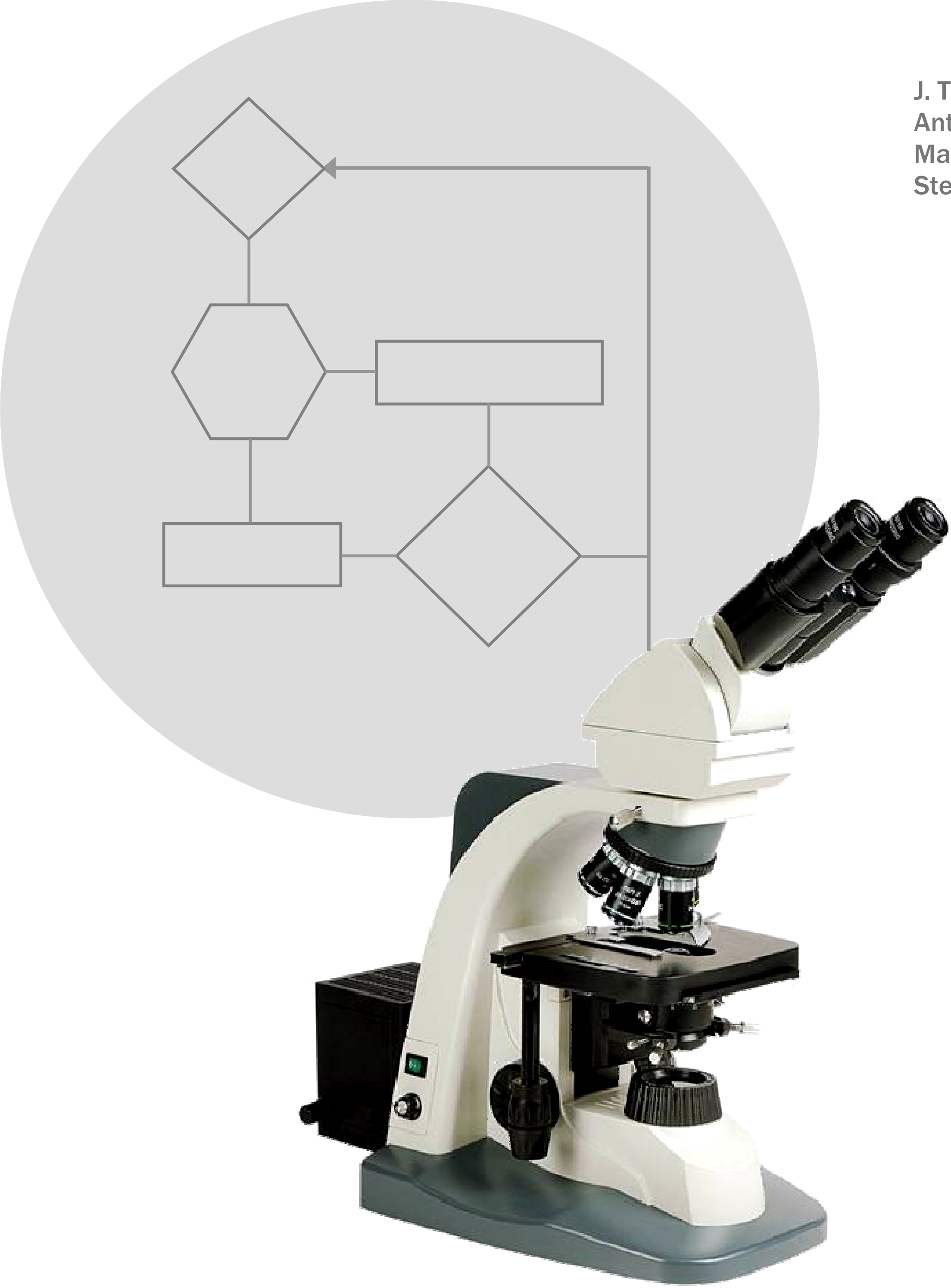
The Use and Regulation of Microbial Pesticides in Representative Jurisdictions Worldwide



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THE USE AND REGULATION OF MICROBIAL PESTICIDES IN REPRESENTATIVE JURISDICTIONS WORLDWIDE

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PREFACE

When there is potential for harm to human and environmental health from the introduction of a pest control product, regulatory procedures for pre-market assessment of safety are common in most countries. An effective regulatory framework provides protection from harm, while still facilitating the availability of useful products. It recognizes the needs of farmers, society, and the commercial interests of the pest control product proponents i.e. registrants. The latter are recognized by being provided clear communication of the terms of the regulatory system and the stepwise process of product registration, and by being charged reasonable costs for registration so products move seamlessly from the application for registration through to the market.

The risks assumed with the introduction of microbial pest control products are related to their toxicity, infectivity, pathogenicity to and displacement of non-target organisms, and the potential irreversibility of introduction into the environment. These are the types of risks which are assessed during a pre-market safety evaluation (Cook et al. 1996; Jaronski et al. 2003; Mensink and Scheepmaker 2007; Organization for Economic Cooperation and Development 2003).

Predictable and efficient regulatory processes ultimately allow registrants to begin to recover their research, development, and registration costs in a timely manner following registration. This is particularly important for industries in the microbial pest control sector, which are mainly small and medium enterprises for whom lengthy regulatory delays may act as a significant deterrent to investment in product development (Cook et al. 1996; Jaronski et al. 2003; Laengle and Strasser 2010).

Regulatory systems for pest control products are designed to achieve several purposes. They must protect human health and the environment from potential risks associated with the use of pest control products. They must also allow nations to meet their needs for food and fibre production by facilitating the availability of pest control products and ensuring that products have acceptable efficacy. In Canada, for example, federal pesticide legislation stipulates that the regulatory system should encourage the development and use of innovative and lower-risk products and pest control strategies, and where consistent with the primary objective to protect human health and safety and the environment, should minimize any negative impact on economic viability and competitiveness (Minister of Justice 2002). An effective regulatory system achieves these goals by putting in place the following elements:

- A system of data requirements to guide the assessment of human health and safety, value (including efficacy), and environmental safety;
- Clear and predictable procedures for assessing the risk and value of pest control products, with sufficient flexibility to allow expert opinion to contribute to the assessment process;
- Mechanisms which afford opportunities for public and industry input into the decision-making process, including the right to appeal decisions;
- Policies which establish reasonable timelines for assessment of various classes of products, and an agency with a good track record with regard to these timelines;
- The flexibility to modify regulatory procedures in line with new scientific information;
- Regulatory fees which are affordable to registrants;
- Enforcement of legislation and regulations related to product use, sale, distribution, and other regulatory requirements.

It is commonly agreed that one way to streamline and speed product registration processes is through international harmonization of a regulatory framework e.g. for data requirements, fees, timelines, criteria for approval, and risk assessments. Indeed, major steps have been taken to increase both the harmonization and transparency of data requirements and the procedures for risk assessment at OECD, North American, and European Union levels. It should be borne in mind, however, that while harmonization is desirable, because microbial agents have a wide range of mechanisms of action, and because their properties are generally poorly understood relative to chemical pesticides, regulatory assessment frameworks must retain a degree of flexibility and reliance on expert opinion in order to comply with the "intra- and interspecific variation of microorganisms and their constituents" (Mensink and Scheepmaker 2007).

The presentation of the regulatory systems in the following chapters provides a means to compare and contrast the variety of approaches taken by selected jurisdictions (countries or groups of countries) in establishing a framework that offers protection from harm, while offering pest control products with utility for farmers, and their degree of success in doing so. Harmonization activities will be apparent, particularly among member states of the European Union and between Canada and the United States, the latter of which is evolving rapidly. Significant gains are still to be made in Africa, but in the meantime, continental harmonization is loosely achieved by following principles of the Food and Agriculture Organization for the registration of synthetic pesticides. The regulation of pesticides among the Newly Independent States of the former Soviet Union has understandably become disjointed, and despite Ukraine being the birthplace of microbial pest control, microbial pesticide use in Eastern European countries is minimal. China demonstrates, however, that a regulatory system can be created rapidly and streamline the inherent complexity involved in the widespread use of unregistered and unformulated active ingredients. India too has experienced a rapid development of microbial pesticide programming in support of poorer farmers and to support the continuing biotechnological advancements arising from Green Revolution of the 1980s. Cuba may in fact be a leader in the use of microbial pesticides under the state mandate of ecologically-based pest management, together with a network of state-supported microbial pesticide production facilities to supply the agricultural sector.

These chapters also indicate the degree to which countries have provided institutional support of microbial pesticides in facilitating their development and adoption, and show the international reach of microbial strains and products. While we recognize that taxonic revisions of certain microorganisms have taken place in recent years, we have retained classifications as they appear on product labels.

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AFRICA WITH SPECIAL REFERENCE TO KENYA

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OVERVIEW AND USE

Africa's agriculture is extensive, ranging from high-input crops for export to low-input crops and subsistence farming. The wide range of crops grown reflects the size and diversity of its vast ranges of geographies and climates: 30,221,532 km^2 divided into 54 countries, including mountains as high as 5895m and straddling the equator.

Crop protection is predominantly based on chemical pesticides, regulated by the respective Ministries of Agriculture broadly following the Food and Agriculture Organization (FAO) principles for their registration and use (see FAO weblink under in References). Most countries have adopted these principles to develop their own regulatory system according to their needs. There are very few examples of harmonized regulations among countries, an exception being the Permanent Inter-State Committee to Combat Drought in the Sahel (CILLSS)[1] in Western Africa. Still, there are formal and informal reciprocity agreements for the acceptance of dossiers and accompanying data, on a case by case basis, from other countries within Africa, as well as from outside the continent, such as the United States and the European Union.

Concerns over the impacts of some of the chemical based pesticide products on both human health and the environment in Africa continue. Concurrent with the removal of some of the more toxic compounds has been the development of alternative crop protection approaches such as integrated crop or pest management (ICM or IPM) which makes use of biological crop protection agents (BCA) (Cherry and Gwynn 2007). Microbial pesticides account for many of these newer crop protection agents and include products based on fungi, bacteria, viruses, and protozoa with activity against insect pests, diseases, and occasionally weeds. Products based on *Bacillus thuringiensis* (*Bt*) are the longest established and most dominant microbial pesticides available. *Bt*-based products are available in many countries in Africa, registered using the chemical plant protection pathway. Dipel, based on *Bacillus thuringiensis* subsp. *kurstaki* (www.valentbiosciences.com), is one such pesticide.

While there has been considerable research focus on microbial pesticides by African-based national and international research organisations, the output of registered products arising from these efforts has been minimal (Cherry and Gwynn 2007; Grzywacz et al. 2008 and 2009). The demand for microbial pesticides is largely driven by the need to support high-value export horticultural crops, at both the large-scale and outsourced small-scale growers. Export horticulture standards now require IPM within their growing protocols as importer countries have had increasingly strict standards for pesticide residues on produce. Because of this, microbial pesticides have become an important tool to serve the need to minimize residues.

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[1] CILSS = Comité Permanent Inter Etats de Lutte Contre la Sécheresse au Sahel whose members include Senegal, Mali, Niger, Burkina Faso, Cape Verde, Chad, Mauritania, Guinea Bissau and the Gambia.

Microbial pesticides are mainly available in horticulture hotspots such as South Africa and Kenya. The microbial based active substances available in South Africa are listed in Table 1. Typically, these actives have been developed into products from indigenous isolates, isolates from other Africa regions, or isolates and products developed elsewhere.

Table 1. Microbial pesticides available in South Africa as of 2010.

	Taxus	Products	Targets
Bactericides			
Agrobacterium radiobacter	Bacterium	Crown Gall Inoculant	Crown gall
Fungicides			
Bacillus subtilis 101	Bacterium	Shelter	Root and leaf diseases
Bacillus subtilis 102	Bacterium	Artemis	Root and leaf diseases
Bacillus subtilis 246	Bacterium	Avogreen	Root and leaf diseases
Bacillus subtilis QST 713	Bacterium	Serenade	*Botrytis* spp.
Ampelomyces quisqualis AQ10	Fungus	Bio-Dewcon	Powdery mildew
Trichoderma harzianum	Fungus	Eco-77 Eco-T Promot Romulus Rootgard Trichoplus Trykocide	Root diseases
Trichoderma harzianum 39	Fungus	Trichodex	Root diseases
Trichoderma harzianum DB103	Fungus	T-Gro	Root diseases
Fungicides/bactericides			
Bacillus subtilis	Bacterium	Defender	Soil-borne fungi and bacteria
Insecticides			
Bacillus thuringiensis subspp. *aizawai* and *kurstaki*	Bacterium	Agree	Lepidoptera larvae
Bacillus thuringiensis subsp. *israelensis*	Bacterium	VectoBac	Mosquito
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	DiPel Rokur Thuricide	Lepidoptera larvae
Bacillus thuringiensis subsp. *kurstaki* H7	Bacterium	Florbac WG	Lepidoptera larvae
Beauveria bassiana	Fungus	Bb Plus Bb weevil Sparticus	Thrips, weevils, whiteflies
Metarhizium anisopliae subsp. *acridum* IMI 330 189	Fungus	Green Muscle	Locust
Granulosis viruses	Virus	-	Lepidoptera larvae
Pseudomonas resinovorans bacteriophage	Virus	Agriphage	Insect pest control
Nematicides			
Paecilomyces lilacinus	Fungus	Bio-Nematon	Nematodes
Paecilomyces lilacinus 251	Fungus	PL Plus	Nematodes

REGISTRATION AND THE REGULATORY SYSTEM

Some African countries are taking a proactive role in developing a capacity for regulating microbial pesticides. Kenya, for example, has developed biopesticide-specific registration regulations, representing a proportional and reasonable system that correctly assesses the safety and risks associated with microbial pesticides. Following this development, a consultative workshop held in Arusha, Tanzania brought together participants from Kenya, Tanzania, Uganda, Ethiopia, and Senegal, and many showed an interest in adopting a similar framework.

Encouraged by Kenya's success, the Ghana Environmental Protection Agency's product registration guidelines have been modified to more reasonably accommodate the registration of biopesticides. Ivory Coast and Togo have also been involved in the Ghanaian process and it is hoped that they too will be encouraged to adopt such guidelines. There are also examples of pragmatic approaches to other aspects of microbial pesticides, such as in Tanzania's exempting from registration local baculovirus isolates for use on particular pandemic lepidopteran pests.

It has been acknowledged in some countries in Africa that registration can represent a hurdle to microbial pesticide product availability. In addition to wanting to encourage import of microbial pesticides, there has been a desire to facilitate in-country development and production. This impetus for development of microbial pesticide-specific registration has been in response to the needs of export growers to access products necessary to support IPM practices.

Biopesticide regulation in Kenya

Agriculture accounts for about 24% of Kenya's gross domestic product (GDP) with an estimated 75% of the population depending on the sector either directly or indirectly. Currently, horticulture is the fastest growing agricultural sub-sector in the country and has become a major foreign exchange earner, employer, and contributor to food needs in the country. Fruit, vegetable, and cut flower production are the main components of horticultural production in Kenya and generate over US $300 million in foreign exchange earnings. The total horticultural production is close to 3 million tonnes, making Kenya one of the major producers and exporters of horticultural products in the world (Export Processing Zones Authority Report 2005).

The success of horticulture, particularly export horticulture, has created an enabling environment to develop microbial pesticides for use in IPM by growers responding to the increasing demand for chemical residue-free crops.

<u>Kenya: the current situation and microbial pesticide usage</u>

In 2002, total pesticide sales in Kenya were valued at approximately $57.4 million, of which $1.15 million (2%) represented sales of all biopesticides, predominantly *Bt* based products (Wabule et al. 2004). Although microbial pesticides can be used in agriculture, at present they are used primarily in high-value horticulture crops. In 2003, in response to horticulture industry needs, Kenya's pesticide regulatory authority, the Pest Control Products Board (PCPB), supported by a range of stakeholders such as the UK Department for International Development (DFID) Crop Protection Programme, developed biopesticide-specific registration pathways, including one for microbial pest control products. This initiative was consolidated with further capacity building in biopesticides for organisations such as the Europe-Africa-Caribbean-Pacific Liaison Committee (COLEACP) PIP programme (www.coleacp.org/pip) and is now supported by a designated biopesticide specialist within PCPB. The increase in the number and range of microbial pesticides now available in Kenya (Table 2) indicates that the development of microbial pesticide-specific registration has been well-targeted.

Table 2. Microbial pesticides registered in Kenya as of 2010.

	Taxus	Products	Targets
Fungicides			
Trichoderma asperellum	Fungus	Trichotech	Soil fungal diseases
Trichoderma harzianum	Fungus	Eco-T Rootgard Promot (temporary registration)	Root fungal diseases
Fungicides/bactericides			
Ampelomyces quisqualis AQ10	Fungus	Bio Dewcon	Powdery mildew
Nematicides			
Myrothecium verrucaria	Fungus	Ditera	Nematodes
Paecilomyces lilacinus	Fungus	PL Plus	Nematodes
Insecticides			
Bacillus thuringiensis subsp. *aizawai*	Bacterium	Florbac Xentari	Coffee giant looper
Bacillus thuringiensis subsp. *israelensis*	Bacterium	Bacticide	Mosquito larvacide
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Biolep DiPel Halt Thuricide	Thrips, African bollworm, *Helicoverpa armigera*, *Spodoptera exigua,* Lepidoptera larvae, diamond black moth
Bacillus thuringiensis subsp. *kurstaki* SA11	Bacterium	Delfin	Diamond black moth, coffee giant looper
Beauveria bassiana GHA	Fungus	Bio-power Botanigard	Aphid, diamond black moth, sucking insect pests
Paecilomyces lilacinus	Fungus	Bio - Nematon	Root knot nematode

Kenya: applications for registration of a microbial pest control product

In Kenya, there is a distinction between indigenous and non-indigenous microorganisms, and regulations were developed to consider both types. For registration of non-indigenous organisms, all applicants intending to import or export live organisms must seek clearance from the Kenya Standing Technical Committee on Imports and Exports on live organisms (KSTCIE) prior to initiating any in-country work with the organisms.

If an indigenous organism is produced in Kenya and is intended for export, notification should be sent to The Convention on Biological Diversity (CBD), which was instigated on December 29, 1993, and to which Kenya is a signatory. The CBD has 3 main objectives: to conserve biological diversity, to use biological diversity in a sustainable fashion, and to share the benefits of biological diversity fairly and equitably (www.cbd.int).

The broad role of the PCPB, the pesticide regulator, includes but is not limited to the following: evaluation of submissions for pesticide and biopesticide approvals, regulation of biopesticide production facilities in terms of safety and production quality, and final product quality control and labelling.

Under the regulations, microbial pest control products include naturally occurring bacteria, protozoa, fungi, viruses, and rickettsia, intended for the control of invertebrate pests, weeds, plant parasitic nematodes, or microbial pathogens of crops. Entomopathogenic nematodes or other nematodes for pest control are regulated under the rules for macrobial pest control products. This regulation does not include the control of vertebrate pests. For registering a microbial pest control product, there are specific forms obtainable from the PCPB website:

(www.pcpb.or.ke/images/stories/PCPBFORMS/Form_A1_Biopesticides_Microbial.pdf).

It is strongly advised that the first step in applying for microbial pest control product approval is to seek a pre-registration consultation with the relevant expert within the PCPB. An applicant who is not a resident in Kenya must appoint an agent permanently resident in Kenya.

To apply for approval (registration) of a microbial pest control product, the applicant is required to complete the relevant summary document along with completed applications for both the active substance (Form A1 List MI) and the product (Form A1 List MII). List MI and MII are supplied as checklists and an index to ensure that the applicant has provided all relevant data and cited material. The application must be accompanied by a technical dossier as per the PCPB data requirements (i.e. Lists MI and MII). In addition, every application must be accompanied by a registration fee and 3 copies of the draft label as per the PCPB requirements. Given the broad range of responsibilities of PCPB, applicants must also submit samples of the technical grade of the active ingredient, the laboratory standard of the active ingredient, the pest control product, and potentially other samples as appropriate.

The PCPB will consider supporting data in the dossier that has been generated outside of Kenya, provided it was carried out to the required standards such as Good Laboratory Practice (GLP). However, it is necessary to carry out at least two seasons of efficacy trials of the product in Kenya. This trial work must be done by a PCPB approved contractor; a list of PCPB accredited contractors, with expertise in different crop groups, is provided on their website. The time from pre-consultation with the PCPB to approval and registration of a microbial pest control product varies with the complexity of the product, and can range from 2 to 4 years.

SUMMARY

The main product demand in Africa has been from export horticulture growers, but this means that products also become available to small-scale growers. There are now examples of distributors preparing microbial pesticide products in packaging sized appropriately and specifically for these growers.

The increase in demand for products that are compatible with IPM systems and that assist growers to manage their chemical pesticide residues has been a strong driver for the development of a microbial pest control product-specific registration pathway. The increasing number of these products submitted for approval indicates that such provisions of proportional regulations can remove significant hurdles to getting microbial pesticides to end users.

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Useful Websites
Biopesticide Industry Alliance – BPIA (www.biopesticideindustryalliance.org)
FAO – IPM information (www.fao.org/ag/AGP/AGPP/IPM/Default.htm)
Food and Agriculture Organisation – Pesticide information (www.fao.org/ag/portal/home/en)
Ghana biopesticide registration – Ministry of agriculture (www.mofa.gov.gh)
International Biocontrol Manufacturers Association – IBMA (www.ibma.ch)
Kenya Biopesticide Registration - Pest Control Products Board (www.pcpb.or.ke)
Kenyan Biopesticide Registration Workshop
(ww.cpp.uk.com/UPLOADS/publications/downloads/1prelimpages.pdf)
Kenya Export Processing Zones Authority (www.epzakenya.com)
South Africa registration – Ministry of Agriculture (www.doa.agric.za)

CHINA

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OVERVIEW AND USE

Biopesticide production in China began in the late 1960s. Encouraged by central and local governments, hundreds of small biopesticide producers appeared. A crude estimate of the production and application of *Beauveria bassiana* alone from the 1970s through to the 1980s is between 10,000 to 20,000 tonnes annually, mainly as unformulated, dried culture, with annual application areas at around 0.7 to 1.3 million hectares and the maximum area of 2 million hectares. Due to a lack of legislative regulation, however, none of these products were registered, and they were sold with no commercial name as most were produced at small scale for local use. It is impossible to accurately assess the number and amount of biopesticides produced during that time period, but the total area of biopesticide application, including agricultural antibiotics, increased from an estimated 800,000 hectares in 1972 to 27,000,000 hectares in 2000 (Ye and Chen 2002; Zhang 2002; Yang 2007).

Since the 1990s, biopesticides have been identified as a promising alternative to chemical insecticides and have received more attention from citizens, scientists, companies, and central and local governments. An increasing number of biopesticides have been developed and marketed, while the regulatory management of their production, sale, and use has also become more rigorous. The Regulation on Pesticide Administration, the first law governing pesticide production and use in China, was issued in 1997. Under this law, biopesticides are required to be registered before entering the market, and since its establishment, increasing numbers of biopesticides have been formally registered.

As of October 2008, there were 327 biopesticides registered in China, accounting for 1.6% of total registered pesticide products (Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA) 2008) (Table 3). Microorganisms used in biopesticide development in China include bacteria, fungi, and viruses. These biopesticides are widely used in various agricultural and forest ecosystems against pests and plant diseases of crops and trees.

Bacteria

Among the 270 bacterial biopesticides from 11 microbial species, 181 are based on *Bacillus thuringiensis* (*Bt*) (ICAMA 2008). They are mainly used against lepidopteran pests of various plants including vegetables, cotton, wheat, corn, tobacco, fruit, and ornamental and forest trees. The annual use of *Bt* products has exceeded 30,000 tonnes in the past 10 years, and the annual application area is over 8 million hectares as of 1995 (Li 2000) targeting lepidopteran insects including *Pieris rapae*, *Plutella xylostella, Helicoverpa armigera*, and some loopers.
Some *Bt* products are mixed with low concentrations of chemical pesticides or avermectin to improve efficacy. *Pseudomonas alcaligenes* and *Bacillus sphaericus* are also used on a smaller scale for insect pest control. The remaining bacterial biopesticides are used for plant disease control, mainly targeting rice false smut, bacterial wilt, rice blast, and root rot of rice and some vegetables.

Fungi

There are 22 registered fungal biopesticide products in China based on 6 fungal species. Products based on *Beauveria bassiana* target mainly the Masson's pine caterpillar (*Dendrolimus punctatus*), and the Asian corn borer (*Ostrinia furnacalis*). *Metarhizium anisopliae* is used mainly against locusts, grasshoppers, and cockroaches. *Paecilomyces lilacinus* and *Pochonia chlamydosporia* are used for plant parasitic nematode control. The active ingredients of a *Conidiobolus thromboides* product for aphid control are bioactives from the fermentation broth and not the spores. The only fungal biological control agent used against plant diseases is a *Trichoderma* sp., which is mainly used for grey mold control.

Specific examples

The largest biological control project in China is the use of *B. bassiana* against the Masson's pine caterpillar (*Dendrolimus punctatus*), the most serious forest defoliator in China and some southeastern Asian countries. The caterpillar infests a minimum of 0.5 million hectares of pine forest each year in China (Qu 2004). The large scale application has a 38 year history which began with support from the Ministry of Forestry (originally the Ministry of Agriculture and Forestry) both financially, and through the provision of an officially assigned market. Application methods include application of conidial dust, launching conidial firecrackers, and releasing artificially inoculated living caterpillars into pine plantations (Li 2007). Due to the long history of *B. bassiana* application in China, along with its practical application methods, it has been adopted as a conventional, and oftentimes preferred, choice for forest farms against pine caterpillars in southern China; the routine use of *B. bassiana* has resulted in a great reduction in chemical insecticide use in forestry (Xu 2004).

Another major target insect for *B. bassiana* is the Asian corn borer in northern China, an aggressive pest with a long history of biological control in China. The control area of the corn borer peaked at 500,000 hectares in 1977 (Xu et al. 1988). Various conidial formulations (dust, water suspension, or granules) were applied to treat corn foliage at the bell-mouthed stage (Xu et al. 1987). However, an easier method is to treat corn straw piles, as corn borers overwinter inside corn stalks. Once corn borers crawl out of the stalk in May to imbibe water (necessary as they move out of hibernation into pupation), they are infected by the conidia that was applied to the corn straw.

The use of fungal insecticides has shown great potential to control *Monochamus alternatus*, the most important insect carrier of pine wilt disease nematode. Non-woven fabric sheets impregnated with conidia and chemical attractants are used to control *M. alternatus*. The application has the potential to significantly decrease the longhorn beetle's life-span, oviposition, and population density. Field applications averaged 50,000 hectares in 2008, mainly in the Anhui and Jiangxi provinces. Spraying *M. anisopliae* conidia oil suspensions to control pasture locusts and grasshoppers has also advanced in recent years. It is reported that the control area reached tens of thousands of hectares in western and northwestern China in 2008.

Viruses

There are 35 registered viral biopesticides, 14 of which are developed from *Heliothis armigera* nucleopolyhedrosis virus (NPV). These biopesticides mainly target lepidopteran larvae on vegetables, cotton, and tea trees. Most viral products are mixed with *Bt*. Viral pesticides were developed and marketed later than fungal and bacterial pesticides, with the first viral pesticide, *Heliothis armigera* NPV, registered in 1993. Viral pesticides have since developed quickly, and annual output is over 2,000 tonnes.

Table 3. Partial list of microbial pesticides registered in China (out of a total of 270 bacterial products, 22 fungal products, and 35 viral products).

	Taxus	Targets
Bactericides		
*Agrobacterium radiobacter**	Bacterium	Crown gall
*Bacillus polymyxa**	Bacterium	
Bacillus sphaericus	Bacterium	
Fungicides		
Bacillus cereus	Bacterium	Bacterial wilt, sheath blight / rice false smut, bacterial wilt
Bacillus licheniformis	Bacterium	Downy mildew, *Fusarium* wilt
Bacillus subtilis	Bacterium	Bacterial wilt, root rot, tobacco black shank, rice blast, rice false smut
Trichoderma spp.	Fungus	Downy mildew, *Rhizoctonia cerealis*, gray mold
Fungicides/bactericides		
Pseudomonas fluorescens	Bacterium	Bacterial wilt, root rot
Insecticides		
Bacillus thuringiensis subsp. *aizawa*	Bacterium	Lepidopteran pests
Bacillus thuringiensis subsp. *israelensis*	Bacterium	Lepidopteran pests
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Lepidopteran pests
Pseudomonas alcaligenes	Bacterium	Locusts, grasshoppers
Beauveria bassiana	Fungus	*Monochamus alternatus, Dendrolimus punctatus*
Conidobolus thromboides	Fungus	Aphids
Metarhizium anisopliae	Fungus	Cockroaches, grasshoppers, locusts
Paecilomyces lilacinus	Fungus	Nematodes
Pochonia chlamydosporia	Fungus	Nematodes
Dendrolimus cytoplasmic polyhedrosis virus*	Virus	Caterpillars
Heliothis armigera nucleopolyhedrosis virus, *Autographa californica* NPV, *Ectropis obliqua hypulina* NPV, *Laphygma exigua* NPV, *Prodenia litura* NPV, *Buzura suppressaria* NPV, *Gynaephora ruoergensis* NPV, *Mythimna separate* NPV*	Virus	Beet armyworm, lepidoptera, looper, *Heliothis armigera*, *Laphygma exigua*
Periplaneta fuliginosa densovirus	Virus	Cockroaches
Pieris rapae granulosis virus, *Mythimna separata* GV, *Plutella xylostella* GV	Virus	*Pieris rapae*, *Plutella xylostella*

*active ingredients of registered products that are currently not marketed

REGISTRATION AND THE REGULATORY SYSTEM

The Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA) is the national authority responsible for pesticide registration and supervision. Its main responsibilities are pesticide registration, quality control, bioassay, residue monitoring, law enforcement, and information exchange on agrochemicals including biopesticides.

Only pesticide companies registered and authorized by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China have the right to apply for the registration of pesticides in China. Research institutes, universities, or other research groups cannot apply for such registration. The registration of a novel biopesticide is at minimum a two year process, requiring three steps: field trials, temporary registration, and formal registration.

Field Trials: Permission for field trials must be obtained from the ICAMA. Documentation required includes a certificate of taxonomic identification by an authorized microbiological unit such as the Institute of Microbiology, Chinese Academy of Science; a report on acute and chronic toxicology by an authorized medical unit such as a provincial centre for disease control and prevention; a report on ecological safety (safety to birds, fish, bees, silkworms, earthworms, algae, daphnia, and some invertebrate natural enemies) by the Environmental Science Institutes of the Ministry of Environmental Protection; and some additional basic documentation about the product.

ICAMA assigns the field trials to 4 out of more than 50 authorized units, mostly provincial institutions. These four selected ICAMA branches carry out two-year field trials and prepare field efficacy reports.

Temporary registration: A successful temporary registration needs the following primary documents: i) basic document on physical, chemical, and biological characteristics of a biopesticide; ii) report on toxicology; iii) report on ecological safety; iv) reports on two year field efficacy in four areas in different provinces; and v) product label and user manual. Only documents i) and v) are supplied by the producer, while the others are provided by the institutions authorized by the Ministry of Agriculture.

Formal registration: One year after a temporary registration is issued, a registrant can apply for a formal registration. This requires documentation similar to that required for temporary registration, along with a supplementary integrated report on the use of the product during the temporary registration period. Biopesticide registrations need to be renewed or extended annually.

SUMMARY

The development of biopesticides has been actively encouraged by the Chinese central and local governments. All public forest pest management projects funded by the Ministry of Forestry are required to use biological control measures, and grants from other origins also encourage biological controls. There is no strict requirement on crop pest management, but many local governments encourage farmers to use biopesticides to ensure safe agricultural practice and crop quality, considering both human and environmental health. It can be expected that biopesticide development will continue to increase, resulting in the emergence of more registered biopesticides in the near future.

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INDIA

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OVERVIEW AND USE

Indian scientific interest in the use of biopesticides for crop protection is long standing, with active research into entomopathogenic fungi being pursued since the 1960s. However, it was probably the impact of the Indian agricultural crisis of the late 1980s, due to widespread failure of chemical insecticides to control *Helicoverpa armigera*, *Spodoptera litura,* and other pests in cotton (Armes et al. 1992; Kranthi et al. 2002), that prompted efforts to develop systematic integrated pest management (IPM) and insecticide resistance management programmes, of which biopesticides are a component. Additional drivers for biopesticide production research include increasing reports of high levels of chemical pesticide residues in fruit, vegetables, mother's milk, and groundwater, and the disappearance of honey bees and other fauna (Chandrasekaran et al. 2008). Subsequently, research into microbial pesticides in India has been substantial and a wide range of pathogens has been investigated in a variety of cropping systems (Rabindra et al. 2001; Ignacimuthu and Sen 2001; Koul et al. 2003).

The Government of India allocates funds for IPM programmes for all major crops, but these funds are mainly implemented at the state government level, through programmes promoting the use of biopesticides to farmers. Major national research programmes such as the National Agricultural Technology project (2000 - 2006) and the current National Agricultural Innovation project also contain important biopesticide research and development components. At the state level, 50% of the plant protection budget is allocated to eco-friendly agriculture (Singhal 2004), to cover both the training of farmers and the procurement of biopesticides for distribution. A website on "Biocontrol strategies for eco-friendly pest management" has been launched recently by the Department of Biotechnology (DBT) (www.dbtbiopesticides.nic.in).

The microbial pesticides used in India are listed in Table 4. The products registered, including *Bacillus thuringiensis* (*Bt*), are sourced partly as imported products, but also include many that are locally produced. Indian biopesticide production is currently dominated by antagonistic fungi and bacteria such as *Trichoderma* spp. and *Pseudomonas fluorescens* (Table 4), but the production of nucleopolyhedrosis viruses (NPV), granuloviruses (GV), and entomopathogenic fungi are also established and expanding (Rabindra 2005). A major goal has been to develop local sourcing of biopesticides as a means of ensuring availability at a low cost to benefit poorer farmers, and as a base for expanding an Indian biotechnology industry. The commercial production of biopesticides began in the 1980s, but expansion became rapid in the late 1990s stimulated by national and state programmes for IPM promotion (Wahab 2004). Other biopesticides currently under development include *Hyblea puera* NPV for controlling teak defoliator (Biji et al. 2006), *Amsacta albistriga* NPV for controlling this pest on groundnuts (Veenakumari et al. 2007), *Nomurea rileyi, Pochomia chlamydosporia,* and entomopathogenic nematodes.

It has been estimated that there are at least 32 commercial companies active in biopesticide production, with an additional 32 IPM centres under the Ministry of Agriculture also producing selected biocontrol agents (Singhal 2004). The state departments of agriculture and horticulture in the states of Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, and Gujarat have established biocontrol laboratories for producing selected microbial biocontrol agents. A few state agricultural universities and Indian Council of Agricultural Research (ICAR) institutions also produce small quantities of microbial pesticides (Rabindra 2005). In total, at least 410 biopesticide production units have been established in India, 130 in the private sector (Singhal 2004).

Table 4. Microbial pesticides used in India as of 2009.

	Taxus	**Products**[1]	**Targets**
Fungicides			
Pseudomonas fluorescens	Bacterium	ABTEC Pseudo Biomonas Esvin Pseudo Sudo Phalada 104PF Sun Agro Monus Bio-cure-B	Plant soil borne diseases
Ampelomyces quisqualis	Fungus	Bio-Dewcon	Powdery mildew
Trichoderma harzianum	Fungus	Biozim Phalada 105 Sun Agro Derma H	Soil borne pathogens
Trichoderma viride	Fungus	Monitor Trichoguard NIPROT Bioderma Biovidi Eswin Tricho Biohit Tricontrol Ecoderm Phalada 106TV Sun Agro Derma Defense SF	Soil borne pathogens
Fungicides/bactericides			
Bacillus subtilis	Bacterium	-	Soil borne pathogens
Insecticides			
Bacillus thuringiensis subsp. *israelensis*	Bacterium	Tacibio Technar	Lepidopteran pests
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Bio-Dart Biolep Halt Taciobio-Btk	Lepidopteran pests
Beauveria bassiana	Fungus	Myco-Jaal Biosoft ATEC Beauveria Larvo-Guard Biorin Biolarvex Biogrubex Biowonder	Coffee berry borer, diamondback moth, thrips, grasshoppers, whiteflies, aphids, codling moth

Table 4 (con'd)

		Veera Phalada 101B Bioguard Bio-power	
Metarhizium anisopliae	Fungus	ABTEC Verticillium Meta-Guard Biomet Biomagic Meta Biomet Sun Agro Meta Bio-Magic	Coleoptera and lepidoptera, termites, mosquitoes, leafhoppers, beetles, grubs
Paecilomyces fumosoroseus	Fungus	Nemato-Guard Priority	Whitefly
Paecilomyces lilacinus	Fungus	Yorker ABTEC Paceilomyces Paecil Pacihit ROM biomite Bio-Nematon	Whitefly
Verticillium lecanii	Fungus	Verisoft ABTEC Verticillium Vert-Guard Bioline Biosappex Versitile Ecocil Phalada 107 V Biovert Rich ROM Verlac ROM Gurbkill Sun Agro Verti Bio-Catch	Whitefly, coffee green bug, homopteran pests
Helicoverpa armigera nucleopolyhedrosis virus	Virus	Helicide Virin-H Helocide Biovirus-H Helicop Heligard	*Helicoverpa armigera*
Spodoptera litura nucleopolyhedrosis virus	Virus	Spodocide Spodoterin Spodi-cide Biovirus-S	*Spodoptera litura*
Nematicides			
Verticillium chlamydosporium	Fungus	-	Nematodes

[1]product names may be local and may not match product names officially registered; some products are pending registration

Source: CIB and RC website, minutes of the Registration Committee meetings, June 2003 – March 2009

Fourteen agents are currently registered and 10 are undergoing registration, including one baculovirus, one entomopathogenic fungus, and two fungal antagonists (Department of Biotechnology 2008). Data on the current production of biopesticides is difficult to assess accurately. In 2008, three larger private companies reported the following total production values: 187 metric tonnes (MT) of *Trichoderma harzianum,* 23 MT of *Trichoderma viride,* 62

MT of *Sendomonas lecanii,* 28 MT of *Beauveria bassiana,* 30 MT of *Verticillium lecanii*, and 25 MT of *Metarhizium anisopliae*.

Despite the progress in establishing a microbial insecticide supply, the scale of biopesticide use in India still remains relatively small in comparison with chemical pesticides (Rabindra 2001). Awareness of microbial products amongst farmers is poor, despite active IPM promotion and training. Much of the current production is sold to government agencies for distribution to farmers in IPM programmes, but distribution systems for biopesticides are underdeveloped in many areas. Market studies have suggested that, apart from the entomopathogen *M. anisopliae,* current production of microbial pesticides meets less than 10% of the identified need (Rabindra 2005).

REGISTRATION AND THE REGULATORY SYSTEM

Biopesticides fall under the Insecticide Act (1968) under which any microbial organism manufactured or sold for pest and disease control should be registered with the Central Insecticides Board (CIB) of the Ministry of Agriculture. To promote registration, biopesticide products benefit from priority processing of registration, simplified registration procedures, and the acceptance of generic registration data for new products containing strains already registered (Kulshrestha 2004). Manufacturers can register their products under either 9(3) B (temporary registration) or 9(3) (regular registration). This system allows commercial producers of those microbial pesticides evaluated as generally safe to obtain provisional registration and continue to develop a market while the product is undergoing full registration; this reduces commercial barriers to product development. The data requirement for registration under 9(3) B is less stringent than for 9(3). For example, efficacy data on specified crops are required from 2 locations over two seasons for 9(3) B, while the same is required from 3 locations for 9(3). Data on product characterization, efficacy, safety, toxicology, and labelling must be submitted while applying for registration. The CIB's established quality standards must be met, with reference to content, virulence of the organism in terms of LC_{50}, moisture content, shelf life, and secondary non-pathogenic microbial load. Protocols for assessing these quality parameters have been prescribed (Rabindra 2005).

A long standing issue is the poor quality and unreliability of some products, which has had a negative impact on farmer confidence, and as a result, farmer demand (Kennedy et al. 1999). Surveys to test the quality of biopesticides are conducted, and while some manufacturers clearly meet accepted standards (Ignacimuthu et al. 2001), other reports indicate quality concerns, especially from new and inexperienced producers. A system of referral laboratories accredited by the DBT for quality testing has been established, but enforcement of standards remains an issue.

SPECIAL CONCESSIONS AND ORGANIZATIONS PROMOTING BIOCONTROL

The national agricultural research system, comprising of the many ICAR institutes as well as state agricultural universities, plays a leading role in promoting biopesticides. The Project Directorate of Biological Control is involved in testing the quality of biopesticides and training the officers of the state department of agriculture in quality control protocols. The National Centre for IPM routinely incorporates the use of biopesticides in its IPM validation programmes and demonstrations, as do the IPM centres of the Directorate of Plant Protection, Quarantine, and Storage. Commodity research boards have also played a role in researching and developing biopesticides for pest control in key crops such as cotton, coffee, tea, and cardamom.

The DBT has had a substantial funding programme for the research and development of microbial pesticides since 1989, with over 200 projects funded (Wahab 2004). This encourages the development of new technology and academic industrial links. The DBT also provides financial support for the generation of toxicological data to promote registration of microbials; data generation has been completed for almost all the currently registered biopesticides.

The state governments play the main role in implementing IPM. Their IPM programmes for purchasing and distributing biopesticides to farmers have been vital to creating a market for and encouraging private commercial production of microbial pesticides. States such as Tamil Nadu, Gujarat, Andhra Pradesh, and Maharashtra have been particularly active in promoting microbial pesticide use. The State Universities of Agriculture have played important roles in biopesticide research, and in a few cases are also producing biopesticides themselves and are advising companies in production. The State Agricultural Universities and other stakeholder agencies, through the Agricultural Sciences Centre (Krishi Vigyan Kendra), are encouraged to take up initiatives to promote local production of microbial pesticides.

Indian companies have formed a biopesticide supplier's association, the All India Biotech Association, to co-ordinate the commercial sector's voice in developing government policy. Other organizations actively promoting biopesticides include non-governmental organizations (NGOs) such as the M.S. Swaminathan Research Foundation, and international research centres based in India such as the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the International Rice Research Institute.

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SOUTH KOREA

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OVERVIEW AND USE

Since the late 1970s, Korean microbial pest control has involved the use of entomopathogenic viruses, bacteria, fungi, and nematodes to control pests in forestry, agriculture, and golf courses. The basic research studies for the development of microbial pesticides focussed on the selection of highly virulent isolates, their characterization, and their efficacy under field conditions. More recently, a university research group has been studying the construction of a recombinant baculovirus by introducing a *Bacillus thuringiensis* (*Bt*) crystal protein gene to improve insecticidal activity.

After establishing the guidelines for experimental protocols, requirements, and reviews of dossiers for microbial pesticides in 2000, insect pathology moved to the development of bioinsecticides. Solbichae, the first commercial bioinsecticide (based on *B. thuringiensis* subsp. *aizawai*), was developed and registered in 2003 to control diamond back moth and beet armyworm in Chinese cabbage. Three new registrations soon followed. Tobagi, also based on *B. thuringiensis* subsp. *aizawai*, was registered to control moths including diamond back moth and oriental tobacco budworm in pepper and rice. The product was registered as both wettable powder and suspension concentrate formulations. Bangsili, based on *Paecilomyces fumosoroseus*, was registered to control greenhouse whitefly and two-spotted spider mite in strawberry and cucumber. Ddangumi, based on *Monacrosporium thaumasium*, was registered to control root knot nematodes in watermelon. A total of 34 microbial pesticide products were registered to control insect pests and plant diseases in Korea by 2009. Among the 18 registered microbial insecticides, 7 products are imported. These include *Bt* subsp. *aizawai*, *Bt* subsp. *kurstaki*, *Beauveria bassiana* GHA, and *B. bassiana* TBI-1 (Table 5). About 20 additional biopesticide products are pending registration.

Research is mainly conducted at universities and governmental or private research institutes. For example, the Insect Microbiology Laboratory at Seoul National University conducts research with entomopathogenic bacteria and viruses, and constructs recombinant viruses. The Biological Control Laboratory at Chungnam National University conducts collection, screening, and characterization of *Bt*. The Insect Virus Laboratory at Chungbuk National University screens and characterizes highly virulent viruses. The Nematode Laboratory at Gyeong Sang National University is studying the behaviour, ecology, and mass production of entomopathogenic nematodes. The Agricultural Microbiology Team of the National Academy of Agricultural Science (NAAS) is studying the use of entomopathogenic fungi to control greenhouse pests, especially aphids and whiteflies. Doubgbu HiTek, Kyung Nong Corporation, Greenbiotech, and Korea Bio Corporation are developing microbial pesticides based on *Bt* and fungi.

Table 5. Microbial pesticides registered in South Korea to control insect pests and plant diseases as of 2009.

	Taxus	Products	Targets
Fungicides			
Bacillus pumilus	Bacterium	Ecosense*	*Phytophthora* blight
Bacilus subtilis		Bibong* Ecogent* Ecosmart* Topsaver Teras Holeinone Ibsalim Greenall Cillus Shootingstar Jaenotan	Powdery mildew, gray mold, *Alternaria* blight, large patch, brown patch, *Pythium* blight, *Phytophthora* blight
Paenibacillus polymixa	Bacterium	Topseed	*Phytophthora* blight, powdery mildew
Streptomyces colombiensis	Bacterium	Mycocide	Powdery mildew, gray mold, brown patch
Streptomyces kasugaensis	Bacterium	Safegrow	Sheath blight, large patch
Ampelomyces quisqualis	Fungus	Cufect	Powdery mildew
Insecticides			
Bacillus thuringiensis subsp. *aizawai*	Bacterium	Biocan* Salchungtan* Scolpion* Solbichae Tobagi	Lepidopteran pests
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Imperial* Tuneup* Gumulmang Biobit Bychung Bigule Samgong BT Shuricide Youngil BT	Lepidopteran pests
Beauveria bassiana	Fungus	BotaniGard* Ceremoni*	Thrips, greenhouse whitefly, two-spotted spider mite
Paecilomyces fumosoroseus	Fungus	Bangsili	Two-spotted spider mite, greenhouse whitefly
Nematicides			
Monacrosporium thaumasium	Fungus	Ddangumi	Root knot nematode

*imported product

Bt products were originally classified as chemical pesticides, but were recategorized as biopesticides in 2004. In 2000, the Korean government forecast that biopesticide sales would rise to $1.1 million by 2005, but sales only reached $0.25 million, including *Bt* products. The Korean government is aiming for a 40% reduction (from the 2004 value) in the use of chemical pesticides by 2013. The number of microbial pesticide related patents pending and/or obtained has been increasing, with an especially sharp rise after 2000. Biopesticides will share 10% of the pesticide market (1,086 billion Korean won) in 2010.

REGISTRATION AND THE REGULATORY SYSTEM

The microbial pesticide regulations from 2000 were replaced in 2005 with new guidelines for biopesticides. The notice was revised in 2006 and 2008 and transferred to the Acts for management of agricultural chemicals in 2009. In the 2008 notice, biopesticides were defined as biological control agents that included living microorganisms and extracts from natural products. They were further subdivided into microbial and biochemical pesticides. Microbial pesticides were defined as agricultural pest control agents that used living microorganisms, including bacteria, fungi, viruses, and protozoa. Biochemical pesticides were defined as natural products that were produced in nature and/or pheromones produced by insects. The Act for registration of agricultural chemicals includes all guidelines for registration of chemical, microbial, and biochemical pesticides. A diagram outlining the current procedure for registration is shown in Figure 1.

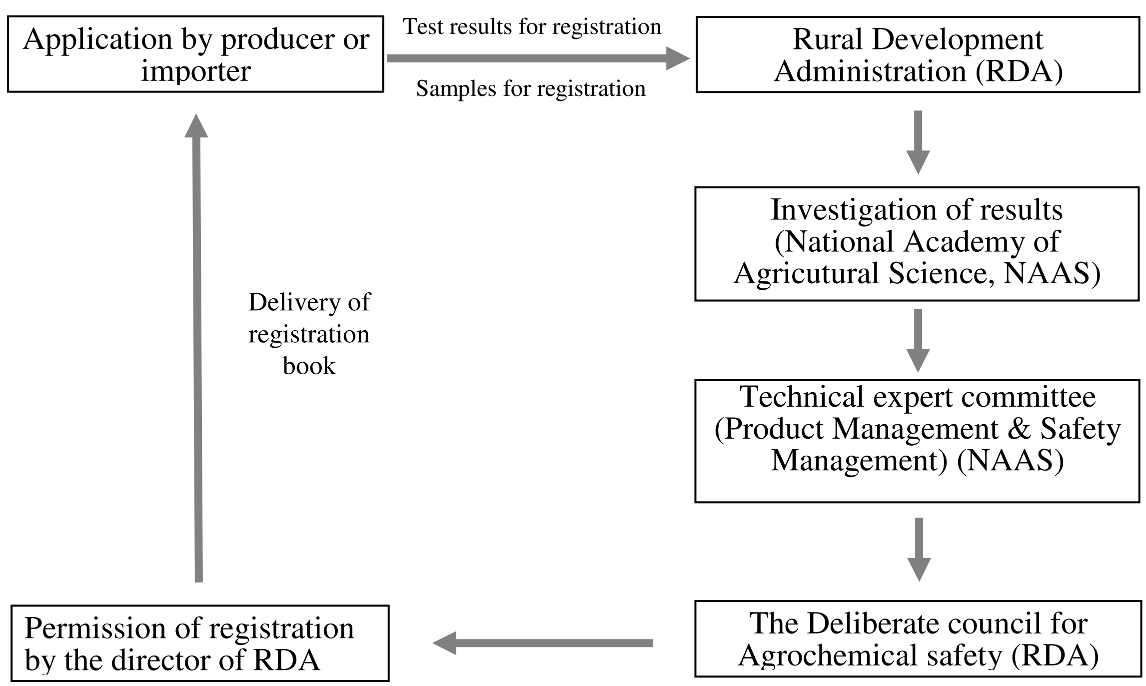

Figure 1. Procedure for the registration of microbial pesticides in South Korea.

Application procedure

A dossier for the registration of a new product is submitted by the applicant to the Agro-materials Management Division (AMD), Rural Development Administration (RDA). RDA directs the examination of the dossier to the Pesticide Safety Evaluation Division (PSED), National Academy of Agricultural Science (NAAS), that evaluates the dossier. Once the dossier is deemed to contain sufficient information, the PSED holds two technical expert committees for product management and safety management, with each committee comprising of 15 members. The committees provide the examination results to the PSED, who adjusts the results and reports to the AMD, RDA. The AMD have a dedicated council for agrochemical safety for the final decision and for reporting to the applicant. This process requires 6 months for new registrations, 30 days for re-registration, and 3 months for amendments/additions such as target pest and crop, and the amount and method of application. The term of validity for a newly registered product is 10 years which must be renewed at least 6 months prior to its expiry.

Requirements of the dossier

To register an agricultural pest control agent, the dossier must include test results in five categories: biological/physicochemical characteristics, efficacy and damage, mammalian toxicity, toxicity against a biological indicator in the environment, and environmental persistence. Each category has different requirements for each of agrichemicals, microbial pesticides, and biochemical pesticides. A test for biopesticide registration in each category is based on a three-tiered test system. Each test must be conducted in a research institute and/or university authorized by the Director of RDA. Dossier requirements for microbial pesticides are detailed below.

<u>Biological / physiochemical characteristics</u>

The final test sample for the biological characteristics test must be the same as that used for the efficacy and damage evaluations, with the identity of the sample confirmed by the NAAS. Requirements for dossiers on biological/physiochemical characteristics are as follows:

i) data on the origin, geographic distribution, and biology of the microorganism
ii) biological characteristics of the active microorganism: host range, mode of action, and propagation
iii) physical properties: color, image, viscosity, physical property of formulation, and stability of application liquid
iv) chemical properties and stability: pH, corrosion, storage stability, storage method, and quality guarantee period
v) manufacturing process and a recipe for manufacture
vi) content, method of analysis, and identification method for the active microorganism
vii) content and method of analysis, if the microorganism produces toxic substances during manufacture or storage
viii) the final formulation and use pattern
ix) contamination tolerances and scientific justification for permissible content

<u>Efficacy and damage</u>

The test for efficacy and damage must be conducted in three field trials in each of two or three years. Efficacy must be over 50% control of the pest compared to untreated control. The modified use of a currently registered product requires two tests, but in the case of the simple addition of a target pest in the same crop, data on crop damage can be omitted. For increased concentration of an active ingredient, only crop damage data and a rationale for the increase are required. For reduced concentration of an active ingredient, only efficacy data are required. Crop damage testing is required for both the recommended and 2X recommended rates. Requirements of dossiers for efficacy and damage include the following:

i) method for use, location (greenhouse, field), application timing and frequency, content for application, and if possible, the method for maximizing efficacy
ii) factors that inhibit efficacy such as chemicals, weather, and cultural environment
iii) pathogenicity and damage to non-target crops

Mammalian toxicity

Toxicity, pathogenicity, and infection to mammals must be shown to be low. Also, the status of registration and regulation in other countries must be indicated. The evaluation follows a tiered system. If the first-tier tests such as the toxicity of oral, dermal, respiratory, intravenous, skin, and eye sensitivity report zero toxicity, second and third tier toxicity tests are not required.

Toxicity to biological indicators in the environment

This also follows a tiered system. In the first step, toxicity tests are conducted on freshwater fish and invertebrates, birds, bees, and soil microorganisms. When the microorganism is reported as safe following the initial tests, further testing, including environmental fate and toxicity against non-target insects, are not required. Once the product/microorganism is shown to have no effect on other organisms in the environment in which the active ingredient is applied, further testing procedures can be shortened. This level of testing is not required if a product is proven to have no possible contact with a particular organism, based on characteristics of the microbial pesticide and its methods of application.

Environmental persistence

Persistence data requirements include the following:

i) persistence such as viability, survival, and propagation within the crop, soil, and water
ii) methods to quantify the microorganism in application environments
iii) dissemination of the microorganism in crop, soil, water, and air
iv) information regarding the donor organism in the case of a genetically modified microorganism

The test must be conducted in the optimum environment of the microorganism under local conditions. If a product is proven to have no toxicity against mammalian and biological indicators in the environment, persistence data can be omitted, except in the case of genetically modified microorganisms. When the microorganism cannot survive in the optimum environment, then all other persistence data may be abbreviated.

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EUROPEAN UNION WITH SPECIAL REFERENCE TO THE UNITED KINGDOM

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EUROPEAN UNION: OVERVIEW AND USE

The European Community or Union (EU) is composed of 27 Member States (countries). Biopesticides are increasingly seen in the EU as important crop protection tools needed to support modern horticulture and agriculture, particularly in integrated crop/pest management (ICM or IPM) systems. There is no standardised European definition of what constitutes a biopesticide. The term generally includes products with active substances based on microorganisms, botanicals (or biochemicals or plant extracts), and semiochemicals (including pheromones). These are regulated under the Council Directive 91/414/EEC[2] and subsequent amendments[3,4] which applies to all plant protection products, regardless of type. However, under this Directive, a microbial plant protection product is specifically defined as 'a microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material. The definition applies to, but is not limited to, bacteria, fungi, protozoa, viruses and viroids.'

Microbial pesticides registered in Europe are listed in Table 6. They are used as crop protection products in conventional agriculture and horticulture, as standalone products, in IPM, and in organic crop production. Across Europe, there has been increasing retailer and consumer pressure on growers to reduce chemical pesticide residues on fresh produce, often to below the levels set and enforced by the European authorities. While a new legislative framework on pesticide residues in food came into force in Europe on September 1, 2008 (Regulation (EC) 396/2005, www.ec.europa.eu/food/plant/protection/pesticides/index_en.htm), growers are being encouraged to produce food without detectable chemical pesticide residues to remain competitive with buyers and to keep their markets. For this reason, microbial pesticides are often considered as suitable alternatives, because their use is unlikely to result in residues on treated produce. Changes such as these continue to stimulate grower interest in microbial pesticides and are driving demand for these types of products. Biopesticide global sales are projected to reach $1 billion by 2010 (Chemistry & Industry, Issue 20, dated 27 October 2008), and $10 billion by 2017 (www.bccresearch.com) and pressure is being felt by both the EU and Member State regulators and producers to fast-track biopesticide authorizations.

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[2] **Commission Directive 91/414/EEC.** COUNCIL DIRECTIVE of 15 July 1991 concerning the placing of plant protection products on the market. (91/414/EEC). (OJ L 230, 19.8.1991, p. 1).
[3] **Commission Directive 2001/36/EC** (16 May 2001) - Amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market (**Microbial data requirements)**
[4] Council Directive 2005/25/EC - Annex VI to Directive 91/414EEC as regards plant protection products containing microorganisms. (Uniform Principles)

Table 6. Microbial pesticide active ingredients registered in the European Union with representative products that are registered in at least one Member State+ as of 2010.

	Taxus	Products	Targets
Bactericides			
Aureobasidium pullulans	Bacterium	Blossom Protect*	Fire blight, postharvest diseases in apples
Fungicides			
Phlebiopsis gigantea (several strains)	Bacterium	Rotstop*	Conifer root rots
Pseudomonas chlororaphis	Bacterium	Cedomon Cerall	*Pyrenophora teres, Pyrenophora graminea, Tilletia caries, Septoria nodorum, Fusarium* spp.
Pseudomonas sp. DSMZ 13134	Bacterium	Proradix*	Root rots
Streptomyces griseoviridis K61	Bacterium	Mycostop	*Fusarium* wilt, *Botrytis* grey mold, root rot, stem rot, stem-end rot, damping off, seed rot, soil borne damping off, crown rot, *Rhizoctonia*, *Phytophthora*, wilt, seed damping off, early root rot
Ampelomyces quisqualis AQ10	Fungus	AQ10	Leaf diseases
Candida oleophila strain O	Fungus	*	Post harvest diseases
Coniothyrium minitans C ON/M-91-05	Fungus	Contans WG	*Sclerotinia sclerotiorum, Sclerotinia minor*
Gliocladium catenulatum J1446	Fungus	Prestop Prestop Mix	Damping off, gummy stem blight, grey mold, root rot, stem rot, wilt, storage diseases, foliar diseases, seed rot
Pseudozyma flocculosa PF-A22 UL	Fungus	Sporodex*	Powdery mildew
Pythium oligandrum	Fungus	Polyversum	Root rots
Trichoderma aspellerum (ICC012) (T25) (TV1) (formerly *T. harzianum*)	Fungus	Tenet	Fungal infections (*Pythium, Phytophthera, Botrytis, Rhizoctonia*)
Trichoderma asperellum (T34)	Fungus	*	*Fusarium* spp.
Trichoderma atroviridae IMI 206040 (formerly *T. harzianum)*	Fungus	Binab T Pellets	*Botrytis cinerea,* pruning wound infection *Chondrostereum purpureum*
Trichoderma atroviride I-1237	Fungus	Esquive*	Fungal infections (*Pythium, Phytophthera, Botrytis, Rhizoctonia*)
Trichoderma gamsii (formerly *T. viride*) (ICC080)	Fungus	Remedier	Fungal infections (*Pythium, Phytophthera, Botrytis, Rhizoctonia*)
Trichoderma harzianum Rifai T-22 ITEM 108 or KRL-AG2	Fungus	Trianum P	Root diseases
Trichoderma harzianum Rifai T-39 (IMI 206039)	Fungus	Trichodex Rootshield	*Botritis cinerea, Collectotrichum* spp.*, Fulvia fulva, Monilia laxa, Plasmopara viticola, Pseudoperonospora cubensis, Rhizopus stolonifer, Sclerotinia sclerotiorum*
Trichoderma polysporum and *T. harzianum*	Fungus	Binab T Vector	Fungal pathogens, fairy ring, *Botrytis, Verticillium, Pythium, Fusarium, Phytophthora, Rhizoctonia, Didymella, Chondrostereum, Heterobasidion*
Verticillium albo-atrum (WCS850) (formerly *Verticillium dahliae*)	Fungus	Dutch Trig	Dutch elm disease

Table 6 (con'd)

Fungicides/bactericides			
Bacillus subtilis QST 713	Bacterium	Serenade	Fire blight, *Botrytis* spp.
Insecticides			
Bacillus thuringiensis subsp. *aizawai* GC-91	Bacterium	Turex	Lepidoptera pests
Bacillus thuringiensis subsp. *israelensis* AM65	Bacterium	VectoBac	Sciarids
Bacillus thuringiensis subsp. *kurstaki* HD-1	Bacterium	Dipel WP	Lepidoptera pests
Bacillus thuringiensis subsp. *kurstaki* ABTS 351, PB 54, SA 11, SA12, and EG 2348	Bacterium	Batik Delfin	Lepidoptera pests
Bacillus thuringiensis subsp. *kurstaki* BMP 123	Bacterium	BMP 123 Prolong	Lepidoptera pests
Bacillus thuringiensis subsp. *tenebrionis* NB 176	Bacterium	Novodor	Coleoptera pests
Beauveria bassiana ATCC 74040	Fungus	Naturalis L	Thrips, whitefly, mites
Beauveria bassiana GHA	Fungus	Botanigard	Whiteflies, aphids, thrips
Lecanicillium muscarium (Ve6) (former *Verticillium lecanii*)	Fungus	Mycotal Vertalec	Whiteflies, thrips, aphids (except the Chrysanthemum aphid: *Macrosiphoniella sanborni*)
Paecilomyces fumosoroseus Apopka 97	Fungus	Preferal WG	Greenhouse whiteflies (*Trialeurodes vaporariorum*)
Paecilomyces fumosoroseus Fe9901	Fungus	Nofly*	Whiteflies
Adoxophyes orana BV-0001 granulosis virus	Virus	Capex*	Summer fruit tortrix (*Adoxophyes orana*)
Cydia pomonella granulosis virus	Virus	BioTepp	Codling moth (*Cydia pomonella*)
Helicoverpa armigera nucleopolyhedrosis virus (HearNPV)	Virus	*	*Helicoverpa armigera*
Spodoptera exigua nucleopolyhedrosis virus	Virus	Spod-X GH	*Spodoptera exigua*
Spodoptera littoralis nucleopolyhedrosis virus	Virus	*	*Spodoptera littoralis*
Nematicides			
Paecilomyces lilacinus PL 251	Fungus	BioAct WG	Common plant parasitic nematodes
Virucides			
Zucchini Yellow Mosaic Virus, weak strain	Virus	Curbit	Yellow mosaic virus

+ In the EU active substances are listed on Annex I then each country registers the associated product, so products listed here are examples and may not be available in all 27 Member States and may have different names in different states.
*active substance provisionally listed on Annex I

EU: REGISTRATION AND THE REGULATORY SYSTEM

Directive 91/414/EEC covers not only authorizations of new plant protection products, but the review of products already authorized in the EU. The Directive aims to harmonise the registration of plant protection products throughout the EU. It is based upon a two-tiered registration system with active substances (or active ingredients) being assessed at the EU level for inclusion on Annex I (sometimes referred to as the 'Positive List') which is the list of substances eligible for use in plant protection products in the EU, and the products subsequently being registered by relevant Member States. The data requirements that need to be addressed are presented in Annexes to Directive 91/414/EEC. Annex IIB is specific to microorganisms and lists the requirements for the *active substance*. Annex IIIB lists the requirements for the microbial pesticide *product*. However, it should be noted that these requirements can be addressed by:

- Submitting new or unpublished data studies; or,
- Submitting a reasoned scientific case as to why the data requirement does not need to be addressed by a study; or,
- Submitting published studies, or other information, from scientific journals or other sources that are in the public domain; or,
- A combination of any of the above three sources.

Limited efficacy data are required in support of applications for active substances. However, a full efficacy data package is required for product registrations.

It should also be noted that for microorganisms, registration is specific to the strain/isolate. When registering an isolate, it must have a unique identifying code and must be stored at a recognised international culture collection. If an applicant intends to make reference to or use data generated for another isolate of the same or a similar species, public domain data for example, it may not be relevant to the isolate being registered. The assumption is that each isolate is different and the phenotypic expression of the isolate's traits may make it unique. Therefore, if data are provided for another isolate, then scientific justification should be provided to explain why it is relevant.

Registration of new active substances

A company with an active substance new to the EU must make an application, accompanied by a complete dossier of supporting data and information, to a Member State of the applicant's choice (termed the Rapporteur Member State (RMS)). The RMS will be responsible for starting the process of checking that the submitted dossier is complete and managing the evaluation and risk assessments. An overview of the process is given in Figure 2.

<u>Completeness of the application</u>

A formal completeness checking process is followed for new substance applications for first inclusion in Annex I. The RMS assesses whether the dossier contains sufficient information to commence the detailed evaluation. A report is sent to the European Commission and is made available to all other Member States and the European Food Safety Authority (EFSA). Copies of the dossier are also provided to all the other Member States, the European Commission and the EFSA. Assuming the dossier is complete, the Commission prepares a draft Decision recognising the completeness which is voted on by Member States at the Standing Committee on the Food Chain and Animal Health (SCFA). Assuming a positive vote, the Decision is then adopted and published in the Official Journal of the European Union (OJ).

<u>Detailed evaluation of the dossier</u>

Once the dossier is regarded as complete, the detailed assessment can begin. The RMS has 12 months from the date of publication of the completeness decision in the Official Journal to complete their Draft Assessment Report (DAR) and submit it to the EFSA.

<u>European decision making process</u>

The process for deciding whether an active substance can be included in Annex I to Directive 91/414 involves a peer review by all Member States, the EFSA, and European Commission. Members of the public and other interested parties can also provide comments for consideration in the process specifically through the public consultation process of the EFSA.

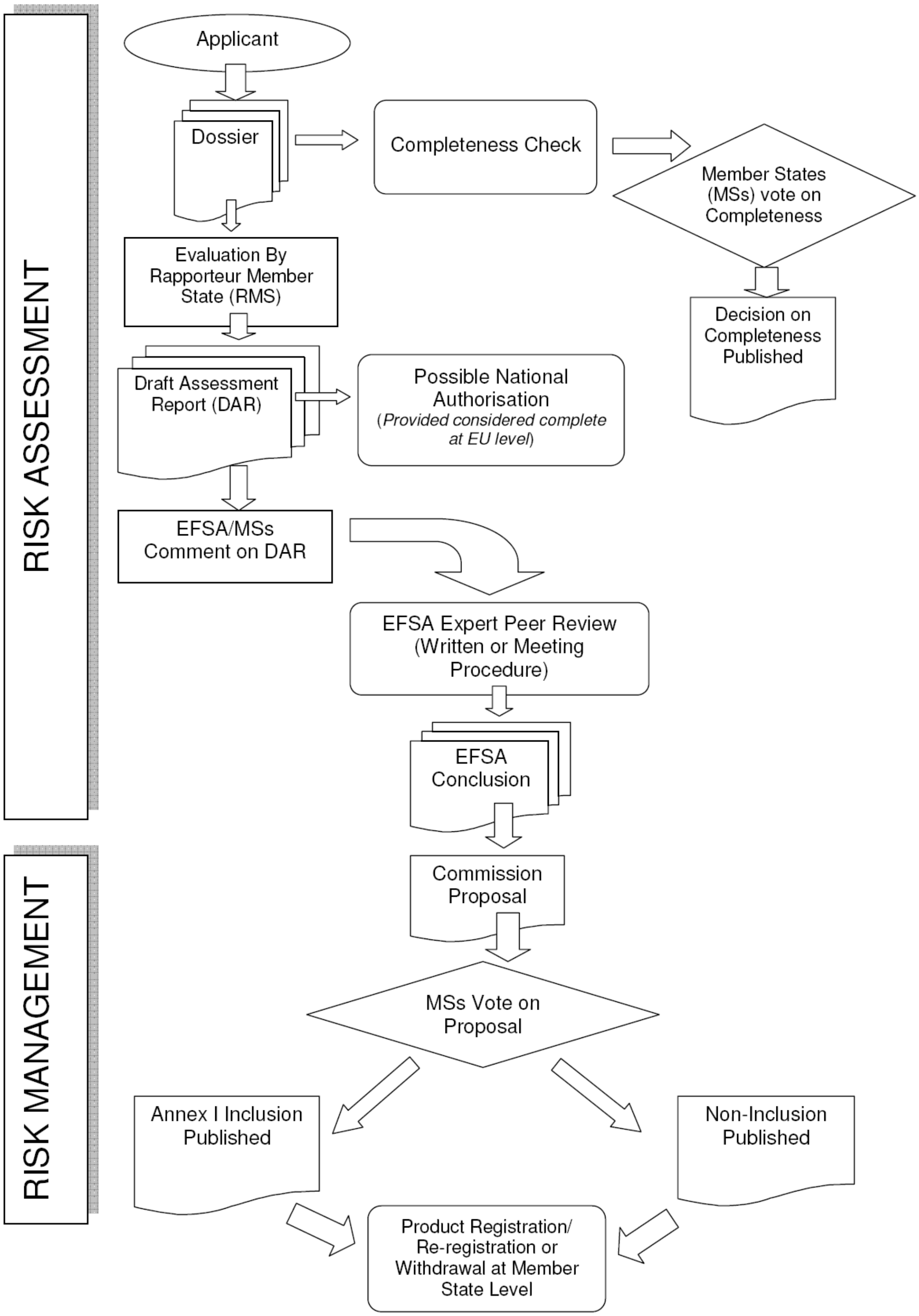

Figure 2. Overview of the EU regulatory process based on a new active ingredient.

Further details on the European Community decision-making process can be found on the European Commission's and EFSA's websites: www.ec.europa.eu/food/plant/protection/evaluation/index_en.htm and www.efsa.europa.eu, respectively.

Active substances listed on the Positive List (Annex I)

Although Annex I listing can be considered a hurdle to placement of biopesticides on the market, there are several active substances now listed, including several microbial pesticides (Table 7). It is estimated that the cost of developing a dossier for a microbial pesticide and submitting it for inclusion in Annex I is around €400,000 but this may depend on the type of product and the risk it represents. The figure could be doubled or in some cases reduced.

Table 7. Microbial pesticide-type active substances on the EU Positive List (Annex I) (as of May 2010).

Product type	Insecticide	Fungicide	Herbicide	Nematicide	Repellant	Other
Microorganism Bt	9	-	-	-	-	-
Microorganism non-Bt, non-baculovirus	5	22	0	1	0	0
Baculovirus	5	-	-	-	-	-
Plant derived	6	0	1	1	2	1
Semiochemical + pheromone	26	-	-	-	-	-
Total	**51**	**22**	**1**	**2**	**2**	**1**

Registration of products

For a new active substance, after it is placed in Annex I, the applicant can then seek a product registration in any Member State where it intends to market the product. To ensure consistency in the evaluations among Member States, Annex VIB of Directive 91/414/EC provides *Uniform Principles* specific for evaluation and authorization of microbial plant protection products. The same principles are also followed when a provisionally authorized product (see below) requires full registration, or other active substances require re-registration.

Directive 91/414/EC also includes a provision for *Mutual Recognition* (MR) of regulatory decisions on products, provided that agronomic, climatic, and environmental factors are similar. In practice, though, the EU does have variable conditions and additional efficacy data is often required for each Member State where the product is to be marketed.

Efficacy requirements

For each Member State where a product is to be authorized for use, an applicant is required to provide data to support the product's label claims for effectiveness and crop safety. These data are likely to be needed for each country/crop/target pest combination and should be generated over two seasons, although exceptions are made in some cases, such as for protected crops. Data should be generated from a number of high quality efficacy trials that address the proposed product use. The trials should be carried out by *Officially Recognised* organisations (i.e. an organisation approved by the respective countries' regulatory authorities as being competent to undertake good quality trials) and to GLP standard. The required number of trials varies among

Member States, but can be reduced by using appropriate laboratory studies and relevant published data. As additional crops and/or pests are added to the label, the number of trials that are needed may also be reduced. Although there are some possibilities to reduce the efficacy data required, including when MR operates, the crop-by-crop, country-by-country requirements can represent a hurdle to product registration.

The cost of product registration per Member State, including development of the efficacy data, is typically about € 50,000 – 100,000, varying according to each country's fees and the amount of data that is needed.

Provisional Authorizations

An application for the *Provisional Authorization* of a pest control product for several Member States can be made at the same time as the application for the active substance to be included in Annex I, and those products containing active substances not yet included in Annex I can currently be granted the *Provisional Authorization.* Such authorizations can be granted for three years with the possibility of extension. Provisional Authorizations are only permitted if, on the basis of a national evaluation and risk assessment, the individual Member State concludes that the substance and product are expected to satisfy the safety and efficacy requirements of the Directive. Provisional Authorizations can only be issued once a decision on the completeness of the dossier has been published. Re-registration in each respective Member State is required once the active substances of Provisionally Authorized products are included in Annex I.

CURRENT STATUS OF MICROBIAL PESTICIDE REGISTRATION IN THE EU

A regulation to replace Directive 91/414/EEC for placing plant protection products on the market was adopted by the EU in November 2009 and applies from June 2011. In this new regulation 1107/2009/EC, there is still no provision for a biopesticide-specific registration process for either active substances or products. However, there is a commitment to continue to provide guidance documents specifically on registration of microbial pesticides.

It is worth noting that, for product registrations there is a category for 'low risk' substances which may be applicable to microbial pesticides. There are listed criteria to meet and if a product qualifies under this categorisation, it is likely to require a reduced amount of data. Such 'low risk' products can be authorized for up to 15 years, will have a longer data protection period, and should be authorized by Member States under shorter time scales.

Currently, while Europe does not have a microbial pesticide-specific directive, guidelines have been developed to assist applicants in navigating through a regulatory system that has been primarily designed for synthetic plant protection products. These guidelines appear in the References section. Some Member States have also introduced policy-led approaches to provide resources to assist applicants who are usually smaller companies lacking knowledge about the regulatory system. For example, as part of Belgium's 'Programme for Reduction of Pesticides and Biocides' the government has constructed web pages as part of their plant protection product registration service (www.fytoweb.fgov.be). The Netherlands established the Gewasbeschermingsmiddelen van Natuurlijke Oorsprong Effectief Gebruiken (GENOEG) (www.genoeg.net/L2/about.htm) project with the following objectives: "to get more natural pesticides registered, to learn about lower risk profiles and apply the knowledge and experience gained in statements for registration purposes". The first phase of this project led to the registration of four natural pesticides for use in glasshouses and the second phase provided support for ten natural pesticides (including microbials) for a variety of uses. This project is now completed and where relevant lessons learnt are being incorporated into on-going microbial pesticide registration processes. The UK has also established the 'Biopesticide Scheme' to assist applicants, described in the next section.

CASE EXAMPLE – MICROBIAL PESTICIDES IN THE UNITED KINGDOM

UNITED KINGDOM: OVERVIEW AND USE

Historically, there have been few authorized microbial pesticide products available in the UK market. Prior to 2003, there were only three organisms authorized (*Bacillus thuringiensis, Verticilium lecanii*, and *Phlebiopsis gigantea*). Since the introduction of the initiatives discussed below, the numbers have grown, along with biopesticides other than microbials. Currently authorized microbial products are shown in Table 8.

UK: REGISTRATION AND THE REGULATORY SYSTEM

In the UK, the regulatory body responsible for plant protection products, including biopesticides, is the Chemicals Regulatory Directorate (CRD) (formerly Pesticide Safety Directorate (PSD). The CRD is a new Directorate of the Health and Safety Executive (HSE), responsible for the regulation of pesticides, biocides, detergents, and chemicals (under the Registration, Evaluation, Authorization, and Restriction of Chemicals Regulation (REACH)). The CRD is a single body made up of policy advisors, project managers, evaluators, and support staff.

In Great Britain, the use, supply, storage, and advertisement of pesticides are regulated by a number of pieces of legislation including the Control of Pesticides Regulations 1986 as amended (COPR) and Plant Protection Products (Basic Conditions) Regulations 1997 (PPPR/BCR). The use of pesticides is also regulated by the Control of Substances Hazardous to Health (COSHH). The statutory powers to control pesticides are contained within Part III of the Food and Environment Protection Act (FEPA). Section 16 of the Act describes the aims of these controls and the COPR provides the national mechanism to achieve the aims. The 1986 Regulations were updated by the COPR (Amendment) Regulations 1997.

Table 8. Microbial pesticides registered in United Kingdom as of 2010.

	Taxus	Products	Targets
Fungicides			
Peniophora gigantea	Bacterium	PG Suspension	*Heterobasidion annosum*
Pseudomonas chlororaphis MA 342	Bacterium	Cerall	Cereal diseases
Coniothyrium minitans CON/M/91-08	Fungus	Contans	*Sclerotinia*
Candida oleophila O*	Yeast	Nexyl	Post harvest diseases
Fungicides/bactericides			
Bacillus subtilis QST713	Bacterium	Serenade ASO	*Botrytis* spp.
Insecticides			
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	DiPel	Lepidoptera pests
Beauveria bassiana ATC74040	Fungus	Naturalis-L	Whitefly, thrips
Verticillium lecanii (=*Lecanicillium muscarium)*	Fungus	Mycotal	Whitefly, thrips, scale insects, mealybug
Cydia pomonella granulosis virus	Virus	Cyd-X Cyd-X Extra	Codling moth

*Registered under national rules not EU

Similar legislation exists in Northern Ireland and the majority of products approved for use in Great Britain are subsequently authorized for use in Northern Ireland.

The Plant Protection Products Regulations (PPPR) is the newer legislation and implements the European Directive (91/414/EEC). As discussed earlier in this chapter, the directive aims to harmonise the registration of plant protection products throughout the European Community. It is based upon a two-tier registration system with active substances being assessed at EC level for inclusion on Annex I, and products subsequently being registered by Member States. COPR will continue until all existing EC active substances are reviewed and placed on Annex I and all products have been re-registered. PPPR applies to new active substances coming onto the UK market and existing EC reviewed active substances that obtain Annex I listing.

Applications for approvals (authorizations) are processed according to specified timelines and the applicants are charged fees for the evaluation. Details of these are provided on the biopesticide section of the CRD's website.

THE CURRENT SITUATION AND MICROBIAL PESTICIDE USAGE IN THE UK

The CRD has introduced a number of measures to help promote biopesticide use. Following discussions with growers, manufacturers, and policy representatives, the CRD launched a Biopesticides Pilot Project in 2003 with the goal of encouraging alternative control measures, including microbial biopesticides. The initiative also reduced registration fees, which had been identified by industry as an obstacle to product availability. The pilot program included discussions with the UK section of the International Biocontrol Manufacturers Association (IBMA-UK, www.ibma.ch), research and development bodies, and academic institutions, leading to pre-submission meetings with potential applicants. From 2003-2006, the pilot program resulted in the availability of three new products based on a pheromone, a viral, and a fungal active substance.

Building on the success of the Biopesticides Pilot Project, the CRD launched a full-scale Biopesticides Scheme (www.pesticides.gov.uk/biopesticides_home.asp) in 2006, the goal being to increase the number of alternative pest control products entering the market. The scheme covers the full range of biopesticides, microbial and otherwise. Other novel alternative active substances and products are assessed on a case-by-case basis. The key elements of the scheme are:

- The appointment of a 'Biopesticide Champion', who provides an initial contact for product innovators/manufacturers, and steers them through the approval process
- Appointment of 'Biocontacts' in each of the specialist areas of risk assessment to provide guidance on specific scientific and regulatory issues
- Encouraging potential applicants to meet with the CRD at the earliest possible stages of product development
- Providing specific guidance to applicants (via pre-submission meetings), flagging possible challenges and identifying the best way forward for their product
- A fee for evaluation that is approximately a fifth of the cost of a conventional pesticide
- A new dedicated Biopesticide area on the CRD website to support those making applications (www.pesticides.gov.uk/biopesticides)

Since its introduction, the scheme has resulted in 12 new biopesticides in the UK, eight of which are microbial. In May 2010, there were an additional six microbial products under evaluation. Several companies are currently discussing applications to the CRD or have submitted draft dossiers as part of the pre-submission process or are discussing applications for new active substances.

The key to the success of the Biopesticides Scheme has been the opening of communication between biopesticide companies and the CRD. This allows for the clarification of the regulatory process and an opportunity for the CRD to hear and respond to the concerns of applicants. Through education, applicants have begun relying on publicly available data as opposed to feeling obligated to generating new data. The CRD continually aims to provide flexibility by having open discussions with industry, and by carefully considering what the appropriate level of regulation should be for each biopesticide product. They also use microbial guidance documents where available, such as those provided by the OECD.

SUMMARY

In Europe, active substances are regulated at the EU level, with product authorizations occurring at the Member State (country) level. While Directive 91/414/EC includes a provision for mutual recognition and data sharing among Member States, this only occurs among some countries, often because of between-country climate variations. The development of efficacy data on a crop-by-crop, country-by-country basis continues to be a hurdle, in terms of time and costs, to the timely delivery of biopesticide products to growers. However, there are the beginnings of an improved understanding of biopesticides, as illustrated by the UK Biopesticide Scheme, the GENOEG scheme in the Netherlands, and Belgium's Programme for Reduction of Pesticides and Biocides. There will also be increased opportunities for mutual recognition of product registrations as more active substances from microbial pesticides are included on Annex I of 91/414/EEC.

There are also potential opportunities in the near future that will be created by the new legislation. The new regulation 1107/2009/EC not only has features such as 'low risk' substances, but also sets shorter deadlines for the evaluation process and also establishes a more formal approach to zonal authorisations across Europe. Procedures for such issues along with a number of the other features of the legislation are currently being explored by Member States. Therefore, more details will become available as countries establish their implementation programmes.

It is widely acknowledged that Biopesticide registration in Europe has been based on a system primarily established for the registration of chemical pesticides and this has not always been appropriate. Therefore, in order to meet the anticipated future demands of modern crop production, the Biopesticide registration process must continue to develop and become streamlined. EU policy makers, regulators, and industries will also need to continue to collaborate more closely to meet consumer and environmental safety standards in a timely and cost effective manner.

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Useful websites

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www.pesticides.gov.uk
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www.fytoweb.fgov.be
www.genoeg.net/L2/about.htm
www.ibma.ch
www.rebecca-net.de

UKRAINE, RUSSIA, AND MOLDOVA

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OVERVIEW AND USE

Highlights of active biopesticide research in the former Soviet Union include the study of *Bacillus thuringiensis* against lepidopteran, coleopteran, and mosquito pests; granulovirus against the codling moth (*Cydia pomonella*); and the fungus *Beauveria bassiana* against various insect pests (Stefanovska et al. 2006). Still, production is minimal, limited to a few small, local laboratories. In general, most pest control products used in eastern Europe are imported, a consequence of a shift from the command economy of the Soviet Union to the market economy of the newly independent states (NIS). This is particularly true for the Ukrainian agricultural sector. Many of the newer synthetic and biological pest control products produced by Russia are marketed in Ukraine and other former Soviet republics.

Despite the limited production of biocontrol products in Ukraine, the country has a rich history of biopesticide research and production, beginning in the 19th century with the work of Illya Metchnikoff, a professor of Microbiology at the University of Odessa. Metchnikoff pioneered the idea that pests could be controlled by the application of a microorganism and discovered the use of the fungus *Metarhizium anisopliae* to control wheat cockchafer (grain beetle) (*Anisoplia austriaca*) (Stefanovska et al. 2006). This led to the establishment of a production plant to mass produce *M. anisopliae* conidia for the control of larvae of the sugar beet curculio. The first of its kind, this production plant initiated industrial-scale production of biopesticides in Europe and North America (Lord 2005). By the 1950s, however, a growing reliance on chemical products stalled biological pest control programmes in Ukraine. It was not until the late 1950s and early 1960s that increasing public concern prompted the re-emergence of biological pest control through the creation of the Institute of Plant Protection (under the Department of Microbiological Plant Protection) in 1957 (Stefanovska et al. 2006) whose focus was to research the combined use of *B. bassiana* and other insecticides to control the Colorado potato beetle (*Leptinotarsa decemlineata*), codling moth, and other insects. Shortly afterward, a Coordinating Committee was formed, bringing together scholars from eighteen universities and research institutions to advance biological plant protection. The establishment of thirteen companies and 268 insectaries to rear insect predators and produce entomopathogens represents the height of biocontrol in the Ukraine. As the Ukrainian economy generally declined beginning in 1991, 40% of the beneficial insectaries were shut down, and have not been recovered to date

(Stefanovska et al. 2006). Currently, the main centre for biopesticide research in Russia is the All-Russian Scientific Research Institute of Plant Protection (VIZR, previously called the All Union Institute), with numerous other government and university level institutes. In Ukraine, biopesticides are developed at the Institute of Plant Protection, Ukrainian Academy of Agricultural Sciences. In Moldova, they are developed at the Institute of Plant Protection and Ecologic Agriculture, Academy of Sciences of Moldova.

Microbial pesticide production at the local level in eastern Europe is significantly more cost effective for farmers compared to imported products, as international market pricing for imported pesticides often exceeds their buying power (Zubenko 1999). Local production is still lower, however, and combined with the expense of imports, pest control has trended to being dominated by the use of synthetic pesticides, with a shortage of pest control products in general. The Ukrainian Ministry of Agriculture Policy estimates that current agriculture practices require a minimum of 22,500 tons of pesticides, but farmers are managing with about 13,000 tons to farm nearly 12 million hectares.

There are over 800 synthetic pesticides on the NIS agricultural market but only 20-30 are commercially available biopesticide preparations (Terentiev 2006). Biological pesticides registered for use in Ukraine, Russia, and Moldova are listed in Tables 9-11. A priority in each country is to minimize the use of synthetic pesticides by encouraging organic farming and the use of non-chemical plant protection products, but biological products are generally not considered a substitute for synthetic products, rather a compliment to conventional methods already in place as part of an integrated pest management (IPM) approach. The biological products include microbial pesticides, growth- and immuno- stimulants, products for improving soil microflora, and biofertilizers. While biopesticide use is generally limited to small farms, this sector still contributes considerably to overall agricultural production in Ukraine, southern Russia, and Moldova. While there is a lack of statistical data, precise biopesticide sales are difficult to estimate, but according to recent information from Moldova, the use of biopesticides appears to be increasing.

Microbial products include fungi, viruses, bacteria, and nematodes. Of the bacterial products, the most common are *Bacillus thuringiensis* and *Pseudomonas* spp., used as insecticides or fungicides. Efficacy of these biological preparations has been shown to be comparable to or even higher than chemical pesticides tested. Examples of other more prominent uses of biopesticides include the control of the codling moth (*Cydia pomonella*) using entomopathogenic bacteria, fungi, viruses and protozoa. The granulosis virus of the codling moth (CpGV) has undergone extensive evaluation as a bioinsecticide in apple orchards (Stefanovska et al. 2005). Fungal-based products including *Beauveria bassiana, Metarhizium anisopliae, and Lecanicillium lecanii* are applied significantly in both greenhouses and field crops.

REGISTRATION OF MICROBIAL PESTICIDES AND THE REGULATORY SYSTEM

The registration process for biopesticides mirrors that for synthetic pesticides, and data requirements are similar among all NIS. It is a process inherited from the former Soviet Union and in general, encompasses all plant protection products. At this time, there are no special regulatory concessions for biopesticides, although rationales for the benefits of bioproducts favorably influence the evaluation decisions by reviewers of registration submissions. Government agencies and departments involved in pesticide regulation, and universities and government agencies involved in biopesticide research and development for each country are described below.

Russia

The state registration of microbial pesticides is overseen by Rossel'khoznadzor (Russian Agricultural Control), which is a federal service of the Veterinary and Phytosanitary Control, the federal executive organization. Rossel'khoznadzor not only administers the registration of pesticides, but also regulates their use, production, sale, transportation, storage, disposal, advertising, and import and export. VIZR in St. Petersburg, falling under the Russian Agricultural Academy (RAN) and is the umbrella organization committed to biopesticide research and development, but it is also involved in various aspects of the registration process. Research and development, and registration roles are provided by VIZR, in addition to numerous other organizations, most of them being research institutes (NII in Russian). They can be categorized as follows:

i) other institutions within the Russian Agricultural Academy: Institute of Cytology and Genetics, Siberian Department of RAN, Novosibirsk; GosNIIGenetika, Research Institute for Genetics and Selection of Industrial Microorganisms, a part of Scientific Centre of the Russian Federation, Moscow; All-Russian Scientific-Research Institute of Biological Plant Protection, Krasnodar, Kuban'; All-Russian Scientific-Research Institute of Phytopathology, Moscow region; Russian Research Institute of Silviculture and Mechanization of Forestry; and ii) scientific-research institutes within various universities, such as: Scientific-Research Institute of Biology (NIIB),

Table 9. Common microbial pesticides used in Russia.

	Taxus	Products	Targets
Bactericides			
Bacillus subtilis	Bacterium	Gamair SP	Bacterial diseases
Fungicides			
Pseudomonas aureofaciens	Bacterium	AGAT-25	Root rots, mildew, septoriosis, brown rust, ear fusariosis, cercosporosis, pseudoperonosporosis
Bacillus subtilis	Bacterium	Alirin-B Phytosporin	Root rot, mildew, bacterioses, phytophtorosis, anthracnose, microsporiosis, seed molds
Pseudomonas aureofaciens	Bacterium	Pseudobacterin 2Z	Root rots
Trichoderma harzianum	Fungus	Gliocladin (Trichodermin T, Z)	Fungal diseases (rots, fusarioses, verticillosis)
Fungicides/bactericides			
Actinomyces levendula	Bacterium	Phytobacteriomycin	Root rots and bacterioses
Bacillus subtilis WG6-14	Bacterium	Bactophyt SP	Mildew, phytophtorosis, root rot, bacterioses
Flavobacterium, *Phytobacteriomycin*	Bacterium	Phytoflavin-300	Bacterioses and fungal diseases
Pseudomonas fluorescens	Bacterium	Planriz KS	Root rots, mildew, bacterioses, phytophtorosis, anthracnose, microsporiosis
Insecticides			
Bacillus thuringensis subsp. *kurstaki* (spore-crystal complex)	Bacterium	Lepidocid	Larvae
Beauveria bassiana	Fungus	Boverin	Insect pests, larvae of Colorado potato beetle
Beauveria bassiana, GHA and *Bacillus thuringensis*	Fungus, Bacterium	Bitoxibacillin	Colorado potato beetle
Entomopathogenic nematode	Nematode	Nemabact	Cabbage fly, thrips

Kazan' State University; Novosibirsk State Agricultural University; and the K.A. Timiriazev Academy in Moscow. Some of the research institutes are former secret institutions of the Soviet Union's Military Defense Complex and their previous role were in the development of biological weapons. The safety aspects of biopesticides are studied at the Research Centre for Toxicology and Hygienic Regulation of Biopreparations, Moscow region.

Ukraine

In Ukraine, the Main State Inspection of Plant Protection is responsible for the registration of biopesticides and all other plant protection products. Similar to Russia, it is the main administrator of state trials, creates the annual State Register of allowable pest control products, and regulates all the organizations and companies involved in the production, use, trade, transport, storage, disposal, and other activities along the post-registration value chain. There are 28 organizations and institutions involved in the certification and approval of pest control products, and like Russia, are the main proponents of biopesticide research and development as well. Most of these organizations fall under the Research Institutes of the Ukrainian Agricultural Academy of Sciences (UAAN), National Academy of Sciences (NANU), or the various agricultural universities, the latter of which are governed by Ministry of Education and Science. The two best known institutions conducting biopesticide research and development are the Institute of Plant Protection (UAAN) and National University of Life and Environmental Sciences of Ukraine (former National Agrarian University of Ukraine).

Table 10. Microbial pesticides registered in Ukraine.

	Taxus	**Products**	**Targets**
Fungicides			
Pseudomonas aureofaciens	Bacterium	Agat 25K	Root rots, mildew, septoriosis, brown rust, ear fusariosis, cercosporosis, pseudoperonosporosis
Bacillus subtilis IPM-215	Bacterium	Bactophit	Mildew, root rots
Klebsiella oxytoca and *Bacillus mucilaginosus*	Bacterium	Kleps	Enhancing of resistance to root diseases
Chaetomium spp.	Fungus	Chetomic	Root molds, grey and white molds, fusariosis, common and silver scrub, rhizoctoniosis
Fomes fomentarius	Fungus	Mikosan	Enhancing resistance to root rots, scrub of leaves and fruits, mildew
Trichoderma viride	Fungus	Mycofungicyd (Trichodermin)	Root rots, white rot, fusariosis, verticillosis
Fungicide/insecticides			
Pseudomonas aureofaciens	Bacterium	Gaupsin	Larvae of harmful insects, scrub, mildew, fruit rots
Fungicide/bactericides/ nematicides			
Pseudomonas fluorescens, *Streptomyces albus,* and *Micrococcus roseus* bacterial complex	Bacterium	Bactophil	Seed germination diseases
Herbicides			
Achromobacter album	Bacterium	Albobacteryn	Sprouting inhibition

Table 10 (con'd)

Insecticides			
Bacillus tenebrionis	Bacterium	Decimid Novodor	Colorado potato beetle
Bacillus thuringiensis	Bacterium	Dendrobacillin Entobacteryn Turingin	Flying insects, web mites, Colorado potato beetle
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Lepidocyd	Various insects
Bacillus thuringiensis subsp. *thuringiensis*	Bacterium	Bitoxibacillin	Colorado potato beetle, various harmful insects
Streptomyces avermitilis	Bacterium	Actofit Astur	Colorado potato beetle, web mites, complex of phytphags
Beauveria bassiana	Fungus	Boverin	Colorado potato beetle, thrips, various insects
Entomophthora spp. (spores and toxins)	Fungus	Mycoaphidin	Pea aphid, peach aphids, other aphids
Metarhizium sp.	Fungus	Metarilin	Soil stages of hard wings insects
Paecilomyces spp.	Fungus	Pecilomin	Larvae of various insects
Verticillium lecanii	Fungus	Verticilin	Flying insects
Nematicides			
Arthrobotrys spp.	Fungus	Nematophagin	Nematodes

Moldova

The executive organization overseeing pesticide registration in Moldova is the State Centre for Attestation and Homologation of Phytosanitary Remedies and Remedies for Enhancement of Soil Fertility of Republic Moldova (CNAOFF). Because of Moldova's smaller population compared to Russia and Ukraine, there are only few organizations carrying out biopesticide research and development: the Institute of Plant Protection and Ecological Agriculture, Institute of Microbiology and Biotechnology and the Agricultural University of Moldova.

In all three countries, an applicant wishing to register a biopesticide must go through several steps to attain authorization for commercial use, including: i) application, including a sample of the pest control product; ii) acceptance of the identity of the control product received; iii) state trials and field experimentation in different types of soil and climatic conditions; iv) approval of the state tests results; v) registration and issuance of the approval certificate; and vi) addition of the product, once registered, into the State Register (State Catalogue in Russia).

The data supporting a registration submission must be provided by the registrant to the Director of the relevant departments for safe use of pesticides and agrochemicals. The departments are Rossel'khoznadzor in Russia, Main State Inspection of Plant Protection in Ukraine, and CNAOFF in Moldova. The data must provide a complete toxicological review of the pest control product and further include: i) methods of application; ii) hygienic evaluation; iii) influence on the environment; iv) physical and chemical properties; v) characterization of the active ingredient; vi) target areas for application; and vii) hazards to humans and the environment. Additionally, the registrant must present i) results from state trials, including a report from the research institute and the location of the trials; ii) recommended use and efficacy; iii) a review of the of suitability of the product and its likelihood of approval from the Ministry of Health Protection and the Ministry of Ecology (Ukraine, or corresponding ministries in Russia and Moldova); iv) methods to evaluate and minimize residue levels in the crop produce and environment (water, soil, air); v) draft instructions for use, transport, storage, personal protection, diagnosis and treatment of poisoning; and vi) a draft product label.

Table 11. Microbial pesticides developed at the Institute of Biological Plant Protection, Moldova.

	Taxus	Products	Targets
Bactericides			
Pseudomonas syringae	Bacterium	Pentafag–M	*Erwinia amylovora, Pseudomonas* spp., *Xanthomonas* spp.
Fungicides			
Pseudomonas fluorescens	Bacterium	Rizoplan	*Fusarium* spp., *Bipolaris* spp., *Helmintosporium* spp., *Ophiobolus graminis*
Trichoderma harzianum	Fungus	Trihodermina Th-7F-BL	*Sclerotinia sclerotiorum, Pythium debaryanum, Rhizoctonia solani, Fusarium* spp., *Botrytis cinerea, Ascochyta hortorum*
Trichoderma lignorum	Fungus	Trihodermina-BL	
Fungicides/bactericides			
Pseudomonas fluorescens	Bacterium	Rizoplan	*Olpidium brassicae*, *Pythium debaryanum*, *Rhizoctonia solani*, *Xanthomonas campestris*, *Erwinia carotovora*, *Pseudomonas tabacii*, *Phytophthora nicotianae*
Insecticides			
Verticillium lecani	Fungus	Verticilina granulara–BL	*Trialeurodes vaporariorum*
Granulosis- and nucleopolyhedrosis viruses of various insects	Virus	Virin CP ABB-3 OS MB HS-2	*Cydia pomonella*, *Hyphantria cunea*, *Scotia (Agrotis) segetum, Scotia (Agrotis)*, *Mamestra brassicae*, *Helicoverpa armigera*
Nematicides			
Arthrobotrys oligospora	Fungus	Nematofagina-BL	*Meloidogyne incognita*

The registrant can also present a case that use of the bioproduct has benefits either in it's own right, or above and beyond those for synthetic pesticides. Examples include increasing crop yield and the quality of agricultural produce, reducing input costs to the farmer, advancing the practice of organic farming, integrating the use of organic materials in farming, and increasing farm profitability. Further arguments that benefit the case for registration include those normally accepted for microbial pesticides: that they are safe for humans and animals when used at recommended rates, they can be used at any stage of plant development, safety is not compromised by the frequency of use, they have a short pre-harvest and reentry interval, they are non-polluting to soil and water, they do not accumulate in plant tissue, and that they can be used when other pesticides are restricted. These arguments are always accepted positively, and favorably influence the decision of state/federal executive organizations in all three countries.

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ARGENTINA

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OVERVIEW AND USE

Bacteria are the most widely used biocontrol agents in Argentina, with the greatest number of registered products (Table 12). *Bacillus thuringiensis* (*Bt*) products were first used in 1950 against *Colias lesbia* in alfalfa (Botto 1996). There are three imported products that are being used for insect pest control in agricultural crops (lepidopteran pests). These imported products, based on *B. thuringiensis* subsp. *kurstaki* (*Btk*), are used for the control of *Rachiplusia nu* and *Anticarsia gemmatalis* (Lepidoptera: Noctuidae): Bactur, Dipel PM, and Vendaval *Bt.* Based on the small-scale and isolated use of these products, the future of the development of pest biocontrol agents in Argentina is uncertain, partially due to a lack of national political strategies focussing on biocontrol programmes (Botto 1996).

The first virus-based product ('Carpovirus plus' based on a granulosis virus of *Cydia pomonella*) was registered in 2000 by Agro Roca. Field applications were initially conducted on apples in the Rio Negro and Mendoza provinces, and afterwards in walnut tree plantations in La Rioja and Catamarca provinces. Basic studies and efficacy trials were carried out by the National Institute of Agricultural Technology (INTA) and the Institute of Agriculture Microbiology and Zoology (IMIZA) in cooperation with two private companies: Agro Roca in Argentina and Natural Plant Protection in France (Sosa Gómez et al. 2008). Currently, *Epinotia aporema*, a granulosis virus, is undergoing the registration process by researchers at INTA and IMIZA.

Published research studies on fungal biocontrol are mostly associated with laboratory and small scale field trials. The use of a product based on *Beauveria bassiana* for controlling *Triatoma infestans* and *Musca domestica* was first published by Alves et al. (2008b). This product is currently undergoing registration by the National Service of Sanity and Agro-alimentary Quality (SENASA) (J. Willemoes, Biagro, pers. comm.). Current field trials using *B. bassiana* are using the product, L-Naturalis, which was formerly used in experiments to control *Trialeurodes vaporariorum*, *Myzus persicae,* and *Aphis gossypii* in tomato and pepper crops. Some oil and water formulations based on *B. bassiana* were also evaluated for control of *T. infestans* by INTA (Alves et al. 2008a and 2008b).

Formulated products based on the microsporidians *Antonospora locustae* and *Nosema locustae* (Canning 1953; Slamovits et al. 2004), were applied to control locusts in natural grasslands in the Buenos Aires, La Pampa, and Chubut provinces (Henry and Oma 1981; Lange and De Wysiecki 1999). These are the only records of protozoans used for pest control in Argentina (Sosa Gomez and Moscardi 1991; Briano 1999; Garcia et al. 2008). Lange (2002) reported that *A. locustae* was naturally established in the locust populations for several years after its introduction. There is no record of the registration of nematodes for insect control in Argentina.

Table 12. Microbial pesticides in Argentina.

	Taxus	Products	Targets
Fungicides			
Trichoderma spp.	Fungus	Biagro TL	Phytopathogenic fungi
Insecticides			
Bacillus sphaericus	Bacterium	Summit-Agro[1] Rosembuch[1]	Mosquitoes
Bacillus thuringiensis subsp. *israelensis*	Bacterium	Chemotecnica[1] Rosembuch[1] Biagro BT[2]	Mosquitoes, black flies
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Dipel[1] Bactur[1] Vendaval [1]	Lepidopteran pests
Beauveria bassiana	Fungus	Biagro Bb-vinchuca[1] Biagro Bb-mosca (registration in progress)	*Triatoma infestans, Musca domestica*
Cydia pomonella granulosis virus	Virus	Agro Roca[2]	*Cydia pomonella*
Epinotia aporema granulosis virus	Virus	Registration in progress	*Epinotia aporema*
Anticarsia gemmatalis nucleopolyhedrosis virus	Virus	Registration in progress	*Anticarsia gemmatalis*
Serum-free media nucleopolyhedrosis virus	Virus	Registration in progress	*Spodoptera*

[1]imported; [2]produced in Argentina

REGISTRATION AND THE REGULATORY SYSTEM

The regulatory institutions in Argentina are as follows: SENASA, Vegetal National Committee of South Cone (COSAVE), Secretary of Agriculture, Livestock, Fish and Food (SACPyA), and the National Administration of Drugs, Food and Medical Technology (ANMAT), the latter of which is exclusively responsible for the regulation of products to control vectors of medical importance. Information relating to pesticide registration, restrictions, commercialization, and use of agrochemicals and biological products is issued by SENSA through their 'Coordination of Agrochemical and Biological Products' department. There can also be province-specific registrations, and these are regulated by individual state departments, for example the Buenos Aires Province Department of Health in the case of Buenos Aires State. The National Agriculture Department and Environmental Policies Secretary is also involved in the regulation process. Specifications for the registration of biological (including microbiological) products are provided in the Resolution 350/1999 (Chapter 12, Agents for Microbial Control), available from SACPyA at www.infoleg.gov.ar/infoleg/internet/anexos/55000-59999/59812/texact.htm

SPECIAL CONCESSIONS AND ORGANIZATIONS PROMOTING BIOCONTROL

Despite the claim by Botto (1996) that a lack of national political strategies focussing on biocontrol programmes makes it's future in Argentina uncertain, some activities are taking place. Several programmes were successfully developed by INTA for controlling *Cydia pomonella* with the granulosis virus in Rio Negro, and for controlling *Epinotia aporema* (soybean borer) with a second granulosis virus that is under experimental registration. *Anticarsia gemmatalis* was also controlled with NPV virus in the Tucuman province by researchers at the Research Centre for the Regulation of Populations of Harmful Organisms (CIRPON) (National Council of Scientific and Technical Research (CONICET), San Miguel de Tucumán). Currently, new control programmes for *Spodoptera frugiperda* with nucleopolyhedrosis virus are underway at the Experimental Station Obispo Colombres, San Miguel de Tucumán, Santa Fe regional INTA stations, and IMIZA- INTA Castelar (IMIZA), Buenos Aires province.

There are a few organizations that are further promoting and developing biocontrol programmes in Argentina. While INTA is one major institution dedicated to biological control programmes in the country, various national universities are also developing research and extension programmes related to insect biocontrol and integrated pest management (IPM). There are also some programmes developed by the Department of Health in the Buenos Aires province, Salta, and Cordoba for the control of mosquitoes using *Bt* subsp. *israelensis* applications, and similarly to control blackflies in the provinces of Río Negro, Neuquén, and Mendoza. INTA agencies recommend *Bt kurstaki*-based products for controlling lepidopteran pests in some crops through their regional experimental stations in several provinces, but often, farmers favour synthetic products because they are more affordable (C. Salto, INTA Rafaela, Pers. Communication).

In recent years, the Centre for Parasitological Studies and Vectors (CEPAVE) (CONICET – University of La Plata), through grants from the University of La Plata, has undertaken biocontrol research and extension projects, culminating in new programmes to educate small farmers in the La Plata Horticultural Belt on the advantages of biocontrol using entomopathogenic fungi. The La Plata Horticultural belt is one of the most important horticultural regions in the Pampeana region, and fungal strains used are those acquired locally. CEPAVE has also engaged in a similar project in cooperation with the Institute for Research on Small-sized Family Agriculture (IPAF) (INTA for the Pampeana region).

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BRAZIL

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OVERVIEW AND USE

Approximately 3 million hectares of agricultural cropland are treated annually with microbial pesticides in Brazil. This use is expected to increase together with the growth of new markets for fruit, greenhouse, and organic crops, as well as for field crops associated with the modernization of dairy and beef production systems. Eight microbial pesticide products are currently registered for agricultural use, with one bacterial, four viral, and three fungal active ingredients. At this time, the products use naturally occurring microorganisms, none of which have been genetically modified.

Despite the considerable area being treated with biopesticides, their use is minor compared to synthetic pesticides. Some exceptions include the viral biopesticides that are used on 10-15% of soybean fields threatened by *Anticarsia gemmatalis* infestation, and the fungus *Metarhizium anisopliae* used on the same percentage of sugarcane fields suspected to be infested by root spittlebug.

Although the currently available mycopesticides show inconsistent efficacy under field conditions, better-quality products and sound recommendations for use by farmers and cattle ranchers will increase demand. The lack of mass production technologies is also a major limitation in the development of biological pesticides in Brazil, as it has not improved significantly in the past 25 years. A stable minimum concentration of viable active ingredient, longer shelf-life, simple application methods, UV protection, and greater field efficacy are all in need of further development. Still, mycopesticides are attractive competitors to synthetic pesticides. Unformulated biological pesticides cost around US $6 per kg and, on average, at least two kilograms are needed per hectare for ground applications. Oil dispersions containing an equivalent quantity of active ingredient are commercialized for US $12-20. Even with the increased cost of emulsifiable oils in recent years, biological pesticides remain economically advantageous in certain pest control situations.

The commercialization and use of different taxa of microbial pesticides in Brazil can be summarized in three specific categories. Viral pesticides that are generally produced by medium- and small-sized companies, and contain formulated products with high efficacy and prices lower than chemical insecticides; mycopesticides produced by small-sized companies, and generally contain unformulated products of moderate efficacy with prices much lower than chemical insecticides; and bacterial pesticides produced by large companies, and contain formulated products with high efficacy and prices higher than chemical insecticides.

Case examples

Fungi

Approximately 40 commercial mycoinsecticides available on the Brazilian market are registered by 19 for-profit companies (see partial list in Table 13). More than 20 laboratories operated by sugar/ethanol mills produce *M. anisopliae* for their own use for control of cercopids in cane fields. Universities, research institutes, non-profit organizations, rubber tree farms, and cattle farms also produce various fungal microbial control agents. The total area treated with entomopathogenic fungi ranges from 600,000 to 1,000,000 ha. These mycopesticides are commercialized mainly as technical grade products referred to as technical concentrates (TK) which are solid substrate plus fungal spores and, on a smaller scale, as technical material (TC) which is pure conidia. The only truly formulated products are oil dispersions (OD), in which conidia are mixed with an emulsifiable oil to render the suspension miscible in water for spraying.

The use of *M. anisopliae* for spittlebug (Hemiptera: Cercopidae) control in sugarcane fields and pastures represents the largest biocontrol programme based on a fungus. In the state of São Paulo alone, *M. anisopliae* subsp. *anisopliae* was applied to 250,000 ha for control of the sugarcane root spittlebug, *Mahanarva fimbriolata (*JEM Almeida, pers. comm.), during the growing season of 2007/2008.

Fungal isolates currently within the complex "*Sporothrix insectorum"* are used on more than 15,000 ha of rubber trees for the control of *Leptopharsa heveae* (Hemiptera: Tingidae). A range of other biocontrol programmes include the use of *Beauveria bassiana* for control of the weevils (*Cosmopolites sordidus*) in banana, *Sphenophorus levis* in sugarcane, *Hypothenemus hampei* in coffee, *Rhyncophorus palmarum* in commercial palm trees, and *Gonopterus scutellatus* in eucalyptus plantations. These are all currently used on a small scale, but in some cases have the potential for significant expansion. Similarly, the commercial use of *B. bassiana* and *M. anisopliae* in protected crops for control of whiteflies, aphids, thrips, and mites is just beginning. *Lecanicillium* sp. is currently being commercialized for the control of aphids and scales.

Trichoderma spp. for disease control

Within an IPM strategy to control witches' broom, a disease caused by the basidiomycete *Moniliophthora* (formerly *Crinipellis*) *perniciosa*, the fungus *Trichoderma stromaticum* is applied annually to about 2,000 ha of cacao trees in the northeastern state of Bahia (Pomella et al. 2007). This mycofungicide was further developed and is currently commercialized by the Executive Commission for the Economic Recuperation of Cacao (CEPLAC), a federal institution responsible for the development of cacao technologies. The product is sold in 2 kilogram packages of ground *T. stromaticum*-colonized rice or sachets with 40 grams of pure spores (Pomella et al. 2007). Problems related to large-scale production and pending registration have limited more extensive use of this product.

According to Bettiol et al. (2008), the fungus *Trichoderma harzianum* is widely used in potting soil in the horticultural and ornamental sectors before seeding, as well as for seed treatment. *T. harzianum* is also commonly used in central pivot irrigation systems in the midwest region of the country to control *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Fusarium solani* in field crops including bean, soybean, cotton, and corn.

A large number of universities and research institutes are involved in entomopathogenic fungal research, including the Brazilian Agricultural Research Corporation (EMBRAPA), Universidade de São Paulo (USP – ESALQ), and Instituto Biológico.

Viruses

Biocontrol of the velvet bean caterpillar, *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), in soybean represents the largest microbial biocontrol programme worldwide. This caterpillar is a key pest in soybean fields across Brazil and approximately 20 million hectares are treated yearly with synthetic and microbial insecticides for its control. On average, two chemical insecticide applications per season are necessary. In 2004-2005, *A. gemmatalis* multiple nucleopolyhedrosis virus (AgNPV) was applied to 2 million hectares, up from 1 million in the 1989/1990 season (Sosa-Gómez et al. 2008). Just one application is enough to control *A. gemmatalis* (Moscardi 1999). Currently, baculovirus production cannot keep up with increasing demand, which is a testament to the trust that Brazilian soy farmers have in its efficacy. Until 1984, dead virus-infected *A. gemmatalis* larvae were hand-picked and used as a non-formulated product, with sprays applied after larvae were ground and filtered. In 1985, a kaolin-based wettable powder was developed by EMBRAPA and this new formulation has been used since. Recently, technology for commercial virus production under laboratory conditions was developed, improving the quality and decreasing the cost to US $0.42 per one ha-equivalent dose, rivaling the cost of chemical counterparts (Moscardi 2007, Sosa-Gómez et al. 2008). The expansion of the use of baculoviruses and bacterial insecticides is challenged by future availability of plants with resistance to the target pests, particularly for caterpillar control.

Bacteria

The use of *Bacillus thuringiensis* in Brazil began in 1991. Approximately 150,000 hectares are treated per year (Souza 2001), with imported products accounting for the majority of sales. The high price of these products compared to chemical insecticides and other biocontrol agents can be partly explained by costs related to their importation, transportation, and distribution (Capalbo et al. 2008). In 2008, however, the first national commercial product for use in organic areas was developed by EMBRAPA (*B. thuringiensis* subsp. *kurstaki* commercialized under the name Ponto Final). High costs and low environmental persistence compared to pyrethroids have limited the adoption of bacterial pesticides on field crops, so their use remains largely restricted to organic and vegetable crops.

REGISTRATION AND THE REGULATORY SYSTEM

A Certificate of Registration, granted by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) after scrutiny by the National Health Surveillance Agency (ANVISA) and the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) is required for all pesticides used in agricultural areas, forests, and pastures. The Certificate of Registration authorizes the marketing of the pest control product in Brazil. Similarly, products for use in urban environments and public health campaigns are registered with ANVISA, after scrutiny by MAPA and IBAMA. Products for use in native forests and water environments must be registered with IBAMA, after meeting the requirements established by the other two registration authorities. The registration of genetically engineered microorganisms is governed by an entirely different set of guidelines.

The publication of Law 4072 in 2002 unified earlier regulations initiated jointly by MAPA, ANVISA, and IBAMA. These three organizations began to differentiate biological products from chemical products and prioritized registration of those with low toxicity and environmental hazard. The end result was that many tests required for chemical products were no longer required for biopesticides.

Table 13. Partial list of microbial pesticides used in Brazil.

	Taxus	Products	Targets
Fungicides			
Trichoderma harzianum	Fungus	Ecotrich (not registered) Trichodermil SC 1306	*Rhizoctonia solani, Fusarium* spp., *Sclerotinia* spp., *Pythium* spp., *Botrytis cinerea, Phytophtora infestans*
Trichoderma stromaticum	Fungus	Tricovab (not registered)	*Moniliophthora perniciosa*
Insecticides			
Bacillus thuringiensis	Bacterium	Agree Bac-Control Bactur Dipel Thuricide Xentari	Lepidopteran pests
Beauveria bassiana	Fungus	Boveril PL 63	Coleoptera (Curculionidae), Acari (Tetranychidae)
Metarhizium anisopliae	Fungus	Biotech (not registered) Metarril E9 Metarril 1037 (not registered) Metarriz (not registered) Methavida (not registered)	Hemiptera (Cercopidae), Acari (Ixodidae)
Sporothrix insectorum	Fungus	No commercial name (not registered)	Hemiptera (Tingidae)
Anticarsia gemmatalis nucleopolyhedrosis virus (AgNPV)	Virus	Baculo-Soja Baculovirus Nitral Coopervirus PM Protege	*Anticarsia gemmatalis*, lepidopterans

Source: Modified from Faria and Wraight (2007) and Ministry of Agriculture, Livestock and Food Supply (MAPA)

The first step for registration of a biopesticide to be used in non-organic fields is to request a Special Temporary Registration (Registro Especial Temporário - RET), authorizing a registering company to carry out field trials to evaluate the efficacy and safety of the product. The request itself includes a preliminary report containing literature on the taxonomy and biology of the microorganism, its host range, target pest(s), safety of the active ingredient (including toxicity, ecotoxicity, mutagenicity, carcinogenicity, and teratogenicity), as well as physical and chemical characteristics of the formulated product. Detailed information on the field experiments should also be included, and they must be carried out at experimental stations with MAPA accreditation. IBAMA and ANVISA have a legal deadline of 60 days to send the toxicological and environmental preliminary evaluations to MAPA, and MAPA has another 15 days to accept or reject the RET application. Once in possession of the RET, the company is authorized to start evaluating the product.

The joint (among IBAMA, ANVISA, and MAPA) Directive #3/2006 establishes the guidelines and procedures related to the definitive registration requirements. The toxicology and non-target organism requirements are satisfied through a process of tier testing. For toxicology, the first of three tiers consists of short-term trials (e.g. dermal irritation, and toxicity and pathogenicity via acute intravenous injections) in which mammals receive a single high dose of the pathogen. Tiers II and III are more complex and expensive, and are unnecessary if results in Tier I are negative. For use in crops intended for human or animal feed, residue studies are needed only when products fail Tier I, but are required in either Tier II or III.

Testing on non-target organisms is divided into four tiers, with non-target organisms being subjected to a single high dose in the first tier. It is mandatory that tests are performed for birds, freshwater fish, and non-target insects. Various other organisms are also identified depending on the nature of the pathogen. If no adverse effect is observed in Tier I, testing of Tiers II to IV are unnecessary.

Furthermore, efficacy data resulting from field trials involving the subject crop and target pest must also be submitted. Once all the documentation is delivered to the official agencies, IBAMA and ANVISA have up to four months to send their evaluations to MAPA. MAPA then has another 30 days to accept or reject the application for the Certificate of Registration.

The registration process does not establish minimum standards of quality for biopesticides, such as guaranteed concentration of active ingredient, maximum moisture content for solid products, maximum levels of contaminants, and so on. Although formulation is required for registration purposes, products which are considered non-formulated according to international literature have been registered, such as mycopesticides commercialized as fungus-colonized substrates.

Provided that a product is subject to Tier I testing only, the cost up to the point of receiving a Certificate of Registration is around US $70,000. This includes charges by federal agencies for the examination of the documents, and the issuing of the RET and the Certificate. Once registered by a federal agency, registration at the state level where the product will be marketed is also required. This step is coordinated by the Department of Agriculture in each state, and is a fairly simple process. Despite recent advances made in the registration process, it is still a bureaucratic process and relatively expensive for small companies.

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CUBA

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OVERVIEW AND USE

Cuba is one of the leading countries in the world in the production of biological agents for pest management (Sinclair and Thompson 2001), with the country's pest management policy considering ecological, economic, and social aspects of pest control (Rosset 1997). 'Plaguicida biológico' or biological pesticide is a term Cuba uses exclusively for products whose active ingredient is a living organism. Their domestic production and utilization is advanced beyond simple substitution of synthetic pesticides, and involves the conservation, biodiversity, and ecological support in pest management (Vázquez 2006). In fact, the broad ecological approach to pest management in Cuba is a defining characteristic, and the history leading to this approach is unlike any other agricultural production system.

Prior to the onset of the Green Revolution in the 1960s, Cuba's agricultural practices included negligible amounts of imported pesticides, but soon after and for the next 15 years, calendar-based pesticide sprays replaced almost all other methods of pest management. By the mid 1970s, resistance issues and new pest problems surfaced, leading to the formation of a national Dirección de Sanidad Vegetal within the Ministry of Agriculture (MINAG), which included an Institute for Research in Plant Protection (INISAV), a network of Provincial Plant Health Laboratories (LAPROSAV) and regional plant protection stations (ETPP) across the country. These institutions were created with the mandate to monitor pest populations and disease presence, provide grower training and education programmes, and conduct research. These efforts, implemented in 1975, reduced pesticide use by over 50% in the first year from 40,000 tons to less than 20,000 tons (Pérez and Vázquez 2002; Pérez 2006). Soon afterward, the comprehensive research programme began to provide the scientific foundation for ecologically-based pest management. In 1982, integrated pest management (IPM) was adopted by the Cuban state as the official pest control policy (Pérez and Vázquez 2002), fuelling the development of methods for mass production of biological control agents (e.g. predators and parasitoids) and biopesticides. With the collapse of the Soviet Union in 1990, Cuba lost its largest trading partner. Combined with the existing trade embargo imposed by the United States, the result was a dramatic decrease in imported synthetic pesticides and a second significant reduction in their use to under 10,000 tons annually (Pérez and Vázquez 2002). The trade crisis also caused an immediate 40% increase in the use of biological controls (Ayala et al. 2008; Jimenez 2010) and dramatic land reform to reorient agriculture, all supported by a government that cooperated at every step including in regulatory matters (Shishkoff 1993).

The greatest pest management successes in Cuba have been obtained using predators, parasitoids, and entomopathogens. Despite Cuba's leadership in the production of biological agents (Sinclair and Thompson 2001), it is not yet meeting its own needs for some products. With the goal of self sufficiency and the establishment of the National Programme for Biological

Pest Control in 1988 by MINAG, two tactics were adopted: i) the initiation of a small number of industrial-scale production centres to provide national standards for quality; and ii) the expansion of the existing network of small-scale Centres for Production of Entomophages and Entomopathogens (CREEs) from 100 to 250, each centre producing biocontrols and biopesticides required locally, along with extension personnel and services to state-run farming enterprises, co-operatives, and independent farmers. CREEs have become the cornerstone of biopesticide production.

The first biopesticide product used in Cuba was *Bacillus thuringiensis* (*Bt*) in the early 1960s. Its success in controlling budworm (*Heliothis virescens*) on tobacco won national recognition and encouraged exploration for other native entompathogens and the initial incentive for the development of CREEs. In 1989, the area treated with biocontrol agents was about 420,000 ha and in 2009, more than 1,300,000 ha. In spite of the difficult economic situation, and lack of infrastructure repair, biopesticide production in the CREEs has continued to increase, reaching 998 tons in 2009 (Jimenez 2010).

Despite the defining feature of the CREE network, production methods vary depending on the area and its resources. For example, the liquid media used for *Bt* production is based on locally available fruit juices; solid media for fungal entomopathogen production can be rice, coffee, or even sugar cane. There is also diversity of the microorganism strains produced: consistent with the agroecological principle of utilizing local biodiversity, strains in production are almost all indigenous to Cuba, with multiple strains being applied to control a single pest (e.g. multiple *Beauveria* strains are used for the control of *Hipotenemus hampei* in coffee plantations).

Biopesticides produced and used in Cuba are shown in Table 14. Certified microbial strains are provided to the CREEs by LAPROSAV. Quality control (QC) protocols are in place and final QC is carried out at each facility on 2% of the daily production. Monthly monitoring of CREEs is carried out by LAPROSAV. It is accepted that quality may be somewhat lower in products from the CREEs (e.g. the *Bt* endotoxin level of product from larger industrial facilities is higher than that of the CREEs) but protocol dictates that the label rate is adjusted according to QC results.

The use pattern for biopesticides can be generalized as follows: a) preventatively e.g. *Trichoderma* applied to soil, seed pieces (sweet potato) or seeds to manage soil pathogens (Stefanova, 1999); b) in combination with other biopesticide and biorational products e.g. *Beauveria* or *Metarhizium* applications around pheromone traps for sweet potato weevil *Cylas formicarius* - traps attract males but those not entering the trap become infected and effectively disperse fungal inoculum throughout the field when they later die (Castellón et al. 2001); or c) as a biological pesticide, applied according to pest levels – e.g. *Bt*, *Beauveria,* or *Metarhizium* against Crysomelidae and *Verticillium* against white fly or aphids.

Biological disease management is beginning to catch up to that for insect pests, and is currently being hastened by, for example, major research initiatives: *Trichoderma* strains for suppression of various diseases (Mesa and Ramirez 2006), *Lecanicillium lecanii* strains against orange coffee rust (*Hemileia vastatrix*) (Gonzalez 2004) and *Pseudomonas aeruginosa* for management of bacterial diseases (Stefanova et al. 2006).

Due to the increasingly complex nature of Cuba's agroecological approach to pest management, the government's Participative Phytosanitary Innovation Programme was established in 2003 to validate a number of agroecological systems using biopesticides and other strategies to control several important pests affecting major crops (Vázquez et al. 2005). Some Cuban experts believe that the use of mass produced biocontrols is an interim phase to achieving ecological equilibrium in the production of food. It will be very interesting to observe how sustainable food production evolves in Cuba in the future.

Table 14. Microbial pesticides used and registered in Cuba.

	Taxus	Products	Targets
Fungicides			
Trichoderma harzianum A-34 (strain for vegetables and ornamentals) (INISAV)	Fungus	Trichosav-34 (use permit only)	Soilborne diseases - *Rhizoctonia solani, Phytium aphidermatum, Phytophthora parasitica, P. capsici, Sclerotium rolfsii, Fusarium* spp.
Trichoderma sp. A-55 (strain for tobacco) (INISAV)	Fungus	Trichosav-55 (use permit only)	Soilborne diseases - *Rhizoctonia solani, Phytium aphidermatum, Phytophthora parasitica, P. capsici, Sclerotium rolfsii, Fusarium* spp.
Insecticides			
Bacillus thuringiensis subsp. kurstaki BT-32 (INICA 050-04)	Bacterium	BT-32	Lepidopteran larvae
Bacillus thuringiensis LBT-1 (INISAV 179-04)	Bacterium	Thurisav -1	Lepidopteran larvae
Bacillus thuringiensis subsp. *kurstaki* LBT-24 and LBT-26 (INISAV)	Bacterium	Thurisav-24 (LBT-24) Thurisav–26 (LBT-26)	*Ascia monuste eubotea*, *Plutella xylostella* (also Strain LBT-1), *Spodoptera frugiperda*, *Heliothis* spp., *Spodoptera* spp., *Trichoplusia brasicae, T.ni*, *Diaphania* spp., *Erinnyis ello*, *Erinnyis alope*, *Davara caricae*, *Hedylepta indicate*, *Mocis* spp., *Liriomyza trifolii*, *Phyllocnistis citrella, Heliothis* spp. (LBT-26 only), lepidopteran pests
Bacillus thuringiensis LBT-13 (INISAV)	Bacterium	Thurisav-13	*Polyphagotarsonemus latus, Tetranychus tumidus, Phyllocoptruta oleivora, Thrips palmi, Liriomyza trifolii* Animal pest control-*Megninia gynglimura* and *Ornithonyssus sylvianum*
Bacillus sphaericus 2362 SC (LABIOFAM 116-03)	Bacterium	Griselesf	Mosquito larvae
Bacillus thuringiensis subsp. *israelensis*, serotype H-14 (LABIOFAM 100-04)	Bacteria	Bactivec	Mosquito larvae
Beauveria bassiana MB-1 (INISAV 182-04)	Fungus	Bibisav -2	*Atta insularis*, *Acromyrmex octospinosus*, *Attamyces bromatificus* (Formicidae)
Beauveria bassiana LBB-1 (INISAV 180-04)	Fungus	Basisav-1	*Cosmopolites sordidus* (banana weevil), *Cylas formicarius* (weevil), *Lissorhoptrus brevirostris* (aquatic weevil), *Pachnaeus* spp. (Curculionidae), *Thrips palmi, Diatraea saccharalis, Hypothenemus hampei, Diabrotica balteata, Pseudacysta perseae, Lagochirus dezayasi, Corythucha gossypii, Tipophorus nigritus*, *Phyllophaga* spp.
Metarhizium anisopliae LBM-11 (INISAV 178-05)	Fungus	Metasav-11	*Lissorhoptrus brevirostris* (aquatic weevil), *Mocis* spp. (lepidopteran larvae), *Prosapia bicincta*, *Cosmopolites sordidus* (banana weevil), *Tagosodes oryzicola, Oebalus insularis, Spodoptera* spp., *Spodoptera* spp., *Pachnaeus litus*, *Thrips palmi*, *Plutella xylostella*, *Hypothenemus hampei, Diabrotica balteata* (Curculionidae)
Verticillium lecanii Y-57 (INISAV 179-05 and 180-05)	Fungus	Vertisav-57	*Bemisia tabaci* (white fly), *Bemisia argentifolia, Frankliniella* spp., *Aleurotracholus tracheoides, Aphis gossypii, Myzus persicae, Lipaphis erizini, Brevicoryne brassicae, Thrips palmi, Bophilus microplus*

Table 14 (con'd)

Nematicides			
Bacillus thuringiensis subsp. *kurstaki* LBT-3 (INISAV)	Bacterium	Thurisav-3	*Meloidogyne* spp., banana nematodes
Tsukamurella paurometabola C-924 (CIGB 001-07)	Bacterium	HeberNem-L	Plant parasitic nematodes
Tsukamurella paurometabola C-924 (CIGB 050-08)	Bacterium	HeberNem-S	Plant parasitic nematodes
Pochonia chlamidospora subsp. *catenulate* (CENSA 047-09)	Fungus	KlamiC	Soil nematodes
Rodenticides			
Salmonella enteriditis subsp. *danysz* (LABIOFAM 101-04)	Bacterium	Biorat G	Rats

CIGB - Centre for Genetic Engineering and Biotechnology, Cuba; LABIOFAM - Laboratorios Biologico Farmaceuticos, Cuba; CENSA - Centro Nacional de Sanidad Agropecuaria, Cuba; INISAV – Institute for Research in Plant Protection, Cuba; INICA – Insecticidas Internationales, Venezuela

Sources: Registro Central de Plaguicidas (Cuba 2007b); Pers. comm. R. Silva and S. Monzón 2010; Carr (2003); Conbiol (2009); Mena et al. (1996 and 1997); Pérez (2006); Pérez and Trujillo (2002); Pérez et al. (2001); Rijo (1996); Vázquez and Fernández (2007)

REGISTRATION AND THE REGULATORY SYSTEM

The Environmental Law (1997) is devoted to sustainable agriculture, and states that the use of all pesticides will be governed by a requirement to minimize environmental contamination, and directs the use of preventative and integrated management of pests and diseases with special attention to the use of diverse biological resources (Pérez and Vázquez 2002). A specific Integrated Pest Management Act (1982) states that IPM is the official pest control policy of the Cuban state (Pérez and Vázquez 2002). Both of these have greatly encouraged the use of biopesticides. The process for biopesticide registration was developed according to standards and guidelines of the Food and Agriculture Organization (FAO 1988), Organization for Economic Cooperation and Development (OECD 1996), and the United States Environmental Protection Agency (EPA 1996) (Díaz 2003), and in 2007, a formal process for registration was published (Gaceta Oficial No. 016; Cuba 2007a).

Regulatory bodies involved

Biopesticides fall under many of the regulations governing synthetic pesticides, industrial chemicals, cosmetics, food additives and veterinary drugs. Four institutions are responsible for regulating biopesticides, and one or more will be involved with any registration:
i) The Central Registrar of Pesticides (CNSV) - a review committee of MINAG, together with the Ministry of Sugar (MINAZ), Ministry of Public Health (MINSAP) and Ministry of Science, Technology and Environment (CITMA). This group receives applications, ensures compliance with data requirements, approves and registers new active ingredients and new products, and changes in any product, formulation or rate, including imported biopesticides; ii) The Centre for Environmental Inspection and Control (CICA, part of CITMA)– regulates environmental aspects of activities involving research on, or for production of biological pesticides; iii) The National Centre for Biological Safety (CNSB, part of CITMA) - regulates research, production, trials, releases, import and export of biopesticides and construction of laboratories and production facilities; and iv) External Quarantine Department of the National Centre of Plant Health (part of CNSB) which regulates the import and export of materials under quarantine, a category which includes biopesticides.

Finally, if toxicological studies are completed in Cuba, they must be from a Good Laboratory Practices (GLP) - accredited lab inspected by the National Centre for Laboratories Using Animals (CENPALAB).

Registration process and regulatory requirements

Five steps characterize the biopesticide registration requirements in Cuba. First, in a pre-submission document, basic product information (description, variety, characterization of metabolites, manufacturing process, formulation, certificates of quality, guarantees, and back up information) is provided to CNSV. The CNSV reviews this information and if deemed to be complete and acceptable, the second step is an invitation to the registrant to submit a full application. The third step occurs once the full submission is made. CNSV conducts a toxicological review. If applications do not meet the toxicological testing requirements for the type of product it is, the evaluation is halted and the application forwarded to a Toxicological Advisory Commission which provides the applicant with a list of required toxicological and ecotoxicological studies and directs the applicant to an accredited lab. Once the Toxicological Advisory Commission has been satisfied, it provides a biological security authorization to the CNSV and the evaluation of the product resumes (Cuba 2007a).

Categories of information required in a full registration application are:

1. Identification and description of the organism e.g. taxonomy, reference collection, morphology, composition
2. Biological properties e.g. target pest, specificity, efficacy, dose, mechanism of action, history of the organism and natural presence, non-target effects, stability of product, genetic stability under expected environmental conditions, presence or absence of toxins, mechanisms to maintain virulence
3. Other data regarding organism e.g. purpose, crops requested, planned areas of application, environmental conditions for use, production methods, probability that the organism will not become infectious, methods for handling, storage, transport, emergency actions
4. Analytical methodology e.g. how to determine purity, variability, control of contaminants, absence of human pathogens, temperature effects, determination of viability
5. Formulation data e.g. type, physical and chemical properties, analytical methods, precautions for storage, transport, dose, frequency, method of application, decontamination and cleaning procedures
6. Toxological data e.g. toxicity and pathogenicity for acute oral, acute skin, acute respiratory, acute parenteral, skin and eye sensitivity, hypersensitivity, sub-chronic toxicology, others such as genotoxicity, reproductive, metabolic. Viruses require cell culture studies with mammal, avian or fish cells.
7. Ecotoxicological studies e.g. on aquatic organisms, non-target plants and insects, and acute toxicity on other non-target organisms potentially at risk. Any adverse effects would require further tests.

The fourth step is the Issue of a Use Permit when the product is approved by CNSV. The fifth and final step is publishing the product in the annual Official List of Authorized Pesticides (Cuba 2007b) with label information including uses, rates, methods and precautions. The Gaceta Oficial (Cuba 2007a) sets out time limits for internal steps in the registration process provided all information is complete, but also sets out exceptions under which the review may take up to 2.5 years:

- From submission of basic product information to decision from CNSV to accept or deny a full applications: 30 days
- Communication between registrant and CNSV regarding cost of registration and data requirements: 30 days
- Agreement to review the submission: 30 days
- Review of physical, chemical and microbiological data: 60 days
- Review of efficacy data: 180 days
- Review by Assessment committee to approve or not: 90 days
- Obtaining permission from MINAG and MINSAP: 30 days
- TOTAL: 450 days

Export/import regulations

Not surprisingly, with the U.S. trade embargo and the loss of the Soviet Union as a trading partner, few pesticides are now imported into Cuba. Still, imported pest control products undergo the same registration process as domestic products except that the External Quarantine Department of CNSB becomes involved because of its regulatory responsibilities for the import and export of materials under quarantine, a category which includes biopesticides. When a biopesticide is intended to be exported from Cuba, a Cooperation Contract with an institution or destination country is necessary and additional product information is required of registrants to protect human and environmental health in the country to which the product is exported (Díaz 2003).

SPECIAL CONCESSIONS AND ORGANIZATIONS PROMOTING BIOCONTROL

While biopesticides are required to undergo detailed studies of environmental impact and toxicological effects before registration, indigenous strains that are specific to a particular group of target pests, and have never been recorded as plant, human, or animal pathogens, have reduced requirements and proceed through the registration process more quickly e.g. *Bt* products (Díaz 2003). This practice arose because of the urgent need for the widespread use of biocontrol agents in response to a food security crisis, and the extensive support for research to develop the new tools domestically. For example, *Trichoderma* strains commonly used in Cuba and which were developed in INISAV research programmes, have not yet gone through formal registration but have Use Permits and Production Licenses. The process to register them now is identical for other biopesticides, but is unlikely to happen unless there is threat of loss of the Use or Production permits.

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CANADA

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OVERVIEW AND USE

Perhaps more than at any other time in history, Canada is engaged in both the research and development of biopesticides, and in their advancement through legislation and regulatory support. Canadians enjoy the benefits that accompany a rigorous regulatory system with respect to human and environmental health. While this rigour is well-represented in pesticide regulation, it can have the paradoxical effect of advancing the number of microbial pesticide registrations due to their preferred minimized risk, but also in restricting their advancement due to a perception of onerous registration requirements i.e. scientific studies and supporting documentation. Nevertheless, the effects of recent initiatives, legislation, and programmes are all being tracked in the interest of increasing the use of low risk pesticides to improve human health, and environmental and agricultural sustainability within the setting of a free market economy.

Demand for biopesticides in Canada is largely driven by conventional agriculture, recent changes to municipal and provincial laws governing cosmetic use of pesticides, and legislation and ensuing promotion for lower risk pest control products (CPL Business Consultants 2010; Minister of Justice 2002). Up until recently, pesticide use has been difficult to accurately assess. However, the most recently ratified Pest Control Products Act legislates the collection of pesticide sales information by registrants, and its provision to the Pest Management Regulatory Agency (PMRA) of Health Canada beginning in 2006 (Minister of Justice 2002). The PMRA is the federal regulator of pesticides in Canada, and they are currently compiling pesticide sales data for public release (PMRA 2006a). This will represent the first accurate picture of pesticide use in Canada and one baseline from which to measure change.

Despite the 'work-in progress' nature of a government-directed comprehensive pesticide use survey, other sources of information currently provide estimates. CPL Business Consultants (2010) reported that end-user sales of pesticides in Canada were valued at $1.4 billion. Of this total, microbial pesticides encompassed about 0.5% ($7.4 million), with 88% of microbials represented by *Bacillus thuringiensis* ($6 million for use in forestry; $500,000 for use in agriculture), 6.7% ($500,000) for other bacteria, 0.67% ($50,000) for viruses, 0.67% ($50,000) for fungi, and 4.1% ($300,000) for nematodes. Thirty eight private companies identified themselves as manufacturers and/or distributors of the associated microbial pest control products. A comprehensive agricultural survey conducted by the federal government in 2001 reported that 73.2% of responding farms used pesticides of some kind. Of the 222,395 reported pest management events considered as alternative to synthetic pesticides (including mechanical, culture, biological, resistant varieties), 2% of those events were an application of *B. thuringiensis*, and 0.1% were the application of other microbial agents (Korol 2004). Bear in mind that these figures represent pest control *events*, and any given farm will employ a range of pest control measures, constituting numerous events per farm. Still, these figures are roughly in agreement to other extrapolations of the use of microbial pesticides. The purpose of the 2001 survey was, in part, to gather baseline data to assess the performance of federal initiatives relating to sustainable agricultural practices in Canada, including initiatives relating to pesticide alternatives. The data from a follow-up survey conducted in 2006 still being compiled will report changes in on-farm pest control practices from 2001 to 2006.

With respect to the taxa of microorganisms used for pest control, in 2004 there were only 13 unique microbial active ingredients registered in Canada. As of 2010, Canada has registered 32; 12 of which are bacterial species, 11 fungi, 6 nematodes (no registration required, but rather Canadian Food Inspection Agency (CFIA) approval), 2 viruses, and 1 protozoan (Kabaluk and Gazdik 2010; Brian Belliveau, pers. comm; Table 15). The number of active ingredients and products is expected to expand significantly in the near future due to government programming aimed at assisting companies with the registration requirements to enter the Canadian market. Without assistance, such registration would likely not occur due to high registration cost:revenue benefit ratio in Canada's limited market, particularly with respect to horticultural crops. Assistance, however, also encompasses the evolution of harmonization of registration efforts with other jurisdictions including the United States under the North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (NAFTA 2009), and the Organization for Economic Cooperation and Development (OECD). Together with increasing global demand for reduced risk pesticides and Canada's role as an agricultural exporter, microbial pesticide sales in are projected to increase to $20 million in the next 10 years.

Table 15. Microbial pesticides registered in Canada as of 2010.

	Taxus	**Products**	**Targets**
Bactericides			
Agrobacterium radiobacter K84	Bacterium	Dygall	Crown gall (*Agrobacterium tumefaciens*)
Pantoea agglomerans C9-1	Bacterium	BlightBan C9-1[1]	Fire blight
Pseudomonas fluorescens A506	Bacterium	BlightBan A506[1]	Fire blight, fruit russeting
Fungicides			
Bacillus subtilis MBI 600	Bacterium	HiStick N/T Subtilex Integral[1]	*Aspergillus*, *Fusarium*, *Rhizoctonia*, *Pythium*
Pseudomonas syringae ESC-10	Bacterium	Bio-Save 10 LP[2]	Blue mold (*Penicillium expansum*), blue mold fruit rot (*Penicillium italicum*), dry rot (*Fusarium sambucinum*), green mold fruit rot (*Penicillium digitatum*), grey mold rot of fruit (*Botrytis cinerea*), mucor fruit rot (*Mucor* spp.), silver scurf (*Helminthosporium solani*), sour rot (*Geotrichum candidum*)
Streptomyces griseoviridis K61	Bacterium	Mycostop	*Botrytis* grey mold (*Botrytis cinerea*), crown rot, damping off, early root rot, *Fusarium* wilt, *Phytophthora*, *Rhizoctonia*, root rot (*Fusarium*, *Phytophthora*, *Pythium*), seed damping off, seed rot (*Fusarium*, *Alternaria*, *Phomopsis*), soil borne damping off, stem rot (*Fusarium*), stem-end rot (*Phomopsis*), wilt
Streptomyces lydicus WYEC108	Bacterium	Actinovate	*Alternaria*, anthracnose, *Botrytis*, downy mildew, *Erwinia,* greasy spot, *Monilinia*, powdery mildew, *Sclerotinia*, soilborne plant diseases (*Pythium*, *Rhizoctonia*, *Phytophthora*, *Verticillium*, *Fusarium*, cotton root rot (*Phytomatotricum omnivorum*), *Aphanomyces*, *Monosporascus*, *Armillaria*, *Sclerotinia*, *Postia*, *Geotrichum*)

Table 15 (con'd)

Coniothyrium minitans CON/M-91-05	Fungus	Contans	*Sclerotinia sclerotiorum*, *Sclerotinia minor*, *Sclerotinia trifoliorum*
Gliocladium catenulatum J1446	Fungus	Prestop	Damping off (*Pythium*, *Rhizoctonia*), foliar diseases (*Botrytis*, *Didymella*, *Rhizoctonia*, *Pythium*, *Phytophthora*, *Fusarium*, *Verticillium*, *Alternaria*, *Cladosporium*, *Helminthosporium*, *Penicillium*, *Plicaria*), gummy stem blight (*Didymella*), grey mold (*Botrytis*), root rot, seed rot, stem rot, storage diseases (*Helminthosporium*, *Rhizoctonia*), wilt (*Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium*, *Phytophthora*, *Plicaria*, *Pythium*, *Rhizoctonia*, *Verticillium*)
Ophiostoma piliferum D97	Fungus	Sylvanex	Anti sap-stain of timber products
Pythium oligandrum DV 74	Fungus	Polyversum[2]	*Alternaria* spp., *Ascochyta* spp., *Botrytis* cinerea, *Fusarium* spp., *Peronosplasmopara* spp., *Phoma* spp., *Phytophthora infestans*, *Plasmopara viticola*, *Puccina* spp., *Pythium* spp., *Rhizoctonia* solani, *Sclerotinia sclerotiorum*, *Unicula necator*, *Verticillium* spp.
Trichoderma harzianum Rifai T-22 (KRL-AG2)	Fungus	RootShield Rootshield Drench	*Cylindrocladium*, *Fusarium*, *Pythium*, *Rhizoctonia*, *Thielaviopsis*
Verticillium albo-atrum WC S850	Fungus	Dutch-Trig	Dutch elm disease (*Ophiostoma ulmi, O. novo-ulmi*)
Fungicides/bactericides			
Bacillus subtilis QST 713	Bacterium	Serenade Rhapsody Serenade Garden	<u>Label common names</u> Angular leaf spot, anthracnose, bacterial fruit blotch, bacterial leaf spot, bacterial soft rot, bacterial spot, bitter rot, black rot, black spot of rose, bot rot, brooks spot, brown patch, bull's eye rot, canker, cedar apple rust, dollar spot, downy mildew, early leaf spot, flyspeck, fruit brown rot, greasy spot, grey mold, gummy stem blight, head and leaf drop, late leaf spot, melanose, mummy berry, onion purple blotch, pink rot, post bloom fruit rot, powdery mildew, rust, scab, shot hole, sigatoka, silver scurf, sooty blotch, sour rot, southern corn leaf blight, southern blight, target spot, white mold <u>Label Latin names</u> *Acidovorax avenae*, *Alternaria* spp., *Aspergillus niger*, *Bipolaris maydis*, *Blumeriella gaapi*, *Botrytis* spp., *Botrytosphaeria dothidea*, *Bremia lactucae*, *Cercospera* spp., *Cerosporidium personatum*, *Cladosporium berbarum*, *Cochliobolus heterostrophus*, *Colletotrichum* spp., *Corynespora cassiicola*, *Diaporthe citri*, *Didymella bryoniae*, *Diplocarpon rosae*, *Elsinoe fawcetti*, *Entomosporium* spp., *Erwinia* spp., *Erysiphe* spp., *Eutypa lata*, *Fusarium* spp., *Gloeodes pomigena*, *Gymnosporangium juniperi-virginianae*, *Helminthosporium* spp., *Lanzia* spp., *Leveillula taurica*, *Moellerodiscus* spp., *Monilinia* spp., *Mycosphaerella* spp., *Myrothecium* spp., *Neofabraea* spp., *Oidiopsis taurica*, *Oidium* spp., *Penicillium* spp., *Peronospora* spp., *Phoma cucurbitacearum*, *Phomopsis viticola*, *Phragmidium* spp., *Phytophthora* spp., *Plasmopara viticola*, *Podosphaera* spp., *Pseudomonas* spp., *Pseudoperonospora cubensis*, *Puccinia* spp., *Pythium* spp., *Rhizoctonia* spp., *Rhizopus arrhizus*, *Schizothyrium pomi*, *Sclerotinia* spp., *Sclerotium rolfsii*, *Septoria* spp., *Sphaerotheca* spp., *Uncinula necator*, *Venturia* spp., *Wilsonmyces carpophilus*, *Xanthomonas* spp.
Pantoea agglomerans E325	Fungus	Bloomtime Biological FD	*Botrytis cinerea*, *Sclerotinia sclerotiorum*, fire blight

Table 15 (con'd)

Herbicides			
Lactobacillus spp. (several species and strains)	Bacterium	Organo-Sol	Clovers, black medick, bird's foot trefoil, wood sorrel
Chondrostereum purpureum PFC2139	Fungus	Chontrol	Resprouting inhibition of cut alder
Sclerotinia minor IMI 344141	Fungus	Sarritor	Dandelion
Insecticides			
Bacillus sphaericus serotype H5a5b strain 2362	Bacterium	VectoLex	Mosquito (*Culex* spp., *Aedes vexans*, *Ochlerotatus melanimon* (*Aedes melanimon*), *Ochlerotatus stimulans* (*Aedes stimulans*), *Ochlerotatus nigromaculis* (*Aedes nigromaculis*), *Psorophora columbiae, Psorophora ferox*, *Ochlerotatus triseriatus* (*Aedes triseriatus*), *Ochlerotatus sollicitans* (*Aedes sollicitans*), *Anopheles quadrimaculatus*, *Coquillettidia perturbans*)
Bacillus thuringiensis subsp. *israelensis* 65-52	Bacterium	VectoBac (Active Ingredient II)	Mosquito, blackfly, nuisance fly, nuisance midge
Bacillus thuringiensis subsp. *israelensis* SA3A	Bacterium	Teknar HP-D	Blackfly, mosquito
Bacillus thuringiensis subsp. *israelensis* serotype H14	Bacterium	Teknar Summit Bti Briquets Mosquito Dunks VectoBac (Active Ingredient I)	Drain fly, filter fly, mosquito, mosquito larvae, psychodid fly
Bacillus thuringiensis subsp. *kurstaki* HD-1	Bacterium	Bioprotec Foray Dipel	Brown spanworm, cabbage looper, cherry fruitworm, cranberry fruitworm, diamondback moth, *Duponchelia fovealis*, early and late season oblique banded leafrollers, eastern hemlock looper, bagworm, eastern spruce budworm, elm spanworm, Essex skipper (European skipper), European corn borer, fall cankerworm, fall spanworm, fall webworm, forest caterpillar, forest tent caterpillar, fruitworm, green spanworm, Gypsy moth, hemlock looper, hornworm, imported cabbageworm, leafrollers (fruittree-, European-, obliquebanded-, three-lined-, omnivorous-), pine budworm, range caterpillar (*Hemileuca*), rangeland caterpillar, satin moth, spring cankerworm, sunflower moth, tent caterpillar, tomato fruitworm, tomato hornworm, western spruce budworm, Jack pine budworm, white marked tussock moth, whitemarked tussock moth, winter moth
Bacillus thuringiensis subsp. *kurstaki* SA-11	Bacterium	Safer Bt Caterpillar Killer Thuricide 48 LV	Alfalfa caterpillar, armyworm, bagworm, black cutworm, budworm, cabbage looper, cabbageworm, webworm, California oak moth, codling moth, cotton bollworm, cranberry blossomworm, cranberry fruitworm, cutworm, diamondback moth, Douglas fir tussock moth, eastern hemlock looper, elm spanworm, fall cankerworm, fall webworm, forest tent caterpillar, fruittree leafroller, green cloverworm, Gypsy moth, hornworm, Jack pine budworm, leafroller, fireworm, looper, *Mimosa* webworm, navel orangeworm, oriental fruit moth, peach twig borer, pecan nut casebearer, southwestern corn borer, European corn borer, pine butterfly, podworm, redhumped caterpillar, saltmarsh caterpillar, soybean looper, spanworm, cankerworm, *Sparganothis* fruitworm, spring cankerworm, Essex/European skipper, spruce budworm, tent caterpillar, tobacco budworm, tomato hornworm, tufted apple bud moth, velvetbean caterpillar, walnut caterpillar, webworm, western tussock moth

Table 15 (con'd)

Bacillus thuringiensis subsp. *kurstaki* SA-12	Bacterium	Thuricide 48 LV Forestry Thuricide HPC	Alfalfa caterpillar, almond moth, bagworm, elm spanworm, banana skipper, budworm, cabbage looper, citrus cutworm, cotton bollworm, diamondback moth, easter spruce budworm, elm spanworm, Essex skipper, fall cankerworm, fall spanworm, fall webworm, filbert leafroller, forest tent caterpillar, fruittree leafroller, grape leaffolder, green cloverworm, Gypsy moth, imported cabbageworm, Indian meal moth, Jack pine budworm, oak moth, omnivorous leafroller, omnivorous looper, orange tortrix, orangedog, podworm, rangeland caterpillar, redbanded leafroller, redhumped caterpillar, rindworm complex, roughskinned cutworm, soybean looper, spring cankerworm, tent caterpillar, tobacco budworm, tobacco hornworm, tomato fruitworm, tomato hornworm, tufted apple bud moth, variegated leafroller, velvetbean caterpillar, western avocado leafroller, western spruce budworm
Bacillus thuringiensis subsp. *tenebrionis* HB 176	Bacterium	Novodor	Colorado potato beetle (*Leptinotarsa decemlineata*), elm leaf beetle (*Pyrrhalta leutola*)
Beauveria bassiana HF23	Fungus	Balance	House flies
Beauveria bassiana GHA	Fungus	Botanigard	Aphid (bean-, cabbage-, cowpea-, green peach-, greenbug-, hop-, melon-, cotton-, pea-, potato-, rose-, Russian wheat-, spotted alfalfa-), leafhoppers and plant hoppers (grape leafhopper, leafhopper, plant hopper, potato leafhopper, leafhopper, variegated leafhopper), mealybug (citrus-, grape-, buffalo grass-, longtailed-), plant pug (Heteroptera) (chinch bug, lace bug), psyllid (pear-, tomato-, potato-), scarab beetle (*Atenius*, green June beetle, white grub), thrip (greenhouse-, Cuban laurel-, pear-, potato-, onion-, palmi-, western flower-), weevils (black vine-, strawberry root-, fuller rose-, root-, rose curculio, billbug), whitefly (banded-, winged-, citrus-, giant-, greenhouse-, silverleaf-, sweet potato-, tobacco-)
Metarhizium anisopliae F52	Fungus	Met52	Black vine weevil
Heterorhabditis bacteriophora	Nematode[3]	Nematop Nema-Green Terranem Larvanem Nemasys G B-Green Nematode HB Heteromask	Asiatic garden beetle (*Maladera castanea*), black vine weevil (*Otiorhynchus sulcatus*), cutworms, dung beetle (*Aphodius* sp.), European chafer (*Rhizotrogus majalis*), garden chafer (*Phyllopertha horticola*), ghost swift (*Hepialus lupulinus*), Japanese beetle (*Popillia japonica*), Japanese beetle grub (*Popillia japonica*), larvae of chafer grubs, larvae of curculionids (*Otiorhynchus sulcatus*, *Hepialus lupulinus*), May/June beetle (*Phyllophaga* spp.), oriental beetle grub (*Exomala orientalis*), strawberry root weevil (*Otiorhynchus ovatus*), Welch chafer (*Hoplia* sp.)
Heterorhabditis megidis	Nematode[3]	Larvanem M Nematode HM Nemasys H	Black vine weevil (*Otiorhynchus salcatus*), strawberry root weevil (*Otiorhynchus ovatus*)
Steinernema carpocapsae	Nematode[3]	Nemastar Nematac C Nematode SC Ecomask	Armyworm, black aetinius weevil, bluegrass billbug, caterpillar, cranberry girdler (*Chrysoteuchia topiaria*), cutworm, girdler, *Hylobius* weevil, leather jacket (European crane fly), mole cricket, mole crickets (*Gryllotalpa* spp.), pine weevil, red palm weevil, sod webworm, weevil grub
Steinernema feltiae	Nematode[3]	Nemasys M Nemaplus Entonem Scia-Rid Traunem Nemacel/ Nemycel	Bibionid larvae, black vine weevil, crane fly, cucumber beetle, cutworm, fungus gnat (*Bradysia* spp.), larvae of sciarid flies (Sciaridae), mushroom sciarids (*Lycoriella* spp.), onion maggot, root maggot, sciarid fly (*Lycoriella* spp.), sod webworm, western flower thrip (*Frankliniella occidentalis*), white grub

Table 15 (con'd)

		Nematode SF Nemasys Scanmask	
Steinernema kraussei	Nematode[3]	Nemasys L	Black vine weevil (*Otiorhynchus sulcatus*)
Steinernema scapterisci	Nematode[3]	Nematac S	Tawny mole cricket (*Scapteriscus vicinus*), southern mole cricket (*Scapteriscus borellii*)
Nosema locustae	Protozoan	Nolo Bait	Grasshopper
Cydia pomonella granulosis virus	Virus	Virosoft	Codling moth (*Cydia pomonella*)
Neodiprion abietis nucleopolyhedrosis virus	Virus	Abietiv	Balsam fir sawfly (*Neodiprion abietis*)
Neodiprion lecontei nucleopolyhedrosis virus	Virus	Lecontvirus	Red-headed pine sawfly (Neodiprion lecontei)
Orgyia pseudotsugata nucleopolyhedrosis virus	Virus	Virtuss TM Biocontrol-1	Douglas fir tussock moth, whitemarked tussock moth

[1] Registered but not currently sold
[2] Registration pending
[3] CFIA approval only – PMRA registration not required in Canada

Sources: Kabaluk, J.T. and Gazdik, K. 2010. *unpublished update of* Directory of Microbial Pesticides for Agricultural Crops in OECD Countries (2007). Catalogue No. A42-107/2007E-PDF; ISBN 987-0-662-47103-5. In English and French; B. Belliveau, pers. comm.

REGISTRATION AND THE REGULATORY SYSTEM

The PMRA is the branch within the federal government department of Health Canada that is responsible for the administration of all regulations associated with the registration of pest control products, with the Pest Control Products Act providing the legislative foundation. The mission of the PMRA is "to protect human health and the environment by minimizing the risks associated with pest control products in an open and transparent manner, while enabling access to pest management tools and sustainable pest management strategies". Within this, their primary objective is "to prevent unacceptable risks to people and the environment from the use of pest control products".

PMRA uses a series of numbered regulatory directives (DIR prefix), proposals (PRO prefix), and other guidance documents to provide a detailed description of the processes, rules, and regulations associated with the registration of all pest control products requiring registration. PMRA's website (www.hc-sc.gc.ca/pmra-arla) is comprehensive, and an excellent resource providing exhaustive information for registrants, regulators, researchers, and the general public. There is a dedicated tab for 'Registrants and Applicants', and the process for the registration of microbial pesticides is provided in DIR2001-02 - Guidelines for the Registration of Microbial Pest Control Agents and Products (PMRA 2001).

By definition in Canada, biopesticides are grouped into the following categories: microbials (PMRA 2001) - a microorganism such as bacteria, algae, fungi, viruses, protozoa, mycloplasma or rickettsia and related organisms to which the effects of pest control are attributed (referred to as microbial pest control agents or MPCAs); semiochemicals (PMRA 2002) - a message-bearing substance produced by plants and animals or a functionally identical synthetic analogue of that substance which evokes a behavioural response in individuals of the same or other species (e.g. pheromones and synomones); biochemicals (PMRA 2007) – as derived from naturally occurring substances by simple processing, or functionally identical synthetic analogues; and other non-conventional pest control products (PMRA 2007) not covered by the previous categories such a food and feed stuffs which are inherently low toxicity to non-target organisms and have low persistence in the environment . Invertebrate biological controls are not registered by the PMRA: nematodes require a rather simple approval for use issued by the CFIA, and macrobiologicals (predator insects), if exotic, require an extensive petition for release, North American-wide review and approval, and final authorization by CFIA; indigenous invertebrates for release have no regulatory oversight.

Data requirements and microbial pesticide registration

PMRA states that a complete submission for pesticide registration contains: "...a covering letter, application form, fees, a product specification form, various letters of support and authorization, draft label, index of supporting scientific data or studies and the scientific data" (PMRA 2001). The cornerstone document itemizing data requirements for Category A (new pesticide to Canada, including both the technical grade active ingredient (TGAI) and associated end-use product (EP)) registrations is the Data Code (DACO) Table. For synthetic pesticides, different groups of DACO tables are available for the major sectors of Agriculture and Forestry, Industry, and Society. Within each of these sectors, the DACO tables are specific to a number of uses, referred to as Use Site Categories (USC) of which there are 33 in total among the major sectors. For microbial pesticides however, there is a separate, and more generic DACO table which is used as a starting point to set data requirements for all microorganisms to be registered. The DACO table for microbials, while described in Appendix III of DIR2001-02 (PMRA 2001), is more specifically designed collaboratively between the PMRA and prospective registrant at the Pre-submission Consultation (described in subsection below).

The purpose of the DACO is to itemize all the information, data and otherwise, that are required for registration. DACOs further associate this information with numeric codes for cataloguing, tracking, reviewing, and relating to corresponding data requirements and codes used in other jurisdictions, particularly the OECD and U.S. Environmental Protection Agency (EPA) whose data submissions are accepted for review in Canada. The organizational structure of the data requirements is similar to that for synthetic pesticides, with major parts including: Index for submission (Part 0); Label, Product Profile, Proposed Use Patterns and International Regulatory Status (Part 1); Product Characterization and Analysis (Part 2); Human Health and Safety Testing (Part 4); Exposure Assessment (Part 5); Food and Feed Residue Requirements (Part 7); Environmental Fate (Part 8); Environmental Toxicology (Part 9); and Value (efficacy) (Part 10). Testing on human health and environmental fate and toxicology are tiered, meaning that if the MCPA is found to produce no effect under the most opportune conditions, then no further testing is required. The efficacy data required includes the performance of the EP compared to conventional control measures and may be obtained from greenhouse and field data. Efficacy data submission requires the description of methods, application rates, artificial inoculation rates or natural pathogen pressures, weather data and the submission of all favourable or unfavourable data. As with other DACO items, data from existing scientific literature are acceptable. The provisions for efficacy information can also be fulfilled with an acceptable value data rationale i.e. there may not be data available but a rationale based on equivalent use in another jurisdiction that has been confirmed to be acceptable and effective. Part 12 of the DACO table provides the option for including information from foreign registration reviews. A schematic outlining the steps for the Category A registration of the TGAI of an MPCA is shown in Figure 3.

Many of the data requirements for an EP are similar to those for the TGAI, although much of these data will have already been provided in the registration submission for the TGAI. The EP may require additional data which demonstrates that the combined product (the TGAI plus other formulation ingredients) does not pose unreasonable risk to humans and/or the environment. Regardless, the TGAI and EP must be submitted for registration separately.

Pre-registration consultation

The application for the registration of a microbial pesticide begins with a Pre-registration Consultation with the PMRA. While not mandatory, the consultation is strongly advised, particularly during the development phase of the product, and then again shortly before a registration application. At the consultation, prospective registrants are advised on data requirements congruent with the DACO table, and on the option for writing justifications (data waivers) if the registrant does not consider certain DACO items as applicable to the target use. Registrants are also advised regarding the application procedure and the processes that can be expected. Pre-submission consultation requires the prospective registrant to submit a proposed product label and a Statement of Product Specification Form which lists all the ingredients in the formulation. Prospective registrants are also required to include additional information which will aid PMRA in setting appropriate data requirements, for example: label information, product description, proposed use patterns, international regulatory status, biological characterization, manufacturing methods and procedures that ensure consistency of the product, preservation methods and quality assurance, ingredients, and product guarantee. The identification of the ecological zone in which the MCPA will be used is critical since a tiered system of evaluation is implemented which determines the level of environmental testing that will be required.

Registration categories

Every pest control product requiring PMRA registration i.e. a submission that is subject to the Management of Submission Policy (MOSP), is assigned to a category from A to E according to the following criteria:

Category A - i) new TGAIs or integrated system products, their related EPs and manufacturing-use products; and ii) major new use of registered EPs.

Category B - i) new EPs containing a registered TGAI; ii) amendments to existing EPs (e.g. product chemistry, labelling); iii) conversion or renewal of a conditional registration; and iv) emergency registrations;

Category C - product registrations and amendments with no data requirements. These applications involve minor label or formulation reviews such as product registration based on registered precedent products.

Category D - submissions within particular programs, including Import for Manufacture and Export Program (IMEP), Own Use Import (OUI), Grower Requested Own Use (GROU), Master Copy Registration Process, Private Label Registration Process, User Requested Minor Use Label Expansion (URMULE), registration renewal, and discontinuations.

Category E - specific to the issuance of field research and testing permissions, and includes i) research authorizations for new active ingredients and new use(s) of registered active ingredients; and ii) research notification for research carried out in Canada.

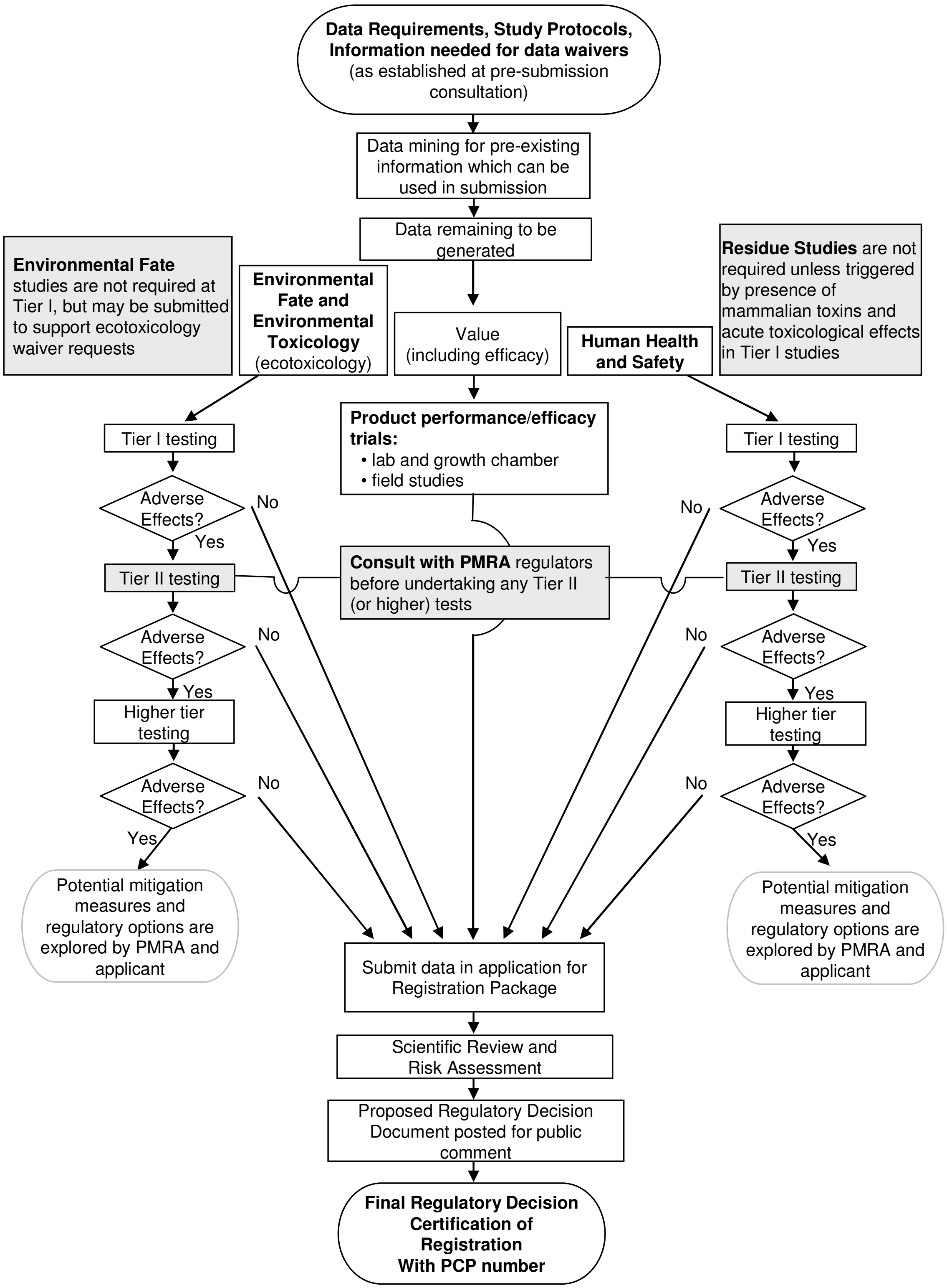

Figure 3. Category A (new technical grade active ingredient) registration process for a microbial pesticide in Canada.

<u>Handling of submission data</u>

While the details of compiling a submission for the Category A registration of a microbial pesticide, and the procession through the review stream of PMRA are described in detail in DIR2001-02 (PMRA 2001), the PMRA is swiftly moving toward an electronic submission process. To facilitate this, they have developed, for use by registrants and reviewers, the 'e-Index Builder' computer software. This electronic interface is designed to index and upload registration-related data provided by the registrant, and organize and house it on PMRA's server so that it can be retrieved in a format conducive for tracking and review. Data entry, where applicable, requires users to code data according to the prescribed data codes (DACO, previously described). DIR2006-05 offers guidance on formatting data for registration applications and the use of the e-Index Builder (PMRA 2006b).

The entire submission package is sent by the registrant to the PMRA electronically, by mail, or both (at time of writing). Within 7 days, PMRA verifies that all fees, forms, labels, and other required information have been provided. If not, then the application is returned to the registrant with a request for the missing information. If provided, then the submission is issued a Submission Number and proceeds to the screening process which verifies that information is provided for the entirety of submission requirements. If deficiencies are found, the registrant is notified and given 45 days to address them, after which the submission and Submission Number are cancelled if they are not addressed. A complete submission package is forwarded for review, and following a review, a regulatory decision is posted for public comment. Following a favourable review, the pesticide is issued a Pest Control Product (PCP) number, permitting its use in Canada. It is required that all pest control products used in Canada display the PCP number on the product label.

International cooperation

Particularly for biopesticides in the horticulture industry, Canada's market is small compared to other developed countries and there is ongoing work with other countries to facilitate and encourage the Canadian registration of pest control products. The challenge is heightened given the high regulatory standard held for pesticide use in Canada.

Significant advances have been made in allowing Canada to accept registration data created in, and submitted to regulators in other countries. The actual data, or data contained within the format of submissions created for the U.S. EPA or OECD are most readily accepted. In fact, 'Crosswalk' documents have been created to cross-reference DACOs with corresponding data codes used by the U.S. EPA and OECD. There are crosswalk documents specific to both microbial EPs and TGAIs, although the data sharing applies to all pesticides. The key to acceptance of data from other countries is that it is generated under conditions in, or relevant for Canada. For example, efficacy data needed for Canada, but collected from the same ecological zone in the U.S., are acceptable. Submissions for pest control products for greenhouses can contain greenhouse data generated from an OECD country.

Further to the acceptance of data from OECD countries, in recent years, Canadian registration requirements for microbial pesticides and pheromones were essentially harmonized with those in the United States. Under the auspices of NAFTA, Canada and the U.S., as represented by the PMRA and EPA, have undertaken the joint review of registration submissions for biopesticides (microbials, semiochemicals, and other biochemicals). From the PMRA website describing the initiative for biopesticides:

> "The procedure entails a joint pre-submission consultation to establish specific data requirements for the product; the proposed use pattern must be common to both countries...The PMRA and the EPA are committed to joint reviews and worksharing of pesticide evaluations on a regular basis. Joint reviews will increase the efficiency of the registration process, facilitate simultaneous registration in Canada and the U.S., and increase access to new pest management tools in both countries".

The NAFTA Joint Review Program (PMRA 2002) allows pesticide manufacturers to apply for registration of a new product for both Canada and the U.S. This reduces the timelines to approximately 12 months for the registration process as the review is shared by both countries. To further address the need to stimulate pesticide registrations in Canada, the User Requested Minor Use Registration (URMUR) program (PMRA 1999) encourages registrants to apply for the Canadian registration of products that are already registered in the U.S. and other OECD countries. Registration through the URMUR program also reduces the review timeline. The NAFTA Joint Review Programme further includes categories for reduced-risk pesticides, non reduced-risk pesticides, and negotiated joint reviews, which include Mexico's Comisión Intersecretarial para el Control del Proceso y Uso de Plaguicidas y Sustanicas Tóxicas (CICOPLAFEST).

SPECIAL CONCESSIONS AND ORGANIZATIONS PROMOTING BIOCONTROL

Special concessions

In a regulatory context, microbial pesticides are afforded special concessions. Evaluation and risk assessment standards remain the same as for synthetic pesticides, but the review timeline is shortened to 12 months for Category A submissions, 6 months for Category B, and 5 months for Category C. Timelines for Canada-U.S. joint reviews are negotiated, but expected to be shorter than those for single-country reviews. To encourage registration submissions and increase the range of candidate reduced-risk pest control products, Canada has accepted the United States definition of a reduced-risk or biopesticide product. The PMRA states that "further reduction of the performance standards (review timeline) may be considered, on a case-by-case basis, for applications that contain biopesticide products registered by the U.S. EPA since 1996 and that have all the U.S. EPA reviews" (PMRA 2002). While the PMRA charges a fee for pesticide registration applications, and annually for the right to sell the product in Canada, biopesticides are exempt, with the exception of the cost charged for reviewing the product label ($262 at time of writing). This can save in excess of $100,000 to a registrant. The exemption is also extended to label expansions of biopesticides for minor use registrations (PMRA 1997). Finally, there are generally no Maximum Residue Limits for microbial pesticides applied to produce, negating the need for crop residue studies.

Organizations promoting biocontrol

<u>Governments, universities, and affiliates</u>

Canada has numerous publicly funding organizations actively involved in either biopesticide research and development or promotion. Many of these groups began taking form following the influential Report of the Standing Committee on Environment and Sustainable Development entitled 'Pesticides – Making the Right Choice' (2000). The report sharply addressed concerns with the use and regulation of synthetic pesticides in Canada, making the following recommendations:

> "that the PMRA, in conjunction with other relevant departments and educational institutions, favour a reduction of pesticide use, develop alternatives to pesticides and promote integrated pest management by developing a pesticide use reduction policy and implementing it in all its activities, including the registration process; that the government allocate appropriate funding year after year to permit full implementation of the PMRA's integrated pest management programme; the government allocate appropriate financial resources to integrated pest management research and public information and, in particular, that Agriculture and Agri-Food Canada increase research into alternatives to pesticides and formulate pest management strategies; and that the government establish a national alternatives-to-pesticides data base and that it be made available to the public through an electronic registry".

The report was so influential that the new Pest Control Products (PCP) Act (2002) actually legislates sustainable pesticide use in Canada. The PCP Act explicitly states its mandate to be:

> "to seek to minimize health and environmental risks posed by pest control products and encourage the development and implementation of innovative, sustainable pest management strategies by facilitating access to pest control products that pose lower risks and by other appropriate measures".

Arising from this mandate was the creation of a joint programme between PMRA and Agriculture and Agri-Food Canada (AAFC) to promote reduced-risk pesticides, with significant support for biopesticides *per se*. AAFC is represented on this front by the Pest Management Centre (PMC), and PMRA by the Policy, Communications, and Regulatory Affairs Directorate (formerly the Alternative Strategies and Regulatory Affairs Division). Support is in the form of research funding for near-market products, assisting smaller biopesticide companies, Canadian or otherwise, with the preparation and completion of DACO tables to encourage pest control product registration in Canada, and in developing other farming practices for reducing the risk of pesticides (Agriculture and Agri-Food Canada 2010; Belliveau 2004). The programme has even hosted a 'biopesticide registration workshop' to explain the regulatory system to registrants and other stakeholders. More recently, the PMC created a biopesticide priority list for products that will receive preferential registration assistance, and plans to renew the list on a regular basis. The PMC houses on its website a directory for microbial pesticides for member countries of the OECD (Kabaluk and Gazdik 2010) so that researchers and growers can take advantage of increasing registration harmonization activities in considering new products for Canada that are already registered other OECD countries. The AAFC/PMRA pesticide risk reduction programme recently began collaborating with the U.S. EPA to evaluate and demonstrate the efficacy of a range of biopesticides of interest to growers in both countries. This joint effort is supported under the auspices of the NAFTA.

The Research Branch of AAFC funds a team of 20 researchers across the country whose activities span research from biopesticide discovery through to registration. While the core funding is moderate, it strengthens Canada's biopesticide community by enabling researchers to more easily collaborate within the group, and to build alliances with outside agencies, often leveraging more funding to advance research beyond that permitted by core levels (Agriculture and Agri-Food Canada 2009).

Provincial governments actively promote biopesticides to growers, most often within the context of extension/outreach by informing growers on biopesticide benefits, availability, and use (e.g. Government of Saskatchewan 2008) through fact sheets and presentations at conferences and trade shows. On a yearly basis, Provincial Pesticide Minor Use Coordinators lobby on behalf of their growers for new pesticide registrations, including biopesticides, to federal government programmes.

Beginning with the Supreme Court of Canada decision in 2001 to permit the town of Hudson, Quebec to enact its own pesticide legislation to ban cosmetic uses, similar bans have swept through Canada, affecting hundreds of synthetic pesticides within local government jurisdictions and even entire provinces (Supreme Court of Canada 2001). This change is expected to drive demand for biopesticides for homeowners and municipalities in the future. Because provincial governments are also entitled to enact similar province-wide legislation, Quebec was the first province to enact province-wide bans on certain pesticides. This has since been followed by Ontario, New Brunswick, and Prince Edward Island, with several other provinces undergoing the same consideration (e.g. see Government of Nova Scotia 2009).

On occasion, special targeted programmes will advance biopesticide research. One example is Canada's Natural Sciences and Engineering Research Council (NSERC) who, through its Research Networks Grants programme, enabled the creation of the Biocontrol Network from 2001 to 2006 and supported 58 biocontrol (biopesticides and natural predators and parasitoids) researchers in governments, universities, and industries from across Canada (see Biocontrol Network in References). While the term of this programme has ended, it significantly advanced biocontrol research, and has resulted in sustained collaborations among several of its members and affiliates.

Numerous universities across Canada are actively engaged in the research and development of biopesticides ranging from botanical to microbial pesticides for control of insects, weeds, and fungi. Kwantlen Polytechnic University in British Columbia recently constructed a stand-alone research laboratory to work with industry and government to develop biopesticide solutions, and is fully equipped for the mass production of fungal, bacterial, and viral pathogens for research and development (see Kwantlen Polytechnic University Department of Sustainable Horticulture in References).

<u>Non-governmental organizations</u>

Although Canada has the Association of Natural Biocontrol Producers (ANBP) to represent natural invertebrate predators for pest control, it lacks European Union and American models for a biopesticide producer and distributor alliance (i.e. the International Biopesticide Manufacturers Association (IBMA; EU) and the Biopesticide Industry Alliance (BPIA; U.S.). However, a recent survey revealed a strong interest among producers and distributors to form an association. Such a group could provide a single voice for lobbying government, sharing information and harmonizing registration activities (particularly with the U.S.), and sharing information for mutual benefit (Dupont 2007). It is anticipated that such a group will form in Canada in the future.

Agricultural producers vary in their support of biopesticides for their respective commodities, but most seem to allocate portions of their research and development budgets for this purpose. A notable example is the growers of greenhouse vegetables who, in recognition of worker safety and pest management and produce marketing advantages, actively support biopesticide research and the pursuit of new registrations.

Other various non-governmental and special-interest organizations across Canada promote biopesticides. In the 1990s, the World Wildlife Fund (WWF - see References) launched an international campaign to reduce the use of synthetic pesticides and began with targeting developed countries. The Canadian office of the WWF began a concerted effort to work with managers in three government departments to influence programmes related to pesticide use: AAFC, Health Canada, and Environment Canada. They informed these departments on aspects of integrated pest management, and are recognized as having influenced biopesticide programmes in Canada.

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UNITED STATES

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OVERVIEW

The United States Environmental Protection Agency (U.S. EPA) is the primary governmental organization responsible for the registration of pest management products. The authority for EPA to regulate pesticides is based on the statutes within the Federal Food, Drug and Cosmetic Act (FFDCA 1938), Federal Insecticide, Fungicide and Rodenticide Act (FIFRA 1947), Food Quality Protection Act (FQPA 1996), and Pesticide Registration Improvement Act (PRIA 2004). The Office of Pesticide Programmes is divided into the Registration Division which handles conventional synthetic chemistries, the Antimicrobials Division which regulates products for decontamination of surfaces and the Biopesticides and Pollution Prevention Division (BPPD) of EPA which regulates biopesticides. The Biopesticides and Pollution Prevention Division was created in 1994. In comparison with registration of conventional chemical pesticides, biopesticide registrations are usually faster, less complex, and less costly when a considerable body of information already exists, and it is presented appropriately to the agency for use in the risk assessment process.

There are numerous terms such as biorational, natural products, organic products, but for regulatory purposes, the term biopesticides usually includes i) microbial pesticides, viral proteins, and their genetic material, bacteria, fungi, protozoa, and algae; ii) biochemicals, including food substances and food additives, pheromones, growth regulators, oils and numerous other substances found in nature; and iii) plant incorporated protectants (PIPs). Unlike other countries, both the U.S. and Canada do not require registration of beneficial insects or entomopathogenic nematodes. Plant growth promoting rhizobacteria, nitrogen fixation or similar type of microbial inoculants do not require EPA registration as long as they do not make a pest control claim.

Microbial pesticide means a microorganism intended for preventing, destroying, repelling, or mitigating any pest, or intended for use as a plant regulator, defoliant, or desiccant, that:

i) is a eucaryotic microorganism including, but not limited to, protozoa, algae, and fungi
ii) is a procaryotic microorganism, including, but not limited to bacteria
iii) is an autonomous replicating microscopic element, including, but not limited to, viruses

Table 16. Microbial pesticides registered in United States.

	Taxus	Products	Targets
Bactericides			
Agrobacterium radiobacter k84	Bacterium	Galltroll - A	Crown gall disease
Pantoea agglomerans C9-1	Bacterium	BlightBan C9-1	Fire blight
Pantoea agglomerans E325	Bacterium	Bloomtime	Fire blight
Bacteriophage of *Pseudomonas syringae* pv. tomato	Virus	AgriPhage	Bacterial speck
Bacteriophage of *Xanthomonas campestris* pv. *vesicatoria*	Virus	AgriPhage	Bacterial spot
Fungicides			
Bacillus lichenformis SB3086	Bacterium	EcoGuard	Fungal diseases

Table 16 (con'd)

Bacillus mycoides isolate J	Bacterium	BacJ	*Cercospora*
Bacillus pumilus GB 34	Bacterium	GB34	Seedling diseases- *Pythium* and *Rhizoctonia*
Bacillus pumilus QST 2808	Bacterium	Sonata Ballad Plus	Powdery mildew, downy mildew and rusts
Bacillus subtilis GB03	Bacterium	Companion Kodiak	*Fusarium*, *Pythium*, *Rhizoctonia*
Bacillus subtilis MBI 600	Bacterium	Histick N/T Pro-Mix with Biofungicide	Damping off
Bacillus subtilis subsp. *amyloliquefaciens* FZB24	Bacterium	Taegro	*Fusarium* and *Rhizoctonia* wilt diseases
Pseudomonas aureofaciens Tx-1	Bacterium	Spot-Less	Turf fungal diseases
Pseudomonas chlororaphis 63-28	Bacterium	At-Eze	Soil and seed borne fungi
Pseudomonas syringae ESC 10	Bacterium	Biosave 10LP	Post harvest diseases
Pseudomonas syringae ESC 11	Bacterium	Bio-Save 11LP	Post harvest diseases
Streptomyces griseoviridis K61	Bacterium	Mycostop Biofungicide Mycostop Mix	Fungi causing damping off, stem and crown rots
Streptomyces lydicus WYEC 108	Bacterium	Actinovate Actino-Iron	Fungi causing damping off, stem and crown rots
Ampelomyces quisqualis M10	Fungus	PowderyGon	Powdery mildew
Aspergillus flavus AF36	Fungus	Aspergillus flavus AF36	*Aspergillus flavus* producing aflatoxin
Aspergillus flavus NRRL 21882	Fungus	Afla-guard	*Aspergillus flavus* producing aflatoxin
Coniothyrium minitans CON/M/91-08	Fungus	Contans	*Sclerotinia minor*, *Sclerotinia sclerotiorum*
Gliocladium catenulatum J1446	Fungus	Prestop	Seed borne, and soil borne diseases
Muscodor albus QST 20799	Fungus	Arabesque	Post harvest diseases
Pseudozyma flocculosa PF-A22 UL	Fungus	Sporodex	Powdery mildew
Trichoderma asperellum ICC 012 and *Trichoderma harzianum* (*gamsii*) ATCC080	Fungus	Tenet Bioten Remedier	Soil borne diseases
Trichoderma harzianum ATCC 20476	Fungus	Binab	Wound healing
Trichoderma harzianum Rifai T-22	Fungus	PlantShield RootShield T-22 Planter box	Seed and foliar diseases
Trichoderma harzianum T-39	Fungus	Trichodex	Soil and foliar diseases
Trichoderma polysporum ATCC 20475	Fungus	Binab T	Soil and foliar diseases
Ulocladium oudemansii U3	Fungus	BOTRY-Zen	*Botrytis* and *Sclerotinia*
Verticillium albo-atrum WC S850	Fungus	DutchTrig	Dutch elm disease
Bacteriophage of *Pseudomonas syringae* pv. tomato	Virus	AgriPhage	Tomato leaf spot
Candida oleophila O	Yeast	NEXY	Post harvest fruit molds
Fungicides/bactericides			
Bacillus subtilis QST713	Bacterium	Serenade	Foliar fungal and bacterial diseases
Herbicides			
Bacillus cereus BP01	Bacterium	MepPlus	Plant growth regulator
Alternaria destruens 059	Fungi	Smolder	Herbicide - dodder
Chondrostereum purpureum PFC 2139	Fungus	Chontrol Paste	Herbicide - stump sprout inhibitor
Colletotrichum gloeosporioides f.sp. *aeschynomene* ATCC 202358	Fungus	LockDown	Herbicide - northern jointvetch
Puccinia thlaspeos woad (dyer's woad rust)	Fungus	Woad Warrior	Herbicide - Dyer's woad

Table 16 (con'd)

Insecticides			
Bacillus popilliae	Bacterium	Milky Spore Powder	Japanese beetle grubs
Bacillus sphaericus Serotype H5a5b strain 2362 ATCC 1170	Bacterium	VectoLex	Mosquito larvae
Bacillus thuringiensis subsp. *aizawai* NB200	Bacterium	Florbac	Moth larvae
Bacillus thuringiensis subsp *israelensis*	Bacterium	BMP	Mosquito and blackflies
Bacillus thuringiensis subsp *israelensis* EG2215	Bacterium	Gnatrol Aquabac	Mosquito, flies
Bacillus thuringiensis subsp. *aizawai* delta-endotoxin in killed *Pseudomonas fluorescens*	Bacterium	M-Trak	Colorado potato beetle
Bacillus thuringiensis subsp. *aizawai* GC-91	Bacterium	Agree WG	*Plutella*
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Thuricide Thuricide Forestry Wilbur-Ellis BT 320 Dust Dipel Deliver Biobit HP Foray Javelin WG Green Light Hi-Yield Worm Spray Ferti-Lome Bonide Britz BT Worm Whipper Security Dipel Dust	Lepidopteran larvae
Bacillus thuringiensis subsp. *kurstaki* BMP 123	Bacterium	BMP123	Lepidopteran larvae
Bacillus thuringiensis subsp. *kurstaki* EG2348	Bacterium	Condor	Lepidopteran larvae
Bacillus thuringiensis subsp. *kurstaki* EG7841	Bacterium	Crymax	Lepidopteran larvae
Bacillus thuringiensis subsp. *tenebrionis*	Bacterium	Novodor	Colorado potato beetle
Bacilus thuringiensis subsp. *kurstaki* EG7826	Bacterium	Lepinox WDG	Lepidopteran larvae
Beauveria bassiana 447	Fungus	Baits Motel Stay-a-while	Ants
Beauveria bassiana ATCC 74040	Fungus	Naturalis L	Various insects
Beauveria bassiana GHA	Fungus	Mycotrol ES Mycotrol O Botanigard 22WP BotaniGard ES	Various insects
Beauveria bassiana HF23	Fungus	balEnce	House fly
Metarhizium anisopliae F52	Fungus	Tick-Ex	Ticks and grubs
Paecilomyces fumosoroseus Apopka 97	Fungus	PFR-97	Whitefly and thrips
Nosema locustae	Protozoan	Nolo-Bait Semaspore Bait	Grasshopper and crickets
Anagrapha falcifera nucleopolyhedrosis virus	Virus	CLV-LC	Lepidopteran larvae
Cydia pomonella granulosis virus	Virus	CYD-X	Codling moth
Gypsy moth nucleopolyhedrosis virus	Virus	Gypchek	Gypsy moth
Helicoverpa zea nucleopolyhedrosis virus (previously *Heliothis zea* NPV)	Virus	GemStar	Cotton bollworm, tobacco budworm
Indian meal moth granulovirus (*Plodia interpunctella* GV)	Virus	FruitGuard	Indian meal moth
Mamestra configurata nucleopolyhedrosis virus (107308)	Virus	Virosoft	Bertha armyworm
Spodoptera exigua nucleopolyhedrosis virus	Virus	Spod-X	Beet armyworm
Saccharomyces cerevisiae	Yeast	Bull Run	Fly attractant
Nematicides			
Bacillus firmus I-1582	Bacterium	BioNem	Nematodes
Pasteuria usgae	Bacterium	Econem	Nematodes
Myrothecium verrucaria	Fungus	DiTera	Nematodes
Paecilomyces lilacinus 251	Fungus	MeloCon WG	Nematodes

Table 16 (con'd)

Virucides			
Zucchini yellow mosaic virus – weak strain	Virus	AgroGuard-Z	Zucchini yellow mosaic virus

The data required to register a microbial pesticide (Data Requirements 2007) are divided into five groups which include A) Product Analysis; B) Residues; C) Toxicology (Health Effects); D) Non-Target –Environmental Effects; and E) Environmental expression. In addition, efficacy testing may be required for public health pests. Residue and environmental expression data are rarely required. Residue requirements are primarily aimed at organisms producing a metabolite of concern. In most cases, there are no numerical tolerances associated with microbial pesticides and they are issued as exemptions from tolerance. Data on environmental expression is also rarely required and are intended to demonstrate whether a microbial pest control agent (MPCA) is able to survive or replicate in the environment.

Product analysis data is always required and includes product identity, manufacturing process, formation of unintentional ingredients, sample analysis methods and certification of limits. In addition, there are data requirements regarding chemical and physical properties. The toxicology test guidelines include acute oral toxicity and pathogenicity, acute dermal, pulmonary, injection, and cell culture. Non-target testing includes avian oral, avian inhalation, wild mammal, freshwater fish and aquatic invertebrates, estuarine and marine animals, non-target plant and insect, and honeybee testing. In addition to these toxicology and non-target test guidelines, there are higher tier studies for toxicology.

Overall, acute oral and dermal, avian oral, non-target insect and honeybee testing are the data that are most frequently fulfilled by toxicology studies while most others are fulfilled by data waivers. Data waivers are a scientifically justified discussion of how the data is fulfilled due to existing knowledge about the organism or why exposure is unlikely to occur. Some of the factors in determining if the data requirement will need to be fulfilled by a toxicology study instead of a waiver include if there is known pathogenicity of the organism to man, animals and plants or proximal relationship to organisms of concern. Other factors include the type of microorganism (fungi, bacteria, virus, etc.), use sites such as food versus non-food crops, outdoor versus indoor application, terrestrial versus aquatic application, etc. The type of application method (such as in-furrow versus airblast sprayer) and product formulation (such as granule versus dust or spray) can also have a profound effect on the potential for worker exposure and consequently the risk.

ORGANIZATIONS, INFRASTRUCTURE, AND POTENTIAL SOURCES OF AID FOR BIOPESTICIDE DEVELOPMENT

Biopesticides may become the preferred alternatives to some conventional pest control agents when used in home and/or commercial settings. In order to facilitate their development, university systems may assist in the pursuit of commercial interests through their Research and Development Office. An Office of Technology Transfer may assist with the patenting process and contacts with potential development partners. University systems vary in their freedom to pursue commercial interests of biopesticides.

Table 17. Departments and agencies with oversight affecting pesticides in the United States

Health and Human Services	Centre for Disease Control and Prevention (CDC) Food and Drug Administration (FDA)
U.S. Department of Agriculture (USDA)	Agricultural Research Service (ARS) Agriculture Plant Health and Inspection Service (APHIS) National Institute of Food and Agriculture (NIFA) IR-4 Project, Rutgers University (IR-04) National Organic Programme (NOP)
U.S. Department of Commerce	National Oceanic and Atmospheric Administration (NOAA) -Estuaries, Coastal Waters and Oceans National Marine Fisheries Service
U.S. Department of the Interior	Fish and Wildlife Service Endangered Species Invasive Species (F&WS) U.S. Geological Survey (USGS) -Surface Waters -Groundwater
U.S. Environmental Protection Agency (U.S. EPA)	Office of Air and Radiation Office of Prevention and Toxic Substances Office of Pesticide Programmes Office of Water

While university scientists are familiar with obtaining grants for basic research, the transition from basic research to developing a marketable product is different. There are specific programmes that can assist with efficacy and registration. The USDA IR-4 Project, based at Rutgers University, typically offers small grants to conduct efficacy research on biopesticides. The research programme is divided into three levels based on their registration status including early, advanced and demonstration stage grants. Early stage projects involve active ingredients that have not completed the tier 1 toxicological data requirements. They are generally well beyond the Petri dish stage and most have indications of commercial interest. Advanced stage projects involve active ingredients that have completed the tier 1 data requirements and usually already have a registered product. Most of the advanced stage projects focus on label expansion such as adding a new pest or new crop to the label. The Biopesticides and Pollution Prevention Division of EPA has the goal of reducing risks associated with pesticide use in agricultural and non-agricultural settings in the United States and advocates adoption of biopesticides through a small grant programme involving efficacy and promoting adoption of biopesticides by their cooperating partners in the Pesticide Environmental Stewardship Programme (PESP). The IR-4 co-funds biopesticide research with the EPA in the Biopesticide Demonstration Grant Programme. This programme involves registered biopesticides and conducts on-farm demonstration of biopesticide efficacy in grower's fields.

One of the barriers to adoption of biopesticides is simply the lack of easily accessible information about what products are registered. The IR-4 Project maintains a label database which is searchable based on crop, pest and state. There is also an option to specify only organic products. Over 31,000 visitors have used the site which aims to bring together growers and manufacturers with biopesticide product information (www.ir4.rutgers.edu/Biopesticides/LabelDatabase/index.cfm). The IR-4 Project also helps public sector scientists and small companies to prepare data packages to EPA for registration.

The USDA has also developed a National Biological Control Laboratory in Stoneville, Mississippi with space for two pilot plants where scientists can cooperate with public and private organizations to test the practical applications of rearing techniques and foster commercial production, especially with small venture capital companies. Other options may be developing a plan on licensing the rights of the product, setting up a campus-sponsored incubator company or continuing technology transfer through federal Small Business Innovative Research (SBIR) grants. The USDA invites science-based small business firms to submit research proposals under the SBIR Grant Programme. The announcement can be found at www.fedgrants.gov/applicants/USDA-GRANTS:07010400/listing.html/. Through this programme, USDA will support high-quality research or research and development proposals containing advanced concepts related to important scientific problems and opportunities that could lead to significant public benefit if the research is successful.

Objectives of the SBIR Programme include stimulating technological innovation in the private sector, strengthening the role of small business in meeting federal research and development needs, increasing private sector commercialization if derived from USDA-sponsored research and development efforts, and fostering and encouraging participation by women-owned and socially and economically disadvantaged small business firms in technological innovation. While not specified in their announcement, many small companies (including university spin-offs) have funded toxicology studies through this programme.

In 1986 and 1989, legislation was enacted as part of the Sevenson-Wydler Technology Innovation Act to enable federal laboratories to enter into Cooperative Research and Development Agreements (CRADAs) with private business and other entities. CRADAs provide the means to leverage research and development efforts and to create teams for solving technological and industrial problems. Through CRADAs, companies or groups of companies can work with one or more federal laboratories to pool resources and share risks in developing technologies.

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AUSTRALIA

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OVERVIEW AND USE

Australia is a vast island continent with a unique flora and fauna. The economy is dependent on bulk commodity exports, and agricultural exports accounted for approximately A$29 billion in 2009, or 4.6% of total exports (Australian Bureau of Agricultural and Resource Economics 2010). However, the Australian pesticide market is small, estimated to be about 2-3% of the total global market for pesticides.

Early experiments with microbial control included field trials in the late 1960s with the granulosis virus of codling moth in apple orchards, and in the 1970s with Elcar, the nucleopolyhedrosis virus (NPV) of *Helicoverpa zea*. Initial success was limited, with poor field efficacy and direct competition with new chemical insecticides. Early large scale field trials with the granulosis virus of potato tuber moth, *Phthorimaea operculella*, gave promising results (Reeda and Springetta 1971), but a commercial product was not registered.

The number of microbial pesticides registered in Australia has increased in the last decade (Table 18), with the widescale use of *Bacillus thuringiensis* subsp. *kurstaki* (Btk). A crisis in insecticide resistance in *Helicoverpa* species in the late 1990s led to adoption of area-wide integrated pest management in the commercial cotton and sorghum industries, where biopesticides are used to manage resistance to chemical insecticides and to reduce secondary pest outbreaks (such as silver leaf white fly) by maintaining beneficial insect populations. Biopesticides are also used in areas of special concern such as national parks, in the expanding 'organic' market, and for export markets such as wine, where the industry restricts the use of synthetic insecticides (Hunter 2010).

The biopesticides market is dominated by Btk products for control of a range of lepidopteran pests, and Bt genes have been incorporated into cotton crops to manage *Helicoverpa* spp. Btk was adopted by the grape and wine industry to control light brown apple moth (*Epiphyas postvittana*), a native tortricid moth. The introduction of Btk in place of broad-spectrum insecticides resulted in a significant reduction in frequency of outbreaks of light brown apple moth in grape vines, presumably as a result of maintenance of natural enemies. *B. thuringiensis* subsp. *israelensis* (Bti) is also used for control of nuisance biting insects and disease vectors in coastal mangroves and housing areas, where application of chemical insecticides is unacceptable.

The success of Bt also demonstrated the importance of good product supply and quality, and established supply chains on which growers could depend. This was also a key factor in success of biopesticides based on NPVs. The brief success with Elcar in the 1970s supported continued research in baculoviruses by the Queensland Department of Primary Industries. Interest was renewed in the late 1990s in the face of a crisis in management of insecticide resistance in *Helicoverpa armigera* and escalating costs of insecticides. The biopesticide 'Gemstar' based on *H. zea* NPV was imported from the USA, and initial trials were conducted under a special permit. The product was registered for use in cotton and sorghum in 1999.

Table 18. Microbial pesticides registered in Australia.

	Taxus	Products	Targets
Bactericides			
Agrobacterium radiobacter	Bacterium	NoGall	Crown gall disease
Fungicides			
Trichoderma harzianum	Fungus	Trichodex Vinevax	*Botrytis* spp. *Eutypa* dieback
Insecticides			
Bacillus sphaericus	Bacterium	VectoLex	Mosquito larvae
Bacillus thuringiensis subsp. a*izawai*	Bacterium	Agree Bacchus XenTari	Lepidoptera larvae
Bacillus thuringiensis subsp. *israelensis*	Bacterium	Aquabac BTI Teknar Vectobac	Mosquito larvae
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Biocrystal Caterpillar Killer DiPel Costar Delfin Full-Bac WDG	Lepidoptera larvae
Metarhizium anisopliae	Fungus	BioCane Granules	Grey-backed cane grub (scarabs)
Metarhizium anisopliae subsp. *acridum*	Fungus	Green Guard	Locusts and grasshoppers
Metarhizium flavoviride	Fungus	Chafer Guard	Redheaded pasture cockchafer
Helicovera armigera nucleopolyhedrosis virus	Virus	Heliocide Vivus Gold Vivus Max	*Helicoverpa* spp.
Helicovera zea nucleopolyhedrosis virus	Virus	Gemstar Vivus	*Helicoverpa* spp.

Demand for NPV was such that in 2000, Ag Biotech Australia established a pilot plant to produce *Helicoverpa* NPV in Australia. The initial product was produced in *H. armigera* using the American isolate from *H. zea* and was branded Vivus. The first commercial sales of Vivus were made in 2003. The American virus strain was then replaced with a native *H. armigera* strain, isolated many years previously by the Queensland Department of Primary Industries and Fisheries, and Vivus Gold was registered in 2004.

Helicoverpa spp. are a major pest of many crops, and the NPV products are now have registered on a broad range of crops including sorghum, cotton and horticultural crops. Around 500,000 hectares of crops were treated in 2008. Vivus Gold is now also registered for application through 'centre pivot' irrigation, a method that has proven to be very successful by a number of innovative sweet corn producers. A concentrate product, Vivus MAX is now registered, containing over twice the number of virus occlusion bodies as previously, thus reducing packaging and improving storage and distribution.

The first fungal insecticide was manufactured and registered in Australia by BioCare Ltd. in 2000. 'BioGreen' (now 'Chafer Guard'), based on *Metarhizium flavoviride*, is a granular product consisting of broken rice on which the fungus is grown and sporulates. It is used to control redheaded pasture cockchafer, *Adoryphorus couloni*, in turf and pasture (Milner 2000). The second product, also registered by BioCare, was 'BioCane' containing *M. anisopliae* for control of greyback canegrub (*Dermolepida albohirtum*). Becker Underwood Pty Ltd. now manufactures both products in Australia.

Metarhizium anisopliae subsp. *acridum* was first evaluated for plague locust control under a collaboration between CSIRO and the UK Commonwealth Agriculture Bureau (CABI) in 1998. An Australian isolate with good production and control characteristics was identified and used initially under a special permit in national parks and 'organic' beef rangeland by the Plague Locust Commission (Milner 2000). The product was registered in 2005 as Green Guard, also manufactured by Becker Underwood, and has been applied to over 100,000 ha between 2000 and 2009 (Hunter 2010).

The success of biopesticides in crops and in locust control has led to significant research in potential controls for emerging pests such as mirids and aphids, particularly in genetically modified Bt cotton, which is susceptible to sucking pests. Trial results have shown good control of aphids and mirids in cotton and pulse crops with native isolates of *M. anisopliae* (Hauxwell, *unpublished*). *M. anisopliae*, *Beauveria bassiana* and *Verticillium lecanii* have also been tested in glasshouse trials (Goodwin and Steiner 2002), though are not currently registered. *M. anisopliae* has also been tested against cattle ticks and sheep lice by the Queensland Department of Primary Industries.

Considerable research has been conducted into use of nematodes against a wide range of pests (including snails, scarabs, weevils, gnats, sheep lice and wood wasps). However, nematodes are considered 'natural enemies' (along with, e.g., parasitoids and predators) if they are visible to the naked eye, and are thus exempt from registration and are not discussed in this review. Those species not visible to the naked eye are classed as microscopic and require registration, but none have been registered.

A small number of anti-microbial pesticides are registered, including *Trichoderma harzianum* against *Botrytis* and *Eutypa* dieback in vines. Becker Underwood has registered NoGall containing the bacterium *Agrobacterium radiobacter* for use against crown gall (caused by the soil bacterium *Agrobacterium tumefaciens*) in stonefruit and ornamental plants.

REGISTRATION AND THE REGULATORY SYSTEM

Registration of pesticides is governed by the Agricultural and Veterinary Chemicals Code Act 1994 and administered by the Australian Pesticides and Veterinary Medicines Authority (APVMA). The importation of a biological agent also requires authorisation from the Australian Quarantine Inspection Service (AQIS) prior to introduction into Australia. If the organism has been genetically altered, approval from the Office of the Gene Technology Regulator (OGTR) is required prior to importation or release.

Approval from APVMA must be obtained for any new active constituent (including an organism), and any new product and all proposed uses of the product must be registered by APVMA. The legislation requires, prior to registering any new product, APVMA to be satisfied that the product, if used in accordance with the instructions for its use:

- will not adversely affect human and animal health and safety,
- will be effective and of consistent quality,

- will not adversely affect the environment, and
- will not affect international trade in commodities.

The APVMA also has a Permit Scheme that allows for the use of pesticides in ways that are different to the uses set out on the product label or for limited ‘emergency’ use of an unregistered product. *M. anisopliae* var. *acridum* for locust control was initially used under permit, later going on to full registration. An application for a permit must satisfy the same criteria as for registration, though as the extent of use is intended to be small, the supporting data requirements and evaluation processes may be simpler. However, in practice, the stringent requirements can be as demanding as a full registration.

The APVMA’s Manual of Requirements and Guidelines (MORAG) sets out the requirements for agricultural and veterinary chemicals to be manufactured or used in Australia (see references for links). The basic requirements for registration of microbial pesticides are the same as for chemical pesticides, including a comprehensive package of data on toxicology, efficacy, storage and (to some extent) field residues. Toxicology and residue analysis should be conducted in accordance with Good Laboratory Practice. Guidelines published in 2005 contain additional requirements for microbial pesticides to include evaluation of potential hazards such as toxin production, pathogenicity and infectivity, host range, and effects on native flora and fauna (APVMA 2005).

Active organisms must be fully characterised and their relationship to other organisms, particularly known pathogens, must be described. Any contaminating microorganisms or preparation by-products must also be identified, quantified and evaluated for pathogenicity, toxicity or persistence. Manufacturing methods and quality control procedures to limit contaminants must be described.

Toxicology testing is modelled on chemical pesticide testing, including requirements to evaluate lethal doses (LD50s) in mg per kg body weight. This may be practically impossible to determine for biological products that do not contain active chemical compounds. Long-term toxicological testing is not normally required unless, for example, the organism produces compounds of concern such as potentially carcinogenic metabolites.

Australian agriculture relies heavily on exports of agricultural commodities. Registration data must demonstrate that the product will not harm crops of importance or "prejudice trade or commerce between Australia and places outside Australia". This is rarely a concern for microbial pesticides, but the potential to affect trade through residues or viable organisms that remain in the commodity must be evaluated.

It is required to demonstrate that the formulated product of chemical pesticides will remain within specification for at least 2 years under typical storage conditions i.e. at around 30˚C in the product packaging. Where this cannot be achieved (as is the case with most microbial pesticides) they can be registered as ‘date-controlled products’ with an expiry date on the label. This may include a requirement for cool storage and transport. Vivus NPV products, for example, have an approved label with a shelf life of 2.5 years when stored at 4°C.

Residue data are normally not required for microbial agents unless the organism produces a metabolite of concern: if not supplied, an exemption must be requested with a sound scientific justification. Residue decline information is generally required when an application is expected to be made close to harvest (usually less than 14 days for most crops), or an application is made after harvest, or there are trade implications for the produce. However, the registration of Green

Guard followed the classic synthetic insecticide pathway, with stringent requirements for residue data.

Residue data may not be meaningful if, for example, there is a persistent, low-level occurrence of the organism in the environment from natural transmission. The short field persistence of some microbial pesticides can be an advantage in this regard. The Vivus range of NPV products, for example, have no withholding period.

APVMA normally requires efficacy data from trials conducted in Australia over at least two years and under a suitable range of pest pressures for each pest and crop combination specified on the label. This should include data from each of the major regions or environmental zones in Australia where the product will be used. Overseas data can be used to support an application if it is applicable to Australian uses and conditions, for example in controlled environments such as glasshouses where conditions are similar to those in Australia. Overseas data alone are rarely sufficient for registration, and thus approval for environmental release must be obtained in order to conduct tests in Australia.

Australia invests heavily in border protection to prevent the introduction of harmful or invasive species and protect its unique flora and fauna. Even endemic species may have adverse effects if introduced into other regions of Australia. Registration submissions for a microbial pesticide must include evaluation of persistence and replication of the organism, including its ability to induce epizootics, and its specificity or potential harmful effects on native flora and fauna prior to introduction into Australia.

The burden of determining effect on native non-target species can be significant, and it may be difficult to anticipate what testing may be requested following review. Non-target studies conducted overseas can be included, however, specific tests on Australian biota may be required where there are concerns about impact on wildlife, flora or ecosystems.

Addressing concerns on potential impact of a microbial pesticides on the Australian environment can be costly, time consuming and difficult. It may be possible to reduce the data required if it can be demonstrated that the agent/organism will not survive in the Australian environment, or if the organism will be effectively contained or is highly host specific. Even where the organism occurs in Australia, requirements for data on natural occurrence and distribution of the organism in Australia are stringent and can be difficult to meet.

The evaluation process

Following submission of an application, APVMA conduct an initial screen to ensure supporting data are complete, and conduct a preliminary evaluation. This may generate requests for further information on technical aspects of the application. After screening, a full evaluation is conducted and data are scrutinised by relevant experts, as outlined in Figure 4.

- Product characterisation, chemistry, production and quality control systems, and residues are assessed by experts within APVMA.
- Toxicology and occupational health and safety are evaluated by the Office of Chemical Safety and Environmental Health (within the Department of Health and Aging).
- Environmental fate and effects are evaluated by the Environment Protection Branch of the Department of Environment, Water, Heritage and Arts.
- Efficacy and stability are evaluated by external experts (usually research scientists) selected by APVMA.

A public consultation process is conducted. Comments are sought from other departments and authorities (such as The Office of the Gene Technology Regulator or the National Health and the Medical Research Council and state departments of agriculture) and public comment is invited through a notice in the Agricultural and Veterinary Chemicals Gazette (available from the APVMA website). A summary of planned approvals is sent to interested members of the public and relevant industry bodies for comment. All comments are considered before the final decision on whether to register the product is made.

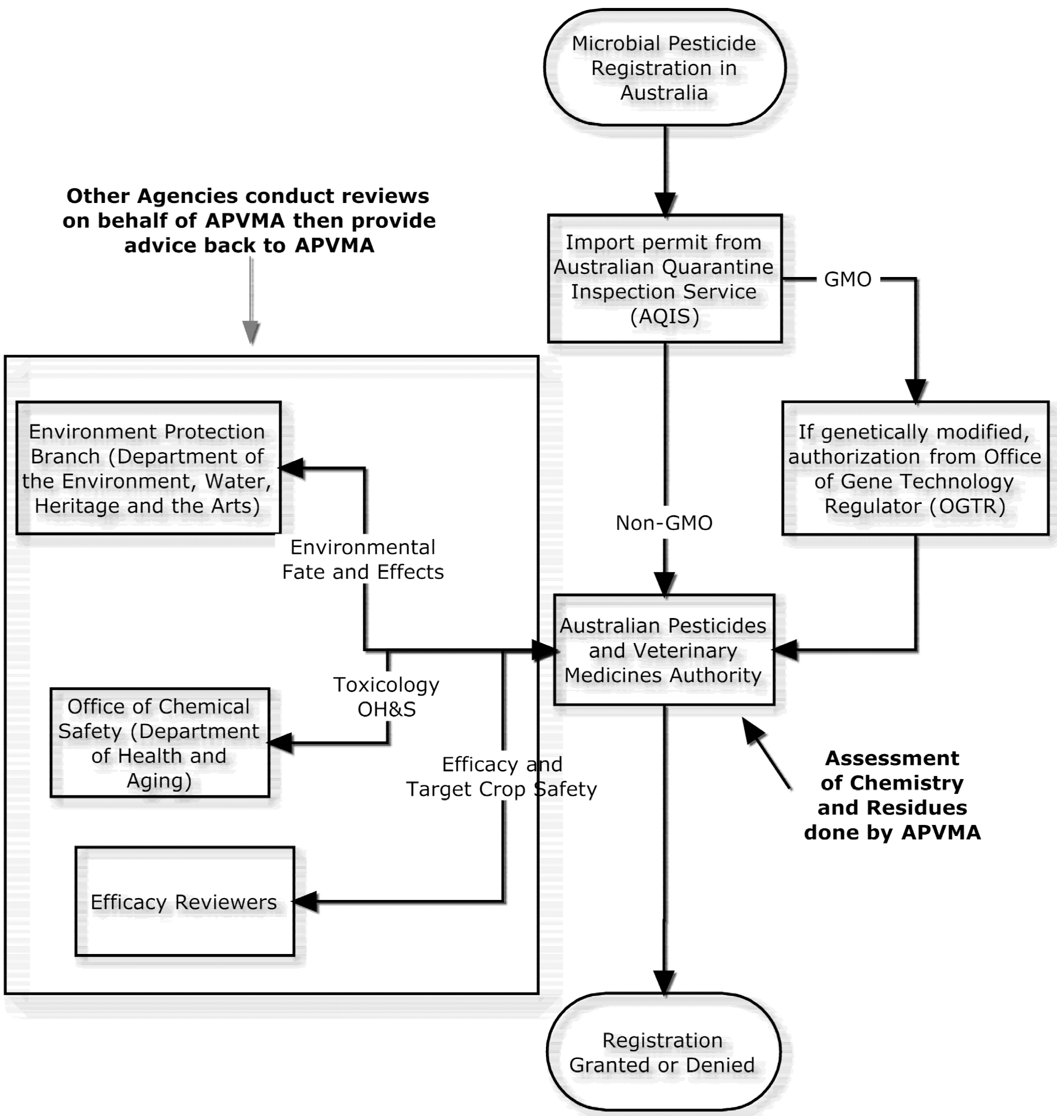

Figure 4. The process for registration of a new microbial pesticide in Australia.

The cost of registration can be prohibitive to microbial pesticides. The Australian market is estimated to be about 2-3% of the total global market for pesticides. Most microbial pesticides are used in niche situations with relatively small sales. Negative cash flows are experienced at the start of the registration process when investment needs to be made to pay the cost of local efficacy studies, additional studies specifically required for registration in Australia, and up-front payment of registration application fees. There are no fee reductions for registration of new products for minor uses, although a system does exist to obtain temporary permits for use of registered products in minor use situations. No income can be earned during the registration process, which can take several years. Thus the cost of registration can be high when comparing projected returns and pay-back times to those for broad-spectrum chemical insecticides.

The regulatory system is focussed on registration of synthetic chemical products, and has limited experience in registration of microbial pesticides due to the small number registered. As a result, applicants often find the process can involve unexpected requests for information not anticipated at the time of submission. These requests for additional, unexpected information can result in delays (which translate into delayed return on investment) as the applicant provides arguments to address the concerns, or can require further investment and time to undertake additional studies.

Assessments of a product application can take 15 months or longer. The registration of one microbial pesticide was delayed by a lack of understanding of the requirement for ingestion and field performance of a biological by the reviewer, which led to rejection of the initial efficacy package. The generation of supporting input from public and private researchers caused the loss of a full season of use and thus of a year's revenue. In the case of Green Guard the registration process took 4 years from the date of submission to the date of registration, and followed the classic synthetic insecticide pathway, with stringent requirements for residue data.

The Australian Government and the APVMA recognise there is a need to improve the regulatory system for novel products, including biopesticides. At the time of writing, the APVMA has established a working group to review the registration process for biopesticides, and the Australian Government is reviewing the operations of APVMA to determine how the process can be improved to authorise use of new products more quickly. It is anticipated that this will address many of the hurdles that currently prevent or delay registration in Australia.

SPECIAL CONCESSIONS AND ORGANIZATIONS PROMOTING BIOCONTROL

No special concessions are given to biopesticides during registration, although APVMA considers the benefits as well as risks of all pesticides. Microbial pesticides have demonstrated benefits in mainstream and niche applications in Australia, particularly in integrated pest management and sensitive environments. They can offer a 'low risk' alternative to chemical pesticides by helping to manage resistance to chemicals and reduced residues. These benefits can be offset against, for example, lower field efficacy. In addition, exemption for some data requirements can be made if a rational scientific case is made.

Consumer demand, workplace safety considerations and increased acceptance of the benefits of integrated pest and disease management will result in increased prospects for biopesticides. Much of Australia's fruit and vegetables are supplied to the consumer through supermarkets, which are demanding 'clean and green' and high quality produce. As a result, there is increasing use of protected structures (e.g. glasshouses, plastic covered poly-tunnels) to achieve the quality of vegetables demanded. There are restrictions on use of many synthetic chemical products in such protected structures due to concerns about exposure of workers to the products. At the same time, such structures commonly provide ideal environments for use of microbial pesticides. The increasing reliance on protected structures will see increasing use of lower risk products, including biopesticides.

Resistance to chemical insecticides continues to be of concern to industry, and biopesticides such as NPVs have demonstrated their value in strategies to minimise selection for resistance, prolonging the effective life of other pesticides. Secondary pest outbreaks are also a concern where disruption of natural enemies by application of broad-spectrum chemistry can lead to large scale and severe outbreaks (e.g. silver leaf whitefly and aphids in cotton). The selectivity of biopesticides enables them to be used without disrupting natural enemies, and so reduce secondary outbreaks.

Industry funding continues to support research into biopesticides, particularly from grains, cotton and horticulture industry bodies. The APVMA systematic review of chemical insecticides has led to withdrawal of many older chemicals, and more are expected to become unavailable for use in the future. The success of biopesticides combined with concerns over resistance and disruption of beneficial insect populations, and health risks to farm workers is leading to greater demand for biopesticides across industry.

Australia has demonstrated that good product quality, supply and distribution can create confidence and significant market demand by growers, which will increase opportunities for new products. Australia shares much in common with crops, pest species and climatic conditions in Asia, and offers excellent facilities and an excellent reputation for testing and data integrity. The Asian and Australasian market for microbial- and nematode-based pesticides was estimated to be worth approximately $132.5 million per annum in 2007/8, and opportunities exist that could raise the total market to $225 million by 2015 (CPL Business Consultants 2010).

SUMMARY

Australia is an island continent with a unique flora and fauna. Consequently, in addition to demonstrating a lack of undue hazard to humans, there is an emphasis on preventing entry of organisms that could have harmful effects on Australia's environment. The fate and specificity of biopesticides, including their capacity to induce epizootics or harmful effects on native species must be considered prior to introducing new microorganisms.

Australia has a small domestic market for insecticides, and microbial products are typically niche products within that market. Small projected returns and the lengthy registration process has limited the registration of microbial pesticides. However, at the time of writing, the registration process is under review with the specific goal of improving registration of such products.

The success of microbial pesticides in managing resistance and outbreaks of secondary pests in mainstream agricultural production such as cotton, sorghum and horticulture has demonstrated a role for biopesticides, and has created confidence and market demand based on quality and supply of products. Consumer demand for quality produce from ‘clean and green’ production systems and organic products, combined with growth in controlled-environment production, suggests there is a favourable future for biopesticides in Australia.

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NEW ZEALAND

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OVERVIEW AND USE

New Zealand is a major producer and exporter of high quality agricultural products and gains value from the "clean green" image of the country and production systems that have minimal chemical inputs. Microbial pesticides can make a major contribution to the production of environmentally sustainable, low chemical residue produce and have been incorporated into horticultural and agricultural production systems (Brownbridge et al. 2008a).

The use of insect pathogens as control agents for insect pests has been considered and attempted for more than a century in New Zealand (Jackson 1991). Early introductions of insect pathogens were inspired by the works of Metchnikoff, and later Steinhaus. Some common strains of viruses, bacteria, fungi and nematodes were introduced for testing, mostly by New Zealand science organisations such as the Department of Scientific and Industrial Research and Ministry of Agriculture and Fisheries (now Forestry) (MAF). By the end of the 1970s, the need for a system of regulation was recognised (Kalmakoff and Longworth 1980), and scientists and regulators developed a set of guidelines that were passed into New Zealand law (Anonymous 1984). By 1991, 11 microbial products were registered. These products were based on two bacteria, *Bacillus thuringiensis* (*Bt*) subsp. *kurstaki* and *Serratia entomophila,* and two entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* (Jackson 1991). Only the *Bt* based products were fully registered for sale at that stage, while the endemic bacterium *S. entomophila* (Enterobacteriaceae) was available under a limited sales permit to allow market evaluation (Jackson 2007). Interest in microbial pesticides increased and, by 2010, 36 products were fully registered for agricultural use in New Zealand (ACVM 2010), including those based on bacteria, fungi, viruses and nematodes (Table 19) (Brownbridge et al. 2008a). Additional microbial products are under provisional registration or have been used in eradication campaigns against mosquitoes where they are registered under the Hazardous Substances and New Organisms Act 1996.

Current status of microbial pesticides in New Zealand

Commercial products are still dominated by *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) (13 products) for use in horticultural production and control of invasive lepidopteran pests. *Bt aizawai* has also been registered for control of brassica pests, either as a single toxic agent or in combination with *Btk*. *Bt israelensis,* while not registered as an agricultural compound, is used in vector control and is being considered for use against nuisance midges (Chironomidae) in oxidation ponds adjacent to urban centres. *Serratia entomophila*, discovered and developed in New Zealand, is used to control the scarab pasture pest *Costelytra zealandia* (Jackson 2007). *Bacillus subtilis* is used as a fungicide and plant growth regulator in vineyards, and the bacterium *Pantoea agglomerans* is registered for application to apple and pear blossom to reduce the incidence of fire blight through pre-colonisation of the susceptible flower parts.

The fungi *Trichoderma atroviride* and *T. harizanium* are registered for a range of products targeting stem and root diseases in various horticultural crops, with a focus on control of fungal disease caused by *Botrytis cinerea* in vineyards and *Armillaria* spp. in pine seedlings. Another fungal biocontrol agent, *Ulocladium oudemansii,* has also been developed and registered for control of botrytis. Indigenous strains of *Lecanicillium lecanii* (designated as *Lecanicillium longisporum* and *Lecanicillium muscarium*) are registered and produced for control of whiteflies, aphids and psyllids in greenhouse and field crops. *Beauveria bassiana* is also registered for control of sucking pests and can be applied either as conidia or blastospores. Four products containing the Mexican strain of the *Cydia pomonella* granulosis virus are registered and imported for control of the codling moth in pipfruit orchards, and the nematode *Steinernema feltiae* is imported for control of sciarid flies in mushroom production as well as thrips and pest Diptera in protected horticulture.

REGISTRATION AND THE REGULATORY SYSTEM

Microbial pesticides in New Zealand must be registered under the Hazardous Substances and New Organisms Act 1996 (HSNO). Since 1998, the importation and release of all new organisms, including microbes for biological control, has been regulated by this Act. Species of microbes unknown in New Zealand prior to 1998 are subject to approval by the Environmental Risk Management Authority (ERMA New Zealand) before release, even if they have been isolated from material collected within the country. Applications may be made for importation into containment or for limited or full release (Pottinger and Morgan 2008). Preparations of microbes already existing within New Zealand can be imported providing they conform to Ministry of Agriculture requirements for identity and purity. Any novel strain of microbe to be developed as a microbial pesticide must be assessed under the HSNO, which is administered by the ERMA. Given that any biopesticide will have biocidal activity, it will be a hazardous substance under the definition of the act. Registration under HSNO will dictate the conditions under which such a hazardous substance can be manufactured and sold. Once approved by HSNO, biopesticides also need to be registered under the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997, which replaced the Pesticides Act 1979. ACVM is administered by the New Zealand Food Safety Authority within the Ministry of Agriculture and Forestry. Biopesticides are registered under the ACVM legislation as Plant Compounds and registration covers conditions of manufacture and sale related to food safety and agricultural biosafety (i.e. minimum residue levels). The formulated product must be registered in accordance with ACVM guidelines (www.nzfsa.govt.nz/acvm).

SPECIAL CONCESSIONS AND ORGANIZATIONS PROMOTING BIOCONTROL

Microbial pesticides are increasingly recognised as key components in integrated crop management systems in New Zealand, promoting sustainable production of a variety of agricultural commodities that can access premium export markets demanding nil chemical residues in agricultural produce. The New Zealand horticulture industry, reliant on international markets, has been faced with the dual problem of producing fruit without contaminant pests while meeting the chemical residue standards (sometimes nil-detectable) of the importers. Kiwifruit producers have risen to this challenge, adopting a “KiwiGreen” strategy for production based on monitoring and use of *Btk*. “Kiwi Green” has succeeded in providing excellent pest control and has gained international recognition for minimal fruit residue (www.zesprikiwi.com/environment_sustainability.htm). A similar need to minimise chemical residues in pipfruit orchards led to Integrated Fruit Production (IFP) and organic production systems where *Btk* and granulosis virus are widely used.

Table 19. Microbial pesticides registered with the Agriculture Compounds and Veterinary Medicines (ACVM) Unit, New Zealand, 2010.

	Taxus	Products	Targets
Bactericides			
Agrobacterium radiobacter	Bacterium	DyGall	Crown gall disease
Pantoea agglomerans	Bacterium	Blossom Bless	Fire blight
Fungicides			
Bacillus subtilis	Bacterium	Donaghys FoliActive	*Botrytis*, powdery mildew
Bacillus subtilis QST713	Bacterium	Sernade Max	*Botrytis*, powdery mildew
Trichoderma atroviride	Fungus	Tenet Unite Vinevax Bio-injection Vinevax Bio-dowel Sentinel	Soil-borne, stem colonising and foliar plant pathogens
Trichoderma harzianum rifai (5 strains)	Fungus	Vinevax wound dressing	Stem pathogens after pruning
Ulocladium oudemansii	Fungus	BOTRY-Zen	*Botrytis*
Insecticides			
Bacillus thuringiensis subsp. *aizawai*	Bacterium	Xentari Agree	Lepidopteran pests
Bacillus thuringiensis subsp. *kurstaki*	Bacterium	Biocrystal kurstaki Dipel Biobit Foray 48B BMP Bactur Bactercide Organic No Caterpillars Delfin	Lepidopteran pests
Serratia entomophila	Bacterium	Invade Bioshield Grass-grub	New Zealand grass grub
Beauveria bassiana	Fungus	Beaugenic Beaublast	Whiteflies, aphids, sucking pests
Lecanicillium lecanii	Fungus	Vertikill Vertiblast	Whiteflies, aphids, thrips
Steinernema feltiae	Nematode	Nemasys	Fungus gnats, thrips
Cydia pomonella granulosis virus	Virus	Carpovirusine Virex Madex CYD-X	Codling moth

For New Zealand meat and milk producers, biological control has been recognized as a means of producing products free of chemical residues, and research has been conducted on the implementation of parasitoid and microbial control in pastures to counter effects of indigenous and invasive pest species (Jackson et al. 2002, Brownbridge et al. 2006, 2008b). Commercialisation of the grass grub control product Bioshield, based on *S. entomophila*, has been carried out by a major New Zealand fertiliser company, Ballance Agri-Nutrients Ltd. Traditionally, low fertiliser costs have allowed farmers to compensate for insect pest damage, but as commodity prices increase and environmental regulations limit the use of such inputs, new economic drivers are supporting the development and increased use of biopesticides to mitigate the impact of pests and diseases on pasture production.

Several small, specialist companies are engaged in development and promotion of biological control using microbes in New Zealand. Agrimm Technologies (www.tricho.com) is based at Lincoln, near Christchurch in the South Island, and produces a range of *Trichoderma*-based products for plant disease control and growth promotion in vineyards and horticultural crops. Crop Solutions, based near Auckland, is developing and producing *Beauveria* and *Lecanicillium* spp. based mycoinsecticides. BotryZen (www.botryzen.co.nz), a company located in Dunedin, has a product based on the fungus *Ulocladium oudemansii,* which is used to suppress the pathogen *Botrytis cinerea,* especially on grapes.

Commercial *Bt* products from international companies have been used extensively by MAF Biosecurity New Zealand, a Government agency, for the eradication of invasive pests. *Bt kurstaki* has been the main component of successful eradication of three invasive lymantriid pests from urban areas (Glare 2009), and *Bt israelensis* has been used as a component of a successful mosquito eradication campaign in environmentally sensitive wetlands (www.biosecurity.govt.nz/media/29-8-08/pest-mosquito).

SUMMARY

The registration of new microbial pesticides in New Zealand is regulated by the ACVM Act 1997 and the HSNO Act 1996, and registration of products with agricultural uses is administered by the ACVM unit of the New Zealand Food Safety Authority. Bacterial and fungal products are manufactured in New Zealand by a range of companies, and additional bacterial, viral and fungal products are imported for pest and disease control. The number of biopesticides registered by ACVM increased from 11 in 1991 to 36 in 2010. *Bacillus thuringiensis* (*Bt*) products play an important role in the management of horticultural pests and have been utilized in the eradication of invasive pests from environmentally sensitive areas. *Bt*-based biopesticides are the most widely used of all microbial biocontrol agents in the country.

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ALTERNATIVE REGULATORY MODELS FOR MICROBIAL PESTICIDES

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It has been claimed that biopesticides are at a relative disadvantage in the regulatory system because they are held to the same standard as the chemical pesticides around which the registration and risk assessment framework was designed. While regulatory agencies realize that biopesticides are fundamentally different than chemical pesticides and should not be held to the same standards of safety and efficacy, it is challenging to design a system for evaluation that is equally fair to both bio- and synthetic pesticides. One part of this difficulty is that regulatory processes assess single products, while microbial pesticides are often designed to be used in the context of integrated pest management. This makes it difficult to articulate efficacy claims because of their dependence on other components, and because of their often more complex modes of action. Because it is part of the regulator's mandate to be accountable for human and environmental health and for the efficacy claims of the products it registers, risk assessors strive to help registrants articulate label claims which clearly state what the product can achieve. In Canada, risk assessors have demonstrated a willingness to assess microbials based on their own values and characteristics, although long discussions and negotiations are required in order to bring this to fruition, and small to medium size enterprises may not have the capacity or endurance to satisfactorily complete this process (Borden 2004). While biopesticides may no longer be held to the same standards as chemical pesticides, and while regulatory agencies seem willing to assess them based on their own merits, the process of implementing this shift is a work in progress. We describe several regulatory models, or their certain aspects, that have been proposed by other authors and organizations for application to microbial pesticides.

Alternative regulatory models for microbial pesticides

It is widely agreed that getting microbial pesticides to market more quickly and in greater numbers would result in economic, environmental and health benefits. A number of proposals exclusive to microbial pesticides have been published, with aspects of some alternative models being taken into consideration by regulators.

On the topic of making regulatory procedures more efficient for microbial pesticides, Mensink and Scheepmaker (2007) suggest that even though data requirements are increasingly transparent and harmonized, there is insufficient guidance on exactly how to evaluate and use submitted data to conduct a pre-market assessment of the environmental safety of microbial pest control products. They present a protocol for evaluation of individual studies submitted to regulatory authorities, a risk decision tree which is designed to generate, amongst other benefits, effective rationales for information waivers. The five-part decision tree encompasses: microorganism characterization, identification and efficacy data, emissions data (noting that data on emissions are scarce and expert judgement may be required), exposure data (same caveat), environmental effect data, resulting in the safety evaluation and decision. They note that in the European Union, decision-making is somewhat more prescriptive, and clear statements on risk criteria with respect to environmental behaviour and fate have been adopted e.g. "no authorization shall be granted if it can be expected that the microorganism and/or its possible relevant metabolites/toxins will persist in the environment in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years, unless a robust safety evaluation indicates that the risk from accumulated plateau concentrations is acceptable" (European Council 2005). Mensink and Scheepmaker (2007) note because "natural background levels" and "robust safety evaluation" are not further defined, that expert judgement is required. They further note that risk criteria which differentiate between acceptable and unacceptable risks have been agreed upon via the Uniform Principles (European Council 2005), which stipulate that in the case of toxic effects, risk characterization is based on Toxicity Exposure Ratios (TERs) that compare no-effect concentrations to predicted environmental concentrations, and that stipulate numerical values for different categories of non-target organisms. There are parallel criteria for pathogenicity, though Mensink and Scheepmaker (2007) characterize them as "less clear."

Mensink and Scheepmaker (2007) stress that their risk decision tree implies the need for additional case-by-case expert judgement, "particularly in view of: i) limited experience with relevant test protocols; and ii) limited knowledge in general of the functioning of microorganisms in (agro-) ecosystems and the particular role of toxins, enzymes and other proteins".

Laengle and Strasser (2010) developed an indicator of environmental risk which can be used to rate both microbial and conventional pest control products, and could facilitate and streamline the introduction of microbials to the Canadian market. Their method generates a single numerical score to represent the environmental risk of each product. The score is based on five separate criteria: persistence, dispersal potential, the range of non-target organisms affected, direct effects, and indirect effects. Each of the five criteria receives a score of 1-5 for both the *likelihood* of effect and the *magnitude* of effect. The authors suggest that the indicator has a wide range of potential uses: helping the regulating authority make decisions on data requirements, fees, and risk mitigation measures; identifying "low risk" and "reduced risk" products in Canada; offering guidance on risk reduction efforts; and helping industry to make decisions on which products to proceed with early in the product development process. Such an indicator, if it lives up to this potential, could have a significant positive effect on the registration and use of microbial products.

Jaronski et al. (2003) suggested that a much less stringent and data-rich environmental assessment process is sufficient to ensure the safety of microbial products, at least for indigenous microorganisms. They argue that, for a variety of reasons, Tier I laboratory testing does not accurately predict field behaviour, and offer numerous examples of laboratory-derived toxicity and infectivity data which do not match field data. While lab data may correctly suggest low levels of risk for microbials with narrow host ranges, data for generalist pathogens such as *Hypocreales* may incorrectly predict risk in field introductions. Following Keller (1998), they suggest that risk assessment should generally capture three areas: i) the origin of the pathogen (indigenous, foreign, genetically altered); ii) the host (original, non-original); and iii) the environment (natural for host and pathogen, non-natural for host and/or pathogen). For non-indigenous microorganisms, laboratory-acquired non-target data is needed, but should be acquired using bioassays that mimic pertinent exposure and environmental conditions as much as possible. They suggest it is the responsibility of public research to develop protocols for such research.

Microbial pesticides are widely used in South and Central America. While it is difficult to register products based on non-indigenous microorganisms, the regulatory regimes in these regions are largely founded on the presumption that “these are naturally occurring, indigenous organisms are much safer than the pesticides they replace” (Jaronski et al. 2003). This is also the case in Cuba and some Asian countries, where government regulatory objectives emphasize protecting consumers and farmers without stifling the thriving local industries that produce the products. There have been efforts to establish a principle of “extended safe agricultural use” for products and pathogens which have been used for many years without causing problems” (Jaronski et al. 2003).

A new approach being considered in Europe is Qualified Presumption of Safety (QPS) (European Food Safety Authority 2007; 2008; 2009). The concept has been accepted by the European regulatory agency, the European Food Safety Authority (EFSA) as a guiding principle for evaluation of microorganisms for a variety of purposes, including plant protection products. The QPS scheme states that microorganisms that belong to certain groupings and that have been granted QPS status are not obliged to undergo individual risk assessments. Rather, their manufacturer simply issues a Notification that the product is about to be marketed. The scheme has the dual benefit of concentrating the attention of regulatory authorities on those microorganisms more likely to present a threat to human health or the environment, and of reducing the time period between investment and return, thereby acting to stimulate the development and marketing of microbial products.

The granting of QPS status is dependent on a microorganism’s characteristics in four areas: i) its taxonomic grouping; ii) whether sufficient information is known about the proposed group of organisms to make a determination regarding their safety; iii) whether the grouping proposed for QPS contains known pathogens; and iv) the intended end use (whether or not it will enter the food chain in food and feed products). If the microorganism(s) cannot be related via existing or historic nomenclature to a taxonomic group with QPS status, then a full safety assessment is required. With respect to the second criteria, the body of knowledge concerning the group of organisms seeking QPS status must be sufficient to provide adequate assurance that any potential to produce adverse effects in humans, livestock or the wider environment is understood and predictable. This determination would be made by an expert group and based on a weight of evidence approach, with the standard of safety being “reasonable certainty of no harm”.

QPS status would, initially at least, be established by EFSA in advance of, and independent of applications by Notifiers. Initially, QPS determinations might centre on the more commonly notified genera e.g. the lactic acid bacteria: *Bacillus* spp. and yeasts; and commonly encountered filamentous fungi. It is thought that consideration of a relatively few fungal and bacterial genera would capture a large majority of potential applications.

Once established and shown to be functional, additions to the QPS list could be made at the request of, and with the help of Notifiers. The decision to award QPS status would remain with risk assessors. A QPS system would not cover product-specific data, including how the product is cultured and what steps are taken to ensure minimal contamination. These kinds of data would continue to be reviewed by risk assessors for each product.

A recent EFSA update on QPS suggests that, though a number of genera have been included in the QPS scheme, applying the concept to pest control products requires further study. The EFSA’s Scientific Committee believes that it may be possible to devise qualifications which would allow a QPS approach to biological control organisms in the future, qualifications that would include a consideration of effects on non-target species (European Food Safety Authority 2007 and 2008).

QPS is generally applied at the species level. For species that produce molecules toxic to humans, QPS status would be granted only if reliable methods exist to differentiate between strains that are toxin-producing and non toxin-producing, assuming that there are no other safety concerns. Strains that produce toxins would be regarded as non-QPS and subject to a full safety assessment. To date, the QPS approach has not been applied to the safety assessment of pest control products (Dr. Tobin Robinson, EFSA pers. comm.).

To address the challenges that face small companies, Dr. John Borden, Chief Scientific Officer at Contech Ltd. (Canada), has suggested that the Minister of Health establish an inter-ministerial task force to develop the terms of reference for a subsidized registration process for biopesticides, in providing services exclusively to small companies (Borden 2004). Delayed cost recovery would be an integral feature of the process, which would incorporate either an up-front fee followed by annual fees (based on a percentage of gross annual sales), or by the latter function alone, with the size of the fees depending on the size of the registering entity. Fees would be low enough as not to hinder development, marketing and sales, and would continue to be charged until the full pro-rated cost of registration is recovered.

Borden suggests that a publicly-funded Biopesticide Agency, independent of the federal regulator, administer the process, which would provide:

a) An *advocate service*: a person appointed by the Agency to work with the registrant to facilitate the registration process;
b) A *substitute service*, where the Agency takes responsibility for registration on behalf of the registrant, who is responsible only for efficacy testing and registration of the end use product;
c) An *intervention service*, wherein the Agency assumes responsibility for registration of promising technical active ingredients that have not been subject to technology transfer to a potential registrant, or have been abandoned by a potential registrant, with the registered technical active ingredient to be offered to potential registrants on a bid basis;
d) An *efficacy testing service*, equivalent to that currently in place for minor use pesticides, with services offered to registrants who lack the resources to conduct the tests necessary to register an end use product; and
e) *Combinations of any of the above services*, e.g. wherein a registrant who acquires rights to a registered technical active ingredient under a substitute service may seek an advocate, who will arrange for efficacy testing in addition to providing advice on registration of the end use product.

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