Delivering biocontrol in the tropics

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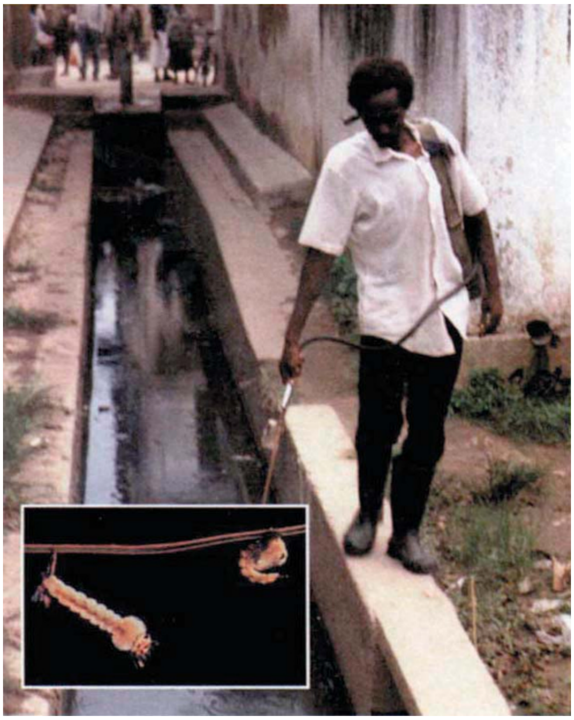

Resisting temptation but tempting resistance. The specificity of biological control means there is no reason for spraymen to divert agents intended for healthcare to lucrative agricultural uses. A major breeding site of *Culex quinquefasciatus* (inset), a vector of filiaris, is being sprayed with *Bacillus sphaericus* in Tanzania.

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Liu et al.[1] from the National University of Singapore report transformation by electroporation of plasmids into the Gram-negative bacterium, *Asticcacaulis excentricus*, of two linked genes from *Bacillus sphaericus*, on p.343 of this issue. The two genes encode the components of a binary toxin that kills the larvae of *Culex* and *Anopheles* mosquitoes if they eat it. The general approach in mosquito biocontrol involves spraying the control agent onto the surface of the waterways and open drains in which the insect larvae live. The authors suggest that transformed *A. excentricus* may have advantages over *B. sphaericus* as a natural production host for a mosquito control agent because the transformed bacterium can be grown on a simpler and cheaper medium without protein supplement, and because it lacks toxin-degrading protease, floats for a longer period, and is more ultraviolet light resistant. Over the last two decades there has been much interest in the toxins of *B. thuringiensis israelensis* (*Bti*) and *B. sphaericus* for mosquito larval control. Their high specificity for their target mosquitoes avoids the risks (real or imaginary, but politically important) of synthetic insecticides. Their specificity also has an important practical consequence: In developing countries where spraymen exist on subsistence wages, the bacterial preparations are not worth stealing from public health organizations to sell for use against agricultural pests. At present, however, most operational use of bacterial toxins is against nuisance mosquitoes in developed countries where use of synthetic larvicides would be politically impossible[2]. The obstacles to their use in the tropics, where mosquito control is of vital importance, include the cost of production and the lack of persistence of the natural bacteria and their toxins. Numerous attempts have been made to transfer the toxin genes into species that could persist in mosquito breeding places. Much of the *B. sphaericus* preparation used currently in experimental mosquito control in developing nations is manufactured in high-technology facilities in the West and requires cold-chain transportation. This latest news from Singapore—where there is a persistent problem of dengue transmitted by *Aedes* mosquitoes—that transformed *A. excentricus* is an efficient producer of certain of the toxins of *B. sphaericus*, is, therefore, very welcome indeed.

There is still some way to go, of course, before transformed *A. excentricus* could be used in tropical mosquito breeding places in the real world. For one thing (as the authors point out), the toxin gene would have to be stabilized by incorporating it into the bacterial chromosome. The tetracycline resistance gene, which was used to select the plasmid transformed bacteria, would also have to be removed.

Probably because of its active flagellum, *A. excentricus* remains in the surface layer of the water. That characteristic may be an advantage in mosquito biocontrol, but not necessarily so. In general, *Culex* larvae do not feed at the water surface[3]. *Anopheles* larvae do, but (as the authors point out) larval control of *Anopheles* is, in practice, largely limited to urban areas with restricted availability of breeding sites. In rural areas, where most of the world's malaria occurs, it is generally better to attack the *Anopheles* adults in village houses where they come to bite, and there are no known bacterial agents for doing that.

The question of resistance to *Bacillus* spp. toxins also needs to be addressed. It cannot just be a fortunate accident that *Bti* and *B. sphaericus* produce powerful toxins against mosquito larvae. Presumably, it is their way of providing a supply of carrion for their descendants to feast on. There is already evidence that *Culex* mosquitoes can become resistant to *B. sphaericus*[4,5]. It seems likely that the production of several different toxins by each bacterial species has been evolved to reduce the likelihood of mosquito resistance because this would require simultaneous occurrence of multiple resistance mechanisms in an individual mosquito. Humans have reinvented the same principle in the use of antibiotic or pesticide mixtures to delay the buildup of resistance. Georghiou[6] has warned against using less than the full natural complement of bacterial toxins in insect control. The present construct in *A. excentricus* lacks two of the natural toxins of *B. sphaericus*. Unless it is further elaborated by the addition of other toxin genes before release into mosquito breeding places, the construct might rapidly select for resistance to itself and also provide a "stepping stone" for evolution of further cases of resistance to natural *B. sphaericus*.

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