Perspectives on the Bacterial Control of Vectors in the Tropics

J-M. Hougard and C. Back

Biological agents have not found wide application in the control of insects that transmit agents of human disease. Fungal pathogens may have a potential for field use, but environmental constraints, lack of knowledge of their life cycle, or dependence on production in vivo, *have prevented their widespread use. In fact, sporogenous bacteria are the only biological agents used on a large scale for the control of mosquitoes and blackflies that transmit diseases in the tropics. Two bacteria,* Bacillus thuringiensis *sp.* israelensis *and* B. sphaericus *are very effective for the control of these vectors. In this article, Jean-Marc Hougard and Christian Back discuss various factors associated with the use of bacteria for vector control, and the prospects for further developments.*

Biological control initially meant the introduction of live predators, parasites or competitors to fight a pest. Nowadays it also includes genetic control, mostly based on the introduction of individuals that are unable to ensure progeny. Finally, in a wider sense, the use of toxic bacteria may be considered to be a form of biological control, as opposed to the use of synthetic poisons. Insecticidal bacteria are now the most widely used biological agents for the control of vectors; there are indeed very few instances, in the tropics at least, of the successful use of predators, of parasites, or of genetic control. Current research on biological control is concerned with all the aspects described above, and here we will deal briefly with the genetic approach and with the search for true pathogens. However, this article will emphasize bacterial control.

Genetic control

The strategy of genetic control is the release of organisms with altered genomes to prevent the target population from ensuring a progeny. For insects of medical importance, this has taken the form of irradiation (tsetse flies), exposure to chemicals and hybridization between strains of the same species (mosquitoes) that have cytoplasmic incompatibilities[1]. Strategies that were also tried or considered include the reduction of fertility through chromosomal translocation, the replacement of wild strains by non-vector strains or the introduction of deleterious genes into the genome[2]. With a few exceptions, genetic control has never reached the operational level, due either to the high costs involved for large-scale implementation or to an incomplete knowledge of behavioral and ecological factors. For instance, the release of sterilized tsetse flies is hampered by the high cost of mass rearing and by the fact that reared flies are less competitive in the natural environment. The recent progress in genetic engineering gives some hope, though, and current research is focused on the genetic factors of vectorial ability and on the engineering of competitive transgenic strains.

Biological control *sensu stricto*

Reviews on biological control *sensu stricto* are regularly published[3–8]. The World Health Organization promotes the dissemination of information on this topic by issuing data sheets on the most promising agents. In retrospect, predators or pathogens such as fish, crustacea, insects[9–11], nematodes[12,13], microsporidia[14,15], spiroplasma[16,17] or viruses[18] have shown promise in the laboratory and on a small scale in the field, but they have failed to reach large-scale application. Four genera of fungi (*Tolypocladium*, *Coelomomyces*, *Culicinomyces* and *Lagenidium*) are more likely to reach that stage[19–21]. They are effective and may recycle to some extent in field conditions[22], but their use is hampered by mass production problems (sometimes *in vivo*), complexity of the life cycle (some demand an intermediate host), lack of selectivity and low tolerance to environmental factors (temperature, pH, water quality).

Bacterial control

Bacterial control of insects was first applied in agriculture, but after the discovery of *B. t. israelensis* (*B.t.i.*) in Israel by Goldberg and Margalit[23], intensive research on vector control followed. It led rapidly to successful field trials against mosquito and blackfly larvae, as reviewed in Ref. 24. One of the major incentives for research was the safety of *B.t.i.* for non-target organisms[25,26].

The Onchocerciasis Control Programme in West Africa (OCP) is the only vector control programme to have used *B.t.i.* on a large scale for a long time (ten years). The main strategy of OCP is to interrupt the transmission of the disease agent through the control of larval populations of blackflies in rivers. Starting in 1975, thousands of kilometers of rivers were treated each week with organophosphorous (OP) insecticides (temephos,

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and chlorphoxim when resistance to temephos arose in some areas). *B.t.i.* was effective against OP-resistant blackflies (Fig. 1), and OCP started using it on a large scale in 1982, when a commercial formulation became available[27]. To avoid problems of resistance, OCP has adopted a strategy of insecticide rotation that now involves *B.t.i.* in conjunction with five synthetic insecticides (three OPs, one pyrethroid and one carbamate). On average, 300 000 liters of *B.t.i.* are used annually by OCP in West Africa, which is more than 50% of the total volume of insecticides applied. The dosage of *B.t.i.* has been maintained since 1985 at 0.72 l of formulation per cubic meter of river discharge. This dosage is six times greater than that for synthetic insecticides, and therefore makes the use of *B.t.i.* relatively expensive because of high application costs (by helicopter). More potent formulations would reduce application costs and would allow OCP to use *B.t.i.* in a wider range of conditions[28].

The interest in *B. sphaericus* grew more gradually than did that in *B.t.i.* as strains with higher toxicity were identified[29]. One of these, strain 2362, was isolated from a blackfly from Nigeria[30] and is now industrially produced. *B. sphaericus* is not effective against either *Aedes* or blackflies[31], but it is very effective against *Culex* spp[32–34], which are a nuisance in urban areas and which may transmit bancroftian filariasis (Fig. 2). On the basis of encouraging trial results in the tropics[35,36] and more particularly in central Africa[37], a large-scale control programme targeted against *Culex quinquefasciatus* has been launched recently in a city in northern Cameroon. Initial results show that the flowable concentrate of *B. sphaericus* (strain 2362) used for treatment is at least as effective as chlorpyrifos, the most widely used OP for the treatment of polluted waters. Considering the safety of *B. sphaericus*[38] for mammals and the extent of mosquito resistance to synthetic insecticides, there is obviously a large potential for the development of similar programmes.

Formulations of *B. sphaericus* and *B.t.i.* can be used for mosquito and blackfly control in the same way and with the same equipment as synthetic insecticides, which can help in their gradual introduction in vector control programmes. They also share the disadvantage of having to be applied repeatedly, since their recycling in treated biotas is usually insignificant[39,40]. There would be an operational advantage in extending the efficacy of treatment through recycling. At the same time, this might increase the risk of resistance.

Unlike synthetic insecticides, *B. sphaericus* and *B.t.i.* can be produced and formulated locally on inexpensive and locally available media[41]. The advantages of local production would be the proximity of the source to the application site, the resulting lower costs and the possibility of precisely adapting formulations to local needs. On the other hand, the market for such products is already small compared to the agricultural one, and local production might deter large companies from investing in product development, which is still needed. Finally, quality control standards for bacterial formulations are difficult to establish and implement, but they remain a *sine qua non* condition for field use, whatever the scale of production. Therefore, a comparison of costs between industrially and locally produced formulations should include quality control.

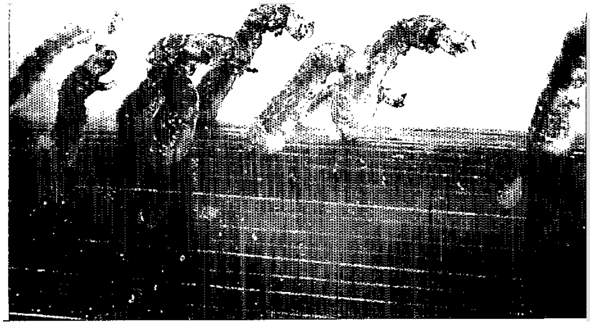

Fig. 1. Blackfly larvae (Simulium damnosum) *lying dead after ingesting* Bacillus thuringiensis *sp.* israelensis.

The isolation of new bacteria with insecticidal properties has become one of the research priorities of WHO, and guidelines have been published with that purpose in mind[42]. Several WHO-collaborating centers in the tropics are trying to find new strains of *B. sphaericus* and *B.t.i.*, new species of *Bacillus* and even new genera. For instance, a new bacterium, *Clostridium bifermentans* serovar. *malaysia*[43], has been shown recently to be toxic for mosquito and blackfly larvae. One of the major advantages of bacteria over synthetic insecticides is that their activity is due, in general, to a complex mechanism where several toxins are involved; the risk of resistance to bacteria is inversely proportional to the complexity of the mechanism. For instance, there has been no change of susceptibility to *B.t.i.*

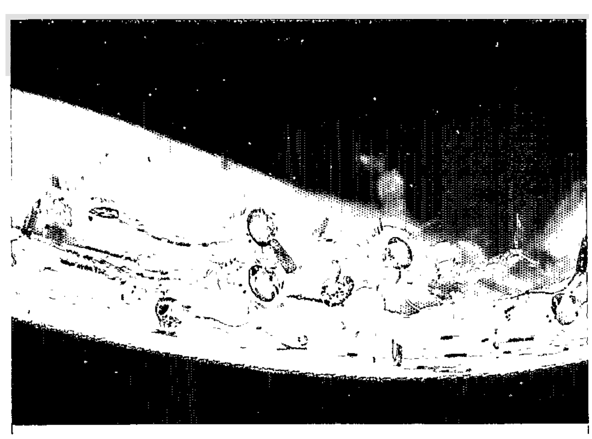

Fig. 2. Mosquito larvae (Culex quinquefasciatus) *lying dead after ingesting* Bacillus sphaericus.

in the OCP area after ten years of intensive use. Studies of the genes responsible for toxin production have prompted the engineering of transgenic organisms (bacteria or algae), with the aim of increasing persistence in the environment or availability to target organisms[44,45]. In view of field applications, the transfer of the full complement of toxins will be important in order to reduce the risk of resistance, and the consequences of introducing recombinant organisms in the environment will have to be assessed.

Conclusion

Biological control *sensu stricto* has been rarely applied in the field, but bacteria do offer a viable alternative to synthetic insecticides for the control of mosquitoes and blackflies. They are effective and environmentally safe, and they can even be competitive in terms of cost. The improvement of formulations could provide additional incentives for preferring them to synthetic insecticides. Moreover, it could promote vector control activities that have recessed because of resistance to synthetic insecticides or because of justifiable concerns about the environment. Bacteria, as control agents, are only one element of large-scale vector control programmes, since financial, logistical and human resources also come into play, but they can be the cornerstone of successful projects.

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