

SAFETY OF *EDHAZARDIA AEDIS* (MICROSPORA: AMBLYOSPORIDAE) FOR NONTARGET AQUATIC ORGANISMS¹

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ABSTRACT. The susceptibility of common nontarget aquatic organisms to the microsporidium *Edhazardia aedis* was investigated in the laboratory. Eight predaceous species along with 9 scavengers and filter feeders were tested. The nontarget organisms were not susceptible to infection by *E. aedis* and there was no appreciable mortality. To measure the relative safety of *E. aedis* to nontarget organisms, a simple mathematical expression was employed where risk is defined as the product of the probability of exposure and the result of exposure (infection) expressed as $\hat{P}_e \hat{P}_i$. In these laboratory tests, the probability of exposure was fixed at 1 (maximum challenge) and the probability of infection was determined to be 0. Therefore, the risk associated with release of *E. aedis* into the environment is considered to be negligible under these conditions. The true risk for nontarget organisms to *E. aedis* can only be determined by careful evaluation of controlled field studies in the natural habitat of the target host.

INTRODUCTION

Microbial pest control agents (viruses, bacteria, protozoa, fungi) are being investigated as alternatives to chemical pesticides. Some of these agents, such as *Bacillus thuringiensis* var. *israelensis* de Barjac (*B.t.i.*) and *Lagenidium giganteum* Couch, are intended for inundative release where they would be widely disseminated into the environment (Lacey and Undeen 1986). Others are intended as classical biological control agents for inoculative release. This latter method would introduce the microbial pest control agent into certain habitats and this success would depend on the ability to disperse and persist in the target pest population. One such organism for inoculative release is the microsporidium *Edhazardia aedis* (Kudo), a promising biocontrol agent for container-inhabiting mosquitoes (Becnel 1990).

Edhazardia aedis was originally observed and described from *Aedes aegypti* (Linn.) in Puerto Rico (Kudo 1930) and rediscovered in Thailand (Hembree 1979). This species has a complex life cycle (Becnel et al. 1989), part of which involves transovarial transmission from females to progeny (Hembree and Ryan 1982). Spores are formed in the fat body of the progeny and this process is usually fatal. Spores released from these dead individuals into the aquatic environment are infectious to *Ae. aegypti* larvae when ingested, yielding infected adults to complete the cycle. *Edhazardia aedis* is known only to infect some species of *Aedes*, *Psorophora* and *Culiseta*; the species of *Culex* and *Anopheles*

tested thus far appear to be refractory (Becnel 1990).

The safety of microbial pest control agents for nontarget aquatic organisms is an important area of research that must be evaluated by both researchers and regulatory agencies (Fournie et al. 1988). Risk associated with the release of a microbial pest control agent for nontarget organisms can be expressed mathematically as Risk = $\hat{P}_e \hat{P}_i$ where \hat{P}_e is the probability of exposure or encounter and \hat{P}_i is the probability of infection. In this study, the probability of exposure is fixed at 1 (maximum exposure) to determine the safety of *E. aedis* for common nontarget aquatic organisms and to evaluate the risk associated with the release of this mosquito pathogen into the environment.

MATERIALS AND METHODS

Aedes aegypti larvae infected with *E. aedis* were obtained from laboratory colonies maintained according to the methods of Hembree and Ryan (1982). Spore suspensions were prepared with a tissue grinder and the concentrations determined with a hemacytometer.

The nontarget aquatic organisms used in these tests are given in Tables 1 and 2. Test organisms from laboratory colonies are indicated; all other organisms were collected from the environs of Gainesville, Florida. Identifications of the nontarget aquatic organisms were made with Pennak (1978) when possible or submitted to the Florida Department of Plant Industries, Gainesville. Predaceous nontarget aquatic organisms were isolated and fed one infected 4th instar *Ae. aegypti* larva (a dose equivalent of 3×10^5 spores) and healthy larvae thereafter. Scavengers and filter feeders were exposed as groups in 100 ml of the spore suspension for 24 h at the doses and numbers indicated

¹ Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the United States Department of Agriculture.

Table 1. Summary of laboratory tests challenging nontarget aquatic organisms with *Edhazardia aedis*

Common name	Order	Scientific name	Life stage	Test parameters		Test results	
				Dose (no. inf. larvae)	n	Exposure (days)	Adjusted % mortality ^c
Mosquito	Diptera	<i>Aedes aegypti</i> ^a	Larva	10 ⁴	100	5	26
Aquatic sow bugs	Isopoda	<i>Astellus militaris</i>	Adult	10 ⁴	100	14	0.0
Scuds	Amphipoda	<i>Hyalella azteca</i>	Adult	10 ⁴ , 10 ⁵	200	7	0.0
Water fleas	Cladocera	<i>Ceriodaphnia reticulata</i>	Adult	10 ⁴	200	12	0.0
Copepods	Copepoda	<i>Macrocypris albidus</i> ^a	Adult	10 ⁴	100	20	2.0
Seed shrimp	Ostracoda	<i>Canthocamptus</i> ^b	Adult	10 ⁴	100	13	0.0
Midges	Diptera	<i>Chironomus</i> sp.	Larva	10 ³ , 10 ⁴ , 10 ⁵	150	7	0.0
Water boatmen	Hemiptera	<i>Corixinae</i> ^b	Nymph	10 ⁴	400	9	7.0
Clam shrimp	Conchostraca	<i>Eulimnadia</i> sp.	Adult	10 ⁵	60	4	0.0
Aquatic earthworms	Haplotaxida	<i>Naididae</i> ^b	Adult	10 ⁴	50	9	0.0

^a Laboratory colony.^b Further taxonomic placement not determined.
^c Mortality adjusted according to Abbott's formula.

in Table 1. Groups were then transferred to pans with 500 ml of the appropriate culture media. Second instar larvae of *Ae. aegypti* were exposed to spores to verify their viability. Control groups were used in all tests and handled in a similar manner but without the addition of spores.

A sample of individuals from the exposed groups was removed approximately 1 h post-exposure; gut contents were examined with phase microscopy for the presence of spores. Mortality was recorded after the exposure time indicated in Table 1 and subsequently was adjusted according to Abbott's formula when control mortality occurred in excess of treatment mortality. All dead individuals were examined for spores, and evidence for infection by *E. aedis* in the nontarget aquatic organisms was determined by examination of Giemsa-stained smears of each survivor.

RESULTS AND DISCUSSION

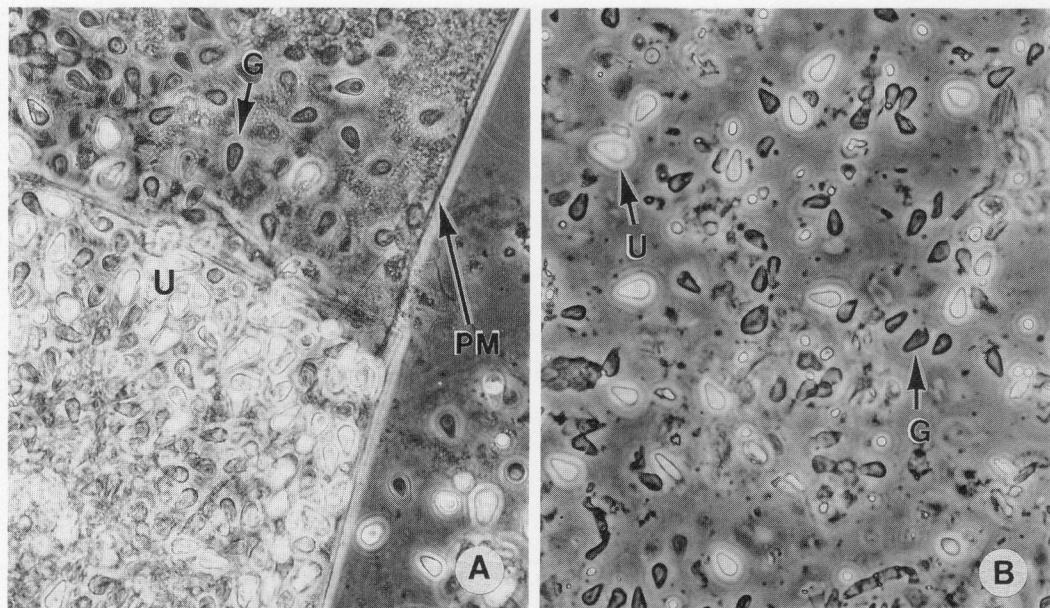
Mature, ungerminated spores were found in the gut contents of individuals from each group of exposed organisms. These spores remained highly refractile as demonstrated in the natural host *Ae. aegypti* (Fig. 1A) and in the nontarget aquatic organisms *Eulimnadia* sp. (Fig. 1B). Germinated spores also were observed in all natural and experimental hosts (Figs. 1A and 1B). These spores were the same shape as ungerminated ones but were dark in appearance with a clearly defined spore wall.

A dose of 10⁴ spores/ml resulted in 100% infection and 26% mortality in larval *Ae. aegypti* 5 days post-exposure (Table 1). There were no infections detected in any of the nontarget aquatic organisms exposed to an equal or greater challenge of *E. aedis* spores (Tables 1 and 2) and only minimal mortality in *Belostoma testaceum* (Leidy) (16.0%), *Hydrochus* sp. (9.0%), *Corixinae* (7.0%) and *Macrocypris albidus* (Jurine) (2.0%).

Few investigations have examined the safety of aquatic microsporidia for nontarget organisms. One exception is the pathogen *Nosema algerae* Vávra and Undeen which infects many species of mosquitoes in a number of genera (Undeen 1976, Brooks 1988). This species has been tested extensively to evaluate the susceptibility of nontarget organisms. *Nosema algerae* has a rather broad host range especially when injected (Undeen and Maddox 1973, Brooks 1988, Fournie et al. 1990). A wide variety of predacious insects, however, were not susceptible to this microsporidium by ingesting infected hosts, the only exception being the hemipteran *Notonecta undulata* Say (Van Essen and Anthony 1977). Based upon the restricted mosquito

Table 2. Summary of laboratory tests challenging nontarget aquatic organisms with *Edhazardia aedis*

Common name	Order	Scientific name	Life stage	Test parameters			Test results	
				Dose (no. inf.)	n	Exposure (days)	Adjusted % mortal- ity ^d	% infec- tion
Phantom midges	Diptera	<i>Corethrella appendiculata</i> ^a	Larva	1 ^c	29	38	0.0	0.0
Planaria	Tricladida	<i>Dugesia dorotocephala</i> ^a	Adult	1	24	14	0.0	0.0
Damselfly	Odonata	<i>Telebasis byersi, Ischnura posita</i>	Naiad	1	59	16	0.0	0.0
Giant water bugs	Hemiptera	<i>Belostoma testaceum</i>	Nymph	1	38	19	16.0	0.0
Water scorpion	Hemiptera	<i>Ranatra</i> sp.	Nymph	1	30	19	0.0	0.0
Predaceous diving beetles	Coleoptera	<i>Dytiscidae</i> ^b	Larva	1	5	19	0.0	0.0
Water scavenger beetle	Coleoptera	<i>Hydrochus</i> sp.	Larva	1	11	13	9.0	0.0

^a Laboratory colony.^b Further taxonomic placement not determined.^c Dose equivalent of 3×10^5 spores.^d Mortality adjusted according to Abbott's formula.Fig. 1. A. Spores of *Edhazardia aedis* in the gut of *Aedes aegypti*. B. Spores of *Edhazardia aedis* released from the gut of *Eulimnadia* sp. G, germinated spore; U, ungerminated spore; PM, peritrophic membrane.

host range and the lack of susceptibility of non-target organisms in this test, *E. aedis* appears to be more host specific than *N. algerae*.

Several of the nontarget aquatic organisms challenged in this series of tests have been shown to be effective predators of mosquitoes. *Corethrella appendiculata* Grabham is a common mosquito predator in both natural and artificial containers with a distribution range from the eastern USA south to northern Argentina (McKeever and French 1991). Although closely

related to mosquitoes, *C. appendiculata* is unaffected by feeding on diseased larvae. Another native control agent of mosquitoes is the copepod *M. albicus* which preys on first instar mosquitoes and has been introduced to control *Aedes* larvae in tires (Marten 1990a, 1990b). While *M. albicus* is not capable of ingesting third or fourth instar larvae containing infective spores, they apparently are not affected by ingesting spores from a suspension. The planarian *Dugesia dorotocephala* (Woodworth) also has been shown

to have potential as a biological control agent of mosquitoes (Legner 1977), and appears to be compatible with *E. aedis*.

Integrated use of microbial pest control agents and predators has received little attention even though it may be effective under certain circumstances. *Bacillus thuringiensis* var. *israelensis* has been found to be compatible with *D. dorocephala* (Perich et al. 1990) and the copepod *Mesocyclops aspericornis* (Daday) (Riviere et al. 1987) in laboratory tests. The possible benefits from the integrated use of *E. aedis* and predators warrants further investigation and field evaluation.

An important finding of this study is how readily spores of *E. aedis* germinated in the guts of a wide variety of organisms. The lack of detectable infection in these organisms is due perhaps to a gut barrier preventing the infective germ from entering the host or some physiological incompatibility preventing parasite development. In the case of *N. algerae*, spores germinated in the guts of all mosquitoes tested but the percent germination was not correlated with susceptibility (Undeen 1976). This appears to be due primarily to a gut barrier because all insects in which spores were injected into the hemocoel became infected (Undeen and Maddox 1973). Regardless, it is clear that by germinating in the nontarget aquatic organisms, a portion of spores would be removed from the environment, reducing the amount of inoculum available to infect the target host. Inoculation of containers with *E. aedis* spores must consider this diluting effect when applying the parasite and evaluating the results, particularly if the nontarget aquatic organism load of the site is heavy. The dispersal and viability of ungerminated spores into the environment after passage through the guts of nontarget aquatic organisms is unknown.

To measure and compare the relative safety of microbial pest control agents requires a standardized method to quantify the risk associated with release. In this evaluation, a simple mathematical expression was employed where risk is defined as the product of the probability of exposure and the result of exposure (infection) expressed as $\hat{P}_e \hat{P}_i$. Employment of this method to determine the safety of microbial pest control agents would provide researchers and regulators a basis on which to evaluate the risk associated with release. Because only one of these parameters needs to be small for the overall risk of release be considered low, an agent with a broad host range would not necessarily be considered unsafe for release. The method of release would also be factored into the equation and if the probability of exposure to the susceptible non-target organisms is low, then the risk of release

would be low. In these laboratory tests, the probability of exposure was fixed at 1 (maximum challenge) and the probability of infection was determined to be 0. Under field conditions, however, the probability of exposure would be considerably less because of the release method (inoculative) and the restricted habitat of the host. Therefore, the risk associated with release of *E. aedis* into the environment is considered to be negligible. The true risk for nontarget organisms to *E. aedis* can only be determined by careful evaluation of controlled field studies in the natural habitat of the target host.

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