Mosquito larva infected by the fungus *Lagenidium giganteum*.

Fungi show promise in biological control

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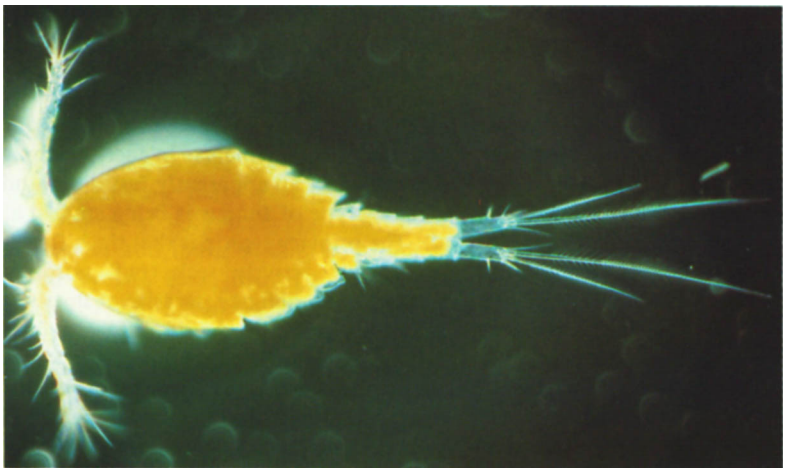
Mosquitoes, like all other organisms, are subject to fatal diseases caused by pathogens such as viruses, bacteria, and fungi. Many of these pathogens have no demonstrable effects on other organisms and thus are excellent candidates for development as biological agents in integrated mosquito control (IMC) programs. Preliminary studies have been conducted in University of California laboratories on the biology, cultivation and mosquito control potential of three fungi: *Tolypocladium cylindrosporum* (class Deuteromycetes), *Lagenidium giganteum* (class Oomycetes), and *Coelomomyces dodgei* (class Chytridiomycetes).

Tolypocladium cylindrosporum was originally described as an uncommon inhabitant of soil but was found recently causing as much as 90 percent mortality in larvae of the treehole mosquito (*Aedes sierrensis*) in Novato, California. The fungus was isolated from diseased larvae and has been grown on readily available artificial media, where it forms blastospores in shake liquid cultures or conidia on semisolid agar substrates. (Blastospores and conidia are the infectious agents of the fungus.) Normally these spores adhere to ▶

Photo by Brian Federici



Cyclops vernalis, intermediate copepod host of a fungus that attacks mosquitoes. Orange color is from carotene pigment in the male gametophyte of the fungus. (Actual size, 0.8 mm)

normal mosquito habitat and stocked with containers for egg laying and containers of rotting fruit and sugar cubes for carbohydrate meals. Rabbits or chickens were periodically provided for blood meals, needed by the female mosquitoes for egg production.

In the tents both normal and sterile males mated with virgin females within 5 minutes after release of the females, and all of the hundreds of females collected from tents and dissected were found to have been inseminated. During each experiment, mosquito eggs were collected from tents each week and then bleached and microscopically examined in the laboratory to determine the percentage of sterile eggs (only a fertile egg contains a developing embryo).

In experiments to evaluate the mating competitiveness of sterilized males, it was found that egg fertility was reduced to 42 percent when 500 sterile males and 500 normal males were put into a tent with 500 virgin females. When 1,000 sterile males were put into a tent with 500 virgin females, and 1,000 normal males were added to another tent with 500 virgin females, the respective percentages of sterile eggs produced were 96 and 3. In still other experiments with 1,000 males and 500 females per tent and sterile-to-normal male ratios of 1:1, 4:1, and 9:1, bleached eggs revealed that the mating competitiveness of irradiated sterile males was equal to that of normal males at a 1:1 ratio (nearly 50 percent sterile eggs), somewhat less than expected at the 4:1 ratio (42 to 65 percent sterile eggs), and again equal to that of normal males at the 9:1 ratio (84 to 94 percent sterile eggs in different weeks).

The most recent series of experiments revealed that even at very low densities per tent (50 sterile males and 50 normal males with 50 virgin females), sterilized males competed equally with normal males in all mating experiments. Survival rates of sterilized males also were equal to those of the normal males released (females released 7 and 15 days after males laid eggs having sterility rates of 49 and 47 percent, the same as males and females released simultaneously).

Under all conditions thus far tested, sterilized males have been equally competitive with normal males when both were released simultaneously into laboratory cages or field tents. Ongoing experiments will determine if virgin wild females from specific localities will mate as readily with sterilized males from a long-established laboratory colony as they will with normal wild males.

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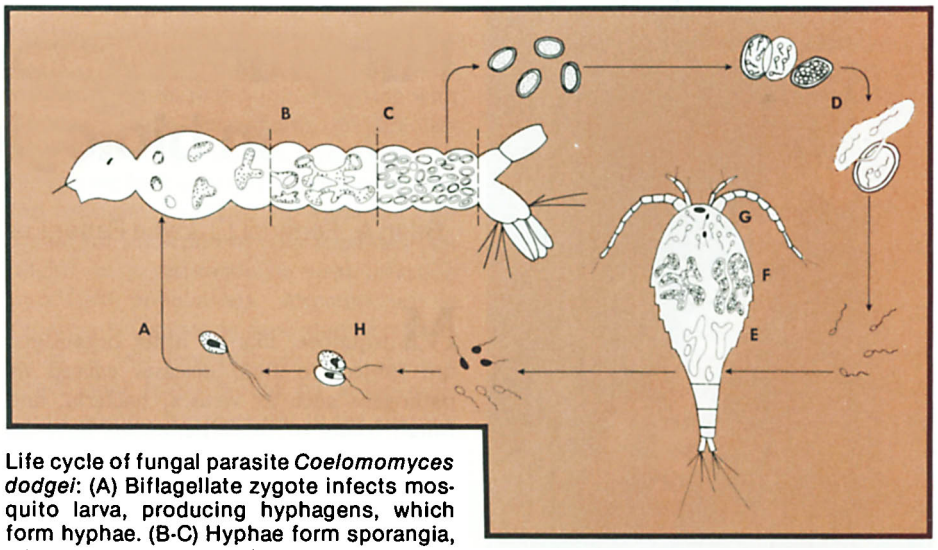
the larval cuticle and germinate, invading the larva through the cuticle. Once inside, the fungus produces an extensive mycelium which eventually kills the larva. After larval death, the mycelium produces millions of conidia.

In laboratory trials with the treehole mosquito, 100 percent mortality was obtained within four days when second-stage larvae were exposed to 5×10^5 blastospores per ml of medium. Conidia were somewhat slower acting although just as effective, causing 100 percent mortality within 10 days of treatment. Similar results were obtained against *Culex tarsalis* mosquitoes with slightly higher concentrations of blastospores and conidia.

Although *T. cylindrosporum* may prove useful against a variety of mosquito species, its greatest value may come in reducing treehole mosquito populations, whose secluded treehole habitat always has been the primary obstacle to control. If adults were attracted to special traps and contaminated with blastospores or conidia, the females would carry spores back to treeholes when they returned to lay eggs. Adults are susceptible to the fungus, yielding as many as 10^7 conidia. Those that die in the treehole would serve to increase the fungal load. If treehole mosquitoes were trapped and contaminated in April, when emergence is peaking and humidity is high, sporulation on adult carriers that are killed would be ensured. The cool shaded nature of the treehole would enhance conidia survival during the summer, and subsequent rains would disperse conidia to infect new broods of larvae.

The fungus *L. giganteum* lives either freely in the aquatic environment or as a parasite of mosquito larvae. Although several strains have been isolated over the past 40 years, current emphasis is on a strain discovered in North Carolina with a broad host range, including species of *Aedes, Anopheles, Culex, Culiseta,* and *Psorophora*. The infectious agent of *L. giganteum* is a motile zoospore that, upon contact with a mosquito larva, encysts on the cuticle, invades the body, and kills the larva by producing an extensive mycelium. Subsequently, through sexual or asexual reproduction, more zoospores are formed, which leave the larva and seek another mosquito or other suitable substrate on which to grow.

Lagenidium giganteum can be grown on artificial media in either liquid or semisolid agar cultures, although Dr. Aristotle Domnas at the University of North Carolina has shown that sterols must be added to artificial media for efficient production of infective spores. The fungus also can be grown on living mosquito larvae in the laboratory.



Life cycle of fungal parasite *Coelomomyces dodgei*: (A) Biflagellate zygote infects mosquito larva, producing hyphagens, which form hyphae. (B-C) Hyphae form sporangia, which are released after larva dies. (D) Meiospores infect copepod, producing gametophytes (E), each of which forms a gametangium (F), which releases gametes. (G) Copepod may contain either male (dark) or female (uncolored) gametes, or both. (H) Gamets fuse, forming zygote.

In rice field trials conducted at Davis with scientists from the U.S. Communicable Disease Center, *L. giganteum* eliminated *Cx. tarsalis* larvae within five days of treatment. Subsequently, the fungus has been isolated from larvae collected at the treated site during four consecutive years. Based on these and other studies, we have concluded that *L. giganteum* is a virulent microbial control agent for *Culex* larvae that survives land management practices associated with California rice fields. However, studies also have shown that high temperatures ($>30^\circ$ C) and high organic and salt concentrations limit infection and that larvae of *Anopheles freeborni* vary in susceptibility to this isolate. Additionally, studies on nontarget organisms from 29 taxa indicate that none is susceptible to infection.

Unlike *T. cylindrosporum* and *L. giganteum, C. dodgei* is an obligate parasite (that is, it requires specific conditions for growth on artificial media, which have not yet been determined). An additional disadvantage to working with this and other species of *Coelomomyces* is that they have complex life cycles in which sexual and asexual generations alternate between two different invertebrate hosts, a mosquito and a copepod (a tiny, fresh-water shrimp). In the mosquito, the fungus forms sporangia, which release uniflagellate spores that infect a copepod. In the copepod, uniflagellate male and female gametes are produced that fuse, forming a biflagellate zygote that invades a mosquito larva.

Research on *Coelomomyces* is in its infancy, largely because of the complexity of the fungal life cycles and their unique nutritional requirements. In fact, one may ask why such an apparently fastidious and complex type of fungus is being studied at all. The primary reason is that the biflagellate zygote actively seeks out mosquito larvae. As an obligate parasite, it does not have the option of developing as a free-living organism; it either finds a larva or dies. This behavior makes members of this group lethal and effective larval parasites. In fact, species of *Coelomomyces* are the only parasites known to cause natural mortalities in larval populations in excess of 90 percent.

To date, only 3 of approximately 50 *Coelomomyces* species have been domesticated. Studies at Riverside are concentrating on *C. dodgei*, a parasite of the common copepod *Cyclops vernalis* and anopheline larvae, as a model system. Laboratory studies have demonstrated that the important California mosquito *An. freeborni* is highly susceptible to *C. dodgei*, routinely succumbing at rates around 90 percent.

Techniques have been developed for limited mass production of the fungus whereby thousands of infected copepods can be produced daily. Currently, these techniques are being improved and expanded, and limited field trials are under way at both Davis and Riverside. These trials will determine (1) the degree of larval control to be expected if techniques were available for true mass production and dissemination of *C. dodgei,* (2) whether this species can be established in new localities, and (3) if so, what levels of mosquito sup-

pression might result on a continuing basis.

Evaluation of these fungi, like most other microbial control agents, is in an exploratory stage. Because these organisms are difficult to manipulate, progress has been slower than with insecticides and will continue to be so. None of the above species is likely to be operational within the next few years. Furthermore, to employ them effectively in IMC programs, new technologies must be developed, which will often have to be tailored to individual mosquito species. These technologies will be more expensive to develop and use than current technologies, but environmental costs will be lower because the fungi, unlike conventional insecticides, are highly specific. Although introduction of these fungi in some locations probably will provide moderate control on a continuing basis due to natural recycling, in most cases they will have to be reintroduced periodically. Nevertheless, it also will be important to evaluate the role each may play in IMC programs, as well as their potential if used unilaterally.

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Nematodes as biological control agents

Edward G. Platzer

A promising biological control agent against mosquitoes is *Romanomermis culicivorax*, a nematode parasite of mosquito larvae. The primary goal of research begun in 1973 was to determine the effects of environmental factors on the life cycle of the nematode so that procedures for mass-rearing and field releases could be improved.

A small scale mass-rearing system was established at University of California, Riverside, and initial studies were undertaken by Dr. B. J. Brown to define the effects of temperature, salinity, and oxygen on the infective stage of *R. culicivorax*. Preparasites infected *Culex pipiens* at temperatures between 12° and 33° C; the optimum range for infectivity was between 21° and 33° C. A temperature lower than 21° C decreased the infection rate but increased the time during which the nematodes remained infective.

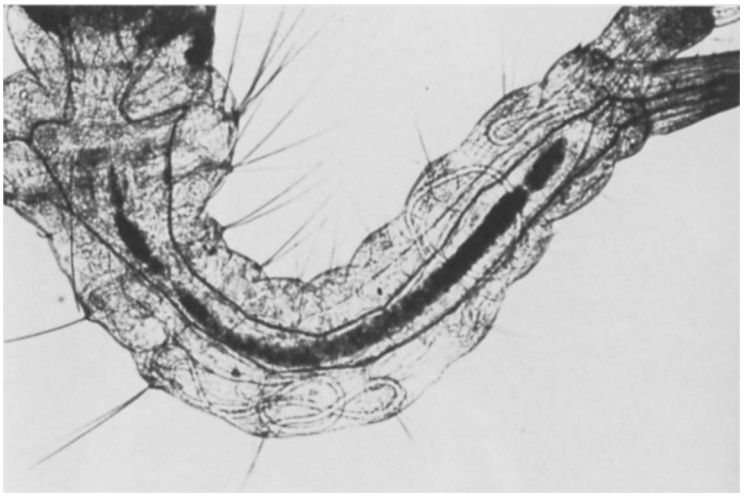
An extensive study showed that at least half of the preparasites were able to infect mosquitoes at concentrations of salts usually found in fresh waters. Elevated calcium, nitrates, nitrites, and phosphates inhibited infection. The findings suggest that use of this parasite would be feasible in most fresh waters in North America.

The infectivity of preparasites depends on adequate oxygen levels. The low oxygen concentration in polluted water drastically reduces infectivity.

The parasitic stage developed within 6 to 28 days at temperatures from 15° to 32° C, a range that corresponds closely to the optimum for preparasite activity. Using heat unit calculations, one can predict the required time for the complete life cycle under natural conditions.

In temperature studies on postparasitic stages in outdoor ponds, adult nematodes developed, and a new generation of infective nematodes was produced within three weeks at an average ambient temperature of 27° C.

Photo by Edward Platzer



Culex pipiens larva 24 hours after exposure to the infective larvae of *Romanomermis culicivorax*. Four parasitic larvae are coiled within the body cavity.

Mass-rearing of the nematodes was greatly improved by eliminating a contaminating fungus infection through an acid treatment of the cultures. This procedure will enable other laboratories and commercial facilities to propagate *R. culicivorax* more effectively.

In artificial and natural sites, *R. culicivorax* was field-tested against four mosquito species to study its effectiveness in California habitats. Infective nematodes were disseminated at approximately 1,000 per square meter of surface area. All species of mosquito larvae were infected; the percentage of infection depended on the mosquito subfamily and test site. In mixed mosquito populations, anophelines were more susceptible than culicines to parasitism. Dense vegetation or algal mats reduced mosquito larva control.

High populations of aquatic arthropods in rice fields prompted investigation of them as predators of the mermithid. Preparasites were attacked by copepods, cladocerans, young gammarids, and ostracods. Further studies with copepods in 1 liter of water demonstrated that 20 copepods significantly reduced mermithid infections of mosquitoes, and 53 copepods reduced the infection level by 50 percent. Thus, high densities of copepods in mosquito-infested waters may interfere with mosquito control by mermithid nematodes. Diving beetles, gammarids, dragonfly and damselfly naiads, and small crayfish avidly consumed *R. culicivorax* postparasites.

These findings indicate that temperature, salinity, oxygen, and the abundance of predators must be considered when selecting release sites for this mermithid. We conclude that *R. culicivorax* has good potential for anopheline mosquito control in California under suitable conditions.

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