16

Impact assessment of the production and utilization of entomopathogens and its effects on public policies

DEISE MARIA FONTANA CAPALBO[1], IRACEMA DE OLIVEIRA MORAES[2], REGINA DE OLIVEIRA MORAES ARRUDA[2,3] AND RODRIGO DE OLIVEIRA MORAES[2,3]

[1]EMBRAPA Environment - Rodovia SP 340 – Km 127.5, CP 69 - 13820 000, Jaguariúna/SP, Brasil; [2]Probiom Tecnologia – Indústria e Comércio de Bioprodutos Ltda. R. Latino Coelho, 1301 CEP 13084 010 Campinas/SP, Brasil; [3]FAPESP - R. Pio XI, 1500, Alto da Lapa, CEP 05468 901, São Paulo, Brasil

16.1 Introduction

The insecticide use since 1940, achieved more than 10 fold increase but crop losses due to insects have nearly doubled in the same period. An estimate by UNIDO places the total loss from agricultural pests about US$ 90 billion per year the world over. This situation has created an awareness even in the most under-developed countries for more sound control methods and to develop integrated pest management (IPM) programmes [1-3]. The well known success of some IPM programmes invariably have three principles in common:

1. Grow a healthy crop
2. Conserve and enhance the beneficial organisms that naturally occur in the agroecosystems, and
3. Use only selective pesticides when an action threshold has been reached.

The use of microorganisms as selective pesticides has had some notable success. At present there are a number of bacteria, fungi and viruses which have been introduced as commercial pesticides, frequently after skilful production and formulation efforts.

Among the many examples [4,5] of effective biological control agents against insects it can be pointed out some in Brazil: virus (*Baculovirus anticarsia* against a soybean catterpilar *Anticarsia gemmatalis*, applied over more than 3.75 million acres or 1.5 million hectars of soybean each year), fungus (*Metharizium anisopliae* against *Mahanarva posticata*, applied over nearly 450 thousand hectar of sugar cane), bacterium (*Bacilus thuringiensis* var. *kurstaki* against many *Lepidoptera* in soybeans, cotton, forestry, nearly US$ 500 000 commercialized each year).

16.2 Commercial development of entomopathogens

Bacteria are the most promising biological control agent. Over 90 species of bacteria which infect insects have been described. A few of them have been produced commercially including *Bacillus thuringiensis (*Bt), *B. popilliae* and *B. moritai*. Others have been proposed as potential candidates for commercial development but no attempts have been made to produce them commercially. The most widely used products are made from Bt. Its unique capacity to synthesize insecticidal protein crystals has promoted investigations into its use as a natural biological control agent in agriculture, forestry and human health for the elimination of disease vectors [6]. Over the last decade research activity into the molecular and genetic make-up of this bacterial group has intensified leading to a comprehensive understanding of toxin gene control and expression, the structure and function of the toxin molecule itself, improved efficiency of biocontrol formulations, knowledge of the ecology of the bacteria and the construction of more effective toxins and delivery systems. However it is the lethal toxins and a parallel in potency with the most active organophosphate insecticides that have attracted industry.

During the 80´s the convergence of new techniques, especially those afforded by recombinant DNA technology, and changing public and political attitudes towards pesticide use, precipitated the increase in Bt research by industry, government and academia alike. Relatively low development costs besides its high natural diversity of strains lead to the development of a new generation of more effective and environmentally acceptable insect control products.

The production of Bt (as well as other bacteria considered as potential bio-agent) could be done by semisolid or submerged fermentation [7,8]. In the former process the bacterium is grown on semisolid substrates, usually moist bran to its ability to hold nutrient solutions on individual particles [9]. Submerged fermentation (also referred to as liquid fermentation) is also an efficient method where the microorganism is put in contact with the substrate in a liquid phase. The fermentors for submerged process are similar to those used for drugs and pharmaceutics, but it has to be pointed out that they are expensive and sophisticated. For semisolid process the equipment could be simpler and less expensive.

Careful controlling and monitoring are necessary for both processes. Some physical parameters have to be followed during the process: pH, temperature, aeration; as well as agitation and sporultation rate. Following fermentation, the beer is concentrated and then formulated into a desired final product. Changes in medium components can lead to increased quantities of endotoxins being produced during fermentation. This, in turn, will lead to a cheaper product to the end user. This is important for expansion of the Bt market, since it is already perceived as expensive relative to chemical insecticides used to control the same insect species.

16.3 Local production for developing countries

Bt proved to be a highly successful weapon for fighting some agricultural pests and some vectors of diseases but its use is still limited in developing countries because of its cost. The high cost of the Bt product is due to production being located in the developed countries where productions costs are higher and also due to expenses paid in transportation to the operational sites. Thus local production should significantly reduce costs of pest control and also could help development of local fermentation industries. Local production is especially adequated for controlling pests in crops with high-cost production and small cultivated area, for specific local pests, and / or for the initial steps in an IPM program [10,11].

In local production some care must be especially followed by technical supervisors and in all of them any decision must be founded in scientific knowledge:

- quality control (including standardization and prevention of contaminants)
- formulation (efficiency and efficacy)
- fate in the environment (possible activity against non target organisms, persistence and dissemination)
- legislation acts (governmental agencies, federal acts, guidelines for registration and / or utilization in pre-stablished areas)

There are a number of advantages in promoting development of local production facilities for microbial insecticides in developing countries:

- Stability: One of the drawbacks in using microbial insecticides has been the instability of the microbial agent and the variation of toxicity of the formulations. It is the result of the lengthy shipping periods and variable storage temperatures before the product utilization in the field. Local production should avoid these problems,
- Formulations: The biopesticide product must have appropriate formulations as it should be present in different habitats where the target insect is. The wide variety of habitats requires appropriate method also. Local production would certainly be highly beneficial in overcoming these issues because it could develop a product for the specific local environmental conditions,
- Substrates: Possible use of many residues and wastewaters, decreasing product costs, and
- Manpower employement: Development of technical capabilities near the production industry.

16.4 The role of Biotechnology

Developed countries are enacting and reinforcing stringent legislation to drastically reduce the use of pesticides in their agriculture, mainly because of environmental concern. On the other hand, third world countries in order to develop their agriculturally based and much indebted economies do not have the protection of the environment as their first priority and, thereby, use any pesticide they can get hold of. To produce a bio-agent in an easy way to be used by the farmers, to overcome some difficulties such as spreading potential, propagation rate, virulence and stability in the field, cost of production as well as biosafety measures, the application of biotechnology to develop new promissing tool based on Bt or other biological agents seems to be the helpful.

The present goal on applying biotechnology is to explore technological concepts to developing molecular biological strategies for insect control. Genetically engineered microbial organisms have been obtained and new mass production technology has been developed. It became possible to synthesize biological molecules by implanting specific genes into various kinds of microorganisms as is the case of the manufacture of Bt toxins using other bacteria as factories.

16.5 Effects on public policies

There has always been the fear that entomopathogens might be selected in such a way and convert to vertebrate pathogen. Before introducing such engineered biopesticides in different control programmes, biosafety studies have to be carried out and it has to be given priority to them in many countries.

The National Center for Environmetal Impact Assessment (Embrapa environment - CNPMA) has for a long time been concerned with issues of environmental sustainability, farmer’s welfare and improved agricultural productivity. Whereas in 80´s the challenge of limited knowledge about biocontrol agents (toxins, mode of action, production and purification) was overcome to a large extend, the challenge nowadays is to integrate that knowledge into an holistic understanding of how these agents interact with its natural environment to provide a basis for the development of useful strategies that are effective and ecologically sound. To meet that challenge, a highly multidisciplinary approach is being required. The success of this effort to address that challenge will contribute in determining whether or not biocontrol full potential will be realized in the near future.

Answering a request from the Brazilian Environmental Protection Agency (IBAMA) a researchers’ group from CNPMA and from some other research institutes have designed special guidelines to establish regulatory requirements for microbial pesticides. This group is also developing some studies to assess the degree of safety of some biocontrol agents (*Bacillus thuringiensis, B. anticarsia, Colletotrichum gloeosporioides* and *Neozygites*) to beneficial invertebrates (terrestrial and aquatic), vertebrates (birds, mammals) and plants.

Guidelines for assessing the safety of such agents adopted by USA / EPA and FAO were followed in some studies. In the resulting proposed Brazilian protocols for biocontrol agents it was realized the usefulness of tiering data. The concept of tiering is risk-based, i.e., there is a basic set of tests that all products must undergo. If no adverse effects are noted, further testing may cease: if adverse effects are identified, then the product may have to undergo further tests at higher tier or level of testing. Data submitted by applicants are designed to allow an assessment of safety by the regulatory agency in order to determine the conditions under which these products can be used.

Researchers fill that these data allow decision-makers (from Health, Agriculture and Environment Agencies) - assisted by technical committes - to evaluate possible effects on users and bystanders, to determine whether residues may occur on harvested food, and if so, the safety of such food for comsumption by humans or animals.

Registration requirements vary considerably from country to country. Usually the purpose of the evaluation is to ensure the 'safety, merit and value' of the candidate product. In Canada, United States and Denmark, for example, data requirements are tailor-made to different types of pesticides, those for microbial pest control agents are less extensive than those for chemicals, yet are designed to answer the same questions of safety, merit and value. As well, performance and effects on environment (including human beings) are evaluated. Even Mercosul is actually stablishing its normative decisions concerning registration, based on this kind of simpler evaluation. It is considered that the registration of a biopesticide preparation will cost at least 10% less of what could be required for a synthetic chemical. In some countries registration requirements for some biorationals have been significantly relaxed in view of their preliminary relative innocuousness to health and the enviroment. This means that the registration process is less costly and quicker for these previous well-studied products, with important consequences for their competitiveness and the speed with which they can reach the market.

16.6 Future possibilities

The future appears impressive with progress likely to continue its exponential course. The ecology and molecular biology should expand with particular verve. Many strain searches are being conducted to find better endotoxins for use in pest control. The resulting extensive culture collections wil probably be screened for many additional materials. Intensive research programmes will have to be embarked upon to explore new avenues and develop non conventional methods of pest control and to advocate and strongly adhere to a sound programme of pest management for sustainable agriculture.

Too many of the cited evaluations of entomopathogens are restricted to judgements based on present knowledge. There are better isolates to be found, if we search for them. There are better fermentation and recovery procedures, if we pursue a logical course in fermentation search. The isolation and selection of new strains that are effective to a wider range of insect pests; developments in production technology reducing costs; and improved formulations to increase persistance at the target and control are some of the tasks for the near future. And of course, developments in genetic manipulation including insertion of toxin genes into transgenic plants and other bacteria; also through conjugation, development of strains active against more than one insect of different orders.

There are multiple possibilites for cooperation programmes and it could be pointed the principal ones for the CNPMA group: alternative fermentation processes, especially those convenient for local production; intensive studies on environmental impacts of biopesticides in order to promote confidence on these organisms and also to improve its utilization; development of methodologies and studies to support decisions for public policies. There are better ways of producing, using and applying these pathogens and toxins, if we seek and develop close cooperation between entomologists, microbiologists, chemists and engineers to find them.

16.7 References

[1] Capalbo DMF. 1989. In: *Development of a semi-solid fermentation process to get Bacillus thuringiensis.* PhD Thesis, UNICAMP, Campinas, Brazil, p 221.

[2] Capalbo DMF. 1994. In: *Memórias do Instituto Oswaldo Cruz. Rio de Janeiro, Brazil* 90:135–138.

[3] Entwistle PF, Cory JS, Bailey MJ, *et al.* 1993. In: *Bacillus thuringiensis, an environmental biopesticide: theory and practice.* John Wiley, Chichester, USA, p 311.

[4] Frankenhuyzen KV. 1993 In: *Bacillus thuringiensis, an environmental biopesticide: theory and practice.* John Wiley, Chichester, USA, pp 1-35.

[5] Meadows MP. 1993 In: *Bacillus thuringiensis, an environmental biopesticide: theory and practice.* John Wiley, Chichester, USA, pp 193-220.

[6] Moraes IO. 1976. *Studies of submerged fermentation to get bacterial insecticide in minifermentors.* PhD Thesis, UNICAMP Campinas, Brazil, p 77.

[7] Moraes IO, Capalbo DMF. 1986. In: *Food engineering and processes applications.* Elsevier, London, pp 377-381.

[8] Moraes IO, Capalbo DMF, Moraes RO. 1990. In: *Engineering and Food: Advanced processes.* Elsevier, London, pp 785-792.

[9] Salama HS, Morris ON, Rached E. 1993. In: *The biopesticide Bacillus thuringiensis and its applications in developing countries.* Al-Ahram Com. Press, Cayro, p 339.

[10] Capalbo DMF, Moraes IO, Arantes OMN, *et al.* 2007. In: *Controle microbiano de pragas na América Latina – avanços e desafios.* FEALQ, Piracicaba – Brasil, pp 239–256.

[11] Moraes IO, Arruda ROM, Moraes RO. 2007. In: *VI Encuentro Latinoamericano y Del Caribe de Biotecnologia Agropecuaria. REDBIO 2007,* Viña del Mar – Chile, p 794.