

Insect versus insect

Company that breeds wasps for use in biological control is ranked among the world's 50 most innovative companies

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A startup company created 11 years ago by postgraduate students at the Luiz de Queiroz College of Agriculture (ESALQ) of the University of São Paulo (USP) is one of the world's 50 most innovative companies, according to a ranking produced by the US technology magazine Fast Company. The company, Bug Agentes Biológicos, is headquartered in the city of Piracicaba, in inner-state São Paulo. Bug Agentes Biológicos operates in the area of biological pest control and has developed an efficient method of breeding insects that can decimate other similar creatures that attack sugarcane plantations and other crops. Fast Company's list, published annually, was headed up in 2012 by major multinational players from the technology sector, such as Apple, Facebook and Google.

Bug is the first Brazilian company to be included in the list, ahead of such giants as Petrobras, Embraer and Grupo EBX. In addition, the company ranks third in the magazine's 'top 10' of the biotechnology sector. "This award from Fast Company came as a real surprise", declared agricultural engineer Alexandre de Sene, one of Bug's partners. "The technology used to breed wasps of the species *Trichogramma galloi* which attack a pest that is commonplace in cane fields,

contributed to this placement. But it was also important that we managed, in just two years of marketing this insect, to treat an area of 500 thousand hectares of sugarcane in Brazil," he added.

The action of *T. galloi* is differentiated by this wasp's method of attacking the eggs of a moth known as the sugarcane borer (*Diatraea saccharalis*) by injecting its own eggs into them, thereby preventing the moth caterpillars from hatching and attacking the plant. Other insects used in biological control parasitize already-hatched caterpillars and adults, which have already had a chance to attack the plantation. There is nothing novel about the breeding and marketing of wasps of the genus *Trichogramma*; in fact, the technique was developed decades ago here in Brazil at the Laboratory of Insect Biology at ESALQ-USP.

Bug's innovation was developing an efficient and economically viable method of breeding the *T. galloi* species. "This is harder to do because this wasp develops very well inside the sugarcane borer's eggs, which require a lot of work to be bred inside the laboratory. To overcome this, we used the eggs of an alternative host, a Mediterranean Flour Moth called *Anagasta kuehniella* that is easy to breed, for breeding the wasps. As a result, we managed to breed them on an industrial scale," explains Sene.

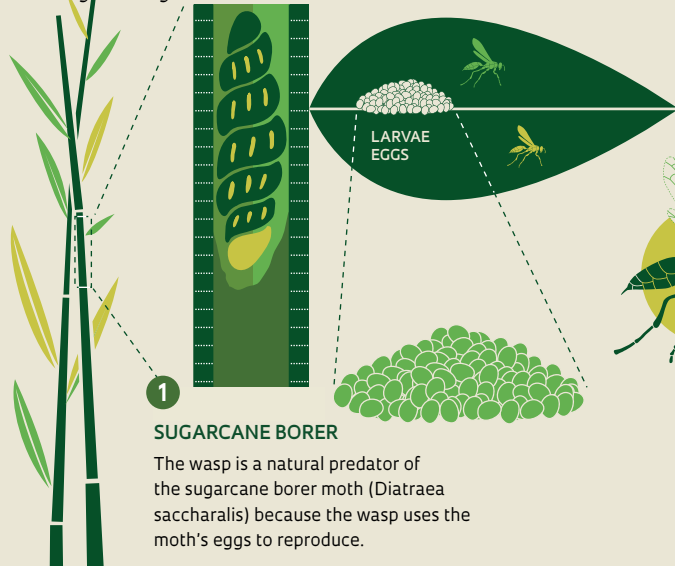
Wasp used in the countryside to control the neotropical brown stink bug (*Euschistus heros*), which attacks soybeans



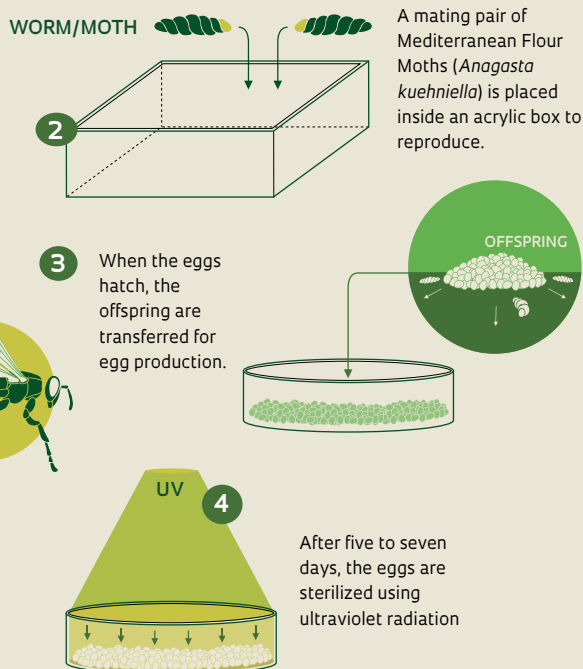
Control of wasps

In only two years, the company Bug used insects to treat 500 thousand hectares of sugarcane. Shown below is the method used to combat the sugarcane borer and the system used to produce the wasps.

Trichogramma galloi



Industrial scale



As the name suggests, biological control employs agents such as insects, acarids, fungi, viruses and bacteria to combat pests that destroy a wide range of crops. In addition to sugarcane, the method is used to treat soybean, corn, fruit plants, vegetables and other crops. Biological control is a component of integrated pest management, a concept that was developed in the US and Europe in the 1960s as an alternative to the application of pesticides for controlling insects and other pests.

The use of living organisms to fight pests is growing in Brazil. Although the precise numbers are unknown, it is estimated that this approach is currently being applied to more than 7 million hectares of crops, producing environmental advantages by reducing the use of insecticides. According to the Brazilian Association of Biological Control Companies (ABCbio), the biological control sector had a turnover of R\$ 250 million in 2010. This figure represents 3% of the pesticide market in Brazil, which posted a turnover of R\$ 8 billion in the same year. "Biological control is one of the few pest control measures that meet the demands of sustainable agriculture, which is so sought after in the world," states professor José Roberto Postali Parra, who coordinates the Laboratory of Insect Biology at ESALQ and is a leading special-

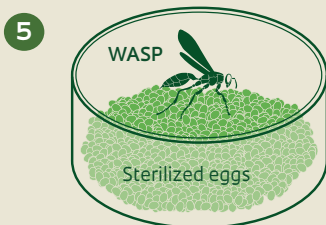
Insects, acarids, fungi, viruses and bacteria are used to combat pests that affect crops

ist on the subject in Brazil. According to Parra, biological control is non-polluting, does not harm farm workers and does not leave residues on food. Furthermore, biological control agents do not need to be applied directly to the pest because they naturally locate their prey in the fields. Finally, this method produces no secondary impacts, such as affecting non-target organisms, and it does not cause the pest to develop resistance.

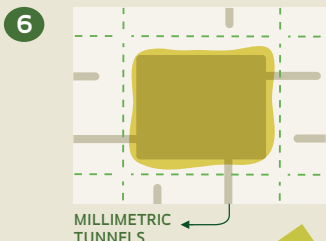
"Most pesticides cannot achieve any of these advantages, and the others fail on some counts, particularly concerning so-called secondary impacts, which can lead to environmental imbalances," states Alexandre Pinto. At present, there are approximately 230 agents used to

biologically control pests throughout the world. In general, these agents are divided into two categories: microbiological organisms (or microorganisms), such as fungi, viruses and bacteria, and macrobiological organisms, which are visible to the naked eye, such as insects and acarids. Macrobiological agents can be classified as predators or parasitoids. Parasitoids are smaller than their hosts, only need one host insect to complete their life cycle, have a free-living adult stage and do not normally kill their hosts until they leave them. Predators, on the other hand, are usually larger than their hosts, require more than one host insect to complete their life cycle and kill their hosts before their life cycle is complete. The technology involved in the breeding and release of macrobiological organisms is usually more complex than that required for the production of microorganisms, which are sold in powdered or granulated formulations.

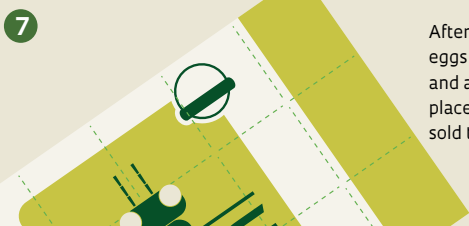
In Brazil, approximately 70 companies market 12 insects and acarids (see page 66), as well as dozens of microorganisms. Another 55 laboratories maintained by sugar mills create macrobiological agents for their own use. Bug's portfolio contains three predator acarids (*Neoseiulus californicus*, *Phytoseiulus macropilis* and *Stratiolaelaps scimitus*) and four para-



Subsequently, the wasps are placed in the box with the eggs of the moth/worm, into which the wasps lay their own eggs



The parasitized eggs are inserted into packages containing miniature tunnels that can hold up to 2 thousand eggs



After six days, the eggs become black and are ready to be placed into packs and sold to farmers



The packs are placed at 20-meter intervals in sugarcane plantations. After the eggs hatch, the wasps leave through perforations in the packs

sitoid wasps (*Cotesia flavipes*, *Trichogramma pretiosum*, *Trichogramma galloi* and *Telenomus podisi*). The company is also currently breeding and investigating four other species: the flower bug

Orius insidiosus, the acarid *Neoseiulus barkeri* and the wasps *Telenomus remus* and *Trissolcus basalidis*, but these have not yet been registered with the official authorities that control this activity.

plastic boxes containing wheat flour and yeast, and the female flour moths lay their eggs in these containers. During a later stage, the eggs are parasitized by the *T. galloi* wasps. The first adult flour moths to emerge are separated, sifted to eliminate the leftover flour and transferred to egg collection boxes. These boxes are productive for five to seven days (the lifespan of the adult flour moth), at which point the moth eggs are collected and sterilized using ultraviolet light, making the embryos unviable. The eggs are then immediately offered to a wasp, which places one or two of its eggs inside each moth egg.

The parasitized eggs are placed in perforated packages made from biodegradable packs with three overlapping layers of cardboard, which were created and patented by Bug. The intermediate layer contains minute “tunnels,” which form capsules that can hold 2 thousand eggs. The packs are sold to farmers, who place them over the plants. The wasps hatch from the eggs and then fly out through the holes in the pack. “The release has to be done every week, for three straight weeks, with an average of fifty thousand wasps per hectare. Since the insect only flies 10 meters during its short lifespan, the packs need to be placed 20 meters from each other,” explains Bug’s partner.

THE PROJECTS

1 Mass raising and marketing of the egg parasitoids *Trissolcus basalidis* and *Telenomus podisi* for the control of soy bugs. 2005/60732-9

2 Study of efficient formulations of conidia of the *Metarhizium anisopliae* fungus for the biological control of pests no. 2005/55780-4

3 Biological control applied to the herbivorous pest *Tetranychus urticae* (Acari: *Tetranychidae*) and the mass production and marketing of *Neoseiulus californicus* and *Phytoseiulus macropilis* (Acari: *Phytoseiidae*) lines that are resistant to agrottoxins no. 2006/56680-6

MODALITIES

1 to 3 – PIPE – Program of Innovative Research in Small Companies

COORDINATORS

- 1 Alexandre de Sene Pinto - Bug
- 2 Ana Lucia Santos Zimmermann – Biocontrol
- 3 Roberto Hiroyuki Konno – Promip

INVESTMENTS

- 1 R\$ 419,460.00 (FAPESP)
- 2 R\$ 42,743.00 (FAPESP)
- 3 R\$ 477,608.27 and US\$ 6,107.56 (FAPESP)

FINANCIAL SUPPORT

In late 2011, Bug merged with another company in the biological pest control sector, Promip, with the aim of increasing the supply of products. As a result of the merger, the company began to sell almost all the species of insects and acarids available in Brazil. Bug and had many commonalities: they were located in the same city, they were both created at ESALQ, and they both had technological innovation projects approved by São Paulo Research Foundation (FAPESP), which helped them become viable. Bug received three rounds of financing from the foundation (see *Pesquisa FAPESP* issue 87), and Promip received one round.

The financial support from FAPESP was largely used to develop breeding schemes for the biological agents, a complex task that also involves breeding the pests. In the case of *Trichogramma galloi*, Bug’s technical specialists had to breed both the wasps and an alternative host, the Mediterranean Flour Moth, in the laboratory. At the beginning of the process, adult flour moths are mated in

When the adult female *T. galloi* finds the sugarcane borer's eggs, she injects her own eggs into them, which prevents the caterpillars from proliferating. In its adult form, the sugarcane borer is a straw-colored nocturnal moth. The females lay their eggs on sugarcane leaves. Subsequently, the caterpillars penetrate the sugarcane, which provides shelter and food, thereby harming the sugarcane fields.

Sugarcane farming is one of the highest adopters of biological pest control in Brazil. "For roughly 50 years, farmers have been using this technology and have included this activity in their production systems," says Parra. The wasp *Cotesia flavipes*, which also parasitizes the sugarcane borer, is the most widely used insect for fighting this pest. The difference between *Cotesia flavipes* and *Trichogramma* is that the former attacks the caterpillar, whereas the latter infects the eggs with parasites before the caterpillars hatch. It is estimated that 4 million hectares of sugarcane fields, approximately half the area under cultivation, are treated with *Cotesia* and *Trichogramma* wasps and with the *Metarhizium anisopliae* fungus. *Metarhizium anisopliae* is also used to fight two other pests, the root leafhopper (*Mahanarva fimbriolata*) and the spittlebug (*Mahanarva posticata*).

Studies show that the combination of *T. galloi* and *C. flavipes* has yielded excellent results. "In areas where the



The parasitoid *Telenomus podisi* in the fight against bug eggs

level of infestation exceeds 15% of the plantation, the simultaneous use of the two wasps is a profitable practice. By releasing the *Trichogramma* for three straight weeks and then the *Cotesia* for two weeks, it's possible to prevent losses of R\$ 935.00 per hectare, discounting the investment. If the farmer chooses to use *Cotesia* alone, the reduction in loss drops to R\$ 674.00 per hectare," states Sene. These calculations, explains the researcher, only consider the value of amorphous refined sugar, the product used by the food industry.

Biological control is also used on more than 2 million hectares of soybean, which is approximately 8% of the total soybean crop area in Brazil. The most widely used product is the *Trichoderma harzianum* fungus, which fights white mold (*Penicillium candidum*), a disease caused by the *Sclerotinia sclerotiorum*

fungus. Along with the *Telenomus podisi* wasp, which parasitizes the eggs of bedbug, the *T. pretiosum* wasp can control leaf stripper caterpillars in an area of approximately 18 thousand hectares. "Soybean growing has terrific potential for biological control, particularly since the ban on the pesticide endosulfan, which was used to control bugs. Without this insecticide, there weren't many chemical options for fighting this pest left open to soybean growers," states Parra.

In an area of 20 thousand hectares (less than 1% of the total area of corn cultivation), corn farmers are using the *Trichogramma pretiosum* wasp against the fall armyworm (*Spodoptera frugiperda*) and the *T. galloi* wasp against the sugarcane borer, which is also frequently found in cornfields. The *T. pretiosum* wasp is employed to control leaf stripper caterpillars in 3 thousand hectares

Army of good

The 12 insects, acarids and worms that are most widely used in biological pest control in Brazil.

* Price of product + freight + application ** Price of product only

Agent	Pest	Crop	Treated area (ha)	Cost (R\$/ha)*
<i>Cotesia flavipes</i> (wasp)	sugarcane borer	sugar cane	3,000,000	25.00
<i>Neoseiulus barkeri</i> (acarid)	broad mites and thrips	vegetable and fruit plants	500	200.00 to 400.00
<i>Neoseiulus californicus</i> (acarid)	two-spotted spider mites	vegetables and fruit plants	500	300.00 to 400.00
<i>Orius insidiosus</i> (beetle)	thrips	vegetables and fruit plants	500	400.00 to 800.00
<i>Phytoseiulus macropilis</i> (acarid)	two-spotted spider mites	vegetables and fruit plants	500	300.00 to 400.00
<i>Deladenus siricidicola</i> (worm)	wood wasps	pine forests	1,000,000	4.50**
<i>Stratiolaelaps scimitus</i> (acarid)	fungus gnats and thrips	vegetables and fruit plants	500	500.00 to 800.00
<i>Telenomus podisi</i> (wasp)	bugs	soybean	8,000	45.00
<i>Trichoderma harzianum</i> (fungus)	white mold	soybean	2,000,000	Unavailable
<i>Metarhizium anisopliae</i> (fungus)	spittlebugs/root leafhoppers	sugar cane	2,000,000	25.00 to 100.00
<i>Trichogramma galloi</i> (wasp)	sugarcane borer	sugar cane	500,000	50.00
	fall armyworms	corn, sorghum	5,000	
	caterpillars and worms	tomatoes	3,000	
	sugarcane borer	corn, sorghum	15,000	30.00 to 60.00
	leaf stripper caterpillars	soybean	10,000	



The wasp *Cotesia flavipes* attacks the sugarcane borer (left), and *Trichogramma pretiosum* parasitizes the eggs of the fall armyworm, a pest that affects corn

planted with tomatoes. Biological controls are also used to combat wood wasps and leaf stripper caterpillars in pine forests. According to Susete Penteado, a biologist with the Embrapa Forest Unit of the Empresa Brasileira de Pesquisa Agropecuária (the Brazilian Agricultural Research Agency) in the State of Paraná, approximately 1 million hectares of pine tree plantations, which is half the national production, is treated with the nematode *Deladenus siricidicola*. This microscopic worm attacks wood wasps by making the females sterile. “We have been breeding this nematode since 1989, and we distribute it to forestry managers/workers in the States of São Paulo and Minas Gerais, as well as in the country’s southern region,” says Susete.

ATTRACTIVE MARKET

Cotesia flavipes is the main insect offered by most Brazilian biological pest control companies, including Biocontrol, which was founded in 1994 in the town of Sertãozinho. “We market *Cotesia* along with the *Metarhizium anisopliae* and *Beauveria bassiana* fungi. These three carry out the work of biological control of pests in the sugarcane fields,” explains Maria Aparecida Cano, a partner in the company who also advises sugar and ethanol mills in the State of São Paulo about the production of insects and microorganisms.

The strength of Brazilian agriculture has attracted multinational agrototoxin companies that are interested in entering the biological products market. These firms include the Japanese com-

“Although they are slower-acting, in the end, biological controls produce the same results as chemical products,” says Santin Gravena

panies Sumitomo and Ihara, the American corporation FMC and the Dutch enterprise Koppert Biological Systems, a global leader in biological agents and pollinators. Koppert was established in the city of Fortaleza in 2009, but last year it switched its base to Piracicaba. “In principle, we set up a branch in the State of Ceará due to its closeness to Europe and its relationship with exporters of melons, a crop that we have a lot of experience in. With the growth in the business, the company moved in order to invest in the creation of products aimed at the Brazilian market,” says agricultural engineer Danilo Pedrazzoli, the company’s managing director and a former founding partner of Bug.

Koppert’s product line includes predator acarids and fungi for controlling pests and diseases, a list that exceeds

50 products in Europe. In Brazil, the company has begun the process of registering 26 products, 5 of which are in the final stage of approval. Pedrazzoli, who is also a director of ABCbio, believes that there is great potential for using biological control in Brazil but that the shortage of efficient companies to meet the demand is a problem.

For agricultural engineer Santin Gravena, the owner of Gravena Pesquisa, Consultoria e Treinamento Agrícola (Gravena Agricultural Research, Consulting and Training), there is another problem: resistance from some farmers. “Brazilian farmers are conservative and were brought up with the mentality of chemical control. Additionally, biological control is slightly slower acting, though in the end it produces the same result as the synthetic chemical product,” states the retired Professor of Entomology from São Paulo State University (UNESP).

Founded in 1993, Gravena specializes in breeding the ladybird *Cryptolaemus montrouzieri*, a predator of the white mealybug, a pest that attacks fruit and ornamental plants. “In the last decade, we have supplied these ladybirds to roughly 20 citrus fruit-producing farms. Right now, unfortunately, we have no more biological control clients. We provide scientific research services and technical studies to around 50 companies and laboratories that hire us to undertake studies of the effectiveness and environmental impact of chemical and biological products used in ecological pest management,” he declared. ■