

Prospects of Microsporidia and Fungi for the Control of Container-Inhabiting Mosquitoes

JAMES J. BECNEL

U.S. Department of Agriculture,
Agricultural Research Service
Medical & Veterinary Entomology Research Laboratory
Gainesville, Florida 32608

Recent trends in the biological control of mosquitoes have focused on *Bacillus thuringiensis israelensis*, *B. sphaericus* and insect growth regulators. These "biorationals" are very effective when used for the inundative control of larval mosquitoes. Other areas of investigation involve the inoculative use of microbes to achieve extended periods of mosquito control. Two important groups of organisms for this purpose are microsporidia and fungi.

Researchers at the USDA/ARS Medical and Veterinary Entomology Research Laboratory are evaluating microsporidia and fungi for the control of container-inhabiting mosquitoes with particular emphasis on *Aedes aegypti* and *Ae. albopictus*. The objective of this program is to devise practical, integrated control methodologies against specific vectors in well defined ecological niches. These control strategies are based on an understanding of the structure of the habitat and the biology of the hosts and control agents.

To meet these objectives, surveys are utilized to identify and characterize habitats and isolate microbial parasites. Candidate organisms are identified for correct taxonomic placement and evaluated as biological control agents through laboratory bioassay. Promising agents are selected and produced for field studies.

Attributes of the "ideal" biocontrol agent depend on the target mosquito and habitat. For container-inhabiting mosquitoes, a successful control agent is: 1) pathogenic and impacts both larval and adult populations 2) specific for the target host 3) able to persist and recycle in the habitat and 4) spread and invade new habitats. Certain microsporidia and fungi possess some or all of these attributes.

Three mosquito-pathogenic fungal

genera, *Coelomomyces*, *Culicinomyces* and *Lagenidium*, have received considerable attention for mosquito control. Each will be discussed briefly followed by an assessment of their biological control potential.

Species of the genus *Coelomomyces* have complex life cycles that require an obligate, intermediate host (Federici et al. 1985). In infected larvae, *Coelomomyces* spp. produce thousands of sporangia which are released after death. Uniflagellate zoospores are then released from the sporangia and infect the intermediate host, either a copepod or ostracod. Uniflagellate gametes are released from the intermediate host after death. These gametes fuse in pairs to form a zygote which seeks out and encysts on the larval mosquito. The fungus penetrates through the mosquito cuticle where a sequence of developmental events produces sporangia and death of the host to complete the cycle. While *Coelomomyces* spp. cause epizootics in many mosquito species worldwide, reliable methods to produce and handle this pathogen are not available. This and the necessity of an intermediate host have hindered development and evaluation for biological control.

Culicinomyces clavisporus is a filamentous fungus (deuteromycetes) which invades mosquito larvae through the cuticular lining of the foregut and hindgut following ingestion of "club" shaped conidia (Sweeney 1985). Following penetration, hyphae branch and proliferate in the coelom of the body. Death of larvae usually occurs in 3 to 6 days post-infection and the body becomes filled with hyphae. After 1-2 days, conidophores exit through the cuticle and eventually produce conidia to complete the cycle. Mosquito larvae of 3 genera (*Anopheles*, *Culex* and *Aedes*) are susceptible to infection by this pathogen and safety

testing indicates that *C. clavisporus* is not infectious outside a limited range of aquatic Dipteran hosts. *Culicinomyces clavisporus* is easily grown on inexpensive media but the conidia are quite fragile and germinate poorly at temperatures above 30°C. New storage and formulation methods are required for mass production but more importantly, field studies of this pathogen's persistence and recycling in nature are needed to evaluate *C. clavisporus* as a biological control agent.

Lagenidium giganteum (a member of the oomycetes) is a facultative parasite of mosquito larvae (McCray 1985). It infects a broad range of mosquito hosts via motile zoospores which can be formed either sexually or asexually. Artificial culture methods have been developed for the sexual oospore stage (the dormant phase) which makes *L. giganteum* amenable to commercial production. *Lagenidium giganteum* was registered by the Environmental Protection Agency in 1991 for the control of mosquito larvae. Therefore, attributes such as broad mosquito host range, its facultative parasitic nature, ability to recycle and storage capabilities makes *L. giganteum* a very promising biological control agent.

Microsporidia are some of the most common pathogens found in mosquitoes. Their usefulness as manipulative biological control agents has been hindered by a paucity of information on modes of transmission and on the host-parasite relationship. Recent breakthroughs in these areas permits evaluation of these pathogens as biological control agents of mosquitoes.

Members of the genus *Amblyospora* are perhaps the most common microsporidia found in *Culex* and *Aedes* spp. worldwide. These parasites have complex life cycles which involves transmission within and between hosts, one of which is a copepod intermediate host (Sweeney & Becnel 1991). Infected female mosquitoes pass the infection to progeny which results in death of fourth instar larvae. Infectious spores from these dead individuals are infectious for a copepod intermediate host. The pathogen develops in and is eventually fatal to the copepod releasing spores infectious to the mosquito host to complete the cycle. Thus, one cycle of these parasites involves 2 mosquito generations and one generation of the copepod. Species of *Amblyospora* studied thus far appear to be host specific and none

of these parasites have been produced in artificial culture. Use of these pathogens therefore, must be directed at specific mosquito hosts in specific habitats to augment the natural infections at critical periods in the cycle.

Perhaps one of the most promising microsporidia for the biological control of *Ae. aegypti* is *Edhazardia aedis* (Sweeney & Becnel 1991). This pathogen was originally observed and described from *Ae. aegypti* in Puerto Rico and rediscovered in Thailand. *Edhazardia aedis* has a complex life cycle, part of which involves transovarial transmission from females to progeny. 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.

The desirable traits of *E. aedis* support the belief that this pathogen can play an important role in developing strategies to control *Ae. aegypti* and possibly other container-inhabiting mosquitoes of medical importance. Host specificity, the potential to persist and disseminate, and the impact on both larval and adult populations are strong positive factors. Ideally, *E. aedis* would be introduced via inoculative releases (rather than inundative releases) lessening the need to mass produce and manipulate the parasite. For this method to be successful, the pathogen must become permanently established or augmented seasonally to maintain acceptable levels of control. Only with controlled field releases can the potential of *E. aedis* be evaluated as a biocontrol agent of *Ae. aegypti* and other container inhabiting mosquitoes.

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