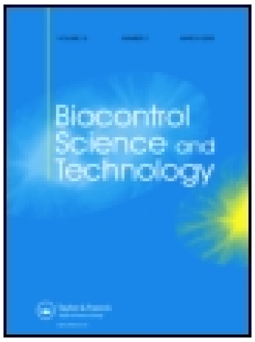

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REVIEW ARTICLE

Biological control of insects in Brazil and China: history, current programs and reasons for their successes using entomopathogenic fungi

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Brazil and China have been successful in the use of microbial control methods to manage several agricultural and forest insects. In both countries, entomopathogenic fungi (EF) have been used for pest management since the 1970s. However, EF production and commercialization have not been constant in either country. Several companies and cooperatives suspended their activities or shut down from the 1970s to the 1990s. This was due to loss of confidence in available mycoinsecticides by Brazilian farmers or due to reduced involvement and government subsidies for biological control in China; and, consequently, mycoinsecticides were largely replaced by inexpensive chemical insecticides. Starting in the 1990s and continuing until today, however, new Brazilian and Chinese private companies have arisen. In Brazil, the area treated with *M. anisopliae* for spittlebug control alone is estimated to be approximately one million hectares in 2008, 75% of which was for control of spittlebugs in sugarcane plantations and the remainder for spittlebugs in pasture grass (primarily *Brachiaria* spp.) and other smaller programs. In China, the fungus *Beauveria bassiana* was used annually in 0.8–1.3 million ha until the 1980s. Several factors were important for the success of these programs, such as: governmental support (at least during the initial steps of biocontrol programs); availability of indigenous virulent fungal isolates; low-cost substrates for mass production; retail prices of mycoinsecticides lower than their chemical counterparts; and sale by contract which allows the products to be immediately available for use, rather than stored. In this report, we discuss the current biocontrol programs using insect fungi in these two developing countries, as well as the future and main challenges they must face to further encourage the adoption of mycoinsecticides.

Keywords: mass production; microbial control; *Metarhizium anisopliae*; *Beauveria bassiana*; mycopesticides; registration

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Introduction

Fungal insecticides (mycoinsecticides) have increasingly enhanced roles in integrated pest management (IPM); and a number of commercial products are making their way to the marketplace, particularly in countries such as China, Brazil, and Colombia. Based on recent statistics of fungal insecticides and acaricides, over 170 products were developed in the last three decades, ca. 75% of which are currently in the process of being registered or are in use without registration (Faria and Wraight 2007).

It is remarkable that 43% of all commercial mycoinsecticides and mycoacaricides are marketed in Latin America, notably in Brazil. Chinese mycoinsecticides were not listed in previous publications due to the noncommercial status of most of the products. As pointed out by Gelernter (2007), Chinese mycoinsecticides usually are produced by very small regional companies whose existence is largely ignored by market surveys; and, therefore, there is a serious lack of official statistics. Also, readers may experience difficulties in citation of Asian references. Nevertheless, these two countries manufacture thousands of metric tons of fungal insecticides yearly, making their microbial control programs among the largest and most successful globally.

The Brazilian history concerning the use of entomopathogenic fungi (EF) has astonishing similarities with that of the Chinese:

a) In both countries, governmental support was essential for establishment of most biocontrol programs, at least in the beginning. For instance, government support was fundamental for initial research, mass production and field applications of the first successful microbial pest-control program in Brazil.
b) Massive use of EF in China and Brazil started in the 1970s.
c) A considerable number of small Brazilian companies and small Chinese fungus-production plants disappeared in the 1980s, although for different reasons. In Brazil there was distrust of mycoinsecticides by farmers, primarily due to low quality of most products, poor dosage recommendations and, therefore, unreliable field performance. In China, on the other hand, there was primarily reduced government involvement and funding, as well as lack of private enterprises during the early stages of the national economic-structure reform.
d) Abundant biodiversity in their vast territories, providing them with indigenous virulent fungal isolates as candidates for control of various pests.
e) Local availability of substrates at comparatively low prices, such as rice, wheat, corn, soybean, types of bran, and powders of silkworm pupae and fish.
f) Seasonal production of mycopesticides and sales under contract for immediate use, with all preparations being conidia-based.
g) Unsophisticated, labor-intensive mass-production techniques, suitable for developing countries with relatively cheap labor. In many instances, this allows sale of mycoinsecticides that are cheaper than chemical insecticides.
h) Details on Brazilian and Chinese commercial production or research on mass production systems are seldom published, with the exception of a few review papers (Feng, Poprawski, and Khachatourians 1994). For example, the entire world literature on mass production of entomopathogenic fungi, based on ISI Web of Knowledge^{SM} as of February 2009, is less than 50 references. In

addition, there has been a small number of papers on mass production published by Brazilian and Chinese scientists in their native languages, some of them appeared in unofficial reports that are not readily available and/or without English abstracts (Li 1974; Guagliumi, Marques, and Villas Boas 1974; Aquino, Cavalcanti, Sena, and Queiroz 1975; Aquino, Vital, Cavalcanti, and Nascimento 1977; Li, Lü, and Tao 1981; Quintela, Yokoyama, and Roberts 1992).

However, some unique differences in the development of fungal insecticides between China and Brazil can be pointed out, some of which are based on differences in the political and economical systems:

a) The main EF in China is *B. bassiana*, and its production is by biphasic fermentation, while in Brazil, the main pathogen, *M. anisopliae*, is mainly produced via solid-culture techniques.
b) Chinese production units are mostly public, while Brazilian companies are all private with the exception of a few municipally-owned plants.
c) In Brazil, the delivery of information to farmers was mainly performed by state scientists and research institutes, while in China it was done mostly by obligatory administrative extension.

History

China (from ancient times to the 21st century)

Antecedent reports

The earliest record of *B. bassiana*-infected silkworms was as a folk medicine and can be traced back to Classic of Herbal Medicine, an ancient Chinese literature (221–220 BC). In the Middle Ages, *Cordyceps sinensis*-infected hepialids, *C. soblifera*-infected cicadas, as well as *B. bassiana* infected-silkworms were all recorded as medicines in the famous Bencao Gangmu (Book of Medicinal Herbs). This book was written by Shizhen Li (1513–1598), an ancient pharmacologist, and published in 1596 with a reedit in 2006 (Li 2006). However, use of EF against insect pests was not recorded in China until the mid 1950s. In 1956, Lin (1956) field tested *B. bassiana* against the sweet potato leaf weevil, *Cylas formicarius*, in Fujian, southeast China, followed by Xu (1959) who tried this fungus against the soybean moth, *Grapholitha glycinivorella*, and Li et al. (1981) against the Masson's pine caterpillar, *Dendrolimus punctatus*, in 1959.

Target pests and biological programs

In the last century, more than 60 pests were targeted with *B. bassiana* with different degrees of success. The main targets were the forest pests: Masson's pine caterpillar and other pine caterpillars, *Dendrolimus* spp.; coast oak tussock moth, *Lymantria xylina*; bamboo zygaenid, *Artona funeralis*; and green elm leafbeetle, *Pyrrhalta aenescens*; as well as the crop and fruit pests: Asian corn borer, *Ostrinia furnacalis*; common rice leafhopper, *Nephotettix cinctictis*; brown planthopper, *Nilaparvata lugens*; small peach borer, *Carpocina nipponensis*; lesser tea leafhopper, *Empoasca* *flavescens*; sweet potato weevil, *Cylas formicarus*, and greenhouse whitefly, *Trialeurodes vaporariorum*. Other fungi have been tried in a few cases against different insects in comparatively small scale field tests or even in laboratory tests. For example, *M. anisopliae* was tried against sweet potato leafbeetles, *Colasposoma dauricum*, and mosquitoes (Xiong and Wu 1981), and *N. rileyi* against noctuids in cotton (Anonymous 1975).

Use of *B. bassiana* against pine caterpillars and the Asian corn borer, with an annual application area in the 1980s usually around 0.8–1.3 million ha, achieved great recognition. The applications were of pure conidia, oil dispersions (conidia in emulsifiable oils), unformulated culture powders containing conidia and substrates, and conidial suspensions made with either milled or non-milled culture powders. The applications were primarily by regular manual or mechanical sprayers and dusters, and rarely by airplane.

In addition to spraying, some unique application methods were developed to reduce costs for forest pest control by *B. bassiana*, including:

1. *Conidial fireworks.* A firecracker was wrapped with pure conidial powder, and manually thrown up to the top of the forest canopy where it exploded, spreading conidial dust over the canopy. For release in very tall crowns or on steep mountains, a mortar-like apparatus was used to launch the firecracker over the canopy. Recently, more sophisticated mortars have been developed specifically for launching bombs containing 1–2 kg of conidial powder up to altitudes of 100–250 m and distances of 70–500 m. A group of three workers normally launched 100–200 bombs covering 33–40 ha of forest once a year (Li 1974).
2. *Use of explosives.* A black powder explosive was used at some forest farms. The explosives were placed on the bottom of a big pit and then conidial powder was poured over the explosives. Exploding the black powder spread the conidia over a wide area. Due to security considerations, this method was discontinued in the mid 1980s. Furthermore, this method may have damaged the conidia, but the damage was never evaluated (Li 1974).
3. *Release of fungus-inoculated caterpillars.* Workers walked in pine plantations carrying the conidial powder or conidial suspensions, collecting healthy caterpillars on their way, dipped them in the powder or in the suspension and then released the inoculated pine caterpillars to induce epizootics.

Mass production

Li et al. (1981) conducted trials on submerged fermentation of *B. bassiana* in the early 1960s and found that the blastospores produced in liquid culture were short-lived and, therefore, with no practical value. Consequently mass production by liquid culture of the fungus for the microbial control of pine caterpillars was discontinued.

In 1969, the pine-caterpillar studies were resurrected in Xinhui County, Guangdong Province, with the development of solid mass production on inexpensive and easily obtainable grain by-products. As a result, nearly 2000 ha of Masson's pine plantation were treated by spraying conidial mists and dusts, as well as releasing living pine-caterpillar larvae pretreated with the fungus. Satisfactory results were obtained (Pu and Li 1996).

In the spring of 1970, in a demonstration of powerful administrative support for biological control, the Ministry of Agriculture and Forestry held a workshop for 13 southern provinces to explain and teach the simple technology needed for use of fungi in pest control. Under the obligatory administrative instruction and the direct (subsidy to producers) or indirect (subsidy to users) financial support of central and local governments, hundreds of small plants (mostly province-owned) of *B. bassiana* appeared throughout China. *B. bassiana* and other fungi were tested against dozens of forest and crop pests, making 1970 a milestone in the Chinese history of microbial pest control. During the 1970s and 1980s, a three-stage solid culture technique was used to produce large quantities of mostly non-formulated fungal insecticides. No official statistics of production and application are available. Since the early 1970s, various other fungi were tried using similar solid culture technology for mass production, including *B. brongniartii*, *M. anisopliae*, *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus)*, *I. farinosa* (formerly *Paecilomyces farinosus*), *Nomuraea rileyi*, *Hirsutella thompsonii*, *Lecanicillium* spp. (formerly *Verticillium lecanii*), *Aschersonia papillata*, and *Zoophthora radicans.*

The first step of the low-tech three-stage solid-culture production used slices of autoclaved white potato (as a substitute for agar slants) in test tubes. After growth and sporulation, the slices were transferred to wide-mouth jars of autoclaved substrate such as wheat bran. Various closed wide-mouth containers or pots were used for production of stock cultures. These stock cultures were used as inocula for production of larger scale shallow-tray culture, or even used as final product for field tests. Several types of bamboo or wooden shallow trays or mats were used for semi-closed or open cultures. Starch-rich grains or grain products were used as media, mainly wheat bran, sometimes mixed with rice bran or corn meal. Two notable simple and cheap techniques were: (1) soil-bed solid culture in the open air: autoclaved substrate was spread on sheets of autoclaved paper on a prepared outdoor-soil bed that was sheltered against direct sunlight similar to seedlings or transplants. The quality of the culture was good, and without obvious contamination. (2) Lime-water culture: 3% lime water, instead of pure water, was added to substrate, which was not autoclaved. The initial substrate pH was up to 11 and gradually went down to 6.5 at the end of the cultivation (Anonymous 1977). No contamination occurred, but sporulation was slightly lower. These techniques were used for a short period, but not widely.

The products were mostly fungus-colonized substrates (technical concentrates), or roughly formulated wettable powders with added fillers, such as gaolinite or yellow subsoil (Li 1974). The cost of low-tech mass production was very low, which allowed the biocontrol program to develop rapidly. Conidial content of some products was fairly high (up to 2×10^{10} per gram dried culture). However, quality was very inconsistent, with conidial yields below 10^9 for some batches and frequent media contamination. In addition, problems related to allergy were serious due to inhalation of conidia when fungus-colonized substrates were ground and packed in inadequately enclosed mills in poorly ventilated rooms.

In the 1980s, however, the situation gradually changed. This was partly due to reduced direct governmental financial support and to the availability of cheap chemical pesticides. The last 20 years of the 20th century were not easy for EF-based programs as technical, economic, and even administrative issues contributed to substantial ups and downs in EF programs. Most EF public plants shut down, with less than 5–10 factories surviving this phase in China. Only 16 companies or plants which produce 11 registered and non-registered fungal insecticides are currently operational in China.

Brazil

Antecedent reports

In Brazil, EFs have been studied since the 1920s when *Penicillium anisopliae* (later identified as *M. anisopliae*) was found attacking two spittlebug species (Pestana 1923). In 1925, an isolate of *M. anisopliae* from Trinidad was introduced into the country. It was tested against the cercopid *Mahanarva fimbriolata*, but without success (Moreira 1925), even though apparent successful results were reported in Trinidad and Tobago years earlier for control of cercopids (Rorer 1910). In 1935, Dr Vera K. Charles received from Dr Charles H.T. Townsend mummified lace bugs (*Leptopharsa heveae* Hemiptera: Tingidae) from Pará state, Brazil, and she identified the fungus as *Hirsutella verticillioids* (Charles 1937). Later, Dr James R. Weir also sent cadavers of lace bugs to Dr Vera K. Charles with the comment that the fungus had practically decimated *L. heveae* over wide areas of rubber tree plantations (Charles 1937). In the 1930s, *Verticillium lecanii* (Zimm.) Viégas (= *Lecanicillium* spp.) was found to be controlling the scale insect *Coccus viridis* on coffee plants (Viégas 1939). In the 1940s, *B. bassiana* was studied to control the coffee borer (Mesquita 1944). *B. bassiana* was also found in several natural epizootics in *Brassolis sophorae*, a lepidopterous pest of coconut trees (Mariconi and Zamith 1954; Habib and Andrade 1977). In the 1960s, several authors reported natural outbreaks of the green muscardine disease caused by *M. anisopliae* on the sugarcane leaf spittlebug *Mahanarva posticata* in Campos, state of Rio de Janeiro (Albert 1964; Ferreira-Lima 1964; Freire, Souto, and Marques 1968; Guagliumi et al. 1974; Alves 1998b). In 1964, there were reports of sugarcane spittlebugs infected with *M. anisopliae* in the state of Sergipe, in the northeastern region (Veiga, Aquino, and Arruda 1972). Starting in 1969, with the introduction of *M. posticata* in other northeastern states, *M. anisopliae* became more intensively studied by several research institutes, such as *Comissão Executiva de Defesa Fitossanitária da Lavoura Canavieira de Pernambuco* (CODECAP), *Instituto Pernambucano de Pesquisa Agropecuária* (IPA), and *Instituto do Açúcar e do Álcool-Programa Nacional de Melhoramento da Cana-de-Açúcar* (IAA/PLANALSUCAR) (Guagliumi et al. 1974; Alves 1998b). Epizootics of the green muscardine caused by *M. anisopliae* in pasture spittlebugs (*Notozulia entreriana* and *Deois* sp.) were also detected in the state of Espírito Santo (Ventura and Matioli 1980).

Target pests and biological programs

The spittlebugs *M. posticata* and *M. fimbriolata* are among the most important pests of sugarcane in Brazil. *M. posticata* is the principal species in the northeastern region and the root spittlebug *M. fimbriolata* has gained importance recently as a sugarcane pest in southeastern Brazil. In 1967, an FAO entomologist, the Italian Pietro Guagliumi, arrived in Brazil to coordinate biocontrol strategies for leaf spittlebug control in sugarcane fields in a few northeastern states. In 1969, a program on the biological control of this pest was started. Initially, the focus was on egg parasitoids and predatory dipterans, but due to poor field results observed in other regions of the world, fungi were not included. This same year, however, a high proportion of nymphs and adults of the root spittlebug in sugarcane fields in the northwestern state of Alagoas were found to be infected by a fungus. Thereafter, following successful laboratory and field trials, *M. anisopliae* was integrated as an important component of this biocontrol program (Guagliumi et al. 1974). Studies on mass production of *M. anisopliae* were sponsored by the state government and conducted by CODECAP and IPA between the 1970s and 1980s (Pinto 2006). In other states, such as São Paulo, farmers founded several cooperatives in the 1970s and produced *M. anisopliae* to control spittlebugs: *M. posticata* and *M. fimbriolata* in sugarcane fields and *N. entreriana* and *D. flavopicta* in pastures. In 1972, CODECAP treated 500 ha with *M. anisopliae* to control the sugarcane leaf spittlebug in the northeastern region. By 1982, the *M. anisopliae* treated-area had increased 44 times to 22,000 ha. In the state of São Paulo, IAA/PLANALSUCAR treated 2,000 ha in 1977 with *M. anisopliae* to control *M. posticata*, and by 1982, this area had increased to 85,000 ha (Alves 1998a; Pinto 2006). Between 1977 and 1991, the cumulative sugarcane area treated with *M. anisopliae* for *M. posticata* control was 670,000 ha in the state of Alagoas (Alves 1998a).

In Brazil, the credibility of EF programs was negatively affected by factors such as low conidial viability. Other technical problems included the short shelf-life under the warm conditions prevailing in the region, poor recommendations to users (of particularly inadequate dosages), and in some cases, contamination by other fungi such as *Penicillium* during spore production (Roberts and St. Leger 2004). These conditions led several companies to close their production facilities. Some Brazilian farmers became dissatisfied with the efficacy of the fungi, especially the lower speed of kill and unpredictable field efficiency as compared to traditional chemical insecticides.

Mass production

The use of rice for mass production of *M. anisopliae* was started in Trinidad and Tobago (Rorer 1910). In 1969, mass production of conidia in Brazil was done primarily in wide-mouth glass milk bottles containing 100 g of rice and 160 mL water (Aquino et al. 1975; Mendonça 1992). The development of mass production technologies was intensified during the 1970s. During 1970–1972, CODECAP produced about 4.6 tons of fungus-colonized rice using 21,000 glass bottles (Guagliumi et al. 1974). In 1977, however, conidial production turned to polypropylene bags each containing 300–400 g of rice grains (Aquino et al. 1977; Mendonça 1992). In the 1990s, some companies started using plastic trays (Alves and Pereira 1989). For *M. acridum* and *M. anisopliae*, reported yields varied from 2.5 to 6.0×10^9 conidia g^{-1} of colonized substrate, respectively (Quintela 1994; Magalhães and Frazão 1996). In large-scale trials using trays, yields reported for *M. anisopliae* varied from 6.1×10^8 to 1.1×10^9, while for *B. bassiana* they reached 6.2×10^9 conidia g^{-1} substrate (Alves and Pereira 1989). Other substrates such as corn flour, cassava flour, rice powder, potatoes, soy bran, wheat bran and others were also tested, but with relatively poor conidial production (Guagliumi et al. 1974).

Current status (21st century)

Despite the fact that the potential of different EF to control a considerable number of pests has been demonstrated in the laboratory, the number of established biocontrol programs using fungal pathogens is somewhat restricted. In Brazil, 40 commercial mycoinsecticides produced by 19 commercial companies were available in 2007 (Faria and Wraight 2007), and an even larger number of manufacturers mass produced *Metarhizium* for their own use. Despite the hurdles, EF-manufacturing plants survived the difficult times. The market nowadays has become attractive to investors, due especially to the small investments needed, simple technology and quick economic return in some regions.

Successful field trials and demonstrations of environmental and economic advantages, have gradually convinced a great number of farmers, technical staff, and government officers that mycoinsecticides have potential and do keep pests below economic injury levels under certain conditions, despite their slower action and lower field efficiency compared to chemicals. This slow cultural change has supported the increasing adoption of these products.

Current mass production, biocontrol programs, and product registration in China

Mass production

EF companies have been attempting to improve the quality of their labor-intensive low-technology mass-production systems. Various biphasic and semi-solid fermentation techniques have been tested in order to increase conidial yield, reduce contamination, and enhance mechanization, but few of them have succeeded. Heat drying is often used, but usually ends with water content in the products of over 10%. In some *B. bassiana* plants, the final culture is exposed to sunlight for quick drying, which may lead to inactivation of conidia. In the mid 1980s, a few Chinese production units developed vacuum extracting equipment that have helped *B. bassiana* plants greatly reduce allergy problems caused by inhalation of conidia. None of the mycoinseticides produced by state-owned plants are registered and most of them have not been formulated, as required by the Regulation of Pesticides of the P.R. China. On the other hand, two new private companies equipped with modern large liquid and solid fermentors or other fermentation facilities have emerged in recent years. As of 2008, six products based on *B. bassiana* and five on *M. anisopliae* have been registered, mainly by these two new companies (Table 1). These products are sold nationwide, in contrast to the local sales of the remaining state-owned plants that are supported by their respective provincial forestry departments through subsidies to users. In recent years, the National Bureau of Forestry has also directly subsidized a few province-owned *B. bassiana* plants to help them improve their mass production facilities.

Since the 1990s, the annual application area with *B. bassiana* for forest pest control is about 500,000 ha and uses approximately 1000 tons of unformulated dried culture (Song and Huang 2006). For locust and grasshopper control with *Metarhizium acridum* in northern China, two large Ministry of Agriculture-supported companies are producing 1000 tons of oil formulation to spray 200,000 ha each year (Zhang, Peng, Wang, Yin, and Xia 2005).

Table 1. Partial list of commercial mycoinsecticides and mycoacaricides developed for control of pests in China.

Trade name[1]	Propagule(s)/Formulation See definitions (last page)	Claimed Target(s)	Manufacturer
Beauveria bassiana			
No trade name	Conidia/Technical material (pure conidia)	Not specified	Hefei Pesticide Co.
No trade name	Conidia/Technical material (pure conidia)	Pine caterpillars	Tianren
No trade name	Conidia/Oil dispersion	Pine caterpillars	Hefei Pesticide Co.
No trade name	Conidia/Technical concentrate (non-woven fabric band)	Pine sawyer	Tianren
No trade name	Conidia/Wattable powder	Pine caterpillars	Tianren
No trade name	Conidia/Oil dispersion	Lesser green tea leafhopper, aphids	Shunhong
Metarhizium anisopliae			
No trade name	Conidia/Technical material (pure conidia)	Not specified	Tianren
No trade name	Conidia/Ultra-low volume suspension	Grasshoppers	Chongda
No trade name	Conidia/Technical material (pure conidia)	Not specified	Kedewude
No trade name	Conidia/Ultra-low volume suspension	Grasshoppers	Kedewude
Keluoka	Conidia/Ultra-low volume suspension	Grasshoppers	Chongda

Source: ICAMA (2008). Pesticide Manual – Electronic Edition for Enterprises. November 28th, 2008. http://www.ny100.cn *in Chinese.*

Definitions (From Faria & Wraight 2007): Technical material (TC). 'An active ingredient isolated (as far as is practicable) from the starting materials, solvents, etc., used to produce it' (FAO/WHO 2002). For entomopathogenic fungi, the starting materials are usually the liquid or solid culture substrates. Technical materials are usually the basis for all other formulation types, although in some circumstances they may be used as end products. According to the CropLife definition, technical materials may include 'associated impurities and small amounts of necessary additives'. Purification 'as far as practicable' is generally considered to result in impurity residues comprising $<10\%$ of the product weight (T.S. Woods, 2006. Chair, Specifications Expert Group, CropLife International, personal communication). In our understanding, conidia or other propagule types isolated from the culture together with associated impurities would fall into this category, also referred to as technical powder (Burges and Jones 1998). Technical concentrate (TK). A material consisting of the active ingredient together with related byproducts of the production process and free of any added modifying agents except for small amounts of stabilizers and free-flow agents, if necessary. This definition is a slight modification of the definition presented by FAO/WHO (2002) for bacterial technical concentrates. According to FAO/WHO, a TK should also be free of 'visible extraneous matter', but the term extraneous matter is not defined, and in our judgment, the FAO/WHO definition fits fungal biopesticides that include components of the spent culture media, e.g., fungus-colonized cereal grains or whole-culture broths. In these cases, there have been no attempts to separate the active ingredient from the substrate. Included in this category, fungus-colonized solid substrates may contain variable proportions of sporulating mycelia and spores, depending on factors such as age of culture and batch. In some countries they are routinely used as end-products through direct incorporation into soil. Alternatively, the active ingredient may be extracted before application (e.g., by washing and sieving, often with the aid of surfactants). In our paper, for all technical concentrates based on solid substrates we consider the propagule type as being conidia + hyphae (C + H), although frequently the vast majority of propagules at the time of sale may be either spores or hyphae. For products produced in liquid media, mixtures of submerged conidia, blastospores, or hyphae may be present. Oil miscible flowable concentrate (=oil miscible suspension) (OF). 'A stable suspension of active ingredient(s) in a fluid intended for dilution in an organic liquid before use'. Ultra-low volume (ULV) suspension (SU). 'A suspension ready for use through ULV equipment'. Oil dispersion (OD). 'A stable suspension of active ingredient(s) in a water-immiscible fluid, which may contain other dissolved active ingredient(s), intended for dilution in water before use'. In practice, oil dispersions contain emulsifiers to render the mixture miscible in water for spraying (T.S. Woods, personal communication). The word 'stable' in this and other of the abovementioned formulations indicates that the active ingredient does not settle out to a nonresuspendable cake during storage (T.S. Woods, personal communication). Here, we consider the definition to include suspensions that tend to settle, but which are designed to be readily resuspendable by the user via manual agitation. Oil dispersions of entomopathogenic fungi have been referred to most commonly in the literature as emulsifiable suspensions or emulsifiable oil suspensions and identified by the abbreviation ES. However, under the Croplife International code, the abbreviation ES refers to emulsions for seed treatments.

Biocontrol programs

Pine caterpillars and corn borers are still the primary targets for fungal insecticide applications (Feng et al. 1994; Li 2007). There are, however, several other fungus-based control programs worthy of note. For example, in this century, grasshopper populations have been one of the main targets for EF. They are controlled by *M. acridum* during their frequent outbreaks on grasslands in northern China, and in paddy rice fields and bamboo plantations in southern China. Fungal control of pine sawyers (Coleoptera: Cerambycidae), the main vector of nematode induced pine wilt disease, have been accomplished through *B. bassiana* applications. *M. anisopliae* has also been tested against the coconut bud leafbeetle (Coleoptera: Chrysomelidae), *Brontispa longissima* (Wu et al. 2006).

1. *Pine-caterpillar control by* B. bassiana. Applications of conidial suspensions are labor-intensive, especially in pine plantations on rugged and water-deficient hills. Comparatively, dust applications are more convenient and economically feasible, and therefore, have been widely used in large areas. Pure conidial preparations are preferred due to their smaller volumes and light weight, which allows the spore to be carried by the wind for long distances and over the tree canopy. Field efficacy varies greatly from very poor to over 80% pine-caterpillar mortality.

Due to inaccessibility of the rugged and thorny forests, it was difficult to inundatively treat the forests using ground sprayers; however, populations of the caterpillars of the whole area declined dramatically, regardless of the application method used, inundative or inoculative. After an application, low insect population densities persisted for at least 3–5 years, showing a strong dispersal capability of *B. bassiana*. This is a very important property for sustainable control. Frequent inoculative releases of *B. bassiana* caused little fluctuation of pest populations and resulted in long-term pest suppression, while inundative releases caused sharp population fluctuations and resulted in shorter suppression (Liu and Han 1995; Han et al. 1996; Han and Li 1997). Accordingly, pine-caterpillar control using *B. bassiana* currently is through inoculative releases. The current cost for one inoculative release is around US$ 2 to 3.3/ha, while one inundative release is around US$ 10/ha.

2. *Corn borer control by* B. bassiana. The Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Pyralidae), is treated annually with *B. bassiana* in hundreds of thousands of hectares of corn fields, especially in northeast China. Dustable powders (conidia with dried media or other filler), conidial suspensions, or fungus-colonized substrates are mechanically or manually delivered to corn foliage at the bell-mouth stage. The dose for

dust applications is 2.5 kg (5×10^8 conidia g^{-1}) per ha. It is easier, however, to treat corn-straw stacks to take advantage of the behavior of corn borers overwintering inside corn stalks: the overwintering insects need supplemental water before they end their hibernation and start pupation. Once they crawl out of the stalks in May, if *B. bassiana* is present, they come in contact with conidia and become infected. Farmers can spray conidia onto corn straws, layer by layer, while they are making up the stacks, or they can spray into the holes in the stacks made by poles pushed into the stacks, at a dose of 100 g culture powder per M^3 stack, with current cost at US$ 5 or more per ha.

3. *Grasshopper control by* M. acridum. Large scale application of this fungus against these pests started at the beginning of the current century. The oriental locust, *Locusta orientalis*, was successfully controlled in ca. 100,000 ha field trials in grasslands of northern China, by ultra-low ground or air application of conidia suspended in oil. The industrial scale mass production of the conidial oil suspensions started with direct support of the central government. Their registered products were also used against rice grasshoppers, bamboo grasshoppers, and Tibetan locusts. In addition, vast areas of pasture in Inner Mongolia and other northern provinces were also treated with different preparations of the fungus.

4. *Aphid and leafhopper control by* B. bassiana. An oil suspension containing 10^{10} conidia/mL was recently registered for control of various vegetable aphids, mainly the peach aphid, *Myzus persicae*, and one of the main pests in tea orchards, the lesser tea leafhopper, *Empoasca flavescens* (Xu, Feng, and Ying 2002; Dun, Feng, and Ying 2003; Ying, Feng, Xu, and Ma 2003).

5. *Pine sawyer control by* B. bassiana. Pine wilt disease caused by the pine wood nematode, *Bursaphelenchus xylophilus*, was first detected in Nanjing, southeast China, in early 1982. It has spread to almost every southern province, resulting in substantial economic and ecological losses. Due to environmental and economic considerations, it is impossible to spray the pest nematode directly. Accordingly, control of pine wilt disease has turned to control of *Monochamus alternatus*, the vector of the pine wilt disease nematode. This system utilizes, in a combination with sawyer traps, a novel preparation based on non-woven fabric bands impregnated with *B. bassiana*. Large numbers of adult sawyers were attracted to the trees with the baited traps, and many of these insects contacted the *B. bassiana*-impregnated fabric bands. This resulted in numerous epicenters of disease with large-area suppression of the sawyers. In 2006, the technique was expanded to ca. 6,000 ha of pine plantations, resulting in obvious beetle-population decline. The treated area was further expanded in 2007.

Product registration

Most mycoinsecticides are not registered in China. Public production facilities have difficulty obtaining official approval to operate as pesticide enterprises, since the central government is limiting the number of pesticide companies. In addition, the procedure is too complex for most small plants. Although the registration agencies treat the registration of microbial pesticides somewhat differently than chemical pesticides, the registration procedure is still slow and expensive. A temporary registration under the guideline published in 1997 will cost between US$ 30,000–50,000. The required tests, run by authorized institutions, include data on safety, toxicology, and ecology. In addition, 2-year field efficacy tests at four different places are specified, suggesting that at least 2 years of research are needed prior to submission for registration. Regulation has not been strictly enforced, and most products are still sold and used without registration.

Current mass production, biocontrol programs, and product registration in Brazil

Mass production

In the last decade, there has been a renewed interest in fungi as biological control agents in Brazil. Several new private companies, grower cooperatives, rubber tree farmers, as well as sugar and alcohol mills have started producing *M. anisopliae*, *B. bassiana* and '*Sporothrix insectorum*'. There is no official data on the production amounts or area treated with entomopathogenic fungi in Brazil. From the most recent published data available (based primarily on the largest companies), it was estimated that total production of fungus-colonized substrates was approximately 2,500 tons, enough to cover ca. 500,000 ha (Alves, Leite, Batista Filho, Almeida, and Marques 2008a). Considering the difficulties in obtaining accurate information on EF production from all sugarcane mills and private and public companies, it is assumed that the area sprayed with EF is much higher, probably reaching around one million hectares during recent years. This is consistent with more recent data from a survey carried out in February 2008 by D.E.N. Rangel revealing that 1,882 tons of *Metarhizium*, 95 tons of *Beauveria*, and 12.5 tons of '*Sporothix insectorum*' were produced per year in just 10 sugarcane mills or private companies in the states of Alagoas and São Paulo (unpublished).

Rice grains continue to be the most used substrate for conidial production. Most companies use from 300 to 500 g of rice per plastic bag. The amount of water per kg of rice is variable. Most commonly, rice is soaked in water, drained and transferred to plastic bags. Following sterilization, conidial suspensions (ca. 5 mL suspension of 10^7 conidia mL^{-1} per 300 g-rice-bag) are used to inoculate each bag of sterile rice. Usually, bags are hand mixed to evenly distribute the inoculum and to disaggregate clusters of rice formed during autoclaving. If plastic bags with substrate are not massaged after spore inoculation, there is an inadequate colonization and poor sporulation results. Incubation temperatures range from 25 to 30°C and incubation time is normally about 14 days. After conidial production, some companies air dry the fungus-colonized substrate on metal trays lined with brown paper at room temperature, other companies just refridgerate the rice in bags.

During the dry season, this procedure takes 5–6 days for satisfactory dehydration, and conidia can then be harvested. In the rainy season, however, this drying process takes longer and increases the chances of contamination. Some companies use intense ventilation in rooms with controlled temperatures (but below 30°C) to reduce the drying time to 4–5 days.

Most companies sell a partially dried fungus-colonized-substrate product. Farmers make conidial suspensions by washing these preparations using a surfactant to remove conidia from rice grains. The cost of this crude product, a technical concentrate, is ca US$ 6 per kg (usually with $1–2 \times 10^9$ viable spores g^{-1} substrate), and the total cost per ha is between US$ 12 (2 kg) to 60 (10 kg), depending on severity of pest infestation and application technology used. Processes for harvesting pure conidia have been developed by certain companies, which sell small (45 g) packages of pure *M. anisopliae* conidia (with $1–3 \times 10^{10}$ viable spores g^{-1}). The cost is equivalent to about US$ 210 per kg. For pure conidia, the recommended dose is two to five packages of 45 g each per ha, which makes the cost US$ 19 to US$ 48, respectively.

Biocontrol programs

Field trials with *M. acridum* for control of the grasshopper *Rhammatocerus schistocercoides* were carried out in Mato Grosso state with an oil-miscible flowable concentrate (conidia in soy oil diluted in kerosene oil before use, sprayed with ULV equipment). Control levels of nymphs were in the range of 65–88% with doses equivalent to $0.5-1\times10^{13}$ conidia ha^{-1} (Magalhães, Lecoq, Faria, Schmidt, and Guerra 2000; Faria et al. 2002). Presently, due to extension of soybean and cotton monocultures in Mato Grosso, populations of this grass-feeding species have been at extremely low levels and control measures are not needed.

The fungus *B. bassiana* has been commercialized for control of pests such as the banana weevil *Cosmopolites sordidus* (Batista Filho, Leite, Raga, and Sato 1990; Batista Filho, Leite, Raga, Sato, and Oliveira 1995), and the mite *Tetranychus urticae* in papaya plantations. A *B. bassiana* product was applied in more than 1,000 ha of commercial papaya production, mostly in the states of Bahia and Espírito Santo (Alves, Pereira, Lopes, and Tamai 2002). However, all *B. bassiana*-based programs are quite localized and do not have the same national impact as biocontrol programs using '*Sporothrix insectorum*' and especially *M. anisopliae.*

1. *Rubber-tree lace bug control*. In the 1980s, natural epizootics of a fungus initially identified as '*Sporothrix insectorum*' were observed in populations of rubber-tree lace bugs, the tingid *L. heveae*, in the Amazon basin. This fungus was found naturally controlling 93% of nymphs and 76% of adults (Celestino Filho and Magalhães 1986). The fungus '*Hirsutella verticillioides*' (initially described by Charles (1937) controlling *L. heveae* in rubber trees in Pará state, Brazil) was also isolated by Junqueira (1990) in French Guiana and in Pará. These isolates found by Junqueira, however, were reidentified later by Richard A. Humber as *Verticillium lecanii* (now *Simplicillium lanosoniveum*) (Rangel 2000). At the same time, another isolate was being produced for rubber-tree lace bug control by Triângulo Agroindustrial S.A. in Pontes e Lacerda, Mato Grosso. This fungus was also misidentified as *H. verticillioides*, and was later classified as *Aphanocladium album* (now: *Lecanicillium aphanocladii*) (Rangel 2000; Rangel and Correia 2003). The identification of *Sporothrix insectorum*, however, has not been resolved. It is possible that the fungus referred to here as '*Sporothrix insectorum*' actually is a group of fungi belonging to more than one genus (K. Hodge 2007, Cornell University, Ithaca, USA, personal communication).

'*S. insectorum*' was first tested to control *L. heveae* in the Amazon in 1987 using 1.5–2 L per tree at a concentration of 1.5×10^{7} conidia mL^{-1}. Mortality rates ranged from 25.5% in the dry season to 93.5% in the rainy season (Junqueira et al. 1999). Presently, this fungus is applied to 15,000 ha annually during the rainy season (Alves, Lopes, Pereira, and Tamai 2008b). Production of conidia is undertaken in solid media using corn, rice, or wheat bran (Junqueira, Lima, Martins, and Magalhães 1987; F. Fonseca 2008, Plantações E. Michelin Ltda, Itiquira, MT, personal communication); or a mix of blastospores, submerged conidia and mycelia produced in liquid media (Leite, Batista Filho, Almeida, and Alves 2003). Conidial suspensions are recommended at doses equivalent to 1×10^{12} viable conidia ha^{-1}, whereas diluted liquid media containing 1×10^{11} propagules ha^{-1} have traditionally been used for population densities below the economic threshold (5–10 insects per leaf). For higher insect densities, it is recommended that the label doses be doubled. Fungal suspensions are applied using ground sprayers (300–400 L ha^{-1}), targeting the underside of leaves where the insects are located. Nymphal mortalities as high as 70–100% within 2 months have been reported (Celestino Filho and Magalhães 1986; Alves et al. 2003). The success of applications is based on inducing early epizootics before the insect has developed large populations.

More recently, applications of 1×10^{12} conidia ha^{-1} of selected isolates of *B. bassiana* have been recommended for areas where the rubber-tree mite, *Calacarus hevea* (Acari: Eriophyidae), occurs. The use of this fungus is increasing because '*S. insectorum*' can infect the lacebug *L. heveae*, but not the mite, whereas *B. bassiana* infects both (Tanzini 2002).

2. *Leaf sugarcane spittlebug control by* M. anisopliae. The leaf sugarcane spittlebug reduces field yields of sucrose by 11% and industrial yields of sugar and alcohol by 15% (Alves, Lopes, Vieira, and Tamai 2008c). The economic threshold varies from 2.5 to 5 nymphs per plant stem. When plant heights allow tractors to enter the crops, ground applications at volumes <200 L ha^{-1} are performed with boom sprayers. Otherwise, aerial applications of 30–40 L ha^{-1} are used. Recommended doses vary from 2 to 5×10^{12} viable conidia ha^{-1}, and reported control levels range from 30 to 60% (Almeida and Batista Filho 2006a; Garcia, Macedo, and Botelho 2006). A total of approximately 300,000 ha of sugarcane in the states of Alagoas, Pernambuco, and Sergipe (all in the northeastern region), are treated annually with *M. anisopliae* to control both *M. posticata* and the root spittlebug (*M. fimbriolata*) (Almeida and Batista Filho 2006, J.E.M. Almeida 2008, Instituto Biológico de Campinas, SP, personal communication).

3. *Root sugarcane spittlebug control by* M. anisopliae. Nymphs of *M. fimbriolata* feed and develop on roots of sugarcane and some forage grasses, and they are less commonly found on stems slightly above ground level. Sugarcane was traditionally harvested manually, which requires burning the leaves prior to harvest, but this is rapidly changing to a fully mechanized system. Due to air pollution problems, a law passed by São Paulo state in 2000 will require complete banning of sugarcane straw burning by 2017. Today, approximately 40% of the sugarcane area statewide, and 30% nationwide, is already mechanized. For other Brazilian states, recent discussions recommend gradual banning starting in 2010 and complete banning by 2020. With the burning restriction, insect populations (especially spittlebugs and borers) have increased dramatically. Canes attacked by *M. fimbriolata* experience losses up to 60% of their weight, and this seriously affects sugar and ethanol production (Almeida and Batista Filho 2006a). Therefore, several sugar and ethanol plants have established their own laboratory facilities to mass produce *M. anisopliae*. This is in addition to their mass production of *Cotesia flavipes* (Hymenoptera: Braconidae), a wasp that controls the stem borer *Diatraea saccharalis* (Lepidoptera: Crambidae).

Although some authors have proposed that the economic injury level in sugarcane is two to eight nymphs per linear meter (Pinto, Garcia, and Botelho 2006; Dinardo-Miranda, Pivetta, and Fracasso 2008), two to three nymphs per linear meter is usually adopted as the threshold for mycoinsecticide treatment (Pinto et al. 2006). This density is usually reached in the beginning of the rainy season, normally between September and November in the southeast and from March to August in the northeastern region. If not adequately controlled during the first and lower population peak, densities in the second and third peaks can be quite damaging to the crop. When plant heights allow tractors to enter the crops, fungal applications at volumes of 300 L ha^{-1} are performed with boom sprayers directed to the base of the plants, trying to reach nymphs located underneath straw. Doses usually range from 3 to 5×10^{12} viable conidia ha^{-1}, and average control levels are around 60% (Almeida and Batista Filho 2006b). Aerial applications using conidial suspensions (30–50 L ha^{-1}) or fungus-colonized rice (8–20 kg ha^{-1}) are also performed by some mills. The efficiency of aerial applications for *M. fimbriolata* control has been questioned by some authors (Pinto 2006; Pinto et al. 2006). Published results for aerial applications are limited, but a recent study reports 62–72% control for nymphs and 34–42% for adults with this application method (Carvalho 2007).

Nationwide, the area cultivated with sugarcane reached 9 million ha in the 2007/2008 season, up from 7.1 million ha in the previous year (CONAB 2008). In the 2006/2007 growing season, the total sugarcane area treated with *M. anisopliae* in São Paulo state, which has 54% of the nation's sugarcane area, reached approximately 250,000 ha (J.E.M. Almeida 2008, Instituto Biológico de Campinas, SP, personal communication), up from 180,000 in the 2004/2005 season (Almeida and Batista Filho 2006b; Alves et al. 2008c). Another 200,000 ha were treated in other states, excluding the northeastern region (J.E.M. Almeida 2008, Instituto Biológico de Campinas, SP, personal communication).

4. *Pasture spittebug control by* M. anisopliae. A complex of spittlebugs attack forage grasses. Approximately 10 million ha are annually attacked by cercopids in Brazil. The main pest species are *Deois flavopicta*, *D. incompleta*, *D. schach*, *N. entreriana*, and *M. fimbriolata*. The majority of cultivated grasses within the genus *Brachiaria* are highly susceptible to one or more cercopid species. *M. anisopliae* is applied as conidial suspensions using boom sprayers. The recommended dose is $3–5 \times 10^{12}$ viable conidia ha^{-1} when insect densities reach approximately 10 nymphs per m^2. Control levels similar to those described for spittlebugs in sugarcane, around 60%, have been reported. However, as also happens in other biocontrol programs using *M. anisopliae*, lack of satisfactory control is common – primarily due to the use of excessively low application rates, questionable application techniques, and quality-related issues of the mycoinsecticide products.

A survey of just four companies in 2001 showed that the amount of *M. anisopliae* sold was enough to treat 86,500 ha per year for pasture spittlebug control (Faria and Magalhães 2001). Currently, the total area is much higher, since new manufacturing units have been opened in remote areas where livestock production is booming, and cercopids now successfully attack the widespread and previously cercopid-tolerant grass *B. brizantha* cv. *marandu*. In contrast to sugarcane mills, technical assistance to cattlemen by fungus-manufacturing facilities is rare. In general, EF plants prioritize technical assistance, when provided, to larger clients such as mill owners. Due to lack of technical assistance, coupled with the lack of recent research on EF for control of pasture spittlebugs, cases of spittlebug-control failure seem to persist among beef and dairy farmers.

Product registration

In Brazil, registration of biopesticides is not subsidized by the government. The first step in registration of a new microbial control product is application by the owner of the product for temporary special registration (RET). This application requests general information on taxonomy and preliminary non-target safety of the fungus in question. The fee for this phase is approximately US$ 400 and the certificate is expected to be issued within 6–10 months. The issuance of the RET enables companies to perform field trials required to obtain definitive registration. The definitive registration, which enables companies to commercialize their products, requires the submission of toxicological data and field-trial results. The whole process is expected to take between 1 and 2 years for completion, at a cost of less than US$ 150,000. As also pointed out for China, most products are sold in Brazil without registration, although enforcement of registration laws seems to be only a matter of time.

Future developments

Despite numerous hurdles to the use of fungal insecticides in China and Brazil, the past 30–40 years has been characterized by large-scale mass production and application by growers, governmental agencies, and commercial organizations. Almost all mass-production procedures in both countries are carried out with simple equipment using many manual steps. This makes fungal production excessively labor intensive; and, therefore, is usually done by small companies at the local level. In China, biphasic fermentation is widely used, but the solid fermentation step is still mostly a labor-intensive low-tech procedure. In Brazil, solid culture is usually the sole step and this may make production even more labor intensive. It is clear that advances in production that significantly reduce costs must be developed and implemented.

Drying of conidia and other fungal products is another serious issue. Due to the high cost of electric ovens, natural drying at room temperature is commonly used. However, faster, more efficient, and economically feasible drying procedures should be made available to mass-production plants.

Mycopesticide formulation is a serious problem in both countries. Although seasonal production based on contracts for immediate use is cheap because it avoids storage of large quantities of fungus-colonized substrate, it has many drawbacks. The short shelf-life under non-refrigerated conditions and inconsistent quality among batches results in products with variable concentrations of infective units and, therefore, inconsistent results under field conditions. The few formulations available are still crude and need further improvements to obtain the necessary physical and chemical properties for long shelf-life and consistent field efficacy.

Regarding safety, allergy problems happen occasionally, showing a need for improved working conditions and improved worker-safety education. In addition to the use of protective equipment in manufacturing facilities, development of dust-free procedures and formulations could help minimize these problems. Therefore, collaboration between mycopesticide manufactures and professionals from other areas, such as chemists and engineers, should be encouraged. Also, specific instructions on the handling of fungal products and proper use of protective clothing must be readily available to personnel involved in production and application of these products.

Considering recent economic developments in China and the rapid increase in sugarcane and beef production in Brazil, it is foreseen that a growing number of commercial products will be available in the near future. We expect that an increasing proportion of fungal products will be of higher quality; and in response to expected law enforcement and increasing demand for sustainable agricultural practices and pesticide free food, many of these products are expected to be registered in both countries.

As with *Bacillus thuringiensis*-based products that are restricted to rather narrow host ranges, e.g., Lepidoptera and Diptera, development of mycoinsecticides for specific pests and market niches seems to be a suitable strategy in Brazil and China, thereby creating opportunities for small companies, while avoiding hopeless competition with large chemical companies.

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