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MEASUREMENT OF THE EFFECT OF MICROSPORIDIAN PATHOGENS ON MOSQUITO LARVAL MORTALITY UNDER ARTIFICIAL FIELD CONDITIONS

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Laboratory tests have indicated that larval mortality is of minor importance when considering the efficacy of the microsporidia *Nosema algerae* Vavra and Undeen and *Vavraia culicis* (Weiser) as biocontrol agents for mosquitoes (Undeen and Alger 1975, Kelly et al. 1981). In the laboratory, infected larvae reach the imaginal stage except after exposure to very high dosages of spores (Savage and Lowe 1970, Haq et al. 1981). Therefore, these microsporidia would be expected to reduce vector population levels primarily by reducing adult longevity and fecundity (Savage and Lowe 1970, Anthony et al. 1972, Undeen and Alger 1975, Anthony et al. 1978, Kelly et al. 1981, Haq et al. 1981).

However, under natural conditions additional biological and environmental stresses might affect the survival of pathogen-infected, possibility weakened larvae. In small scale field tests, *N. algerae* was evaluated against *Anopheles albimanus* Wiedemann in Panama (Anthony et al. 1978) and *An. stephensi* Liston in Pakistan (Maddox, personal communication). In both tests, larvae from the test sites were reared to adults in the laboratory, then checked for infection. Results were expressed only in terms of percentage infection, which was fairly high in some test plots. If, as was suspected in the Panama tests (Anthony et al. 1978), treatment with spores increased larval mortality in situ, percentage infection would be underestimated because the larvae which died in the breeding habitat or prior to eclosion in the laboratory evaluation were not represented in the samples. Thus, a series of tests was devised to determine if it is possible to detect low or intermediate levels of mortality among infected larvae in outdoor situations.

Larval mortality assessments are generally made by pretreatment and posttreatment population estimates. These data would be difficult to interpret in cases where mortality is expected to be low and occur long after treatment. The present study reports the effect of *V. culicis* and *N. algerae* on *An. quadrimaculatus* Say and *Culex salinarius* using a method which eliminates the estimation of population.

First instar *An. quadrimaculatus* and *Cx. salinarius* Coq. larvae were exposed in the laboratory to spores of *V. culicis* at a spore concentration of 1×10^6 spores/ml. First instar larval *An. quadrimaculatus* were exposed to *N. algerae* spores at 5×10^4 spores/ml. The larvae remained with the spores for 24 hr and then were rinsed and counted, 150 into each laboratory rearing pan (2–3 pans/exposure) and into the pools. The larvae in the pans were fed a mixture of hog chow supplement, yeast and liver powder until pupation. Uninfected larvae were reared in like manner as mortality controls and to check for microsporidia in the colony mosquitoes. Pupae from each pan were counted and placed in cages. After eclosion was completed, the adults were individually crushed under coverslips and microscopically examined for microsporidia spores. Percentage infection in adults from the pans was used as the expected percentage infection for comparison with the observed percentage infection in adults from the pools.

Sod-lined, 1.85 × 1.50 m concrete test pools (Focks and Bailey 1983) were used as simulated field sites. The 4 pools used in each test were covered with pyramidal emergence traps to retain emerging adults. No additional food was provided for the larvae in the pools. Each pool contained known numbers of infected and uninfected larvae and therefore was a paired test, eliminating the variability between pools. The first 3 tests evaluated the effect of *V. culicis* on *Cx. salinarius* and *An. quadrimaculatus*. In the first, 500 *V. culicis*-exposed and 500 unexposed larvae were placed in each of the 4 pools. In the 2nd and 3rd tests, the 4 pools contained: (1) 500 exposed and 500 unexposed *An. quadrimaculatus* larvae, (2) 500 exposed and 500 unexposed *Cx. salinarius* larvae, (3) 500 exposed *An. quadrimaculatus* larvae and 500 unexposed *Cx. salinarius* larvae, and (4) 500 exposed *Cx. salinarius* larvae and 500 unexposed *An. quadrimaculatus* larvae. Infections in the previously uninfected

species would reveal transmission of the microsporidia between larvae, a source of bias inherent in the experimental design. The infection rates, as revealed by the percentage infections in the pans, were sufficiently below 100% that, even when unexposed conspecifics were not added, differences between laboratory pan- and pool-emerged adults could be obtained. The effect of *Nosema algerae* was evaluated in 2 tests (8 pools) only in mixtures of exposed and unexposed *An. quadrimaculatus* larvae because *Cx. salinarius* is large refractory to *N. algerae.*

Adults emerging from the pools were collected at 1–3 day intervals and microscopically examined for infection. The percentage infection in the adults from each pool of a test series was subtracted from the percentage infection observed in adults that had experienced the same pathogen exposure as larvae and had been reared in the laboratory. The null hypothesis: $\bar{x}$ lab − $\bar{x}$ pool = 0 was tested by Student's *t*-test.

The temperature was uncontrolled and varied widely by time of day and season. During the cool season (December–January) only *Cx. salinarius* and *V. culicis* were used as the temperature (10–20°C) was generally too cool for *An. quadrimaculatus* and *N. algerae.* In the fall and spring the pool water varied between 15°C and 30°C, high enough to work with both *An. quadrimaculatus* and *Cx. salinarius* without remaining too hot for the latter. The development rate of both the larvae and microsporidia was greatly affected by the temperature, ranging from 55 days for *Cx. salinarius* in the cool season to 14 days for *An. quadrimaculatus* under warmer conditions. This temperature and developmental time variability was disregarded in the subsequent analysis of pooled, paired test data because each experimental pair was subjected to similar variations.

The uninfected larvae of one species, when reared with infected larvae of the other, never became infected. The larval development times were longer and mortality rates were higher in the pools than in the laboratory (Table 1). Although the mean daily mortality rates appear higher in the infected than uninfected groups, these differences were not statistically significant. However, the means of the differences between the percentage infection of the larvae put into the pools and adults collected from them were significantly greater than 0 for the combined results of *V. culicis* and for *N. algerae*-infected *An. quadrimaculatus* (Table 2), indicating higher total mortality among infected than uninfected larvae in the pools. This mean percentage infection shift provides a more precise measurement of mortality than the mean observed mortality rate because of the paired design which minimizes environmental variables.

The specific environmental stress factors associated with the shift in infection rate were not identified, but the percentage of larvae reaching the adult stage might be a cumulative measure of all of them. If so, the difference between expected and actual percentage infections should be negatively correlated with emergence success. This relationship was significant with *N. algerae* in *An. quadrimaculatus* ($r = -0.81$, prob $> R = 0.02$) but not for *V. culicis* in either host.

These tests show that infection with either *N. algerae* or *V. culicis* is associated with some larval mortality in the field that does not occur under less stressful laboratory conditions. The data do not allow extrapolation regarding the

Table 1. Effect of microsporidian infections on *Anopheles quadrimaculatus* and *Culex salinarius* (means 3–8 replications).

	Laboratory pans		Outdoor pools	
	Exposed	Unexposed	Exposed	Unexposed
	V. culicis in *An. quadrimaculatus*			
Infection in adults (%)	35.4 ± 5.6	0	15.8 ± 3.2	0
Eclosion (%)	69.6 ± 0.8	65.0 ± 2.2	11.7 ± 3.1	14.1 ± 2.9
Days to eclosion	13.3 ± 0.3	12.8 ± 0.6	21.5 ± 4.3	22.1 ± 2.9
Daily mortality (%)	2.7 ± 0.02	3.4 ± 0.3	11.5 ± 3.1	9.8 ± 1.7
	V. culicis in *Cx. salinarus*			
Infection in adults (%)	74.0 ± 11.5	0	37.0 ± 3.8	0
Eclosion (%)	74.8 ± 5.8	72.0 ± 4.4	31.6 ± 5.4	32.6 ± 6.5
Days to eclosion	12.4 ± 0.6	12.3 ± 0.6	35.3 ± 4.9	33.3 ± 4.4
Daily mortality (%)	2.4 ± 0.5	2.6 ± 0.9	4.9 ± 1.5	5.0 ± 1.3
	N. algerae in *An. quadrimaculatus*			
Infection in adults (%)	95.4 ± 2.3	0	30.1 ± 6.3	0
Eclosion (%)	84.7 ± 2.7	83.5 ± 3.5	8.7 ± 2.9	11.5 ± 2.3
Days to eclosion	11.0 ± 1.2	10.3 ± 0.8	22.3 ± 2.9	22.8 ± 2.2
Daily mortality (%)	1.6 ± 0.3	1.9 ± 0.5	14.4 ± 3.2	10.7 ± 1.8

Table 2. Differences between expected and actual percentage infection among adult mosquitoes from the pools.

Host	*n*	Mean difference	*t*	Probability
		Vavraia culicis		
Cx. salinarius	8	3.22 ± 1.55	2.08	0.076
An. quadrimaculatus	4	4.17 ± 1.34	3.12	0.052
Both species combined	12	3.54 ± 1.09	3.23	0.008
		Nosema algerae		
*An. quadrimaculatus**	8	14.90 ± 5.73	2.60	0.036

* Mean difference = Σ[(% Infection in pans/2) − % Infection in pools]/number of pools.

possibility of the occurrence of even greater mortality among infected larvae under more deleterious field conditions. However, the methodology developed for these tests provides a means of further studying the question. Containment of infected and uninfected insects in the same environment is shown to be a sensitive method for assessing differential mortality rates, provided that no transmission occurs during development and the infected individuals can be detected in the adult stage.

REFERENCES CITED

Anthony, D. W., K. E. Savage, E. I. Hazard, S. W. Avery, M. D. Boston and S. W. Oldacre. 1978. Field tests with *Nosema algerae* Vavra and Undeen (Microsporida, Nosematidae) against *Anopheles albimanus* Wiedemann in Panama. Misc. Publ. Entomol. Soc. Am. 11:17–28.

Anthony, D. W., K. E. Savage and D. E. Weidhaas. 1972. Nosematosis: Its effect on *Anopheles albimanus* Wiedemann, and a population model of its relation to malaria transmission. Proc. Helminthol. Soc. Wash. 39(Special issue):428–433.

Focks, D. A. and D. L. Bailey. 1983. An outdoor test pool for evaluating mosquito (Diptera: Culicidae) larvicides. J. Med. Entomol. 20:224–225.

Haq, N., W. R. Reisen and M. Aslamkhan. 1981. The effects of *Nosema algerae* on the horizontal life table attributes of *Anopheles stephensi* under laboratory conditions. J. Invertebr. Pathol. 37:236–243.

Kelley, J. F., D. W. Anthony and C. R. Dillard. 1981. A laboratory evaluation of the microsporidian *Vavraia culicis* as an agent for mosquito control. J. Invertebr. Pathol. 37:117–122.

Savage, K. E. and R. E. Lowe. 1970. Studies of *Anopheles quadrimaculatus* infected with a *Nosema* sp. Proc. Int. Colloq. Insect Pathol. IV: 272–278.

Undeen, A. H. and N. E. Alger. 1975. The effect of the microsporidian *Nosema algerae* on *Anopheles stephensi* Liston. J. Invertebr. Pathol. 25:19–24.