) examined for infection.

Approximately 7,000 1st- and 2nd-instar *Ae. cantator* larvae were added to each of the 6 steel drums on March 29. The *Amblyospora connecticus*-infected copepods were released 1 week later on April 5. Larval densities in each control and treatment drum were estimated weekly. These were based on larval counts obtained from 100-ml water samples (n = 2) and calculated as follows: total no. larvae/drum = no. larvae/ml \times vol. water/drum ($\pi r^2 h$, where, r (drum radius) = 25.4 cm and h (water depth) = variable).

Once a week, 25 *Ae. cantator* larvae were collected from each treatment and control drum (with the exception of 28 larvae from treatment 1 on April 26 and 19 larvae from treatment 2 on

May 3), transferred to the laboratory and microscopically examined for *Amblyospora connecticus* as described above. This was initiated 2 weeks after the release of infected copepods (April 19) and was continued until the onset of pupation (May 17). Emerging adults obtained from field collected pupae were similarly examined so that prevalence rates could be determined for each host sex.

RESULTS

Of the 200 female *A. vernalis* that were examined from the pool surrounding the treatment drums, only one (0.5%) was found to be naturally infected with *Amblyospora connecticus* (Table 1). No infections of any type (transovarially or horizontally transmitted) were detected in the resident *Ae. cantator* population that was sampled from the pool during the course of the experiment.

Amblyospora connecticus was successfully transmitted to *Ae. cantator* larvae that were exposed to infected copepods (Table 1). Initial observations made 2 weeks after release (April 19) revealed benign infections in 4-12% of the 2nd- and 3rd-instar larvae present within the 3 treated drums. Marked increases in larval infection were detected in all treatment drums by the 4th week (mean = 17.8%). These rates leveled off thereafter, and maximum infection rates, ranging from 16-24%, were obtained in the 3 exposed populations by the time of pupation (May 17). An analysis of the overall prevalence of infection in larvae from the 3 replicated treatments revealed no significant difference ($P <$

Table 1. Phenology and percent infection with *Amblyospora connecticus* in female *Acanthocyclops vernalis* and larval *Aedes cantator* developing within a salt marsh pool and within copepod-treated and control steel drums^a

Date	Within pool			<i>Ae. cantator</i> within steel drums								
	<i>A. vernalis</i> percent infection	<i>Ae. cantator</i>		Treated				Control				
		Stage	Percent infection	Stage	1	2	3	mean \pm SE	1	2	3	mean \pm SE
Feb. 23	0											
Mar. 1	0											
9	0	L ₁	0									
15	4.0	L ₁ , L ₂	0									
22	-	-	-									
29	0	L ₁ , L ₂	0	L ₁ , L ₂	-	-	-	-	-	-	-	-
Apr. 5	0	L ₂	0	L ₂	-	-	-	-	-	-	-	-
11	0	L ₂ , L ₃	0	L ₂	-	-	-	-	-	-	-	-
19	0	L ₃ , L ₄	0	L ₂ , L ₃	12.0	8.0	4.0	8.0 \pm 2.3	4.0	0	0	1.3 \pm 1.3
26		L ₃ , L ₄	0	L ₂ , L ₃	21.4	16.0	16.0	17.8 \pm 1.8 ^b	0	4.0	0	1.3 \pm 1.3
May 3		L ₃ , L ₄	0	L ₂ -L ₄	16.0	10.5	16.0	14.2 \pm 1.8 ^b	0	0	0	0
10		L ₄ , P	0	L ₃ , L ₄	20.0	16.0	8.0	14.7 \pm 3.5 ^b	0	0	4.0	1.3 \pm 1.3
17				L ₄ , P	20.0	24.0	16.0	20.0 \pm 2.3 ^b	0	0	0	0

^a No entry indicates host(s) not present; - indicates host(s) present but infection with *Amblyospora connecticus* not determined.

^b Significantly greater than control mean by two-way ANOVA ($P > 0.05$).

0.05). The overall prevalence of infection in unexposed larvae from each of the control drums was less than 1% ($n = 125/\text{control}$), and with one exception on April 19, the average weekly rates were significantly lower ($P > 0.05$) than those observed in larvae from the treated drums. An examination of adult *Ae. cantator* emerging from the treated drums revealed a combined infection rate of 22.8% ($n = 44$) for males and 16.2% ($n = 74$) for females. No infections were observed in adults emerging from the 3 untreated control drums ($n = 10$ males and 10 females/control).

Steady weekly reductions in larval density and prolonged larval development were observed in all drums regardless of treatment (Fig. 3). Treated populations, which initially ranged from 5,403 to 11,426 larvae/drum on April 5, were reduced by 92.6% to an average of 656 larvae-pupae/drum by the time of adult emergence (May 17). Untreated control populations similarly declined by 95.9% (mean of 5,189 to 212 larvae-pupae/drum). Although some weekly variation in larval density was observed within the various drums, statistical analysis (two-way ANOVA) revealed no significant differences ($P < 0.05$) between the treated and control populations ($F = 2.60, 1,36 \text{ df}$) or among the replicates within each group ($F = 0.12, 2,36 \text{ df}$).

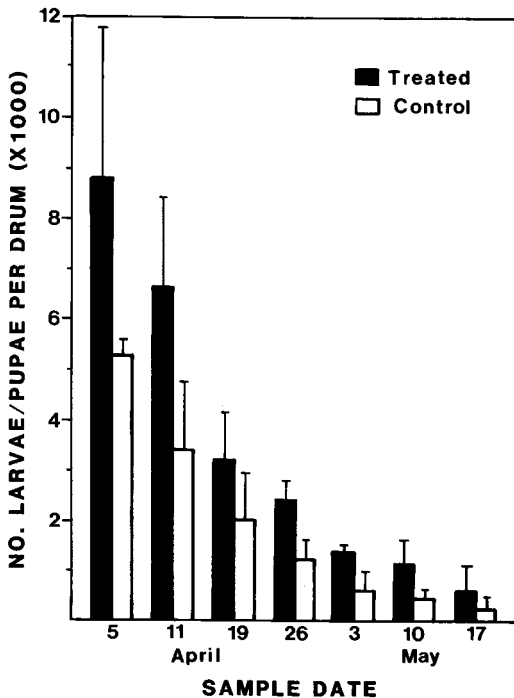


Fig. 3. Weekly estimated density (\pm SD) of *Aedes cantator* in the 3 treatment and control drums.

DISCUSSION

These experiments demonstrate that *Amblyospora connecticus* can be introduced into a larval field population of *Ae. cantator* via the release of live infected copepods. The use of naturally infected *A. vernalis* in 2 of the 3 treatments further indicates that infections can be induced in resident mosquito populations by simply releasing infected copepods from an adjacent pool. Female *Ae. cantator* thus infected, will subsequently lay infected eggs and the disease will be expressed in the F_1 generation when infected larval progeny die as 4th instars (see Fig. 1) (Andreadis 1983a, 1986, 1988b). Unfortunately, the latter could not be measured in the present study because of the simultaneous emergence of naturally infected *Ae. cantator* from several adjacent pools and the inability to control the selection of specific oviposition sites by infected females.

The 20% infection rate achieved in this field trial was substantial and compares favorably with the 30–40% infection usually observed in wild mosquito populations (Andreadis, unpublished data). Results further indicate that most infections were acquired by 2nd- and 3rd-instar larvae within 3 weeks of release. The 10-fold greater inoculum of spores released in treatment 1, however, did not appear to result in higher infection rates in that test group of mosquitoes.

It is unclear whether the containment of mosquito larvae and infected copepods within the designated treatment areas may have been an important contributing factor that facilitated transmission. This would appear to limit the dispersal of both hosts and thereby increase the potential number of encounters made between larval mosquitoes and infectious spores of *Amblyospora connecticus*. Further quantitative studies will therefore be required to determine the precise level of inoculum necessary to infect unrestricted larval mosquito populations. This might present some problems, however, because of the need to monitor a comparable, untreated control population simultaneously.

The high preadult mortality and prolonged larval development observed in both the treated and control mosquito populations were consistent with field studies of other mosquito species (see Barr 1985 and Service 1985 for reviews). According to Service (1985), high preadult mortality is common among mosquito populations and appears to be a density dependent process caused mainly by overcrowding and subsequent competition for limited food resources. The degree to which larval confinement within the drums contributed to the observed mortality is difficult to assess. It does appear that there was

stress associated with the confinement since there was prolonged larval development, and this would be expected to result in some increased mortality. However, reductions in larval *Ae. cantator* populations were also detected within the pool surrounding the treatment area, but no attempt was made to quantify this mortality due to the clumped distribution of larvae and fluctuating water levels.

In conclusion, I believe this study represents progress towards the effective utilization of a naturally occurring microsporidian parasite to help suppress a mosquito host. Infected copepods can be used to infect a susceptible mosquito population through their release at the appropriate time. Further studies are needed to determine how and when periodic releases may be specifically employed to augment natural infection processes, and under what conditions infected copepods might be used to introduce and establish a particular polymorphic microsporidium as a permanent natural suppressive agent.

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