with an insert of males carrying the heterozygote translocation, and to recover the interchange in progeny collected in the field. Quality control tests of release mosquitoes indicated that 99.4 percent of the males released were carrying the translocation. The number needed for a successful release was again ascertained by computer simulations based on previous population estimates. Male adults instead of pupae were released in the field to ensure against unwanted introductions of females.

In 1978, 180,000 translocated males were released. The progeny of females collected in the field were genetically tested to determine male parentage. In 22 cases tests confirmed that released translocated males had mated with native females. Unfortunately, parallel tests in outdoor cages revealed that the release stock had become less competitive with time; thus again, the release did not have sufficient impact to cause a continuing population reduction in the field. The data, however, did show a four-week delay before the population reached a peak, when compared with that of previous years, and the insert may have contributed toward keeping the population down during the release period. The population increased considerably once the release ended, and the peak population was twice that of the previous year.

A new method for rapidly identifying homozygote translocations was perfected. Currently, simulated field releases of various combinations of homozygous lines are being conducted in large outdoor cages. The advantages of homozygotes over heterozygotes would allow use of a field-replacement mechanism in addition to a self-destruction scheme.

One desirable genotype to be carried into field populations is decreased competence of *Culex tarsalis* as a vector of encephalitis viruses. A strain that rejects infection with western equine encephalitis (WEE) has been isolated by selection techniques. Although we lack conclusive genetic data on this resistance to WEE, the strain is a candidate for induction of translocations that might carry resistance to viral infection into field populations. Attempts are in progress to isolate a gene that acts as a lethal at low temperatures only. Such a conditional lethal would be a desirable "time bomb" for insertion in the summer to kill overwintering populations.

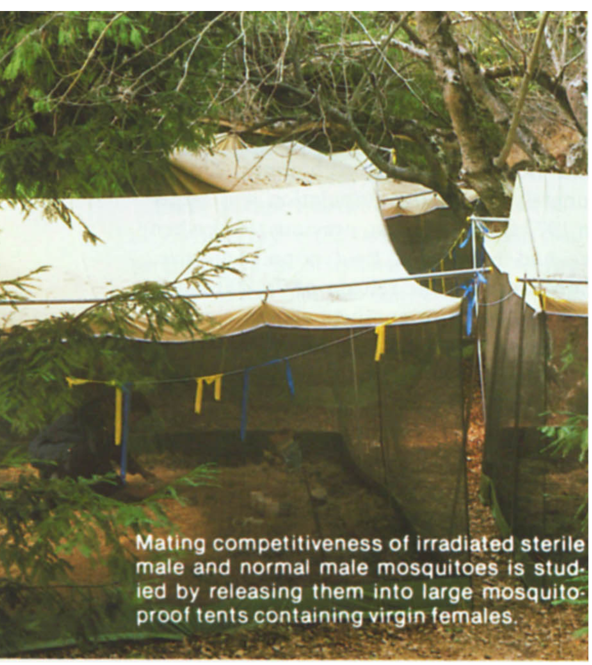
In studies on the feasibility of using the sterile male technique to control *Cx. tarsalis*, radiation sterility curves were established, mating competition tests were performed in the laboratory, and extended tests were conducted in walk-in cages outdoors. Egg hatch was reduced to 43 percent when irradiated males and nonirradiated males competed in a 1:1 ratio as compared with a 92 percent hatch in the nonirradiated control cage, and a 3 percent hatch in the irradiated control cage. In August 1979, a small-scale field release of sterile males reared from field-collected pupae was made in an isolated area outside Bakersfield. Only 1 of 50 egg rafts from females collected before the release had hatch rates lower than 70 percent, while 22 of 112 females collected during the first four days after the release laid rafts with low or medium hatch rates. Such results warrant further research with this method.

The behavior and ecology of *Cx. tarsalis* in the field, primarily in relation to reproduction, have been investigated continuously. Studies on reproductive vigor of laboratory-adapted and field-collected populations indicated that laboratory populations were more successful in insemination, oviposition, and the production of hatching egg rafts in small cage tests. The results also documented a substantial reproductive disadvantage of newly colonized populations during the initial colonizing process.

Current priorities are to keep field populations that are captured and colonized for genetic studies biologically and genetically close to the original stock and to improve the fitness of genetically altered strains to withstand field releases. A strict quality-control program has been established to ensure that specific attributes essential for normal behavior in the field are not lost during or after colonization. Representatives of translocated stocks and wild-type populations are being reared as continuously as possible in large outdoor cages under overwintering conditions.

Studies have been initiated to identify genetic selection factors that reduce competitive fitness. Study of genetic markers for several enzymes in *Cx. tarsalis* strains are under way. Analysis of the wild-type populations as they become colonized or become less competitive because of other selection factors will be made. The enzyme markers can also contribute to our formal genetic information, to release experiments where we can follow these "fingerprinted" strains, and to the study of reproductive behavior.

Sister Monica Asman is Associate Research Entomologist, Paul T. McDonald is Assistant Research Entomologist, and Frank G. Zalom is a Graduate Research Entomologist. All are with the University of California, Berkeley, CA 94720.



Mating competitiveness of irradiated sterile male and normal male mosquitoes is studied by releasing them into large mosquito-proof tents containing virgin females.

Using sterile males to reduce mosquito numbers

John R. Anderson ☐ Sister Monica Asman

Control of the treehole mosquito, *Aedes sierrensis*, is difficult, because it develops in hard-to-find treeholes. For this reason, the release of sterilized males is being researched as one possible component of an integrated management program. Initial laboratory experiments determined the most effective sterilization doses, and established that Gamma-irradiated, sterilized males competed equally with normal males in all mating experiments. Since females mate only once, regardless of whether mating with a fertile or sterilized male, sterilized males could be released and used to help reduce populations of this common pest.

In experiments at the University of California Russell Tree Farm Field Station (near Briones Regional Park, Contra Costa County), laboratory-reared mosquitoes were released into large tents having screened sides and natural turf floors. For these experiments, males mass-produced in the laboratory were sterilized by exposure to 7 kR of Gamma irradiation when less than a day old. The tents were set up in a

Mosquito larva infected by the fungus *Lagenidium giganteum.*

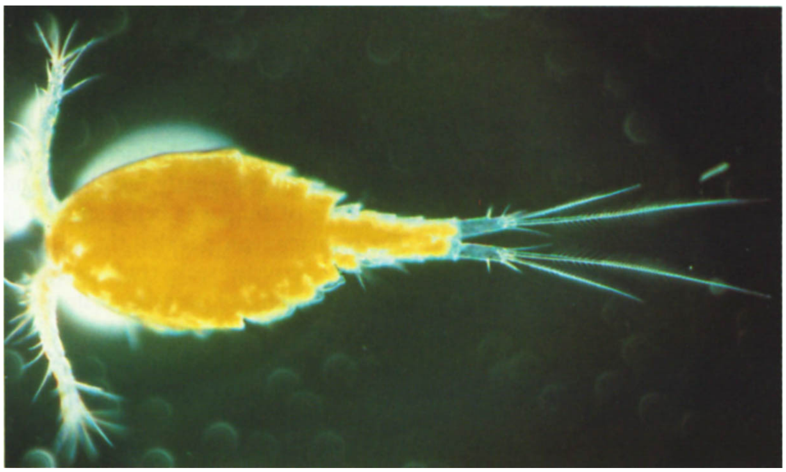
Fungi show promise in biological control

Brian A. Federici ☐ Joyce Fetter-Lasko ☐ George Soares ☐ Pamela W. Tsao

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.

John R. Anderson is Professor of Entomology, and Sister Monica Asman is Associate Research Entomologist, University of California, Berkeley, CA 94720.