Quarantine facilities and legal issues of the use of biocontrol agents in Brazil

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Abstract – The objective of this work was to address the classical biological control of pests in Brazil, regarding procedures to import and export native biological material. A brief introduction will be given on the current legal issues on the use of biocontrol agents, as well as some considerations on the existing quarantine pests and actions already carried out in the country. The safety in the introduction of exotic organisms is important for Brazilian phytosanitary defense and for a higher adoption of classical biocontrol, making it available for integrated pest management (IPM). Legal and normative aspects establish the procedures that must to be adopted, not only to protect bioprospecting and native organisms, but also to minimize risks to the national genetic patrimony associated with the introduction of exotic organisms. Furthermore, the import/export procedures adopted for vegetal and useful organisms for pest biological control and for other genetic material must be subjected to phytosanitary measures performed in government-certified quarantine facilities and diagnostic laboratories. Finally, the quarantine activities listed here are strategic for safeguarding the country from potential problems arising from border transit of living organisms.

Index terms: biological control, plant protection, IPM, legal issues, natural enemies.

Estações quarentenárias e aspectos legais do uso de agentes de controle biológico no Brasil

Resumo – O objetivo deste trabalho foi abordar o controle biológico clássico de pragas no Brasil, quanto aos procedimentos para importação e exportação de material biológico nativo. Será dada uma breve introdução sobre os aspectos legais do uso dos agentes biológicos, bem como feitas algumas considerações sobre as pragas quarentenárias existentes e as ações já realizadas no País. A segurança na introdução de organismos exóticos é importante para a defesa fitossanitária brasileira e para uma maior adoção do controle biológico clássico, tornando-o disponível para o manejo integrado de pragas (MIP). Aspectos legais e normativos estabelecem os procedimentos a serem adotados, não somente para proteger a bioprospecção e os organismos nativos, como, também, para minimizar os riscos ao patrimônio genético nacional associados à introdução de organismos exóticos. Além disso, os procedimentos para importação/exportação de organismos vegetais e úteis para o controle biológico de pragas e de outros materiais genéticos devem estar sujeitos a medidas fitossanitárias realizadas em estações quarentenárias e laboratórios de diagnósticos certificados. Por fim, as atividades de quarentena listadas aqui são estratégicas para o País, para o salvaguardarem de problemas potenciais advindos do trânsito fronteiriço de organismos vivos.

Termos para indexação: controle biológico, proteção de plantas, MIP, aspectos legais, inimigos naturais.

Introduction

Interest in the use of biological control techniques has grown worldwide as an alternative to chemical control, especially due to the adverse effects of chemicals on the environment and human health. In this context, international attention to agricultural production has come hand-in-hand with the use of alternative and less environmentally aggressive means of pest control, aiming at favoring the sustainable use of agroecosystems (Waage, 1996).

The classical biological control of pest organisms involves the introduction and the establishment of alien biological control agents. Some factors leading

to an increase in the use of classical and other types of biological control include: the rapid growth in the international trade of agricultural products, stimulated by the General Agreement on Tariffs and Trade (GATT), with a consequent tendency for an increase in the introduction rate of new exotic pests; the global commitment to new international agreements based on Agenda 21, the standard document for development and environment, as well as for environmental policies from international research funding agencies for the use of integrated pest management (IPM); the growing concern with environmental conservation and biodiversity, according to the Convention on Biological Diversity, which emphasizes the use of biological control; and the growing number of biofactories, making biocontrol agents available for mass release (Waage, 1996).

Applications of biological pest control in Brazil have been highlighted by several authors (Gassen & Tambasco, 1983; Alves, 1998; Bento et al., 1999; Oliveira et al., 2000; Parra et al.; 2002; Martins et al., 2004; Bueno, 2009; Sá, 2010; Sá & Pessoa, 2015). One of the first applications refers to the introduction, in 1921, of the parasitoid *Encarsia berlesei* (Howard) (Hymenoptera: Aphelinidae) for the control of the white peach scale, *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Hemiptera: Diaspididae) (Parra et al., 2002). Successful cases of biological control against aphids on wheat (*Triticum aestivum* L.), in the South Region of the country, in the 1970s, include: the introduction of parasitoid species of the genera *Aphelinus* (Dalman), *Aphidius* (Nees), *Ephedrus* (Haliday), and *Praon* (Haliday); and of predator species of the genera *Hippodamia* (Guérin-Méneville) and *Coccinella* (Linnaeus). Until now, the most outstanding initiative is the biological control of the sugarcane (*Saccharum officinarum* L.) borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae), since 1975, using the parasitoid *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae).

When introducing alien organisms, safety is of vital importance, both for Brazilian phytosanitary defense and for a higher adoption of classical biological control, making it available for IPM. Legal aspects establish the procedures to be adopted, not only to protect native organisms, but also to minimize risks to the national genetic patrimony associated with the introduction of exotic organisms. Some of these organisms will be presented and discussed subsequently.

Procedures for the importation and exportation of biological material

The processes to import and export biological material are determined in specific norms and procedures established by Ministério de Agricultura, Pecuária e Abastecimento (Mapa), the Brazilian Ministry of Agriculture, Livestock and Food Supply (Brasil, 1994).

In general, the first step to import beneficial organisms as agents of biological control of pests is requesting an importation permit to Mapa. In order to do this, public and private institutions must submit one the form shown in the flowchart presented in addenda I and II of Ordinance 74 (Brasil, 1994). The parties interested in importing the beneficial organism must also justify the reason for doing so. Other information that must also be informed include: the number of shipments and of organisms to be received per shipment; the possible suppliers; and locations where the organisms to be introduced will be collected. Additionally, the interested party must previously contact the responsible quarantine facility, which must issue a written document agreeing to import and process the organism.

Regarding the exportation of organisms, the interested parties must also take into consideration the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Cites), which regulates the exportation/importation of animal and plants, including their parts and derivatives. In Brazil, the exportation of organisms requires permits issued by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (Ibama), the national environmental protection agency (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, 2015).

In case the quarantine facility agrees with the introduction of the exotic organism, operational procedures and predefined actions must be outlined in a quarantine technical document that will be sent to Mapa. The activities developed in the quarantine facility are performed to safeguard the country against the introduction of contaminants and/or the escape or dispersion of possible undesirable organisms, such as

arthropods, mites, plant diseases, hyperparasitoids, and microorganisms in general, which can be associated with the beneficial organisms or with the substrate in which they are transported in. Therefore, the quarantine of biological control agents is a screening process to technically subsidize Mapa's final authorization for the release of exotic organisms in a new region, while simultaneously excluding all contaminants. Since Mapa may authorize the final release of an exotic organism. When considered inappropriate, the final release is prohibited and the imported organism is incinerated in the quarantine facility (Sá & Pessoa, 2015).

Current legal issues of the use of biocontrol agents in Brazil

Certain legal and normative aspects establish the procedures to be adopted in order to safeguard the use of prospecting and native organisms, but also to minimize risks to the national genetic patrimony associated with the introduction of exotic organisms. Therefore, the legal requirements in Brazil were elaborated to assure that all introductions, related to research activities or to commercial purposes, are done using official criteria, conducted by certified institutions and people responsible for specific activities, as defined both by Mapa and Law 13,123 of May 20th, 2015 (Vasconcelos, 2012a, 2012b; Brasil, 2015).

Normative Instruction 03 of Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), for the conservation of biodiversity, dated March 1st, 2014, sets the rules for the collection of biological material and enables researchers to obtain permanent or temporary authorization to perform the collection, capture, and transportation of said material. When required, the researcher must request authorization to collect the material through one of the following systems of ICMBio or through equivalent state agency: "Sistema de Autorização e Informação em Biodiversidade" (Sisbio), an authorization and information system for biodiversity; or "Sistema Nacional de Gestão da Fauna Silvestre" (Sisfauna), an authorization and information system for fauna. In short, the interested researcher should perform the following steps:

First, register the following data in the SisFauna or Sisbio systems: personal information, including name, individual taxpayer registration number, and mailing address; name of the scientific institution to which he or she is linked to or for which he or she was nominated; and curriculum at the Lattes platform, a curriculum database of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the national council for scientific and technological development.

Second, describe the research proposal, including, at least: objectives; activities to be carried out; methods to be used; taxa that will be collected, captured, marked, or transported; intended use of the collected material; areas and periods for sampling collection; and whether or not the access to the genetic resource or to the associated traditional knowledge is planned, as provided by Law 13,123/2015 (Brasil, 2015).

Third, inform names and individual taxpayer registration numbers of team members, except field assistants and vehicle drivers, for which the authorization should also be issued to.

After this, a temporary authorization may be given for a specified period, according to the schedule of activities, and may be renewed, on an annual basis, upon the submission of a report on the activities conducted during this time. The authorization will be valid as long as the researcher remains with the same institution to which he was bond to when the permit was requested. Permanent authorizations will not be valid for the following activities: collection and transportation of species officially listed as endangered; temporary maintenance of specimens of wild animals in captivity; receiving or shipping biological material abroad; and research performed in a federal conservation unit or natural underground cavity.

However, authorization is not required for the following activities, except when conducted in a conservation unit or natural underground cavity: observation and recording of images or sounds; collection and transportation of feces, regurgitation, fur, feathers, and teeth, when catching the specimen is not involved; and collection and transportation of botanical, fungal, and microbiological material, except of vegetables living in water or species listed as endangered.

Collection of biological material

Fauna in private areas

Authorization from ICMBio, through SisFauna, or from equivalent state agency, is mandatory to collect fauna in private areas. For collections in indigenous

areas, an authorization from Fundação Nacional do Índio (Funai), the national Indian foundation, is required. In addition, when there is an overlap of indigenous land with a conservation area, prior authorization from Ibama, or equivalent state agency, is also necessary.

Fauna, botanical, fungal, and microbiological material from conservation or protected area

Authorization is mandatory to conduct the collection of fauna, flora, fungi, or microorganisms in a conservation unit or protected area. This permit is issued by ICMBio, through Sisbio or SisFauna, or by equivalent governmental agency. There is no possibility of obtaining a permanent authorization in this case.

Botanical, fungal, and microbiological material from private areas

Authorization from ICMBio, or equivalent state agency, to collect and transport botanical, fungal, and microbiological material from private areas is not necessary, except for aquatic plants or species listed as endangered. However, researchers should use the voluntary registration, provided by Sisbio, to issue a certificate with guidelines to the correct collection and transportation of the material, in case of inspection during these processes. The registration needs to be done only once.

Foreigners' participation in collection activities

The participation of foreign researchers in expeditions to collect samples must be authorized in advance by Ministério da Ciência, Tecnologia e Inovação (MCTI), the national ministry of science, technology, and innovation. This participation is regulated by: Law 6,815, of August 19th, 1980; Decree No. 98,830, of January 15th, 1990; MCTI No. 55, of March 14th, 1990; and Resolution No. 82, of Conselho Nacional de Imigração (CNIg), the national immigration council, of December 3rd, 2008. It should be noted that one of the prerequisites for MCTI to issue an authorization is a joint project between a Brazilian institution – responsible for implementing it – and a foreign institution – responsible for employing researchers, who will participate in the sampling expedition.

The Brazilian institution must monitor and supervise the activities developed by the foreign researchers in Brazil. To do that, the Brazilian institution should: have high and recognized technical and scientific achievements in the field of research related to the work being undertaken; delegate the degree of participation and responsibility, including financial ones; monitor and supervise the activities carried out by the foreigner institution; provide the necessary support to the foreign participants; carry out prior recognition, screening, and selection of the collected material to ensure that the required specimens or their parts are deposited in a Brazilian institution; send partial and final reports to CNPq; ship abroad part of the collected material, after authorization from MCTI or before its delegation; and suspend any activity breaching the current law and immediately report it to CNPq and MCTI.

Foreign researchers must have a formal bond with the foreign institution counterpart in the project. Besides this requirement, the foreign institution must also follow these obligations and commitments, directly or through its researchers: submit a "résumé" in any format; be responsible for implementing financial activities; have knowledge of the rules governing collection activities in the country, especially regarding the shipment of the material collected; authorize MCTI and the Brazilian institution to translate, publish, and disseminate, in Brazil, the work produced; return to Brazil any material collected; report to the Brazilian institution, on a periodic basis or upon request, on the development of the work produced abroad with the material collected, providing partial or final scientific results.

These documents must be forwarded to CNPq, which will review formal aspects and issue technical opinions, forwarding the case to MCTI for final decision regarding an authorization or not. There is no need, however, to obtain authorization from MCTI for collections conducted by foreigners under scientific exchange programs linked to agreements on cultural, scientific, technical, and technological cooperation, signed by the government of Brazil, or to international programs approved in the country.

Biological control of agricultural pests in Brazil

The international exchange of beneficial organisms in the country, from 1991 to 2013, was reported to be around 773 species of organisms introduced for biological control and other purposes, responding

to requests from up to 14 Brazilian states (Table 1). Although most species were introduced only once, some of them were introduced up to three times. Furthermore, 31 species of beneficial organisms were exported up to six countries in the same period (Table 2). In Brazil, the potential use of exotic natural enemies for the biological control of localized or widespread pests is high (Sá & Pessoa, 2015). Some exotic pests registered in the country were successfully controlled by native natural enemies, whereas others demanded more efficient ones brought from their respective countries of origin. Below are some examples of cases when the introduction of exotic biocontrol agents was necessary.

Huanglongbing (HLB, ex-greening), a citrus (*Citrus* spp.) disease caused by the bacterium *Candidatus* Liberibacter spp., was detected in Brazil in 2004 (Sá & Pessoa, 2015) and is currently the most important citrus phytosanitary problem. IPM strategies are focused on the control of the disease vector, the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). Its parasitoid *Tamarixia radiata* (Waterston) (Hemiptera: Eulophidae) was scheduled to be introduced in Brazil, but it was later found in the country, making its importation unnecessary. High levels of psyllid parasitism have been reached in the state of São Paulo, but low levels in northeastern Brazil (Sá & Pessoa, 2015). For this reason, the exotic parasitoid *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae) was imported from the United States for studies aiming the increase in psyllid parasitism rates. The interaction of *D. aligarhensis* on *T. radiata* will also be studied in quarantine conditions.

The citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), another exotic pest of citrus, was first found in Brazil in 1996 (Sá & Pessoa, 2015). Its parasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was introduced in 1998 from Florida, in the United States, reaching high levels of parasitism (almost 90%).

Some species of predatory mites have also been introduced, including the following phytoseiid (Acari: Phytoseiidae) species in the 1990s: *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* (McGregor), *Typhlodromus pyri* Scheuten, and *Acarophenax lacunatus* (Cross & Krantz) (Acari: Acarophenacidae). Research activities in the Brazil, with the first of these species, suggested that native species showed better performance as a control agent

Table 1. Groups of organisms imported from 1991 to 2013 through the Costa Lima Quarantine Laboratory of Embrapa Meio Ambiente, located in the municipality of Jaguariúna, in the state of São Paulo, Brazil.

Group of organisms	Number of species	Purposes and crops	States of destination[1]
Mites	29	Stored grains, cassava, apple, vegetable, and tomato crops	BA, SP, SC, MG, RS, PR, RR
Bacteria	186	Seeds, antiserum, taxonomy, diagnostic kit, soybean, forage palm, and sugarcane	DF, PR, SP, CE, RJ
Fungi	485	Biofertilizers, morphological characterization, human consumption, enzymes, plant diseases, seeds, heavy metals, bioremediation, antimicrobial agents, cattle, taxonomy, industry, laboratory tests, coconut, cassava, forage, soybean, cotton, bean, garlic, and onion	AM, DF, SP, PR, SE, BA, RS, MG, RS, RJ
Predators	5	Tomato and citrus	SP, BA
Parasitoids	44	Laboratory tests, sugarcane, tomato, fruit, cassava, coffee, maize, forest, and citrus	AM, AP, SP, PE, BA, ES, PR, MG, MS
Nematodes	14	Edaphic pests, laboratory tests, as well as morphological, biochemical, and molecular characterizations, and forest	SP, PR, DF
Virus	1	Maize	PB
Protozoa	1	Biological control of *Aedes aegypti*	DF
Ants	1	Fire ant for parasitoid specificity tests	SP
Fruit flies	1	Mass production of insects, by the biofactory Moscamed Brasil	PE
Inoculants	3	Liquid inoculants for maize and soybean	RS
Biofertilizers	3	Bacteria-based biofertilizers	RS
Total	773	-	-

[1]Brazilian states: AM, Amazonas; BA, Bahia; AP, Amapá; CE, Ceará; DF, Distrito Federal; ES, Espírito Santo; MG, Minas Gerais; PB, Paraíba; PE, Pernambuco; PR, Paraná; RJ, Rio de Janeiro; MS, Mato Grosso do Sul; RS, Rio Grande do Sul; RR, Roraima; SE, Sergipe; and SP, São Paulo.

of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) (Watanabe et al., 1994).

The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), was considered an A1 quarantine pest until 2010. Its exotic predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) was introduced from Chile in 1997, being available for IPM (Nardo et al., 1999).

Regarding forestry pests, the red gum lerp psyllid, *Glycaspis brimblecombei* (Moore) (Hemiptera: Psyllidae), one of the most serious pests of *Eucalyptus* spp., was first reported in Brazil in 2003 (Sá & Wilcken, 2004; Ferreira Filho et al., 2015). Its parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) was introduced in 2006 from Mexico (Sá & Pessoa, 2015; Wilcken et al., 2015b). The effectiveness of the control reached rates varying from 25 to 94%, depending on the region. Since 2008, other exotic eucalyptus pests have been detected in Brazil, namely the bronze bug, *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae), and the eucalyptus gall wasp, *Leptocybe invasa* Fisher & LaSalle (Hymenoptera: Eulophidae) (Wilcken et al., 2010, 2015a). Both pests have severely damaged eucalyptus forests. The successful introduction of the exotic parasitoid *Cleruchoides noackae* Lin & Huber (Hymenoptera: Mymaridae) from Chile, in 2011, led to the effective control of *T. peregrinus* (Wilcken et al., 2015a). The parasitoid *Selitrichodes neseri* Kelly & LaSalle (Hymenoptera: Eulophidae) was selected in 2014 for the control of L. *invasa* and was introduced from South Africa in 2015 (Sá & Pessoa, 2015).

Two scale insects, *Orthezia praelonga* Douglas (Hemiptera: Ortheziidae) on citrus and *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae) on pineapple [*Ananas comosus* (L.) Merr.], have already been controlled in Brazil since 1998 by the imported predator *C. montrouzieri*. An ongoing eradication program targeting *Bactrocera carambolae* (Drew & Hancock) (Diptera: Tephritidae) has been using the exotic parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Moreover, the parasitoid *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae) from Hawaii was also introduced in the country to control fruit flies, such as *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Sá & Pessoa, 2015).

Other predaceous phytoseiid mites were introduced from 2012 to 2014 for the biological control of the red palm mite, *Raoiella indica* (Hirst) (Acari: Tenuipalpidae) (Moraes et al., 2012). Among these mites are populations of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) from La Reunion Island and Thailand; and of *Amblyseius cinctus* Corpuz & Rimando (Acari: Phytoseiidae) from Thailand (Moraes et al., 2012). A commercial population of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) from the Netherlands and another population from the Republic of Benin were introduced for laboratory studies envisioning the control of the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Cavalcante et al., 2015). In the same period, to control the tomato (*Lycopersicum esculentum* Mill.) russet mite, *Aculops lycopersici* (Massee) (Acari: Eriophyidae), there were also introductions of *Euseius emanus* (El-Banhawy) (Acari: Phytoseiidae) from Peru, *Euseius flechtmanni* Denmark & Muma (Acari: Phytoseiidae) from Paraguay, *Euseius fructicolus* (Gonzalez & Schuster) (Acari: Phytoseiidae) from Chile, and *Euseius vivax* (Chant & Baker) (Acari: Phytoseiidae) from Costa Rica.

Regarding the exportation of beneficial organisms, one example is the participation of Brazil in an extensive international project on the biological control of the cassava green mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) (Table 2). Cassava is one of the major sources of carbohydrate for many

Table 2. Groups of organisms exported from 1991 to 2013 through the Costa Lima Quarantine Laboratory of Embrapa Meio Ambiente, located in the municipality of Jaguariúna, in the state of São Paulo, Brazil.

Group of organisms	Number of species	Purposes and crops	Importing countries
Parasitoids	19	Greenhouse, golf courses, laboratory studies, and fire ants	United States, Holland, and Japan
Predatory mites	11	Cassava and coconut	Africa, Sri Lanka, and Colombia
Fungi	1	Laboratory studies and cassava	Africa and Colombia
Total	31	-	-

countries, especially in the Americas and Africa. The cassava green mite and the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), were first found in Africa in the early 1970s (Neuenschwander, 2001; Yaninek & Hanna, 2003). Within a short time, the cassava green mite became a major problem to cassava growers. An international program for the biological control of the pest was very successful, resulting in the establishment of three species of predaceous phytoseiid mites that controlled the pest (Yaninek & Hanna, 2003). It should be noted that this is one of the most important projects of classical biological control of agricultural pests.

Concluding remarks

This work deals specifically with the classical biological control of pests, which involves, in its initial phase, the discovery of prospective natural enemies to be used for control of exotic organisms, followed by their actual introduction and field colonization. All these activities are regulated by Brazilian norms aiming to protect national interests related to the use of biodiversity, in this case for biological control purposes and for environmental protection against possible detrimental effects of introduced organisms.

The increasing importance of biological control in Brazil has become evident by the growing interest of the private sector. Recently, a few companies involved with the production of biological control agents were established in the country and are now objectively implementing another important class of biological control, i.e., augmentation, with mass production and releases of biological control agents. The practical use of these biological control agents by many growers gives a new impetus and credibility to this type of pest control, and also promotes the general interest in continuing the use of this technique.

However, there are aspects that still refrain from the wider use of biological control, specifically the country's legislation. Constant progress in the field of biological control has raised the need to: create specific norms to allow its further development, produce biological control items, and make its use feasible by growers. The main concern refers to the current legislation regulating the registration, approval, use, and residue levels of the adopted control measures, which is the same for biological control and agrochemicals, despite the huge differences between these two methods of pest control. Therefore, exclusive norms for the production, registration, and commercialization of biological control items are urgently needed for the wider implementation of biological control in Brazilian agriculture.

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