TRANSGENIC MOTHS ON THE HORIZON

The fall armyworm (*Spodoptera frugiperda*) is responsible for losses of up to 50% on maize farms

PRODUTORA SÃO PAULO / OXITEC

New technology developed to combat the main threat to maize crops

Frances Jones

PUBLISHED IN MARCH 2023

Brazilian farmers will soon be able to use a new tool to combat what is considered by agribusiness as maize farming's worst pest. The company Oxitec do Brasil is preparing for the commercial launch of its genetically modified moth, which can be released in maize fields to combat the fall armyworm (*Spodoptera frugiperda*). The armyworm is found in every maize-growing region of Brazil and can cause crop losses of up to 50%. In 2021, Oxitec's transgenic moth, called Spodoptera do Bem, was granted approval by Brazil's National Biosafety Commission (CTNBio), the office of the Ministry of Science, Technology, and Innovation (MCTI) responsible for authorizing the release of genetically modified organisms in Brazil.

"Spodoptera do Bem is a safe and effective product", says geneticist Natalia Ferreira, executive director at Oxitec do Brasil. "We are in the phase of engaging farmers, talking to distributors, and continuing with trials on farms to understand how the product fits into the agricultural producer's routine," she explains. The company says that the product's commercial launch will take place within the next few years.

Spodoptera do Bem is the commercial name of the genetically modified OX5382G strain, developed by the original company in the UK and tested on two Brazilian farms, one in Mato Grosso and another in São Paulo. Oxitec was founded in 2002 as a spinoff from the University of Oxford and is now a subsidiary of the American company Third Security, based in Virginia.

Brazil is the first and only country in the world to release transgenic moths into the field. The genetically modified version of the fall armyworm carries two different genes that were introduced in the laboratory. One of these genes, known as tTAV, prevents the development of females so that only males hatch from the eggs of the next generation, drastically affecting the insect's ability to reproduce.

"In the lab, we improved a gene that already exists in *Spodoptera* and other insects and arachnids, inserting a promoter [a specific DNA sequence] that tells the cell to produce a lot of that gene," said Ferreira. "It's like an overdose. Like if instead of producing organ cells, my entire body started producing only collagen," he says. "The result is that I would no longer produce blood, saliva, or anything needed to sustain my life; I would die due to a lack of these substances."

The second inserted gene, DsRed2, is a marker derived from a species of marine coral that produces a fluorescent protein, helping to distinguish modified animals from wild insects.

The technique for combating the pest consists of releasing genetically modified males into the field to breed with wild females. These pairings only produce male larvae, which after the pupal stage become moths carrying the self-limiting gene in their DNA that in the future will again prevent any female offspring from being born. Thus, within a few generations, the insect population will significantly decrease, according to the company.

The same technology is used in Aedes do Bem, sold by the company in Brazil since 2021 to reduce *Aedes aegypti* mosquito populations. The objective is to reduce cases of dengue and other diseases transmitted by mosquitoes, such as Zika and Chikungunya (*see report on Page 54*). The small group of transgenic animals approved for

sale in Brazil by CTNBio includes the modified *Spodoptera*, two versions of *Aedes aegypti* created by Oxitec, and a salmon developed by the Canadian company AquaBounty.

Maria Lúcia Zaidan Dagli, a veterinarian from the Experimental and Comparative Oncology Laboratory at the School of Veterinary Medicine and Zootechnics of the University of São Paulo (FMVZ-USP) and a member of CTNBio, sees the release of Spodoptera do Bem in Brazil as a positive move. She was involved in the decision to approve Oxitec's first version of *Aedes aegypti*.

Dagli explains that to be authorized for sale by CTNBio, a product must be approved by the agency's four sectors, which verify its impact on humans, animals, plants, and the environment, certifying that it is safe based on data and studies presented by the applicant company. After approval is granted, the product is monitored closely for five years, during which time the company has to submit annual reports to CTNBio.

"It's the same process that takes place with new drugs released by other regulatory agencies. If a problem is reported, depending on the severity, sale of the product may be suspended," underscores the researcher. She points out, however, that no CTNBio-approved product has ever been suspended.

There are approximately 200 chemicals available on the market in Brazil to help farmers combat the fall armyworm, according to the Brazilian Agricultural Research Corporation (EMBRAPA). However, *Spodoptera* has demonstrated resistance to conventional insecticides. There is also concern about the unwanted effects these pesticides may have on the health of nontarget organisms and the environment.

In addition to insecticides, there are nine biological products registered in the country and another four soon to be launched. Transgenic maize that expresses proteins from the bacterium *Bacillus thuringiensis* (Bt) to kill larvae has also been used since the 2008/2009 harvest. However, the insects already show resistance to the modified crop.

"When we use insecticides or transgenic plants to control a pest, we end up involuntarily selecting individuals capable of surviving these technologies in the wild," explains Alberto Soares Corrêa, head of the Molecular Ecology of Arthropods Laboratory at USP's Luiz de Queiroz College of Agriculture (ESALQ). "A single female *Spodoptera frugiperda* can lay up to 1,500 eggs in its life cycle. It's an extremely complex species to address due to its polyphagy [ability to feed on different plant species] and dispersal capacity. Native to the Americas, it has recently become a cosmopolitan pest with reports of its detection in countries in Africa, Asia, Europe, and Oceania," says Corrêa.

To delay the insect's resistance to transgenic maize, farmers are advised to reserve some land—between 10% and 20% of the crop, although there is no consensus on the exact area—for the cultivation of conventional, nontransgenic plants,

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In addition to maize, the moth feeds on 50 other plant varieties

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Oxitec employees in the field conducting a pilot study of Spodoptera do Bem

known as a refuge. The idea is that the resistant moths will breed with those from the refuge that do not have the alleles (different forms of a given gene) that confer protection. "The problem is that farmers often do not bother planting a refuge area, and as a result, the insects develop resistance faster," says the ESALQ researcher.

According to Oxitec, transgenic *Spodoptera frugiperda* is a highly effective method of controlling resistance to Bt maize. "Spodoptera do Bem has never seen insecticides, it has never seen Bt in its life, it's totally susceptible," explains Ferreira. "When transgenic males on a farm mate with nonmodified females, any male descendants inherit the part of the father's genome that does not provide resistance. The effect of all insecticides, pesticides, and Bt maize is thus restored. This is a technology that will allow farmers to use less pesticide and to recover or extend the lifespan of biotechnological seeds."

Corrêa explains that autocide—when a genetically modified insect is used to control the population of a species through breeding—is an old technique. "The classic example is the screw-worm fly [*Cochliomyia hominivorax*], which was eradicated in the United States after millions of sterile insects were released starting in the 1950s," he says.

The important difference is that instead of transgenic versions, males rendered sterile by gamma-ray irradiation were released. It is hoped that the use of transgenic insects will overcome some of the previous method's weaknesses, at least initially. "Exposure to radiation can harm the insects in various ways, impacting their biological characteristics and behavior, which can make the strategy less successful. The idea is that with transgenic insects, individuals can better compete with wild males, mating with more females that do not produce any offspring, reducing the population of the target species."

Corrêa is reluctant to speculate on the risks and potential ecological consequences of releasing a transgenic insect into nature. "There are no scientific data available in the literature to answer the biggest questions. This has never been done before on a large scale," says the researcher. "In the case of *Spodoptera frugiperda*, if CTNBio approved it, then they must believe it meets the minimum safety criteria for the technology to be applied."

He notes that the same questions arose with transgenic plants. "Today, we know that they are extremely safe. Thus, their use has expanded worldwide. With animals, however, there is a large difference in reproductive and biochemical issues and with regard to genome structure. We can't simply say: it worked with transgenic plants, so it'll work with transgenic animals."

Biologist José Maria Gusman Ferraz, a visiting researcher at the Laboratory of Ecological Engineering of the University of Campinas (UNICAMP), studied *Spodoptera frugiperda* during his PhD. He sees the new technology as adding another string to the bow to help fight the pest but has doubts about its efficiency, since adult fall armyworms can travel long distances and maize is generally planted in large open areas. "The history of this type of technology is that it only works well in isolated areas, like islands," he says.

Ferraz would also like to see more data on possible damage to parasitoids (the moth's natural enemies) and the risks of transgenic DNA remaining in the environment. "New technologies can work in a short time frame, but they can also

PHOTOS 1 EDUARDO CESAR/ PESQUISA FAPESP MAGAZINE **2** PRODUTORA SÃO PAULO / OXITEC

have negative effects and then stop working," he points out. "The basic principle of life is diversity, and when we reduce that diversity, the system becomes fragile."

Another advantage of genetically modified organisms (GMOs) over irradiated organisms is practicality and cost, explains Margareth Capurro, a biochemist from USP's Institute of Biomedical Sciences (ICB) and technical coordinator of a study on transgenic Aedes mosquitos carried out in Bahia. According to Capurro, 44 countries are preparing to release sterile males to control insect populations, although no other countries are using GMOs to do so.

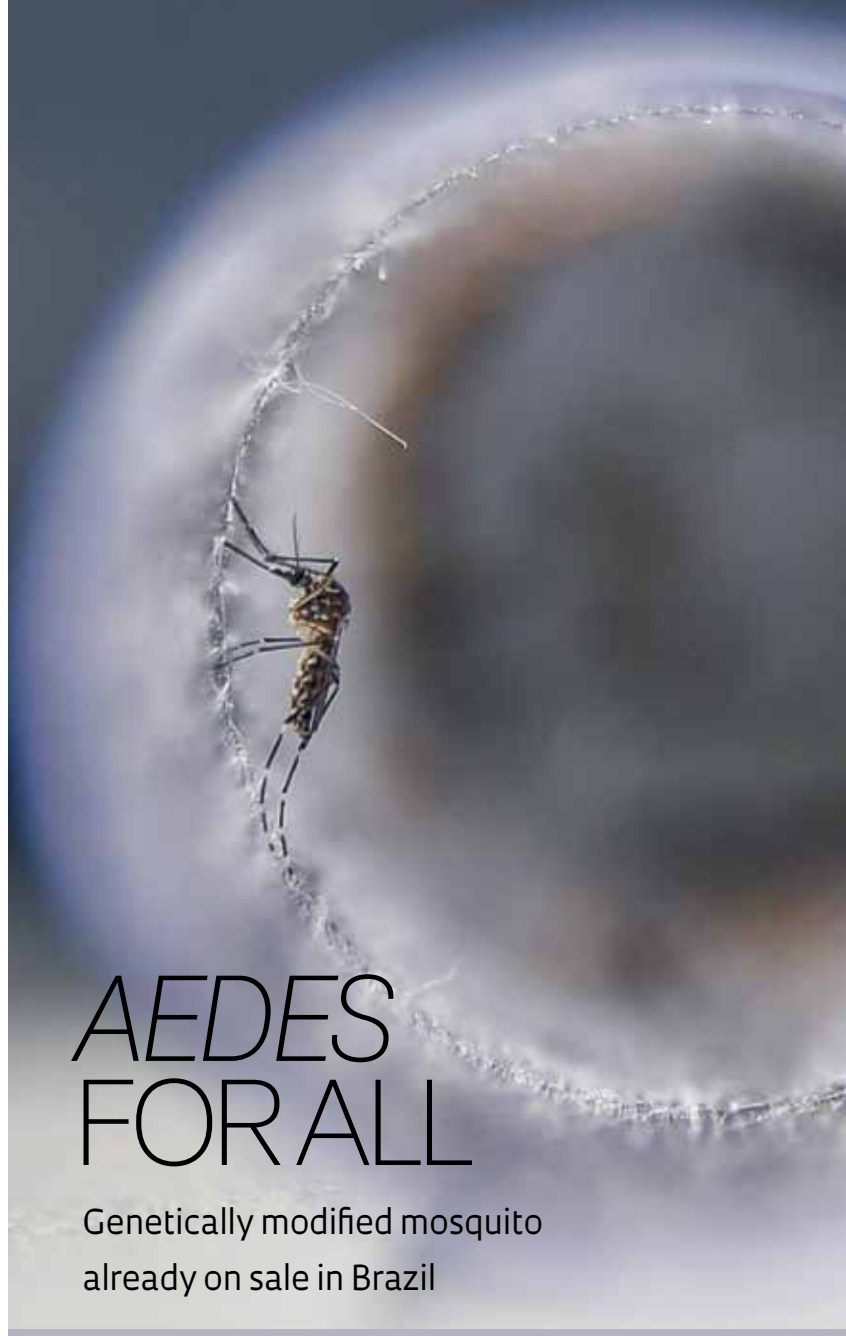
"For sterile males, all you need is to set up a biofactory and pay ongoing production costs; for transgenic insects, you need to pay the company that manufactures them. Transgenic versions, however, make life easier because they eliminate the need for equipment that costs between one and two hundred thousand dollars. How would you have an irradiator in every state in Brazil?" she asks. "It's not feasible. The logistics of the sterilized male *Aedes aegypti* mosquitos requires that they be produced near the irradiator and transported and released within 24 hours."

One difference between the screwworm fly eradicated from the United States last century and *Spodoptera frugiperda* is that the former is monogamous—females mate only once and with only one male. The latter can breed multiple times. Unlike *Aedes aegypti*, which is an exotic mosquito from the region of Egypt, the fall armyworm is native to the American continent. In addition to attacking maize, *Spodoptera* also causes problems for other important crops, such as cotton, soy, wheat, rice, and beans. It feeds on approximately 50 plant varieties from more than 20 botanical families, according to EMBRAPA data.

To eradicate the pest, a public policy would be needed that would promote action across the national territory and even in neighboring countries of the American continent. "Brazil is an enormous country with an extremely long border. We have problems uniting government agencies, companies, and farmers to implement pest monitoring and control strategies," ponders Corrêa. "Eradicating the pest in Brazil would be practically impossible. I don't think that is the company's objective." ■

Scientific article

REAVEY, C. E. *et al.* Self-limiting fall armyworm: A new approach in development for sustainable crop protection and resistance management. **BMC Biotechnology**. Jan. 27, 2022.



AEDES FOR ALL

Genetically modified mosquito already on sale in Brazil

Brazil pioneered the release of transgenic *Aedes aegypti* mosquitoes, with testing carried out in towns in the states of Bahia and São Paulo over the last decade. One controversial article was published in the journal *Scientific Reports* in 2019 about a study in Jacobina, Bahia. The article concluded that the modified insects transferred the transgenic genes to the wild population, generating hybrid mosquitoes. After criticism, the journal attached a note of editorial concern to the paper in 2020, supported by six of its 10 authors (*see* Pesquisa FAPESP *issue no. 285*).

Transgenic *Aedes aegypti* mosquitoes could help contain dengue outbreaks

APU GOMES/ AFP VIA GETTY IMAGES

Two years after the controversy, the Brazilian government authorized Oxitec to sell the genetically modified mosquito throughout Brazil based on a favorable opinion from the Brazilian National Biosafety Technical Commission (CTNBio). "We are introducing Aedes do Bem to a large customer base of both companies and individuals, and it is being adopted by many of them," says Matheus Valério, a biologist at pest control company Detecta. "We have to demonstrate this new concept, because most people are used to conventionally applied products." Headquartered in Campinas, São Paulo, Detecta started selling the product as an Oxitec distributor in October 2022.

The company recommends releasing the mosquitoes for at least eight consecutive months, preferably including October to March. The basic kit consists of two boxes, enough for 5,000 square meters (m²). Each box contains approximately 2,300 eggs, but only 1,200 male mosquitoes are born from each—the females do not survive. The boxes have to be refilled with new eggs every 28 days.

The kit also includes four sachets used to control water quality, which is essential to the mosquito's development. Each box sold by Detecta costs R$460, and a refill costs R$196. Valério highlights, however, that the total cost varies widely. "It depends on the size of the area, whether the product needs to be moved around, and who is taking care of the boxes. Each project is unique."

In the USA, the mosquitoes are still being released on an experimental basis and only in Florida. In 2020, the USA's Environmental Protection Agency (EPA) authorized Oxitec to carry out pilot tests of the technology with its second-generation mosquito (of the OX5034 lineage) in parts of Florida and California.

The American agency, however, took an extra precaution, prohibiting the company from releasing mosquitoes less than 500 m from possible sources of the antibiotic tetracycline, such as sewage treatment plants, farms producing apples, pears, and citrus fruits, and cattle or pig farms. Tetracycline is commonly used in both animal and human health care, as well as in agriculture. Because it can sometimes pass through the body without being metabolized in the digestive tract, it can contaminate the surrounding environment, even at low concentrations.

Oxitec's transgenic mosquito is raised in a tetracycline-rich environment. Similar to *Spodoptera*, the lethal gene introduced is tTAV, made from synthetic DNA based on a fusion of sequences from the bacterium *Escherichia coli* and the herpes simplex virus. "In the lab, high concentrations of tetracycline can inhibit expression of the lethal gene. In the field, the male transgenic insect mates with the female, who then lays the eggs. The eggs hatch and become larvae, which are aquatic. The offspring do not encounter high concentrations of the antibiotic in this medium, so the lethal gene is expressed, and the larvae die. This is a general summary of the process," says ESALQ agronomist Alberto Soares Corrêa.

In a 2022 statement, the EPA argued that there is a "remote chance that environmental sources of tetracycline could have enough tetracycline present to act as a counter agent to the OX5034 female mosquito-lethal trait." The aim is to reduce any chance of transgenic females surviving and reproducing. Only female *Aedes* bite humans and transmit disease.

In Brazil, there are no restrictions on where transgenic moths or mosquitoes can be released. Fernando Hercos Valicente, an agronomist, entomologist, and researcher at EMBRAPA Maize and Sorghum who joined CTNBio while the first version of Oxitec's *Aedes aegypti* was under approval, says the subject was discussed at the time. "Someone mentioned dog food, which can contain tetracycline. But there was a big difference between the amount that can be in dog food and how much is needed for the insect to survive. A much larger dose would be needed. It's not a problem," says Valicente. For him, "the positive results of the tests carried out in Brazil demonstrate the benefit of the technology."

José Maria Gusman Ferraz, a biologist who was also working at CTNBio and voted against approving the transgenic mosquito, says the authorities have been extremely careless and much more research on the impacts on the ecosystem as a whole is needed. "The precautionary principle is not being followed. The release will only be reevaluated if a highly serious problem occurs. But by then it might be too late. When we authorize a product, we don't just authorize the transgenic plant or insect. We authorize a technology that could cause environmental changes." ■ **Frances Jones**

Scientific article

EVANS, B. *et al.* Transgenic Aedes aegypti mosquitoes transfer genes into a natural population. **Scientific Reports**. Sept. 10, 2019.