Against the one hundredth locust: the commercial use of insect pathogens

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> *And a locust unto Mahomet said: We are the army of the great God; we produce ninety-nine eggs; if the hundred were completed, we should consume the whole earth and all that is in it* – Arab legend

Insect pathology has a long history dating back to the earliest studies in microbiology. A select few of the many known insect pathogens can be produced and used on an industrial scale as biopesticides and are championed for their low environmental impact. The commercial application of microbial insecticides has been limited competition with chemical insecticides. However, the advent of gene technology led to a multibillion dollar industry incorporating bacterial toxins into insect resistant transgenic crops, and in the development of expression vectors. Recent use of insect pathogens in Australia has demonstrated that these biopesticides can play a significant role in mainstream commercial agriculture for the management of multiple-insecticide resistant pests and maintenance and in integrated pest management.

There are several thousand known pathogens to anyone who has tried to keep an insect colony alive, including ancient cultivators of silkworms. Lethal fungal pathogens are very easily observed sporulating on cadavers and were amongst the first microorganisms to be recognised. As early as 1835 Agostino Bassi demonstrated that microbial *Beauveria bassiana* could be transmitted readily from a diseased insect to a healthy one, and in 1888 Isaak Krassilstschik established an industrial plant for production of conidia of *Metarhizium anisopliae* (on a substrate of beer mash) for control of the sugar beet curculio, thus founding the industrial production of microbial pesticides [1].

Of the many diseases of insects, several are closely related to mammalian and plant pathogens, but many bacteria, viruses, fungi and nematodes are specific to arthropods, some to a single insect genus. They are championed as biological insecticides or 'biopesticides' for their selectivity and low impacts on humans, the environment and the predators and parasitoids that naturally reduce pest populations. However, commercial success of a biopesticide also requires viable industrial production and field efficacy in comparison to the chemical insecticides used widely in agriculture.

Many commercial biopesticides are 'biorationals', insecticidal compounds derived from microorganisms, such as Avermectin and Spinosad (fermentation products of *Streptomyces avermitilis* and the Actinomycete *Saccharopolyspora spinosa*). The most successful are the insect-specific *Cry* protein toxins of *Bacillus thuringiensis* (Bt). These are easily produced by industrial fermentation and are applied as either the spore or the purified toxins. The isolation of the first genes for the *Cry* proteins in the 1980s led to a proliferation of new recombinant insecticidal bacteria and a multi-billion dollar industry in incorporation of Bt toxins into insect-resistant transgenic crops such as cotton and maize [2].

Obligate pathogens, those that require living insects for replication, face greater challenges as microbial insecticides, though both pathogenic viruses and fungi were early candidates. Baculoviruses are insect-specific double stranded DNA viruses with a protein occlusion body that makes them relatively stable (for a virus) in sprays and the environment, though they are

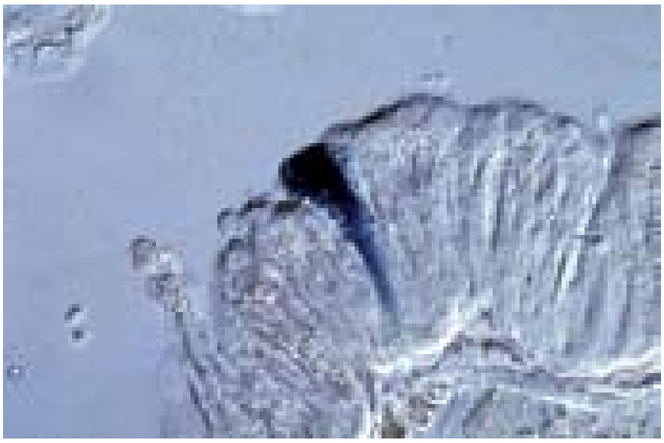

Figure 1. A section through the midgut of *Trichoplusia ni* infected with a genetically modified baculovirus showing localised *LacZ* gene expression in columnar epithelia cells.

rapidly inactivated by UV light. They are typically highly host-specific, making them particularly safe for non-target organisms, including farmers. One or several occlusion bodies must be ingested by the host, initiating an infection in the cells of the mid-gut that spreads through the insect tissues (Figure 1). The insect host continues to feed for several days before liquefying on death to release around over a billion occlusion bodies per cadaver (Figure 2).

Baculoviruses are often regarded as too slow, too specific, too difficult to produce, too inefficient and too sensitive to UV to be a viable alternative to chemical controls which is a significant challenge to commercial production. However, significant work in Brazil, Canada and the USA led to semi-mechanised production based on mass rearing of insect hosts. Successful products were produced and used in forestry, where the slow speed of kill is more tolerated than in agricultural crops, and in Brazil as local product for control of *Anticarsia gemmatalis* in millions of hectares of soybeans[3].

The 'golden age' of baculovirus research began with the identification of permissive insect cell lines and the discovery that genes for late-expressed proteins such as the occlusion body protein polyhedrin are under the control of powerful promoters but are not essential to replication in tissue culture[4]. Insertion of new genes into the dsDNA of the baculovirus under the control of these promoters led the development of baculoviruses as expression vectors, which became an important tool in protein research. Genetic modification also offered the potential to improve performance of baculovirus insecticides through the insertion of insect-specific toxins. Though these were shown to reduce the time to death and damage by the insect compared to unmodified virus, the environmental release of genetically modified viruses incorporating a toxin has remained highly controversial and highly regulated[5].

The development of permissive insect cell lines also led to research in large-scale production of baculoviruses in tissue culture. Though there are significant difficulties in maintaining stable occlusion body production, continuing work at the University of Queensland shows considerable promise[6] and may yet lead to industrial *in vitro* production of baculovirus insecticides or expression vectors.

In Australia, baculoviruses have been successfully adopted in mainstream intensive cropping for control of *Helicoverpa armigera*, a serious pest of food crops and cotton that was estimated to cost up to A$300 million per year in damage and control and rapidly develops resistance to chemical

Figure 2. A cadaver of *Helicoverpa armigera* liquefies to release billions of baculovirus occlusion bodies.

Figure 3. The entomopathogenic fungus *Metarhizium anisopliae* sporulates on a cadaver of the green vegetable bug, *Nezara viridula*.

insecticides [7]. Work by the Queensland Department of Primary Industries and Fisheries identified baculoviruses of Helicoverpa as a potential control agent, leading to the registration of three commercial baculovirus insecticides that are widely used in sorghum, cotton and horticulture.

The success of baculoviruses in Australia is in part due to the high quality and reliable supply of these products, but also to their use not as a direct competitor to chemical insecticides but as an effective tool in integrated pest management and resistance management. They provide growers with a 'soft' option that controls insecticide resistant Helicoverpa larvae but doesn't kill the many beneficial predators and parasitoids [8] that naturally reduce pest pressure and help to prevent further pest outbreaks. By timing control against early instars and to early crop stages, growers are able to tolerate the small amount of feeding damage caused before death and avoid generating resistance to available chemicals that can then be used for more urgent control. Efficacy has been improved by good spray application and the use of a simple formulation containing sugars or molasses [Hauxwell, unpublished].

The use of entomopathogenic fungi was significantly boosted by research on *M. anisopliae* for the control of locusts under the LUBILOSA programme in West Africa, where chemical insecticides to control locust plagues were a significant problem for human and livestock health [9]. Significant progress was made in production of fungal pathogens on grain based media, formulation in oil and timing of application [10]. Related work by CSIRO led to the registration of Metarhizium for plague locust control in Australia [11].

Fungal pathogens of insects also have significant potential in modern intensive agriculture. The adoption of integrated pest management and introduction of transgenic cotton in Australia has led to a reduction in Helicoverpa occurrence but an increase in threats from sucking pests, and in particular the multiple-insecticide resistant silverleaf whitefly, which are not susceptible to Bt toxins. There is wide industry concern that application of insecticides against sucking pests such as mirids and the green vegetable bug and thus the destruction of natural enemies could trigger outbreaks of resistant whitefly that would devastate crop production.

Viruses and bacteria that must be ingested cannot be used against sucking pests. However, fungi such as Beauveria and Metarhizium infect through germination of conidia on the cuticle of the insect (Figure 3). Spores applied to the plant can be picked up by the target insect without the need to be ingested, and can thus be used to manage sucking pests. Our work has selected Metarhizium and Beauveria isolates with significant impact on a range of pests, including aphids and mirids, but little impact on beneficial predators. Fungal pathogens thus offer options to growers as a tool in the evolving integrated management of pests in broad acre cropping.

Commercial use of insect pathogens has moved in a large and progressive spiral, from the early work of Metchnikoff and Krassilstschik, through the advances of gene technology, back into mainstream commercial agriculture. The requirements for re-registration and the withdrawal of many of the cheaper, and less sustainable chemical insecticides, the need to manage insecticide resistance and the increasing adoption of integrated pest management and transgenic crops have all created broad niches for biopesticides. While increasing regulation for registration may slow adoption, Australian growers have already demonstrated a successful model for the mainstream use of microbial insecticides.

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