Environmental risk assessment of genetically modified insect viruses for the control of *Helicoverpa* species

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Genetically modified insect viruses as bioinsecticides

One approach in the drive by the cotton industry to introduce more effective and more sustainable methods of bollworm control than are presently available, has been to develop a new generation of insecticides based on naturally occurring insect pathogens such as the nuclear polyhedrosis viruses (NPVs). From an environmental standpoint, these biocontrol agents have the advantage of a much narrower target range, leave no toxic residues, and have no adverse affects on human or animal health. Although NPVs have been applied as insecticides since the 1940's, the relatively long time it takes to kill the insect target (often several days) has generally limited their use to low value, mostly perennial cropping systems e.g. forest plantations.

This is no longer the case. With the advent of genetic engineering technologies in the late 1980's, has come the opportunity to significantly enhance their speed of kill[(1)]. As a consequence both the type of insect pests which can be controlled and the cropping systems which can be targeted have now broadened. In 1988, CSIRO's Division of Entomology initiated a programme to genetically modify an Australian NPV isolated

from the cotton bollworm, *Helicoverpa armigera* (HaNPV) for increased speed of kill (see Christian & Richards in these proceedings).

Risk assessment and regulation

Before these genetically modified organisms can be released into the environment as biological insecticides, regulatory authorities first require the provision of a detailed environmental impact statement in order to identify any risks the GMO may pose to public health and safety, agricultural production and quality of the environment. In Australia, the Genetic Manipulation and Advisory Committee (GMAC), state National Registration Authorities (NRAs) and Environmental Protection Agencies (EPAs) are responsible for overseeing and assessing the risk assessment work. GMAC guidelines[2] for any GMO comprise a comprehensive list of core and specific questions, ranging from the ecology of the unmodified (wild-type) organism to the genetics of the GMO, its stability, survival and transfer, to the reliability of monitoring procedures and contingency protocols put in place before its release into the environment.

Risk assessment of genetically modified HaNPVs

In 1994, the Co-operative Research Centre for Sustainable Cotton Production and the CSIRO Division of Entomology began a project to explore the risk factors associated with a planned release of a modified *Ha*NPV. The project thus far has been solely concerned with the wild-type viruses in a series of laboratory studies, field experiments and surveys.

The authors take this opportunity to report on the project's findings in five critical areas: (1) Host range -which species can these viruses infect? (2) Stability -how persistent are these viruses in the environment? (3) Dispersal -what potential is there for these viruses to disperse from a release site? (4) Distribution -what is the natural habitat and range of these viruses in Australia? (5) Monitoring - what methods and protocols are in place to follow the fate of the GMO once released?

Host range

NPVs belong to a diverse group of DNA viruses known as the Baculoviridae which have only been isolated from invertebrates. While the vast majority of baculoviruses infect insects, mostly moths and butterflies (lepidoptera) and sawflies (hymenoptera), a few have also been isolated from the Crustacea e.g. Penaeid prawns. Most NPVs tested so far are not specific to one host

Table 1. Susceptibility of 11 candidate species to *Ha*NPV

	Species	Susceptibility
Heliothinae	*Helicoverpa armigera**	++
	*Helicoveropa assulta**	++
	*Helicoverpa punctigera**	++
	Helicoverpa zea	++
	Heliothis subflexa	+
	Heliothis virescens	++
	*Heliochilus eodora**	+
	*Heliochilus pallida**	+
Noctuidae	*Anticarsia gemmatalis*	-
	Chrysodiexis argentifera	-
	Trichoplusia ni	-

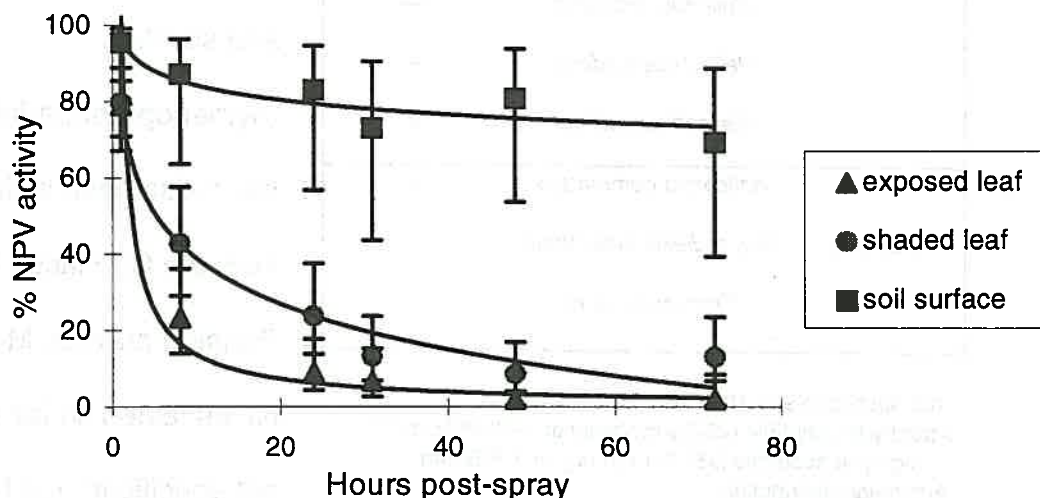
- not susceptible at 100 PIBs/mm^2
+ poorly susceptible (<50% mortality at 1-10 PIBs/mm^2)
++ highly susceptible (>50% mortality at 1 PIB/mm^2)
*Australian distribution

species but cross-infective to other members of the genus of the original host and often across several genera within a family. This is the case for NPVs isolated from species represented in the sub-family Heliothinae (Lepidoptera: Noctuidae), to which some of the world's most notorious pest species including *Heliothis virescens*, *Helicoverpa zea, H. armigera* and *H. punctigera, belong.* Infectivity of NPVs in species from different families appears to be the exception but this is known for some NPVs. To date, data indicate that the *Ha*NPV (A44EB1) is not one of these (Table 1).

Environmental stability

The most important environmental factor influencing a pathogen's probability of survival is exposure to ultra-violet (UV) radiation. In a series of field experiments conducted between January-March, 1995, we applied a formulation of the wild-type *Ha*NPV to a commercial cotton crop (Siokra, variety L22) at three successive crop growth stages, and used a sample

Figure 1. Pattern of HaNPV inactivation in the canopy of a flowering Siokra cottoncrop (Myall Vale, 02/95)

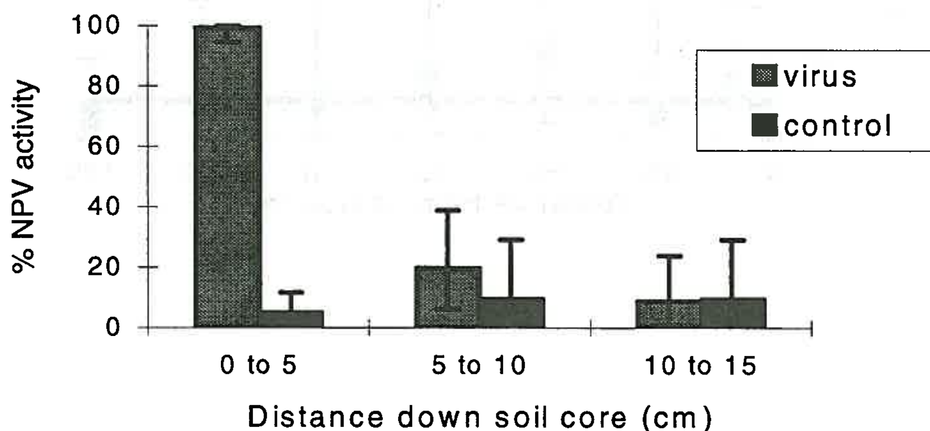


incorporation bioassay to measure the rate of virus inactivation at exposed and shaded locations in the crop canopy, and at the soil surface directly below the canopy. Figure 1 illustrates the temporal pattern of virus inactivation at each location in a flowering crop (intermediate canopy development). The data clearly illustrate how rapidly the virus is inactivated in the leaf canopy compared to virus at the soil surface, with significant differences also observed within the canopy at exposed versus shaded positions. This effect was consistent at all crop growth stages. In fact, extrapolation to the time point at which 90% of virus activity has decayed, at the leaf surface (hours) and on the ground (years) represents a difference of 2-3 orders of magnitude.

Virus dispersal in irrigated cotton

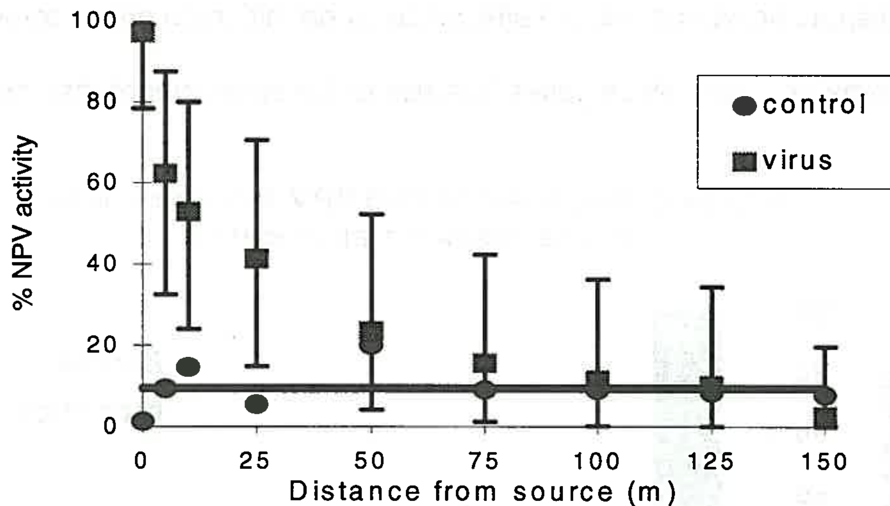
A further concern associated with a release of a GMO is the risk of it dispersing from a release site, since by definition an organism which remains in one place poses less risk to the environment than one which is

Figure 2. Dispersal of HaSNPV through the soil profile following an irrigation



highly dispersive. Although NPVs are non-motile, movement off a cotton field could feasibly occur by water-borne advection through the soil profile and/or by surface wash-off during irrigation. In a series of laboratory and field experiments, we have shown that little or no vertical movement of virus through the soil profile occurs, at least over the short-term (Figure 2). Bioassays of soil samples taken at various distances downstream from the virus source, both before and after irrigation, illustrate that while virus can be vectored across the field surface the logarithmic decrease in virus activity with distance observed (Figure 3) indicates such a route to be of little concern over the course of a single irrigation. These data should prove a valuable aid in designing a planned GMO release strategy to minimise the risk of such an occurrence.

Figure 3. Distance-dispersal pattern for HaSNPV across the field surface following irrigation



Distribution of HaSNPVs in the Australian agro-ecosystem

It is the protection from UV radiation offered by the soil environment which promotes the long-term stability of these viruses in nature. In order to understand how these organisms are distributed in the Australian agro-ecosystem we have quantified a soil incorporation bioassay method to measure virus loading in soils. After constructing regression lines of log NPV concentration against logit NPV mortality for different rates of soil incorporation, we have used a maximum-likelihood computation to estimate the number of NPV PIBs per gram of soil. Blind tests carried-out to assess the reliability of this method indicate these regression models provide an accurate measure of virus loading in a range of soils. A soil survey conducted in eastern Australia in October 1995, encompassing all of the cotton growing regions in NSW and Qsld, and a large area of south-west Qsld important in the build up of *H. punctigera* populations, has shown that wild-type NPVs are essentially limited to cultivated sites (Table 2).

Table 2. Distribution wild-type HaNPVs in the Australian cotton agro-ecosystem (October 1995)

Land use	No. +ive sites	Range for +ive sites (PIBs/g soil)	Mean for +ive sites (PIBs/g soil)
Uncultivated (inland *Helicoverpa* survey region)	7/49 (14%)	18-73	32
Cultivated (cotton regions of NSW & Qsld)	37/87 (43%)	15 - $3.1x10^4$	$3x10^3$

Although we do not yet fully understand the reasons for this stark delineation, any explanation will certainly involve the effect of crop type on *Helicoverpa* larval density how this will influence the rate of virus transmission and in turn, the level of virus production (Table 3). Clearly, conditions in the natural environment act to delimit their distribution.

Table 3. Abundance of *Ha*NPVs in east Australian soils according to standing or previous crop (October 1995)

Crop	No. of sites	Mean NPV PIBs/g soil
Lucerne	4	$2.2x10^4$
Sorghum	10	$1.2x10^3$
Corn	3	$7x10^2$
Cotton	8	25

Monitoring

The soil incorporation bioassay method designed to measure the distribution of wild-type *Ha*NPVs in Australian soils, also gives us the capability to follow the fate of a novel *Ha*NPV following its release. Using this method we can detect NPV down to a level of 12 virus PIBs per gram of soil. Moreover, through the application of standard molecular techniques e.g. the polymerase chain reaction (PCR) and restriction endonuclease analysis (REN), we are able to distinguish the DNA profiles of individual NPV isolates which we know differ from each other by less than 2% at the nucleotide level. For this reason, a novel virus presents a much easier task than do wild-type viruses since the foreign DNA provides an unambiguous marker. The project is presently conducting further risk assessment studies in several

other areas before a release of a modified HaNPV can be contemplated. Nevertheless, we are now well advanced in our goal to contribute to the implementation of safe and effective biopesticides for *Helicoverpa* control in Australian crops.

References

[1] Stewart LMD, Hirst M, Lopez Ferber M, Merryweather AT, Cayley PJ and Possee RD (1991) Construction of an improved baculovirus insecticide containing an insect specific toxin gene. Nature. Vol 532 pp. 85-88

[2]Guidelines for the Planned Release of Genetically Manipulated Organisms (1993) Genetic Manipulation Advisory Committee (GMAC). Commonwealth Department of Administrative Services. Canberra. Australia.