MICROBIAL INSECTICIDES - AN INDUSTRY PERSPECTIVE

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ABSTRACT

Within the Agrochemicals Industry, microbial insecticides are relatively new technology needing a quite different approach to development and marketing. The biological segment has tended to suffer from a lack of patent protection leading to low-quality business with many small companies competing on price. This has created considerable instability within the biological control agent market and ongoing rationalisation of specialist companies, especially over the last two years.

Within microbial insecticides, the main area of current business is *Bacillus thuringiensis*. Products based on these strains are under threat however from insect resistance and competition from transgenic plants expressing *B.t.* Cry genes.

A substantial research focus for a number of companies is recombinant baculoviruses which have been genetically engineered to express an insect-specific toxin gene. These materials offer probably the best opportunity for microbial insecticides to exhibit chemical-like levels of efficacy and crop protection effect, and break through to become major products. However, considerable challenges need to be overcome in areas such as formulation and production technology before they can fulfil their commercial potential.

INTRODUCTION

The Crop Protection Industry ranges from small, venture capital-funded organisations selling perhaps a couple of million dollars-worth of products per annum through to the AgChem 'majors' with annual sales of more than $2.5 billion. As such, there cannot be one all-encompassing Industry perspective. A small biological specialist may enthusiastically pursue a BCA (biological control agent) niche product, which would hold no interest to a 'major' due to a perceived lack of sufficient market potential.

This paper therefore presents a view that would be more typical for one of the top 5 agrochemicals companies in the area of microbial insecticides.

CHALLENGES FACING BCA DEVELOPMENT IN AN AGCHEM MAJOR

Many of the main agrochemicals companies, or their predecessors, have been active in the chemical pesticides business for fifty to sixty years. Within these organisations there has developed a wealth of experience covering areas such as chemical synthesis, formulation, performance biology, environmental impact, toxicology, chemical engineering, and sales/marketing. When a new active area of chemistry is discovered, their depth of experience is such that a sound and accurate measure of the potential of a new molecule can be put together relatively quickly. Performance can be quantified, a reliable formulation produced and process chemistry used to estimate cost of goods. Chemical pesticides often need a dedicated production plant manufacturing perhaps one compound, or a single class of chemicals. This demands substantial up-front investment, and the flexibility to reduce manufacturing costs over time is relatively limited (Harris, 1993). This necessitates that the new compound must have substantial market potential, with sales exceeding $50 million per annum to justify the enormous development and plant construction costs. As such, this tends to breed a 'volume' culture where profitable niche products are largely ignored.

Microbial insecticides on the other hand are a relatively new technology. As yet, formulation technology is in its infancy for these types of insecticides and even the existing commercial products frequently suffer from short shelf life and brief persistence in the field, as a result of wash-off or U.V. degradation. Microbial insecticides are also quite unlike chemical pesticides in their manufacturing characteristics and requirements. Most are produced by deep liquid fermentation processes in specialised production facilities that are not usually present within a chemical company. Additionally, market penetration has as yet been quite modest. Even the most successful microbial insecticides, based on strains of *Bacillus thuringiensis*, have only captured about 1% of the global insecticide market. All these features tend to work against major agrochemical companies developing such products in-house.

MARKETING OF MICROBIAL INSECTICIDES

Historically, many microbial insecticides have tended to be marketed by concentrating on safety issues. It is not unusual to read within a product brochure, statements about safety to non-targets, beneficials, applicators, consumers etc. Claims are often made about the product being ideal for use in an integrated pest management (IPM) system as a result of its specificity and preservation of predators.

These are undoubtedly all very attractive features but what seems to be frequently ignored is that the end-user (grower) predominantly chooses which products he uses on the basis of cost-efficacy. Safety is of much lesser concern unless there are specific residue issues centred around a particular crop or there is political intervention to encourage growers to use softer products.

Perhaps as a consequence of this, BCA's are often viewed as safe products that only work well under certain limited circumstances. Their image has not always been helped in that there are also some truly weak products on the market, with poor efficacy and questionable quality control.

The challenge has to be the development of microbial insecticides that are cost-effective and offer chemical-like levels of activity with all the inherent safety benefits.

Only then will microbial insecticides 'break-through' to become major market opportunities for insect control.

MICROBIAL INSECTICIDES AND PATENT PROTECTION

Patent protection is crucial for a company to have a serious business interest in a product. Without the ability to protect an invention, there is the real risk that other companies will plagiarise it resulting in a number of basically identical products competing against each other for the same area of business. Historically, most work on BCA's has been conducted in Universities or Government Laboratories. When discoveries have been made, the tendency has been to rush to print as quickly as possible. This is quite understandable as continued individual or project funding is frequently dependant upon the regular publishing and presenting of papers. However, the consequence of this is that for most of our current microbial insecticides there is no effective patent protection. Using *B.t.* (*Bacillus thuringiensis*) products as an example, the non-proprietary nature of this business means that as market entry is relatively easy it has become extremely competitive and very much a commodity business. Local production initiatives, although usually short-lived due to being economically unsound, create significant market disruption and discourage the major companies developing new products.
To survive and grow in such market conditions requires a number of key strengths but clearly one of the most important is fermentation expertise. In the long term, the companies that survive in this area will be those that are the low-cost producers who have high volume, state-of-the-art fermentation and recovery facilities with stringent quality control procedures. However, this has not prevented a number of smaller companies entering this market relying on toll-manufacture but most of these ultimately fail.

In recent times however, the value of patent protection has become more widely recognised within academic environments and these bodies are increasingly protecting their inventions prior to publication. Although this is undoubtedly a well-advised strategy, this in itself is presenting difficulties in the commercial development of new technologies. For example, particularly in the area of biotechnology / genetically engineered organisms, individual pieces of enabling technology are widely dispersed amongst different groups/companies. This restricts the freedom to operate of companies wishing to develop and commercialise such products as they must take licenses to utilise key pieces of technology. Although this is not a problem in itself, the patent holders are often commercially very inexperienced and as a consequence sometimes have completely unrealistic expectations as to the monetary value of the invention and demand high prices for the issue of a license. The need for multiple licenses can quickly erode the profitability of a new product to zero, killing commercial interest. It is to the benefit of both inventor and commercial company to negotiate realistic license conditions such that the patent holder can gain a fair return on the invention whilst enabling the company to earn acceptable profit on the resulting product.

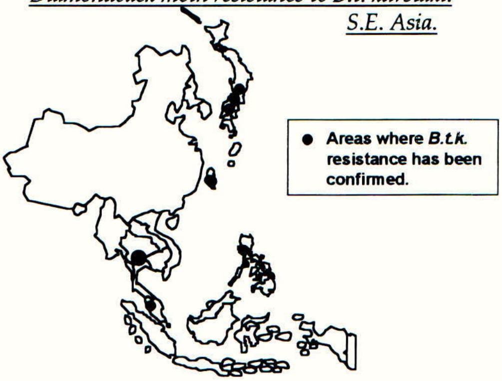
MICROBIAL INSECTICIDES AND THEIR POTENTIAL

Of the current microbial insecticides available, the success story has undoubtedly been *B.t.* (*Bacillus thuringiensis*). Compared with other biological control agents, *B.t.*s dominate the current market. Strains that are in commercial usage include;

B.t. subsp. *kurstaki* ...	for Lepidoptera control in agriculture and forestry.
B.t. subsp. *aizawai* ...	for control of *B.t.k.*-resistant Lepidoptera, especially DBM.
B.t. subsp. *tenebrionis* ...	for Colorado Potato Beetle (CPB) control.
B.t. subsp. *israelensis* ...	for mosquito larviciding.
Bacillus sphaericus ...	for mosquito larviciding in polluted water, for *Culex* control.

Dramatic steps forward in the quality of the leading *B.t.* products mean that today, *B.t.*s are increasingly cost-effective as well as being very specific to the target pest species, and safe to people and the environment. It is important to note that as described earlier, it is the improved cost-efficacy (making them more competitive with chemical insecticides) that has driven this market growth. There are signs though that the foliar *B.t.* market is probably close to maturity and that future growth may be limited. One cloud on the horizon is resistance to *B.t.* subsp. *kurstaki*. Resistance has been slow to develop probably due to a combination of low product usage and short persistence in the environment, limiting insect selection pressure. However, increasing sales have led to resistance in some pests, most notably in the Diamondback Moth (*Plutella xylostella*) on crucifer crops in South East Asia (Harris, 1995).

<u>*Diamondback moth resistance to B.t. kurstaki.*</u>
<u>*S.E. Asia.*</u>

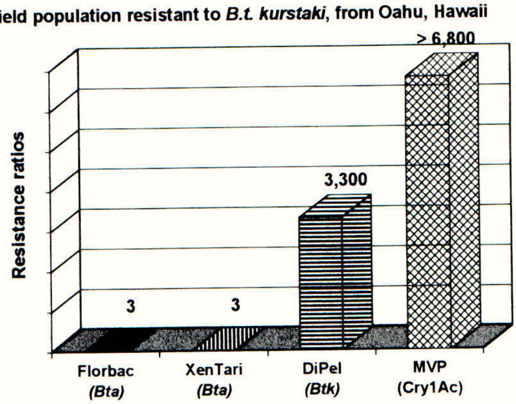


Products based on the *B.t.* subsp. *aizawai* strain have proven to continue to be field effective, even when insects have developed resistance to the *B.t.* subsp. *kurstaki* strain (Tabashnik <u>et al</u>, 1993). This is due to the presence of a different range of *cry* proteins in the two strains, in particular the strong activity exhibited by *cry1C*.

Cry 1 and 2 gene profiles, by PCR analysis.

Product	Cry1Aa	Cry1Ab	Cry1Ac	Cry1C	Cry1D	Cry1G	Cry2A	Cry2B
DiPel (*Btk*)	+	+	+				+	+
Florbac (*Bta*)	+	+		+	+	+		+

DBM ... resistance ratios to different B.t. strains.



There is recent evidence though, that field strains are now starting to become less sensitive to *B.t.* subsp. *aizawai*. Wright et al (1996) recently reported on a field population of Diamondback moth from Serdang in Malaysia which in laboratory tests had exhibited reduced sensitivity to *B.t.* subsp. *aizawai*. Although this was not yet at a level where field failures would occur, there clearly is the potential for a shift in sensitivity within the population and the risk of future field resistance occurring unless sound resistance management strategies are employed.

The other major threat to the use of *B.t.* foliar sprays is the advent of transgenic crops capable of expressing *B.t.* cry proteins. Many such crops are currently being commercialised, and projected dates of introduction are shown below;

Transgenics for insect control.

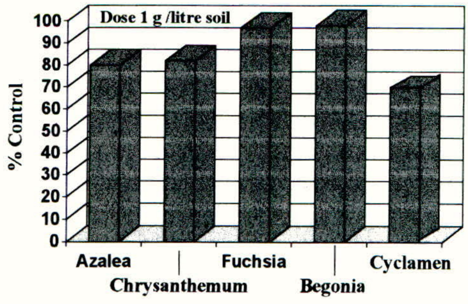
Technology	*Introduction*	*Company*
Bt cotton	**1996**	**Monsanto**
Bt potatoes	**1996**	**Monsanto**
Bt maize	**1996**	**Various**
Bt tomatoes	**1997**	**Monsanto**
Pyramider Bt cotton	**1999**	**Monsanto**
Bt rice	**2000**	**Monsanto**

B.t. when expressed in a plant has a number of advantages compared to foliar application. Firstly, it is present in the 'activated' form in the plant whereas in foliar products the crystal has to dissolve in the gut of the insect to release the active peptides thus taking somewhat longer for feeding inhibition to be initiated. Secondly, neonates are more susceptible to *B.t.* than older instars and with a transgenic crop the insects are exposed to the *B.t.* from their first mouthful catching them at their most vulnerable stage. Thirdly, other than when the plant is maturing or under environmental stress, transgenic plants produce *B.t.* throughout most of the season and therefore there are no issues around correct application timing or U.V. degradation. All these features suggest that *B.t.* transgenic crops will tend to supplant foliar usage of *B.t.* in a number of their key markets, such as cotton, tomatoes, and potatoes.

Furthermore, there is the distinct risk that *B.t.* transgenic plants will accelerate resistance occurring to *B.t. cry* proteins. Whereas with foliar use, selection pressure on the insects has been limited, transgenics expose the populations to a single *cry* protein continuously over the season, thus applying enormous selection pressure. To date, resistance management strategies for *B.t.* transgenic plants have centred around the use of refugia theoretically creating a massive influx of susceptible individuals. The practicality of this approach has yet to be comprehensively proven however. Most of these refugia strategies are based on computer simulation models, which are of course only as good as the parameters written into the models. Furthermore, it is far from certain how refugia requirements can be effectively enforced in practice. It seems very possible that unless oversprays of effective insecticides with an alternative mode of action are employed, field resistance could be a real possibility within a short timescale. Indeed, some workers believe that resistance to transgenics is inevitable, it is just a question of how fast it occurs (Baum, 1996).

Entomopathogenic fungi have also been heavily researched as potential microbial insecticides. In particular, *Beauveria* and *Metarhizium spp*. have been investigated because of their ability to be active against a wide range of insects including aphids, whiteflies, leaf miners, weevils, cockroaches, and grasshoppers. Unlike bacteria and viruses the most common route of infection is by conidial germination followed by penetration of the insect cuticle rather than by ingestion. As such, this gives these strains a certain degree of contact activity and feeding is not required in order to achieve mortality. Hyphal growth occurs throughout the body of the insect leading to organ destruction and death. One example that has been investigated by Bayer AG is BIO 1020, a strain of *Metarhizium anisopliae* for the control of *Otiorhynchus sulcatus* (black vine weevil) (Reinecke et al, 1990). This pest is a major problem on several ornamentals crops in glasshouses. The product consists of dry granules with a claimed shelf life of 6 months if kept in cool conditions. The granules are admixed with soil and following water uptake by the granules the fungus starts to produce large numbers of conidia which are viable for many months. Insects become infected when they come into contact with these conidia. This does mean however that the application must be of a preventative nature but due to the long survivorship of the conidia, satisfactory protection should be achieved for the whole duration of the crop. The recommended rate is 1 g/litre soil, at which good levels of control of *O. sulcatus* have been seen on a number of glasshouse crops.

Effectiveness of BIO 1020 against *Otiorhynchus sulcatus* eggs and larvae in ornamentals under glasshouse conditions 28 days after treatment.



However, entomopathogenic fungi are slow acting compared with chemicals and formulation is a particular challenge. The lack of patent protection and differentiated products in this area is again key to limiting market potential, and thus company interest. For example, it is believed that Bayer are discontinuing their activities with BIO 1020 because the market value is considered inadequate (Schnorbach, 1996).

Despite this, products based on *Beauveria* and *Metarhizium spp*. are probably the BCA area with highest business potential for smaller companies, due to the large number of available strains active against a wide range of insect pests. For example, the use of *Metarhizium anisopliae* has been reported for the control of Western Flower Thrips, *Frankliniella occidentalis* (Vestergaard et al, 1995), and a number of new products have recently been introduced such as Bioblast termiticide from EcoScience, Mycotrol from Mycotech, and Naturalis from Troy Bioscience.

Some other microbial insecticides also include *Verticillium lecanii*, which has seen some usage for aphid and whitefly control in glasshouses but is disadvantaged by requiring a relative humidity of 95-100% to function. *Nosema locustae* (protozoan) has been reported to give about 50% control of grasshoppers when applied as a bait but is only effective against young larvae up to 3rd instars (adults not affected). As such, these materials would probably have to be regarded as having niche potential only.

BACULOVIRUSES

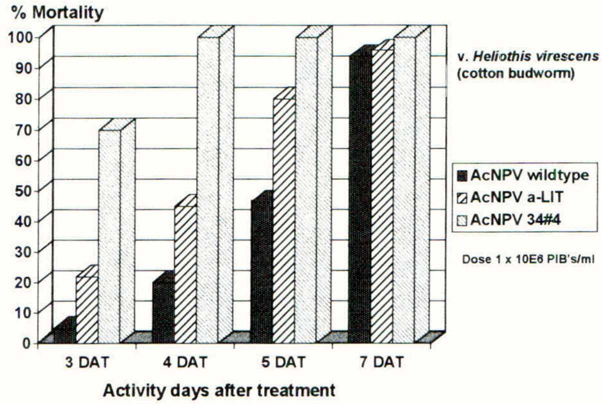
Arguably, the most promising area for future microbial insecticides are nucleopolyhedroviruses. Such baculoviruses can be isolated from Lepidoptera (butterflies and moths), Hymenoptera (sawflies only), and Coleoptera (beetles) (Cory & Bishop, 1995).

Examples of commercial products include *Spodoptera exigua* NPV (Biosys) aimed at controlling the beet armyworm on a range of crops, and *Lymantria dispar* NPV (USDA / Cyanamid) for aerial application to control gypsy moth in forestry. Such wildtype baculoviruses have given good results when carefully applied, and pest pressure is not too high. One disadvantage though is that baculoviruses can take some days to kill or inhibit feeding, sometimes resulting in unacceptable crop damage before mortality is achieved.

Recent efforts from a number of companies have centred on genetically engineering baculoviruses with a gene expressing an insecticidally active toxin. Most commercial activity has centred around lepidopteran-active baculoviruses, in particular *Autographa californica* nucleopolyhedrovirus (AcNPV). This virus is relatively broad spectrum being permissive to some key lepidopteran pests such as *Heliothis/Helicoverpa spp*., *Spodoptera exigua*, and *Trichoplusia ni*. Additionally, it is fairly amenable to genetic engineering techniques and is frequently utilised as a model system for research.

The figure below demonstrates some of the potential benefits of 'arming' a baculovirus with a toxin gene. Two constructs are compared with the wildtype AcNPV. The first construct has received a gene encoding for alpha-laterodectus insect toxin (Watkins et al, 1997). The second construct has received a gene described as Tox 34#4, which is an RT-PCR generated cDNA clone encoding a protein with a high level of homology (94% identity) to the original TxP-1 itch mite (*Pyemotes tritici*) toxin (Tomalski & Miller, 1991). Both of these constructs have the toxin gene driven off the P10 viral promoter.

Wildtype AcMNPV versus constructs.



As can be seen, there is a substantial benefit in terms of speed-of-kill especially with AcNPV + Tox 34#4. This virus is used solely as a research standard within Zeneca.

It is believed that highly effective recombinant baculoviruses offer the best likelihood of microbial insecticides achieving chemical-like levels of efficacy and crop protection effect. Consequently, a number of major companies have been actively researching in this area including Cyanamid, Zeneca, and DuPont.

However, there still exist a number of challenges that must be addressed before recombinant baculoviruses can reach their commercial potential. Baculoviruses are very prone to deactivation by U.V. light and certain leaf exudates, and these weaknesses must be addressed by specialist formulations designed to protect the virus on the leaf surface as well as offering acceptable shelf life. Safety, and the public perception to the release of genetically engineered organisms will need to be handled thoroughly and sensitively. The differential attitude of regulatory authorities to engineered organisms, with some being pragmatic whilst others are less so, will cause development delays in some cases. And finally, there are considerable production challenges. Recombinant baculoviruses produce only a fraction of the virus yield within an insect compared with a wildtype baculovirus, thus eliminating *in-vivo* insect production as a viable economic option. Instead, commercial production will need to be undertaken *in-vitro* utilising optimised insect cell lines grown in large-scale bioreactors. It is believed that *in-vitro* production technology for baculoviruses is currently at about the 250 litre bioreactor level. In order to attain the necessary economies of scale for a successful commercial product, reliable production will ultimately need to be carried out in large reactors of 16,000 litre capacity and above.

SAFER CHEMICAL INSECTICIDES

One feature of the chemical pesticide industry is the tendency to introduce newer molecules that are more specific and intrinsically less toxic to mammals and non-target organisms. An example would be tebufenozide, which is an insect growth regulator which accelerates moulting (Heller et al, 1992). It is highly selective to Lepidoptera and possesses a very favourable toxicology and environmental profile, to the point where it is almost considered as a 'biological' chemical. Regulatory authorities are encouraging the development of such 'low-risk' compounds by providing accelerated and cheaper registration. It is inevitable that this will continue to focus agrochemicals companies on the search for safer, more specific compounds. As such, this offers a considerable threat to many microbial insecticides, because as chemicals become perceived as being safer, they will blur the distinction between chemicals and biologicals.

BUSINESS POTENTIAL FOR MICROBIAL INSECTICIDES & CONCLUSIONS

In terms of business opportunity, today's first generation biological control agents have only modest potential. Including parasites/predators, such products are currently worth about $200 million in sales per annum, with half of this coming from *Bacillus thuringiensis*. Growth is slightly ahead of the overall agrochemicals market.

In terms of the focus of this conference, as to whether microbial insecticides are 'novelties or necessities', I believe that they probably fall somewhere in-between. In some cases, there is the potential to use them in a true IPM approach where all available tools including cultural practices, resistant varieties, chemical insecticides, and microbial insecticides are used in a programmed approach to prevent or minimise resistance development whilst enabling the highest quality and cost-effective crop to be produced by the grower.

From an industry point-of-view, the main areas of interest are likely to be *B.t.* insecticides, entomopathogenic fungi (especially for small BCA specialists), and recombinant baculoviruses. Other areas are likely to remain insignificant, and of low interest to the industry as a whole.

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