DEVELOPMENT OF VIRAL INSECTICIDES FOR CONTROLOF *HELICOVERPA* SPECIES: BIOLOGICAL CONSIDERATIONS

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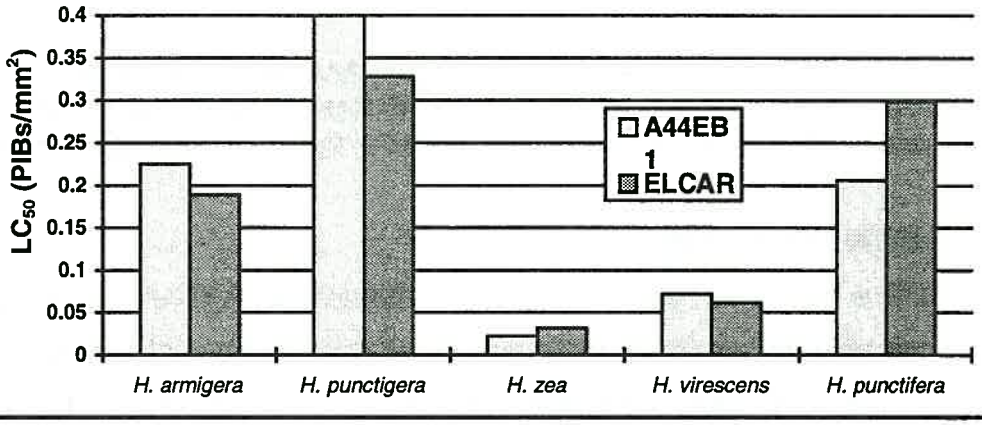
INTRODUCTION

The last five years has seen considerably renewed interest in the development and use of biological insecticides and strategies for the control of *Helicoverpa* and *Heliothis* species on cotton. This interest has been precipitated by several factors operating primarily outside of the industry, most notable being the development of resistance by *Helicoverpa armigera* to a number of currently available chemical insecticides. Among the new generation of biological insecticides for *Helicoverpa* and *Heliothis* control are sprayable formulations the entomopathogenic bacterium *Bacillus thuringiensis* (Bt) and transgenic cotton plants that are capable of expressing the δ-endotoxin from this same bacterium.

At CSIRO's Division of Entomology, for the last several years we have been pursuing the development of a number of alternative biological insecticides for *Helicoverpa* and *Heliothis* control based around insect pathogenic viruses. One of these aims to exploit a small RNA-containing virus isolated from *Helicoverpa armigera* as a source of insert genes for transgenic plants, protecting the crop against *Helicoverpa* attack. A second approach aims to develop sprayable insectcides from a large double-stranded DNA-containing virus known as an entomopoxvirus (EPV). The third strategy utilises a different type of double-stranded DNA-containing virus, known as a nuclear polyhedrosis virus (NPV).

To date, NPVs have been the most successful group of insect viruses used as biopesticides. The first registered NPV based biopesticide was produced for control of *Helicoverpa* and *Heliothis* species in the late

Figure 1. Activity of A 44EB1 and ELCAR™ against a range of heliothine species



1970's. Released under the tradename of ELCAR™ it was aimed primarily at the cotton industry. Although it achieved some commercial success, its slow-rate of kill, susceptibility to inactivation by UV radiation and problems with formulation led to its eventual removal from the market place in the early 1980's. Despite these problems, what undoubtedly contributed most to the demise of ELCAR™ was that its appearance coincided with the commercial release of the synthetic pyrethroids (SPs). With the demise of ELCAR™ commercial interest in viral insecticides waned for a number of years until in the late-1980's. However, In those years following the demise of ELCAR™ there were several important developments that eventually contributed to renewed interest in NPV insecticides.

First genetic manipulation technology was developed for NPVs that offered a solution to some of the problems associated with the slow speed of kill that ELCAR™ had experienced. This technology allows for the insertion of foreign genes into the NPV genome to produce viruses with faster activity. Second, there were some successful demonstrations of the use of baculovirus insecticides for insect pest control, the most notable being an NPV to for the control of soybean looper, *Anticarsia gemmatalis*, in over 2 million ha of soybean per year in Brazil.

NPV Bioinsecticides

We have been actively involved in the development of faster acting strains of NPVs for use as *Helicoverpa /Heliothis* control agents in cotton for the past several years. This program has been underpinned by the development of a genetic engineering technology suitable for NPVs that have activity against *Helicoverpa* and *Heliothis* species and the isolation and insertion of genes likely to improve the speed of action of the virus. However, this is only one aspect of the development of a suitable NPV insecticide for *Helicoverpa* and *Heliothis* control. Equally important is selection of a virus isolate with which to engineer faster acting NPVs. It is this last component that we will discuss in this paper.

Selection of a suitable Isolate

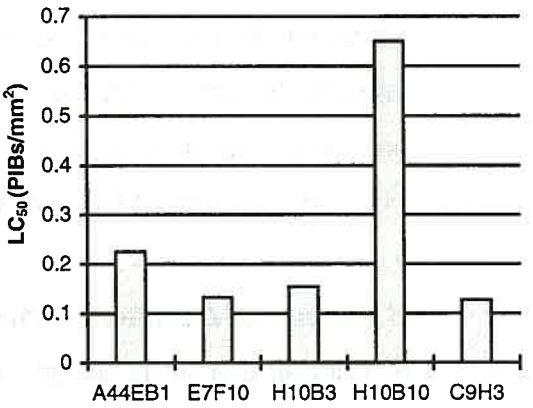
In choosing a virus around which to base our engineering technology it was important to choose an isolate that was:

1) Australian in origin - making the eventual process of registration easier than if a foreign isolate was chosen
2) Active against the *H. armigera* and *H. punctigera* particularly, but also with good activity against the North American pests (*H. zea* and *Heliothis virescens*),
3) Able to replicate in cell culture; not only because this is perceived to be the ultimate production process but also to assist in engineering the virus.

In the first instance, we obtained a number of Australian isolates of NPV from Dr Bob Teakle (Centre for Tropical Pest Management, Brisbane). These isolates were recovered from field populations of *Helicoverpa* species, passaged through *H. armigera* larvae and subjected to an *in vivo* cloning process to separate "individual" genotypes. From this procedure several isolates were selected and preliminary bioassays carried out against *H. armigera* larvae. One of these isolates, termed A44EB1, appeared to perform slightly better than the other isolates and was

selected for further study. The selected isolate, A44EB1, was then tested for its ability to replicate in cultured *Helicoverpa* cells, where it was found to perform as well as ELCAR™ (originally isolated from *H. zea* larvae in Texas).

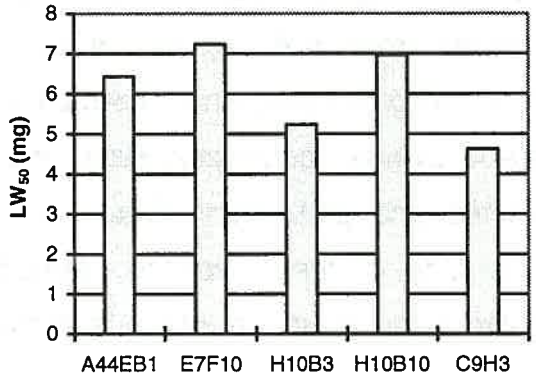
Figure 2. Activity of four *in vitro* isolated clones of A44EB1 relative to A44EB1



Biological Characterisation of A44EB1

In the next phase of the project, A44EB1 was bioassayed against a range of heliothine species to ascertain it's relative efficacy against the Australian heliothine pest species. In addition, studies were also carried out in conjunction with Drs Art McIntosh and Carlo Ignoffo at the USDA's Biological Control of Insects Research Laboratory in Columbia Missouri, to test it's activity against the North American heliothine pests *Helicoverpa zea* and *Heliothis virescens*. In all of these studies an isolate of ELCAR™ was compared alongside A44EB1. A summary of the results from these bioassays are shown in Figure 1. Activity is expressed as LC_{50} i.e. the concentration that kills 50% of the test insects. These results show that the activity of A44EB1 is at least equivalent to that of ELCAR™.

Figure 3. Mean weight (mg) at which 50% of test larvae(LW_{50}) are killed by clonal A44EB1 isolates



In the course of our genetic studies with A44EB1 we found that the *in vivo* isolate was composed of a number of genotypes. A number of these genotypes (clones) were

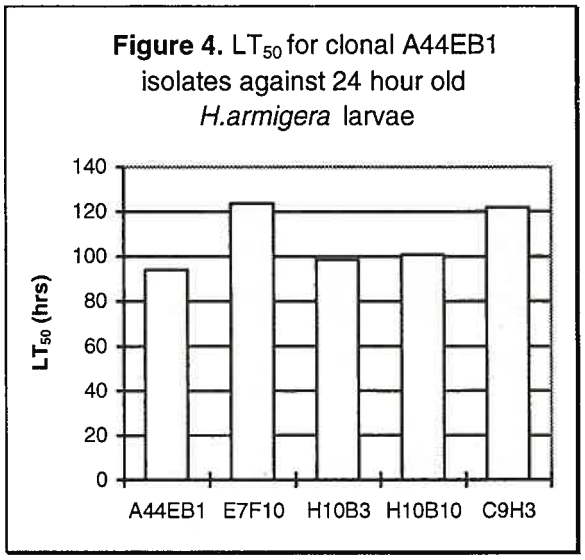


Figure 4. LT_{50} for clonal A44EB1 isolates against 24 hour old *H.armigera* larvae

isolated by an *in vitro* process and then assessed for their biological activity *against H. armigera* and *H. punctigera*. The results for some of the clones included in these tests are shown in Figure 2. From these data we can see that not all of the clones had the same intrinsic levels of activity (the lower the LC_{50}, the better the intrinsic activity of the isolate). Indeed, between the best (E7F10) and the worst isolates (H10B10) there is over a 6 fold difference in their intrinsic activities ($P<0.001$).

We then posed the question, did these isolates differed in any other characteristic other than intrinsic activity? We tested each of the isolates against different larval instars and measured LT_{50} - the time at which 50% of the test insects are killed, and their relative performance against different larval instars. A summary of the results are shown in Figures 3 and 4. The results of the two experiments are interesting as they suggest that the best isolate in terms of its intrinsic activity (LC_{50}) does not necessarily perform best with respect to other biological characters such as its speed of action (LT_{50}) and the size of larvae it is capable of infecting at particular dose (LW_{50}).

Improvement of Intrinsic Virus Activity

Once a suitable isolate has been decided upon, suitable production and formulation systems then need to be selected. In the case of the former, the primary aim is to reduce the overall product cost while the formulation strategy aims to maximise the control achieved per unit cost. However,

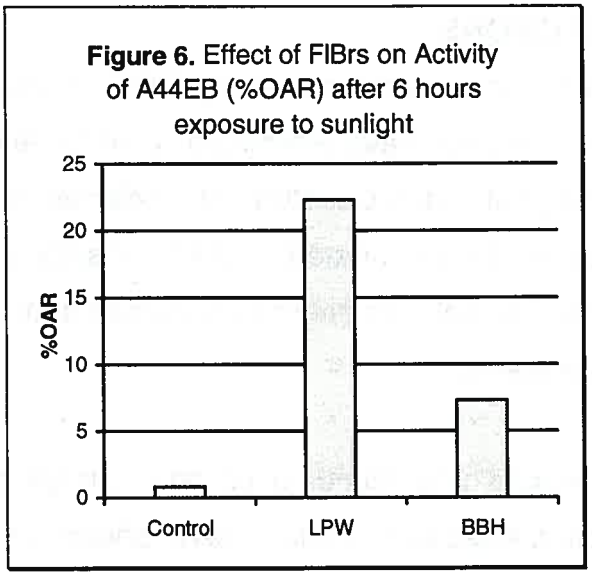


Figure 6. Effect of FIBrs on Activity of A44EB (%OAR) after 6 hours exposure to sunlight

that can lead to rapid inactivation of virus. Under Australian conditions, UV inactivation of virus is very rapid (see Richards *et al.* in these Proceedings). Therefore, an important part of any formulation for a biological insecticide is an effective UV-screen. We have begun to look at compounds that might prove suitable as sunscreens for NPVs. One such group is the fluorescent brighteners (FIBrs) based around stilbene disulphonic acids. These compounds are used widely in the paper and textile industries as whitening agents and we have been working with Dr Marty Shapiro from the USDA in Beltsville (Insect Biocontrol Laboratory, Beltsville, Md) to assess whether any of these compounds can act as effective sunscreens for NPVs. Preliminary results from these studies are shown in Figure 6. As can be seen from these data, the FIBrs can give relatively high levels of protection to the normally highly labile NPVs ($P<0.001$).

In addition to the UV protection that FIBrs can provide they also have the intriguing property of being able to enhance the activity of some viruses. We have also looked at the ability of FIBrs to enhance the intrinsic activity of HaNPVs and some of these data are provided in Figure 7. As this figure demonstrates, these compounds can also enhance the activity of the HaNPVs (in the region of at least a four-fold improvement) as well as providing UV protection ($P<0.001$).

DISCUSSION and CONCLUSIONS

As part of our program to develop novel faster acting NPV insecticides for control of *Helicoverpa* species, we have attempted to more accurately define the relevant biological characteristics of *Helicoverpa* NPVs (HaNPVs). In particular we have concentrated most of our studies around an Australian isolate of HaNPV that was originally collected from infected *Helicoverpa* larvae in Queensland.

This naturally occurring isolate was found to contain a large array of genotypic variants, which subsequent studies have shown vary in a number of their biological characteristics. It is worth remembering that the isolates that we have generated first by an *in vivo* cloning process and second by an *in vitro* process, all came from the same original field sample. In fact, we have observed over 20 different genotypic variants just from the *in vivo* cloning process alone. Recent studies (see Richards *et al.* in these proceedings) have found that NPVs are abundant in cropping systems. These naturally occurring viruses represent a huge as yet untapped reserve for future biopesticides.

While selection of a suitable isolate/strain is an important part of the process of developing novel viral biopesticides, equally important is the development of suitable production and formulation strategies. In our results presented here we have attempted to demonstrate that particular attention has to be paid to the biology aspects of both the product and the production system. Furthermore, we also believe that by the use of novel and innovative formulations great

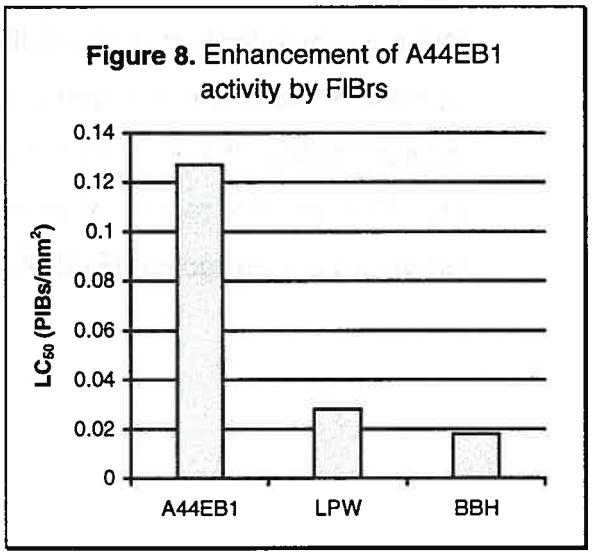


Figure 8. Enhancement of A44EB1 activity by FIBrs

improvements can also be made on the intrinsic activity of the product and how the product can be made more stable in the environment.

As a new generation of biopesticides begin to reach the marketplace, there is an obvious need for us to work towards developing products and formulations that are specific for Australian conditions. Biopesticides need to have the right activity spectrum to deal effectively with Australian pests in Australian cropping systems under Australian conditions. It is towards these goals that we are currently working.