

# Bioinsecticide made of microorganisms

Worms and bacteria acting together are raw material for a product for commercial use in agriculture

**A**fter 15 years of research, a new technology for biological pest control is ready for commercial use. It is a biopesticide made from nematodes, tiny worms, less than a millimeter in size, that live in the soil. They are to be used in combating insects and other organisms that attack crops such as sugarcane, ornamental plants and eucalyptus. The new biological insecticide was developed by agronomist and entomologist Luís Garrigós Leite, of the Campinas unit of the Biology Institute, under the São Paulo State Department of Agriculture and Food Supply. He began his work in 2002, and the following year, he continued it in partnership with the company Bio Controle, located in Indaiatuba (São Paulo State); the company engages in the monitoring and control of agricultural pests, through a project of the Innovative Research in Small Businesses Program (PIPE), supported by FAPESP. The company is about to market the product to farmers. The nematodes will be sold with the worms wrapped in diatomite, a mineral powder that leaves the worms

moist and in a dormant state. The worms only become active when the product is diluted in water.

“Nematodes are used in the United States and Europe mainly to control soil pests that attack the root, and in cryptic environments, which are enclosed with little light, such as drainage holes made by drills in plants, for example,” says Leite. To create the biopesticide, step one was to isolate and select nematodes of interest. Many of these worms are harmful to a host of agricultural crops such as soybeans and sugarcane. Leite selected the species he considered would be useful, such as worms of the genera *Steinernema* and *Heterorhabditis* and bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively, which form a natural symbiosis to destroy crop pests. “For sugarcane we work mainly with *Steinernema brazilense* to control the weevil [*Sphenophorus levis*] that attacks this plant. For protected cultivation of ornamental plants and mushrooms, we use *Steinernema feltiae* and *Heterorhabditis indica*, and *Steinernema rarum* to control the fungus gnat [*Bra-*

*dysia* sp.]” Despite the name, the fungus gnat is an insect.

Leite says that when the nematodes encounter the insects, they enter the insect’s body through its natural orifices, and once inside, they release bacteria that causes septicemia, which kills the insect within 48 hours. “The symbiosis is an advantageous combination for the two species,” he says. “Bacteria cannot survive in an open environment, only in the intestines of nematodes. In addition to housing them, they can carry them to a new host. In return, the bacteria produce enzymes that digest the insect tissue, providing food for the worm.”

## SCALING UP

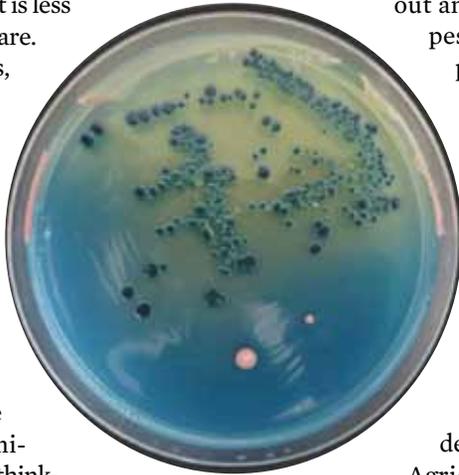
One of the biggest obstacles in creating the new biopesticide was developing a way to produce the nematodes on a large scale, at a cost that would make its price competitive with chemical insecticides. To overcome this obstacle, Leite spent a year, between 2014 and 2015, at the United States Department of Agriculture (USDA). “I worked on developing culture media and processes for the in

vitro production of entomopathogenic nematodes, which have a symbiotic relationship with bacteria; I was trying to make the production viable at a low price and had in mind major crops such as sugarcane,” he says.

Using a culture medium consisting of egg yolk, vegetable oil and yeast extract, Leite says that the nematode production cost is less than R\$10 per hectare. Labor and logistics, for example, must be added to that amount, but even so the product is still price competitive with agrochemicals. “Many Brazilian farmers think that biological control should be cheaper than chemicals,” he says. “That thinking is not prevalent in Europe, where farmers place more value on biological control, because of the restrictions placed on the use of chemicals due to their harmful effects.”

Leite cites other advantages of using nematodes instead of agrochemicals. These include the fact that the insects are not resistant to them, and their use is safe for the environment,

for farm workers and consumers, since the nematodes are not harmful to human health. “The worms are extremely tenacious in the insect environment. When 100 of them invade an insect, they feed and multiply within its body for up to three generations, reaching up to 100,000 individuals. After exhausting this food source, they go out and look for another pest to invade and repeat the process,” says Leite.



“Nematodes are widely used around the world, but still very little in Brazil,” says José Roberto Parra, a specialist in biological control at the Luiz de Queiroz College of Agriculture of the University of São Paulo (ESALQ-USP).

“They are difficult to produce in bulk, because we have not completely mastered the production technology. Once this problem is solved, nematodes will have the same advantages of any microorganism used in biological control,” says Parra. “The technology has been determined and is open to any interested company, but large-scale pro-

duction has yet to evolve. For example, the life span of commercial nematodes is two to three months, and their use depends on the presence of rain, which complicates the logistics of distribution in periods of drought,” says Leite. Bio Controle primarily focuses on sugarcane. “We are the only company that has and is registering a product in Brazil based on entomopathogenic nematodes,” notes Fábio Silber Schmidt, a researcher with the company. “The product is Bio Bacteriophora, which is based on the nematode *Heterorhabditis bacteriophora*; one of its targets will be the sugarcane weevil.” The company anticipates that the product’s final registration will occur between 2017 and 2018, at which time it can be marketed. ■

## Projects

1. *Evaluation of methodologies and techniques for the industrial production of entomopathogenic nematodes and study of market for commercialization of these agents* (No. 2003/02137-1); **Grant Mechanism:** Innovative Research in Small Businesses Program (PIPE); **Principal Investigator:** Carmen Maria Ambros Ginarte (Bio Controle); **Investment:** R\$337,818.00.
2. *Entomopathogenic nematodes: mass production and potential use in biological pest control* (No. 2002/09506-0); **Grant Mechanism:** Regular Research Grant; **Principal Investigator:** Luís Garrigós Leite (Biology Institute-Campinas unit); **Investment:** R\$17,400.00.
3. *In vitro mass production of entomopathogenic nematodes: selection of media, biphasic production, formulation and use of the industrial waste* (No. 2014/00651-4); **Grant Mechanism:** Scholarships abroad – Regular; **Principal Investigator:** Luís Garrigós Leite (Biology Institute-Campinas unit); **Investment:** R\$119,425.19.



Nematode production at the Biology Institute of Campinas. Above, bacteria cultivation