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> INTEGRATED MANAGEMENT OF INSECT VECTORS OF HUMAN AND ANIMAL DISEASES

Developing genetic control

Jérémy BOUYER

The evolution of insecticide resistance in insect vectors of human and animal diseases and the introduction of exotic vectors to new territories, in a context of tighter regulations on approved molecules, call for the development of new pest control methods.

Among these new methods, genetic control shows promise, as demonstrated by the tsetse fly eradication project in Senegal. It nevertheless requires complementary studies combining public and private research. Moreover, it must be combined with other methods of pest control – chemical, physical and biological – within an integrated management framework.

Awareness of the toxicity of insecticides to living organisms and ecosystems is leading a growing number of countries to reduce the number of approved molecules. For example, in 2014, the European Union was still authorising only four classes of vector control insecticides, and only three new classes are being developed, for operational use no earlier than 2019. Moreover, resistance to pyrethroids, the most common class used against insects, is spreading, which could result in its disuse in the short-term.

These regulations are being tightened amid growing pressure from insect vectors of human and animal diseases. This pressure is explained

by greater resistance to insecticides, but also by global factors, such as climate change or growth in trade. These global factors foster the invasion of new territories by exotic vectors. For example, the midge *Culicoides imicola*, a vector of blue-tongue disease native to tropical Africa, is invading Europe from the south. This disease caused an unprecedented health crisis in the French ruminant sector between 2006 and 2008. The tiger mosquito, *Aedes albopictus*, which is native to Asia and a major vector of arboviruses in humans, caused indigenous cases of dengue and chikungunya in mainland France in 2014. Innovations are therefore urgently required in the field of vector control.

perspective

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Neutralising females

Genetic control is one of the methods that could replace insecticide use. It consists in the large-scale rearing of insects – whether genetically modified or not –, with the subsequent release of males in order for them to sterilise females or to transfer to females mutations that are either lethal or impede their ability to transmit a disease. One variant consists in contaminating females with symbionts (organisms living in symbiosis with insects) – whether modified or not –, which sterilise females or block the transmission of the disease.

The sterile insect technique (SIT) is the first method based on this strategy: the males are irradiated and transmit to the females with which they mate sperm cells carrying lethal mutations. This technique has been used successfully in the control of a number of insect pests or vectors, such as species of fruit flies, the screw-worm fly and the tsetse fly.

The use of genetically modified insects – or transgenic males – to transmit a mutation to females has the advantage of avoiding the need for irradiation, which is often based on the use of radioactive sources. Mutations can block the ability to transmit a disease, turn females into inoffensive males, or even destroy the targeted insect. However, this technique is less acceptable to society because of its potential biological risks, especially the transfer of genes to non-targeted insects. This risk depends on the genetic mechanisms used, and in particular on their potential for dissemination. It is thus lower for the transfer of dominant lethal mutations (similar to that of the sterile insect technique) than for the use of symbionts.

Supporting genetic control through field research

In Senegal, a tsetse fly eradication project has been implemented in the Niayes area, based on an integrated strategy, the approach underpinning the IVEMA research project, “Integrated vector management: innovating to improve control and reduce environmental impacts” (see box p. 4). Because of its climate, this area near Dakar is suitable for the intensification of cattle farming, for which there is high demand, especially for milk (the zone is home to almost 80% of the population of Senegal). However, an isolated tsetse fly population maintains the transmission of trypanosomiasis, reducing milk and meat

production and animal labour by around 30% and hindering intensification. By controlling the disease, the project aims to enable communities of livestock farmers who so wish to adopt more productive breeds and to reduce herd sizes in a context in which land constraints limit this size and pose feeding problems.

Each stage of the project involved methodological and technological developments. Habitats suitable to the tsetse fly were identified by remote sensing based on a population distribution model. Using this model, the number of traps needed per km² to suppress tsetse populations by more than 95% was reduced by 30%. This model was also used to set the densities of sterile males to be released (10 sterile males per km² if the habitat is unsuitable; 100 if it is suitable). These sterile males were produced and irradiated in Burkina Faso at CIRDES (Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide), and in Slovakia, by the Slovak Academy of Sciences. They were transported to Senegal by express delivery under regulatory and optimal conditions to guarantee their survival (in particular, a temperature of 10°C). They were then reared for six days in an insectarium at ISRA (Institut Sénégalais de Recherches Agricoles), before being released by an automatic machine loaded onto a gyrocopter (see Photo, next page). This machine, developed by a Mexican company, is piloted by a geographic information system (GIS) that can adjust the density of sterile males to be released in order to achieve the objective. Installed on an android tablet, this GIS enables the pilot to concentrate on following release lines, with the machine automatically beginning the release upon entering the target zone, and ceasing upon exit.

This eradication campaign opens up economic and environmental opportunities, and an ex-ante cost-benefit analysis has demonstrated its profitability. For a total cost of 6 400 euros per km² to the government of Senegal and international donors for permanent tsetse fly eradication, after 10 to 20 years (depending on the innovation scenario), the project would generate 2 800 euros per km² per year in additional sales of animal products (milk and meat) for the community of beneficiary livestock farmers. IVEMA places integrated pest control at the core of its strategy, associating chemical, biological and mechanical methods according to the conditions for action, and optimising their association

➤ **Integrated control: a core challenge associating chemical, biological and mechanical methods, with help from modelling**

➤ **Use of transgenic insects requires a case-by-case analysis**



• In Senegal, tsetse fly eradication improves cattle productivity (pedal of gyrocopter releasing sterile tsetse males over transhumant zebus between baobabs in the Niayes area in Senegal) © J. Bouyer.

• through a significant modelling element. By supporting genetic control through field research, this project has succeeded in reducing the use of insecticide treatments (in terms of their frequency or dosage) and in developing the sterile insect technique.

• The innovative elements of this project are currently being transferred to other tsetse fly eradication projects in Africa, within the framework of PATTEC (the African Union Pan African Tsetse and Trypanosomosis Eradication Campaign). Many of them will also help to improve mosquito control in other parts of the world.

• However, implementing SIT requires sufficient quantities of sterile males to overwhelm the target population, and also a number of sterile males exceeding that of wild males. To overcome these obstacles, a variant of SIT, known as “boosted SIT”, has been developed. It aims to reduce by 90 to 99% the quantity of sterile males needed to control or eradicate a target population. In this variant, sterile males are considered as a specific means of contaminating females with a control agent. This agent may be an active substance, bacteria, a fungus or a virus, or even recombinant versions of these pathogens. But this is dependent on two conditions: avoiding contamination of species other than the target species; and avoiding dissemination after the

> New cooperation strategies are needed, implying attitude changes among partners

insect’s death. Developing this kind of biocide implies a risk assessment, guaranteed by close cooperation between public research and industries.

Involving public research and industries

Collaboration between public research and private research is needed not only for the large-scale use of transgenic insects, but also for the development of new control methods and to guarantee their safety and sustainability. All partners will benefit from this.

The large-scale use of sterile insects implies mobilising extensive research and development programmes, such as the ones leading to the eradication of the screw-worm, an insect of veterinary concern, in North America, Central America and Libya, and of the Mediterranean fruit fly, of agricultural concern, in Mexico – this fly is the subject of an SIT pest control programme in Spain. However, public research cannot implement these programmes alone, but can contribute to them by conducting the operational research essential to their optimisation and their success.

As for industries, they are faced with stricter regulations limiting the number of approved molecules and with the shorter lifespan of these molecules because of resistance. It is therefore in their interest to invest in the development of new non-toxic control methods, and to deploy these within an integrated pest control framework, taking into account lessons learned from past experience. Public research can make significant contributions to both processes.

Another area of collaboration is the assessment and management of the impact of chemical molecules used and of risks associated with the use of transgenic insects. To ensure that chemical molecules can be used in the long term, their effects on vector populations, as well as the changes caused, must be evaluated. As for transgenic insects, their use is booming. Releases of *Aedes aegypti* mosquitoes (another major vector of arboviruses) that have been genetically modified to atrophy the wings of female descendants and to prevent them from flying, have succeeded in eliminating more than 90% of target populations in parts of the Cayman Islands, Panama and Brazil. Testing in natural habitats has been authorised in Malaysia, Mexico and the United States, while trials under controlled conditions

A few words about...

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• have been authorised in four European countries including France, and an application is pending for testing in natural habitats in Spain. Moreover, in France, the Plan Santé Environnement (PNSE 3 – environmental health plan) 2015-2019 encourages the development of research on the sterile insect technique for mosquitoes. The use of transgenic insects therefore requires a case-by-case analysis of risks, since the risk depends on the genetic mechanisms used, and in particular on their potential for dissemination. In Europe, this analysis can be based on EFSA (European Food Safety Authority) recommendations.

• New collaboration strategies are therefore required, involving a change of attitude among

partners. Industries are invited to take risks and to accept the need to phase out their reliance on chemicals in favour of diversified strategies. And researchers need to open up to industries in order to turn their inventions into innovations.

Although genetic control has potential that must be exploited, no technique should be automatically ruled out, and it is essential to analyse the advantages, disadvantages and risks associated with each one. This implies gaining further knowledge of the ecology of target vector populations, but also of the socio-ecosystems concerned by vector control. It will then be possible to achieve an optimal combination of several different methods, with the help of modelling processes. <

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At Expo Milano 2015, the tsetse fly eradication project won second prize in the category "Best sustainable development practices in small rural communities", as an example of ecological intensification.

This research has resulted in the following publications:

Bouyer F., M. T. Seck, A. Dicko, B. Sall, M. Lo, M. Vreysen, E. Chia, J. Bouyer & A. Wane, 2014. Ex-ante cost-benefit analysis of tsetse eradication in the Niayes area of Senegal. *PloS Negl. Trop. Dis.* 8: e3112.

Bouyer J. & T. Lefrançois, 2014. Boosting the sterile insect technique to control mosquitoes. *Trends Parasitol.* 30: 271-273.

Dicko A. H., R. Lancelot, M. T. Seck, L. Guerrini, B. Sall, M. Lo, M. J. B. Vreysen, T. Lefrançois, F. Williams, S. L. Peck & J. Bouyer, 2014. Using species distribution models to optimize vector control: the tsetse eradication campaign in Senegal. *Proceedings of the National Academy of Sciences* 111: 10149-10154.

It has also given rise to a presentation (www.isntd.org/#/isntd-bites-15-bouyer/4589861215) and an interview (www.isntd.org/#/isntd-bites-15-interv-bouyer/4589652779) that reflects the opinion of the author and not that of CIRAD, and can be found online on the website of the International Society for Neglected Tropical Diseases.



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• Alphey L., 2014. Genetic Control of Mosquitoes. *Annu. Rev. Entomol.* 59: 205 (for a review of the potential for dissemination of different genetic mechanisms).

• McGraw E. A. & S. L. O'Neill, 2013. Beyond insecticides: new thinking on an ancient problem. *Nat Rev Microbiol* 11: 181-193 (for a review of credible alternatives to insecticides).

• Suckling D. M., P. C. Tobin, D. G. McCullough & D. A. Herms. 2012. Combining tactics to exploit Allee effects for eradication of alien insect populations. *J. Econ. Entomol.* 105: 1-13 (for a review of the integration of different pest control methods).