INTEGRATED PEST MANAGEMENT AND ENTOMOPATHOGENIC FUNGAL BIOTECHNOLOGY IN THE LATIN AMERICAS: I-OPPORTUNITIES IN A GLOBAL AGRICULTURE

by

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Resumen

Valencia, E. & G.G. Khachatourians: Integrated pest management and entomopathogenic fungal biotechnology in the Latin Americas: I- Opportunities in a global agriculture. Rev Acad, Colomb. Cienc. **22**(83): 193-202- 1998. ISSN 0370-3908.

Los hongos entomopatógenos (EPF) han sido ampliamente utilizados en el control biológico de plagas, tanto individualmente como en forma integrada (IPM). Tal manejo ha causado un impacto agrícola en beneficio de los cultivos. Los hongos entomopatógenos pueden jugar un papel significativo en la preservación y en el balance natural de la biota útil, así como en su expansión activa, como ocurre en el bosque húmedo tropical, en la selva andina y en otros ecosistemas agrícolas de Centro y Suramérica. De todos los EPFs conocidos, cerca de media docena cumplen con los requisitos para ser utilizados industrialmente en biotecnología de tipo R y D. Latinoamérica ejerce un liderazgo en el uso de EPF en IPM en las prácticas agrícolas y en el diseño racional de bioinsecticidas. Para mayores beneficios, deben aumentarse tanto la investigación como los esfuerzos por industrializar los EPF en el manejo integrado de plagas, dado que su potencial como micoinsecticidas está en pleno auge y desarrollo.

Abstract

Entomopathogenic fungi (EPF) have been used as insect biocontrol agents separately and in integrated pest management (IPM) worldwide including in the Latin Americas. The proper use of these agents along with other elements and concepts of IPM has significant impact on the provision of crops, fruits and other agricultural products Entomopathogenic fungi can play a significant role in the preservation of the natural delicate balance of beneficial biota and their active spreading from the surrounding natural habitats, such as the tropical rain forest, the

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humid cloudy forest in the Andes and other important ecosystems, to the agricultural lands in Central and South America. Of all known EPF, there are only about half a dozen for which prerequisite aspects of fungal biotechnology for industrial R and D are in place. Because of its precedent setting leadership in the use of EPF in IPM and agroecological practices, Latin America stands to gain immensely here and more so if the concept of rational design of bioinsecticides (RADBIO) is employed. The future prospects will depend on a balance of discovery research coupled to a strong and dependable industrialization effort to develop the framework for illustrating public acceptance, commercialization potential and widespread use of EPF in IPM. The potential for the next generation of mycoinsecticides is now looming on the horizon.

1. Introduction and scope

The 21st century comes along with big challenges and opportunities for humanity. The production of food and fiber and the obtaining of energy, shelter and bioactive substances, represent universal needs of people worldwide. These needs must be satisfied in spite of shrinking resources and the increase in human population.

In most countries of the world, the agricultural lands have been pushed to their physical limits. Further expansions can not be attained without impairing remaining important ecosystems. Most of such lands are the border of total destabilization due to human activities (**Ruttan,** 1996).

Industrialized countries were directly involved in the implementation of the so called "Green Revolution" during the past 30 years. These countries have been responsible for a big proportion of the mass production of food, fiber and other resources for the world population. However, really dramatic increases in crop productivity are unlikely to occur for all agricultural sectors at the same time, regardless of powerful technological tools such as molecular biology ana biotechnology. This fact strongly suggests that non-industrialized countries will have to participate more actively in the production process within a global base, to meet the needs of the human papulation already estimated at 6.3 billion people for the year 2010 (**Ruttan,** 1996).

Non-industrialized countries, especially those located in the tropics, have a significant advantage in terms of potential agricultural productivity over countries located in temperate regions. This is in part due to the lack of seasonality and the high intensity sunlight which make possible the continuous outdoor cropping year round.

Among non-industrialized tropical regions, Latin America emerges with unique conditions for production of food, fiber and bioactive substances. Because of the characteristics of these agricultural environments, the high contents of volcanic ash in the soils, the amount and regularity of rainfall, the diversity of thermal levels due to variation of altitudes and the direct or indirect influence of the abundant tropical rain forests, Latin America stands out as a privileged region for agricultural production This continental region holds a long tradition of agroecological practices, which create a favorable environment for the design and introduction of new strategies of crop protection and production. Latin America should be seriously considered to significantly contribute to meet human needs in the next century.

Latin America has a big proportion of important ecosystems which are still conserved. Many of these ecosystems occur along the borders of farming lands. Consequently these lands are susceptible to the impact of low-technology agricultural practices within the countries of this region, as well as to the detrimental effects of subsistence agriculture. For this reason, Latin American governments must be committed to achieve sustainable agricultural systems, by switching from resource-based to knowledge and science-based agricultural production (**Nene,** 1996).

However, the rapid implementation of novel technologies will initially be expensive. Further, considering the limited economic resources for most of the countries in Latin America, the challenge of sustainable agriculture will probably require balancing the use of traditional/ conventional technologies and the most innovative modern technologies now available. This balance is fundamental to make possible that big producers as well as small farmers can equally benefit from the technological inputs.

A key requirement of crop production is crop protection, including the management of arthropod pests. This is definitely a major issue within the strategies for ensurig healthy harvests. Many countries in Latin America have implemented successful practices for the management of key pests. Nevertheless, due to the arthropod biodiversity of most of these agricuttural ecosystems, the occurrence of potential and secondary pests is a permanent risk. This would further be aggravated if a coherent approach for managing the phytophagous insect-complex is not put in perspective. Because of biodiversity, there is also a significant prevalence of beneficial arthropods in most cropping systems, which are offering an ongoing in expensive natural control of the phytophagous complex (**Valencia,** 1994).

Some of the methods for the control of key pests often disrupt the agroecosystem by a drastic reduction of the beneficial species. This quickly switches the situation of phytophagous insect complex to the occurrence of an insect pest complex, complicating our approach to sustainable agriculture. These disruptions are particularly significant for methods such as the non-selective use of wide spectrum chemical insecticides, the total eradication control of weeds, the clearing out and destruction of plants around crops and some traditional practices like burning residues on the soils during or after the harvest (**Altieri,** 1994).

2. Current Latin American practices

Latin America because of its large natural resources and vast numbers of plant and insect species, harbors a very rich environment and niche for the discovery and use of EPF for IPM. The interest in biodiversity of species and genetic diversity, has a special value in the context of biotechnology and its practice in Latin American countries. As described in the volume, "Biotechnology in Latin America: Politics, Impacts and Risks", 1995 (**Peritore and Galve-peritore,** 1995), the editors N Patrick Peritore and Ana Karina Galve-Peritore, in their preface, express caution regarding the 1 or 2% of risky procedures or substances which will emerge through biotechnology and have extensive effects on the environment. However, on the opposite side, the benefits of biotechnology are greater. Many Latin American countries have indeed used and done this very successfully, so far, as the naturally occurring microorganisms and specially EPF for biocontrol of insect pests, are concerned.

To give two examples, one has to look at the development of some 228 Biological Pest Control Production Centers in Cuba (**Feinsilver,** 1995), or use of fungi in IPM control of the coffee berry borer (CBB) in Colombia (**Valencia,** 1995). In Colombia, the National Federation of Coffee Growers launched a massive program for the control of the CBB using mainly the filamentous fungus *Beauveria bassiana* Bals. (Vuill) and cultural practices. As a result of this program more coffee growers use *B. bassiana* as an important part of the IPM of the CBB. The entrepreneurship of private companies has been important for the widespread use of EPF in this country. Trans-national companies such as Hoechst Schering AgrEvo Colombia and Laverlam have started to scale-up the production and commercialization of mycoinsecticides. So far, AgrEvo S.A. Colombia is undergoing an intensive research and development effort, for the introduction of mycoinsecticides as an innovative component OfI PM strategies in the most important commercial crops.

Further, through international umbrella organizations, collaborative initiatives such as CGIAR (Consultative Group on International Agricultural Research) have generated newer diffusion of technology in pest management. Entomopathogenic fungi, particularly *Metarhizium anisoplieae* and baculoviruses, have been commercially used for several years in Brazil for the control of major pests in important crops such as sugar cane and cotton (**Cardona,** 1995). Other Latin American countries such as Costa Rica, El Salvador, Chile, Mexico, Ecuador, Peru, Nicaragua, Panama, Honduras and Guatemala have made significant developments in IPM concepts and programs, including an extensive utilization of biological control agents (**Andrews and Quezada,** 1989). The Escuela Agricola Panamericana in El Zamorano, Honduras, in cooperation with the University of California in Riverside, has made huge contributions to the implementation, adoption and widespread use of IPM in Central and South America. The application of commercial products derived from *Bacillus thuringiensis* and the releasing of the egg-parasitoid wasp Trichogramma *spp*, have played a majar role for IPM, mainly of lepidopteran pests in several countries of Latin America.

3. The potential of EPF for IPM strategies

Integrated Pest Management, first proposed more than 20 years ago (10), is the better approach to ideal management, as a concept compatible with the sustainability of agriculture and the protection of biodiversity, within the terms defined at the Earth Summit in Rio de Janeiro in 1992.

According to the fundamental philosophy of IPM, different, compatible, and complementary methods of pest control must be combined in harmony to meet the economic, sociological and ecological needs of modern agriculture (**Valencia,** 1993). Methods such as chemical, physical and biological control as well as cultural practices have been well recognized for the IPM of major pests worldwide. Due to the emphasis in sustainability given to extensive agriculture during the last years, ecologically oriented methods, such as biological control, varieties of plants resistant to pests, agroecological practices and cultural practices, are gaining momentum as pest control measures compatible with the protection of the environment.

Insecticides derived from bacteria, fungi and viruses definitely play a major role among the different biocontrol agents. Many microorganisms can be easily produced and fomulated on an industrial scale and they can be applied by means of conventional application equipment used for chemical pesticides (**Burgerjon and Dulmage,** 1977).

Most of the commercially available microbial insecticides are derived from bacteria. *B. thuringiensis* has dominated the bioinsecticide international market with a market share consistently higher than 90% during last few years A number of *B. thuringiensis*-derived insecticides have been targeted primarily for the control of lepidopteran larvae. However, in certain examples, lepidoptera have become resistant to *B. thuringiensis* toxins. Additionally, many major pests of several important crops belong to other orders of insects including Coleoptera, Diptera, Homoptera, Hemiptera, Orthoptera and Thysanoptera. Many of these insects occur as pests in the adult as well as in the immature stages. Furthermore, some important agricultural pests do not even belong to Insecta but to Arachnida class, such as a big group of tetranychidae, eriophyidae and tarsonemiidae pest mites, which do have natural fungal enemies such as *Hirsutella spp*. In these cases, *B. thuringiensis*-based products and baculoviruses, having a fundamental ingestion mode of action, present serious limitations to be incorporated in the IPM strategies for the control of these pests.

Considering the importance and versatility of microbial insecticides as a part of the IPM approach, it is clear that new alternatives are now required. This is needed in order to widen the feasibility of using microbial insecticides in crop protection in Latin America, where many crops present a complex of pests during different plant developmental stages.

Entomopathogenic fungi occur naturally in the environment and are responsible for periodic control of many pest species. The Deuteromycetous or asexual fungi contain over 700 known species with pathogenecity to insects alone spanning some 85 genera (**Khachatourians,** 1991). Although 20-30 EPF are the subject of intense laboratory research, only few are entering commercial production and use as mycoinsecticides. Given this, the process of marching the development of EPF as biocontrol agents should not be too difficult.

In this respect, EPF represent an interesting challenge. Due to their particular *contact mode of action*, these filamentous fungi are able to effectively act against a wide range of insect species, including those with unique feeding habits (i.e. sucking insects belonging to Homoptera, Hemiptera and Thysanoptera) which are unlikely to be controlled by using naturally accurring microbials which act through ingestion. The contact mode of action of EPF enables them to control even the non-feeding stages of insects such as pupae and eggs (**Rodríguez-Rueda and Fargues,** 1983). As well, new-hatched larvae and non-feeding adult and quiescent stages which occur in some pest species, can be efficiently controlled by EPF. Despite the relatively wide spectrum of pathogenicity of EPF species, they can be made highly specific through selection of strains for the control of insect pests. These isolates have a significantly lower impact on non-target organisms as a result of their specificity to the target host (**Sitch and Jackson,** 1997).

Beyond these advantages, many native species of EPF are facultative saprophytes and therefore can grow although to a limited extent in organic matter inside cropping areas. This factor offers the possibility that a low pressure of inocula can be maintained under field conditions for certain periods of time, thus reducing the potential of some pests to initiate out-breaks. Fungal species with such a profile actually have a huge potential to be used within agro-ecosystem, as a part of a generally accepted practice in pest control programs.

Additionally, considering that fungi are the most ubiquitous eukaryotes, there are many possibilities of finding EPF practically in every environment where pests species occur. This situation represents a good chance for the isolation of fungal pathogens in the original habitats of exotic pests, thus allowing these pathogens to be used in classical biological control programs, once the basic studies of impact on non-target organisms have been completed. On the other hand and due to the ubiquity of EPF, there is a tremendous opportunity for the discovery of native fungal isolates for the control of indigenous pests. In this case, EPF have a huge potential as microbial agents within biological control strategies such as augmentation and conservation.

Although naturally occurring isolates of entomopathogenic fungi can be directly used in crop protection strategies, they may have a few limitations in their performance under field canditions, including a restricted window of action at unfavorable climates. Other limitations include problems with mass production and formulation of particular fungal strains and propagules, mainly due to hydrophobicity of their spores. Some of the most important limitations, whether perceived or real of EPF are their moderate efficacy under field conditions, their speed of action, dependence on environmental factors for survival and expression of pathogenecity. Equally restricting are the difficulties in finding or selecting fungal strains simultaneously having desirable traits of pathogenecity, physiological fitness and rusticity under field conditions.

Most of these limitations can be partially or totally overcome by means of the scientific tools of biotechnology and molecular biology within a coherent strategy of IPM. It is very important to point out that one of these limitations, the moderate efficacy, is not necessarily incompatible with a cost effective strategy of pest control. As a matter of fact, the modern concept of *biological regulation*, indicates that biocontrol agents are able to effectively regulare pest papulations (**Rodríguez del Bosque,** 1994).

This is particularly interesting for EPF, when they are applied to low levels of infestation of the pest, below the action threshold defined for the application of chemical insecticides, thus increasing the possibility to efficiently prevent future out-breaks of key or potential pests, for a reasonable period of time (**Rodríguez del Bosque,** 1994). This option is interesting not only to maintain the pest populations at a low level, but mainly because it facilitates the rational use of other pest control alternatives, such as chemical insecticides and cultural measures (**Valencia,** 1995). The concept of the use of biologicals for insect pest regulation is visionary and fully compatible with the preventive philosophy of the good agricultural practices. However, it requires qualified and permanent technical assistance as well as a change of mindset of traditional farmer expectation from this method of pest control.

Considering that moderare efficacy and slow speed of action can still create difficulties of acceptance of mycoinsecticides by some farmers, all attempts to increase the levels of biological efficacy of EPF or their speed of pest control, will certainly contribute to widening their levels of utilization. The biological efficacy and the speed of action of EPF are functions of several physiological events. Key amongst these events are, attachment of the spores, both specific and non-specific, to the insect cuticle, germination of the spore, formation of the appressoria, penetration through the cuticle, neutralization of the immunological defenses of the host, production of fungal toxic metabolites, growth and reproduction of the fungal propagules inside the insect and dispersal after the host death (**Hegedus and Khachatourians,** 1995; **Khachatourians,** 1996).

Remarkably, almost any of these traits of EPF are amenable to improvement by selection of species or strains and the tools of biotechnology and molecular biology (**Hegedus and Khachatourians,** 1995; **Khachatourians,** 1996; **Khachatourians,** 1986). As well, the tolerance of EPF to unfavorable climatic conditions and their profile for production and formulation on an industrial scale, can be likewise improved by means of the same tools. Additionally, an IPM model especially designed for a pest, crop and geographical zone, will certainly maximize the possibilities of success of an IPM program using wild type or improved EPF. This comprehensive strategy must be camplemented with a very careful approach to achieve the genetic stability of the already selected isolates (**Khachatourians,** 1991). The key point inside the strategy, is to identify *the limiting factor or factors* determining the speed of infection, the pathogenecity or other biological traits. These factors are probably very different for each insect-fungal pair isolate interaction and therefore they should be established for the control of each pest.

4. Two case studies and new approaches to EPF in Latin America

Latin America holds a long tradition in the practice of IPM and EPF have been extensively used due to the particular characteristics of the region. Among the outstanding characteristics of most agro-ecosystems in Latin America, is the abundant fauna of beneficial insects present in their crops (**Valencia,** 1995). If this fauna is maintained, it offers a complementary and inexpensive effect of pest control, in combination with other control measures including bioinsecticides (**Rodríguez del Bosque,** 1994).

To be fully adopted by farmers mycoinsecticides must be complimentary to modern strategies of crep protection. There are few studies documenting an increase in net profits as a result of the implementation of IPM practices, as compared with conventional pesticide programs under field conditions (**Trumble, Craosn, Kund,** 1997). The use of EPF itself may not be sufficient however, in today's economic environment. Of many options it is only IPM practical models that can enhance EPF to meet the needs of traditional and/or highly technical agricultural ecosystems (**Valencia,** 1995).

The IPM model for our purpose is defined as: combination of factors and selection of conditions giving to each method of pest control the highest possibility of success, for a pest or a pest complex, crop, and specific environmental conditions. Eight major components are incorporated into the model:

1. The phenology of the plant and level of insect damage during cropping.
2. Seasonality and fluctuations of environmental conditions.
3. Life cycle, population dynamics, habits and behavior of the pests.
4. Key abiotic-climatic factors affecting the pest populations.
5. Key biotic factors, e.g. beneficial insects and biocontrol agents affecting the pest.
6. Key control actions for the management of individual pest insects.
7. Action thresholds and sub-thresholds required to apply the control actions.
8. Sociological, cultural and economic factors.

As an IPM model has to be designed for specific situations the model or its elements can not be extrapolated from one crop or one region to another, without prerequisite tests and validations. Two principal examples, involving two major pests of cotton, are used to illustrate how B. bassiana, and an IPM model can be designed. The first example is an IPM model for the cotton boll weevil Anthonomus grandis Boheman A second model for the tobacco bud worm, Heliothis virescens, (F.) will be briefly introduced. The interaction of these pests with the crop is rich and complicated enough, to challenge the potential utility of any IPM modeling systems.

The IPM model for A. grandis involves the use of B. bassiana, for which efficacy has been previously established (**Wright**, 1993). The idea is to design an IPM program giving the highest opportunity to the fungus to achieve a cost-effective control of the cotton boll weevil, while optimizing the performance efficacy of other control measures.

Anthonomus grandis can present itself in four different populations: immigrant, establishing, resident and emigrant boll weevils. The IPM objective in this case is the management of population dynamics, based on the control of immigrant and establishing post populations through early applications of B. bassiana. This objective can be attained by optimizing the circumstances for contact between the pest and the pathogen.

At first, the behavior of the pest as soon as it appears in crop, needs consideration so as to elaborate the initial steps of the model. Immigrant A. grandis adults may appear in cotton fields before or soon after cotton buds are formed depending on the characteristics of the zone. The pest occurs initially in a focus, and the first activities of the immigrant adults are walking around and inside an area and feeding on these structures. This behavior provides the opportunity to start the IPM program by means of terrestrial applications of B. bassiana with the appearance of the pest in the first focus of the field.

At this point the application of Beauveria should be done at infestation levels lower than an equivalent situation in which chemical insecticides are recommended. For this reason we describe an action sub- threshold, the level of infestation to start the applications of the pathogen. These applications should be directed to the squares where the immigrant post occurs. The strategy also offers additional protection to the EPF spores as the cotton buds sepals should reduce the exposure of conidiospores to solar radiation. The application of B. bassiana in this instance diminishes the potential of the adult females to oviposite in the buds. As a result of this approach there is; 1) a reduction of water volumes and amounts of B. bassiana conidiospores used per hectare, 2) a lower application costs, and 3) reduced costs of sampling and counting to evaluate the efficacy of the treatment.

Once the boll weevil adults have oviposited on a number of cotton buds, the buds fall, releasing the first new generation of boll weevils. These new adults will climb up to the remaining buds and to the cotton flowers to walk, feed and make ovipositions. Therefore the manual collection of infested-fallen buds is one of the main cultural practices to control A grandis in several countries (**Murillo and Cifuentes** 199?). The emergence of the new adults from the buds in the soil, represents another prime opportunity to affect the population dynamics of the pest by means of Beuveria applications as the new adults walk around on the soil in the fallen squares. During the first hours after their emergence, weevil's cuticle will be brown-reddish and softer than in the case of the older adults. Thus, the application of the EPF spores to the soil, at proper time can impact and reduce the population of the post.

When the levels of infestation of the pest are low and its distribution is generalized, aerial applications of B. bassiana can be made using small size droplets to ensure a good covering inside the plant structures where the insects rest or feed.

Once the infestation of establishing boll weevils increase and the pest is totally generalized in the field, applications of B. bassiana alone may not be enough to offer a good protection to the crop. Here the initiation of chemical applications must be made on the basis of an action threshold already defined for the pest (**Andrews and Quezada,** 1989). If the infestation pressure of the pest is particularly high, the chemical insecticide can be applied in mixture with B. bassiana to attain a combined effect whether synergistic or additive.

A major advantages of the use of mycoinsecticides is that the utilization of products at the beginning of the season, favors the establishment and increase of the visitor and resident beneficial insects, as applications of wide spectrum chemical insecticides can be delayed (**Valencia,** 1995).

On the other hand, the use of EPF into an IPM model for A grandis in cotton, has to be linked to the traditional cultural practices for the IPM of this pest. Special importance must be given to the establishment of early and late trap crops aside the cotton fields (**Murillo and Cifuentes** 199?). Equally importants is the uprooting and destruction of ratoons after the harvest, in order to prevent the survival and reproduction of the boll weevils in these materials (**Murillo and Cifuentes** 199?). The trap crops represent an additional opportunity for applications of Beauveria spp for the control of early immigrant or emigrant insects. Since the total area of these traps normally is only 1% of the crop area, higher dosages of B. bassiana can be applied with partial eradication, without greatly impacting the overall costs of the program.

We illustrate next how this IPM model relates to some of the strategies of improvement of EPF by selection, formulation or by means of biotechnological and molecular biology approaches introduced above.

First of all, the very selection of B. bassiana strains with an increased efficacy against boll weevils, implies a cerlain level of specificity of these isolates to attack the pest. This specificity will probably contribute to diminish the impact on non target organisms or NTOS, as it has been reported for other fungal species and strains (**Rodríguez-Rueda and Fargues,** 1983). Since the central goal of the IPM model is the optimization of every ene of the control measures in the system, the rational use of the biological agents is also desirable, as it is a determinant to guarantee the cost-effectiveness of the program.

There could be many improvements of EPF strains as related to the production process and the development of special formulations, depending on the site of application within the crop Although oil or water-based formulations can be appropriate for the applications on the aerial parts of the cotton plants, the applications to the soil would probably require formulations with specific characteristics. Granular, dust powder or pelletizec forms would be more favorable than liquid formulations to be applied to the soil (**Morales,** 1995). Under ideal conditions, the active ingredient (i.e. fungal spores) should remain in the outer soil profiles for a longer time where the producti is required to achieve control. Furthermore, as the soil might be a more favorable environment for Beauveria than plant surfaces, special fungal propagules such as blastospores or mycelia can be used (E. Morales, personal communication). In Latin Americas, the soil is generally a more humid micro environment than the crop canopy; this factor can facilitate the eventual establishment of the fungal pathogen on the soil upper profile. Finally, applications of these formulations between the crop rows as well as at the base of cotton plants, should provide a good level of protection to the fungus from sum radiation. Improvements to the formulations related to the use of UV- filters and the selection of strains on low-water activity media would increase the "rusticity" and survival of isolates in the field. These improvements would be a significant contribution to the use of EPF in cotton areas, where high sun radiation, high temperatures and drought, are predominant climatic conditions.

The second IPM model, is proposed for the tobacco budworm, Heliothis virescens one of the most limiting pest of cotton, exhibiting a good capacity for developing resistance against several groups of chemical insecticides (**Valencia,** 1993) by the EPF, Zoophthora radicans. Further, physiological resistance due to high levels of activity of esterases, carboxyl esterases and the mixed function oxidase system has been confirmed (**Valencia,** 1993) . The IPM model for Heliothis using EPF is aimed at the management of the early infestations of the pest, as it occurs at the upper and middle thirds of the cotton plants. The ultimate purpose is to reduce the population dynamics of the pest during the period of establishment in the crop, while the native beneficial fauna is maintained.

Considering the habits of H. virescens to colonize the cotton fields, the first actual stage of the pest on the plants are the eggs. For this reason, the mass release of the egg parasitoid Trichogramma spp. has a paramount importance within the strategies aimed to control the budworm. Remarkably, Trichogramma spp mass release is able to reduce the total number of applications against Heliothis up to 90%, under favorable conditions (**García,** 1995) . The larvae are the actual damaging stage of the budworm because they sequentially destroy young leaves, buds, flowers and bolls of cotton, as they develop from the first to the fifth larval instar. But more importantly, since older larvae (fourth and fifth instars) are extremely voracious, the overall strategy of population management relies on the control of the younger larvae preferentially.

It has been found that Z. radicans is highly pathogenic to lepidopteran larvae belonging to the Heliothis oomplex. This Zygomycete presents a series of biological characteristics which make promising its R & D for pest control in cotton. Z. radicans is adapted to a range of temperatures (0-36°C) wider than most EPF (**Glare, Milner, Chilvers, Mahon, Brown,** 1987) . Additionally, its infective and transmission capacities increase during dew periods. Most cotton growing areas are located in very hot regions and usually have abundant dew on the crop, especially at nights and during the early mornings. These conditions are very favorable for the activity of Zoophthora.

An IPM model for the control of young larvae of Heliothis including Z. radicans can be effective under field conditions. Normally the action threshold for the chemical control of the tobacco budworm in cotton, is around 15% of infestation of first to third instar larvae in young leaves and buds. We propose here that in case of applications of Z. radicans for the control of these larvae, a series of sub-action thresholds (i,e. 7 and 10% of infestation) should be also tested under experimental conditions.

Several biotechnological improvements could be introduced to facilitate the utilization of Z. radicans within the IPM model proposed for H. virescens. The first and more important, is the optimization of the mass production and development of an adequate formulation for this EPF. Emphasis must be given to the induction of the protoplast stage of the fungus, which would significantly facilitate its mass production. Formulations such as wettable granules or liquids should be preferred to powders or dusts, because the spores of most Entomophthorales are very fragile to physical agents.

The dissection of the pathogenic process of Zoophthora in Heliothis, should provide powerful insights to determine which type of interactions are taking place, but more importantly, to determine the limiting steps of pathogenesis. Some of the biotechnology approaches already discussed in the case of the cotton boll weevil, would be also valid to develop a Zoophthora-based product for Heliothis.

The cuticle of lepidoptera larvae is generally very soft although elastic, to allow the tremendous growth attained by these larvae during development. This characteristic suggests that proteins might be a predominant moiety in the cuticle of immature stages of lepidoptera. In such a case, the tool box of molecular biology can be valuable to determine the participation of extracellular proteases in the process of penetration in Heliothis.. Indeed, the selection of protease defective mutants would be a practical demonstration of this limiting step (**Khachatourians,** 1996). Again, if predominant proteases correspond to single gene products, the isolates could be engineered for an over or constitutive expression of these enzymes.

Heliothis virescens is non particularly susceptible to B.thuringiensis especially in cotton and baculoviruses have offered erratic controls under field conditions (**Cardona,** 1995) , thus EPF stand a good chance for the implementation of new IPM programs. Therefore, the development of an IPM model for this pest involving Z. radicans, appears as an opportunity. Nevertheless,the commercial development of Zoophthora would require a more intensive research than in case of Deuteromycetes, as the fundamental knowledge about the genetics, physiology and mass production of Z. radicansis currently being gathered.

5. RADBIO concept

What Will make a significant shift in the business paradigm of EPF and IPM is a new concept which we Call rational design of bioinsecticides (RADBIO). After considering all issues confronting the use of whole microbial organismic agents induding EPF as a key element of IPM, it is RADBIO concept which finally offers the most positive contributory effect. RADBIO approach would overcome many limitations for the use of wild-type EPF under field conditions, while efficiently addressing concerns about environmental impact and human health For RADBIO contribution in IPM, we need the adoption of four combined strategies:

1. Natural selection: The selection of EPF for the desired insect host, crop and environment, based on the concept of ecopathological unit (EPU). This concept relates to the fact that biological behavior and pathogenic potential of a microbial insecticide depends not only by the pest species, but also by the crop where the pest exists as well as by the environmental conditions (biotic and abiotic) under which a triad of plant-insect-entomopathogen interactions occur. The EPU also provides special emphasis to local IPM strategies involving either indigenous or in-

troduced fungal strains, selected for their particular profile for the pest of being the best option in a specific crop/pest and under particular environmental condition(s).

2. Mutation and selection: Obviously, in the absence of natural selection of more potent variants, mutagenic agents can generate strains with characteristics of virulence and adaptability to particular host or environment (**Hegedus and Khachatourians,** 1994).

3. Hybridization. Strains having complementary desirable characteristics, can be hybridized (**Khachatourians,** 1991). The use of auxotrophs as "tagged" strains which are not within the category of recombinant or genetically engineered microorganisms (GEMS), should further facilitate registration processes. Finally, hybrid strains are safe for the environment and proprietary for commercial purposes.

4. Genetic engineering: Any additional traits such as lethal marker genes or enhanced performance, as for example gene amplification in a background of environmental sensitivity to high or low temperatures (**Hegedus and Khachatourians,** 1994) will depend on rDNA techniques to yield panicular EPF as GEMs.

The four principal avenues of research implementation of RADBIO concept, should create new R & D opportunities and renew the IPM theory and practice. Further, the availability of improved EPF for IPM, will facilitate the introduction of new and highly efficient biocontrol agents with a lower dependence on optimal environmental conditions for optimal activity. This achievement would bring huge benefits for the widespread use of mycoinsecticides in IPM. Indeed, the more dependable and reliable these bioinsecticides are, the less the supervision, extension and labor costs, required for the successful use and commercialization of these products. These kinds of improvements will directly add to the profitability of IPM programs not only for farmers, but also for the companies interested in production, formulation and commercialization of EPF.

In the second part (**Valencia and Khachatourians,** 1998) we will illustrate prerequisite R & D for the commercial development of mycoinsecticides. In general and in particular cases, EPF need new enabling technologies and strategies, such as RADBIO to serve IPM, in diversity of agricultural and agro-forestry systems in Latin America and elsewhere.

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