

CROP PROTECTION PROGRAMME

**Entomopathogenic viruses for control of African armyworm
Spodoptera Exempta in Tanzania**

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FINAL TECHNICAL REPORT

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Acronyms

ACP	the Armyworm Control Project
DLCO.EA	Desert Locust Control Organisation for East Africa
HV	High volume (sprayers)
NPV	Nucleopolyhedrovirus
OB	Occlusion body
PCR	Polymerase chain reaction
PCS	Pest Control Services
RT-PCR	reverse transcriptase PCR
SpexNPV	<i>Spodoptera exempta</i> NPV
MoA	Ministry of Agriculture
ULV	Ultra low volume
IARI	Ilonga Agricultural Research Institute
IIBC	International Institute of Biological Control
LD/LC ₅₀	Lethal dose/Lethal concentration
CABI-ARC	CAB International Africa Regional Centre
IPARC	International Pesticide Application Research Centre
IBDC	International Biopesticide Consortium for Development
NERC	Natural Environment Research Council
LUBILOSA	Lutte Biologique Contre les Locustes et Sauteriaux
DFID	Department for International Development

Executive Summary

The purpose of this project was to develop and evaluate the use of a natural biological agent, an insect virus, *Spodoptera exempta* nucleopolyhedrovirus (SpexNPV), as a safe alternative to chemical insecticides for control of the African armyworm (*S. exempta*) in Tanzania. Dr Cherry of NRI led the project from its commencement in October 1996 until the summer of 1997 when the present author took over as project leader.

In the first year (1996-97) a stock of the SpexNPV from 53,000 infected insects was produced at NRI for use in field trials. A PCR test for rapidly diagnosing NPV infections was developed as a tool for monitoring field trials and for virus production quality control. It had been planned to conduct field trials over two years to optimise and evaluate the NPVs. However, in only one of the three field seasons (1999) was there an opportunity to conduct field trials on a major outbreak of armyworm

Trials in that year showed that simple, high volume, application of NPV could successfully kill >95% of armyworm in outbreaks. These trials also showed that the simple knapsack sprayers universally used by farmers in East Africa could be used successfully to apply the SpexNPV. ULV application techniques were however found to give ineffective control. The potency of the Spex was very high and the application rate needed to achieve control is at 1×10^{11} infective particles per hectare equivalent to the virus content of 50 infected larvae. This virus is effective at a rate 10 times lower than existing commercial virus biopesticides.

Monitoring of trial sites showed large numbers of SpexNPV killed armyworm were recoverable for up to two months after a single application. This raised the serious possibility that SpexNPV could be produced locally and cheaply without the need for a dedicated NPV production facility. Selected outbreaks of armyworm on low value rangeland could be managed and sprayed to maximise the production of virus-infected insects. These insects, each containing hundreds of millions of virus particles, could then be collected for making new SpexNPV formulation. Such a system has already been developed for a similar pest in Brazil, and at a cost of US\$ 1.50 per hectare the product is significantly cheaper than chemical insecticides.

The project has achieved its key output of producing and field testing SpexNPV as a control tool for African armyworm. However some important questions remain to be addressed before it is ready to be promoted to farmers on a large scale. It needs to be tested on a full range of cereal crops in farmer participatory trials to ensure it is compatible with existing farmer practices. Local production techniques need to be developed, evaluated and costed. There also needs to be socio-economic analysis to determine if this new technology can be successfully integrated into the existing national and regional armyworm control.

Background

The Armyworm problem

Tanzania is a country in which agriculture plays a dominant role in the livelihood of the rural poor. The African armyworm is a migratory pest of cereals, pasture and rangeland which is widely distributed in Africa south of the Sahara and exhibits great variability in the extent and severity of infestations (Scott, 1991). The areas from which infestations have been reported and the frequency of attack have increased since 1970 (Haggis, 1987). Tanzania has long suffered from the widespread depredations of armyworm outbreaks that appear to be increasing in regularity and seriousness as agricultural production continues to expand. Outbreaks normally occur during the early part of the main rainy season December- March, just after the main food crop of maize has been planted. Yield losses within an outbreak are up to 50% although 30% loss in recently-planted maize has been estimated as an average (Scott, 1991).

Currently poor farmers have two strategies to deal with armyworm outbreaks. The first is to use chemical insecticides but these are beyond the financial means of most farmers at a cost of at least 6000 Tanzanian shillings (10 US\$) per ha. The Ministry of Agriculture (MoA) does supply some insecticide to the poorest farmers in each district. However due to the need to import pesticide and hard currency restrictions, it is only able to supply between 10-30% of the needs of these poor farmers during outbreak years (M. S. Marawita, MoA Tanzania, per comms). Increasingly farmers are encouraged to buy their own insecticides, with government support being provided only during emergencies. In surveys of 272 farmers in 9 regions of Tanzania only 30% sprayed chemicals and of those who did not spray, 94% said it was due to financial constraints (Scott, 1991). The common strategy for subsistence farmers is to wait for the outbreak to die off, often from natural NPV epidemics, then to replant. If the rains continue this can be effective in minimising losses. If the rains are short this can lead to the higher rates of reduced yields, while at the same time, extra seed may not be available, or if it is then the farmers often cannot afford to buy it. Thus highest yield losses due to armyworm tend to occur during drought periods when crop production is already poor and this exacerbates food shortages.

Armyworm can also have a damaging effect on livestock production because armyworm feeding continuously on grasses such as *Cynodon* spp. stimulates the production (cyanogenesis) of high levels of cyanide in the plant making such pastures unfit for cattle (Georgiadis & McNaughton, 1988). Normally cattle feed through light peripatetic grazing that does not stimulate cyanogenesis in pasture grasses. The indications are that this phenomenon is mainly a feature of drought years when pasture is already under stress and the consequences may be severe on already weakened livestock.

The high cost and limited availability of insecticide has also prevented the implementation of strategic control of primary outbreaks that often occur in small areas on low value rangeland. These foci of armyworm outbreaks later spread to larger areas of cereal crops. This project sought to reduce dependence on

environmentally damaging chemical insecticides and replace them with more acceptable and locally-produced alternatives by developing new, biorational technologies.

Research on Armyworm Control

An almost continuous programme of research concerned with the African armyworm has been underway in eastern Africa since 1962 (Iles, 1993). Because of the regional importance of this pest DFID and the EU have funded operational research at the Desert Locust Control Organisation for East Africa (DLCO.EA) under the regional armyworm programme (RAP) in Nairobi. This resulted in a strategic control strategy evolved from the development of monitoring and forecasting techniques. DFID, with the Government of Tanzania, also jointly funded the Armyworm Control Project (ACP) based at the Pest Control Services in Arusha and this reflected the importance of Tanzania as a breeding area and potential source of outbreaks for other countries (Iles *et al.*, 1987). As a component of the RAP, production and use of *Spex*NPV for armyworm control was investigated in both Kenya and the UK. This demonstrated the high pathogenicity and specificity of NPV to populations of E.African *S. exempta* in the laboratory, confirming results published by Odindo (1981). Parallel DFID-funded research (Project A0198) continued at NRI and included studies on cross infectivity of *S. exempta* NPV and also provided optimised NPV production parameters (Cherry *et al.*, 1994).

In 1992, the EC funded a six-month collaboration between DLCO.EA, International Institute Biological Control (IIBC) and NRI to investigate the feasibility of virus production in Kenya, which included the construction of a small virus production facility and insectary. An evaluation of this study concluded that development of virus for armyworm control in E. Africa should continue (Jones, 1992). The final review of the DLCO.EA RAP conducted by the Institute for Project Planning also endorsed the recommendation that a programme for the development of armyworm virus in Kenya be continued. Further support from the EC to pursue these recommendations was not forthcoming however and work was discontinued.

There are several examples of the large-scale use of insect viruses in developing countries. The largest of these is the use of NPV to control *Anticarsia gemmatalis* on soybean in Brazil. In 1998 nearly 2 million hectares of soybean were sprayed with NPV (Moscardi, 1999). During 1993-98 there was a programme to control the early season migration of Heliothine species *Heliothis zea* and *H. virescens* into cotton in the southern USA. This involved spraying 214,000 acres of wild hosts annually with *H. zea* NPV (Street *et al.*, 1998) to kill the over-wintering insects. In China, viruses were sprayed onto 16,000 ha to control *Pieris rapae* (Huber 1986) and the NPVs of *Spodoptera litura*, *Helicoverpa armigera* and *Mythimna separata* have been developed for use on several crops and local production established (Hunter-Fuijita *et al.*, 1998, J. M. Vlak pers. comms.).

Current armyworm control strategies rely on conventional chemical insecticides. Although many outbreaks go uncontrolled, at the farm level in Tanzania the government has previously met most of the costs of control (Iles *et al.*, 1987). Whilst

these have been shown to be effective they can lead to problems of environmental contamination and increased insect pest problems resulting from destruction of natural enemies. Alternative control measures that do not have these associated problems are desirable.

Demand for the project

A number of studies have underlined the need for armyworm control including those of Scott 1991, Cheke & Tucker 1995, Iles *et al.*, 1989). The potential for using SpexNPV as an alternative control agent was identified by Rose *et al.* (1995). A recent report by Dewhurst (1999) on the 1998/99 outbreaks in Eastern Africa and the Great Lakes Region underlined the continuing scale of the problem. It pointed out that with the exception of Ethiopia none of the affected countries (Tanzania, Kenya, Rwanda and Burundi) were able to run efficient control operations with existing control technologies. While much of this was due to poor monitoring, forecasting and financial constraints, the non-availability of locally produced cheap alternative controls is an important part of the problem.

Current strategic and local control relies entirely on chemical insecticides although these are considered inappropriate for outbreaks in ecologically sensitive regions such as pasture and nature reserves. In severe outbreaks pesticides may be provided by the state but otherwise the farmers buy them themselves. However, unavailability of materials is the most frequently cited reason for not treating outbreaks. Demand for alternative technologies is in evidence from many countries within the armyworm distribution range. Baculoviruses can be produced locally using sustainable materials at costs often well below those of conventional chemical insecticides

Project Purpose

The project was to develop, demonstrate and promote the use of baculoviruses as alternative insecticides for control of the African armyworm in Tanzania.

Additional agreed objectives.

Project amendment 1 17 November 97

To undertake an additional pre-season visit to Tanzania to ensure all field trial equipment and transport was ready and to liaise with collaborators to plan trials, brief district officers and train staff ready for the trials.

Project amendment 2 13 April 99

To conduct additional field trials of NPV on the armyworm outbreaks as these were the largest and most extensive since 1984.

To provide additional funds to maintain armyworm pheromone trap network used for forecasting outbreaks, this was previously the responsibility of CPP project R6762 (Decision tools to aid armyworm surveillance) that had finished on 31 March 1999.

Research Activities

Output 1 Specific polymerase chain reaction (PCR) test developed as diagnostic tool in armyworm virus detection.

Activity 1.1 Complementary PCR primers designed and ordered based on polyhedrin gene sequences.

It was proposed that in the first year of the project a protocol would be developed using the polymerase chain reaction (PCR) as a diagnostic tool to detect NPV infections in *S.exempta*. This would serve three purposes:

1. To rapidly screen the insect culture for the presence of active SpexNPV.
2. To detect successful infections in field treated insects.
3. To detect latent infections in field or laboratory cultures

It had originally been proposed to develop specific SpexNPV primers. After consideration it was decided to design a more general primer that would detect as many NPV isolates as possible. This would have the advantage of detecting infections in *S.exempta* caused by NPVs other than known SpexNPV. Experimentally it has been shown that this insect is susceptible to such cross-infections. (Andy Cherry, unpublished data). In addition the polyhedrin gene of NPV was found to be highly conserved (Zanotto *et al.*, 1993) and there is little data on other areas of the SpexNPV genome. The lack of information on genetic variability of SpexNPV found in the field would have made it difficult to test a specific primer against a range of isolates. Collecting, purifying and analysing these was likely to take several years and be very expensive. Other molecular options for specific detection were also considered to be too expensive.

A number of primers were designed using published sequence data (Zanotto *et al.*, 1993) and appropriate PCR protocols were developed. The most promising primers were then evaluated against a wider range of species and isolates.

Activity 1.2 Validation of SpexNPV primer pair.

The PCR test was evaluated using *S.exempta* infected with SpexNPV inocula and the larvae tested after infection in both symptomatic and early asymptomatic stages. To evaluate ability of the test to detect latent SpexNPV was more difficult as there was no direct test for latency to validate the PCR test against. However a culture was obtained that, while showing no symptoms of NPV normally, could be caused to

express SpexNPV and produce identifiable virus when subject to environmental stresses (overcrowding, heat or cold). The PCR test was therefore validated against this culture.

Output 2 Production and formulation of SpexNPV

Activity 2.1. *S. exempta* colony boosted ready to supply larvae for infection.

A small disease free-culture of *S.exempta* was available at NRI prior to starting the project. This was established in 1991-92 using insects originally from Tanzania. It was first necessary to build this up to produce the required 2-4,000 III instar larvae per week needed to support NPV production so that it was not until January 1997 that SpexNPV production commenced.

Activity 2.2 Mass production and formulation of SpexNPV at NRI.

The strain of SpexNPV used in this project was a wild type multiply-enveloped (containing more than one virus capsid within each virion envelope) strain had originally been isolated from Tanzania in 1974. This NPV is found naturally in armyworm outbreaks (McKinley, 1975) and the DNA had been physically characterised (Harrap *et al.*, 1977). Cross infectivity studies indicated that this strain was specific to *S.exempta* (McKinley *et al.*, 1981; A.C. Cherry unpublished). This strain had been safety tested following FAO/WHO recommended protocols and no evidence of toxicity in non-target hosts was found (Harris, 1973). This agrees with the findings from an extensive body of testing that NPVs do not infect vertebrates, plants or beneficial arthropods, and are very safe for humans and livestock (Groner,1990, Saik *et al.*, 1990, Black *et al.*, 1997). It was not known if this was the same strain investigated earlier by Odindo (1981 & 1983) as these studies had been carried out before cloning techniques and DNA REN analysis were in widespread use for characterising NPV strains.

It was the objective to produce enough SpexNPV for at least two years of field trials on up to 100 ha total area. As the expected maximum application rate, based upon data from other NPVs, was 1×10^{12} occlusion bodies (OB) of NPV per hectare this indicated a production target of 1×10^{14} OB in total or the equivalent to the NPV found in 50,000 infected armyworm larvae. The build up of the colony started in late 1996 and production commenced in January 1997. This continued from January to September 1997 though NPV production was not continuous over this period . The procedure for *in vivo* production were those detailed in the paper on NPV production in *S.exempta* by Cherry *et al.* (1997). Thus:

1. Infect larvae of a susceptible species at an appropriate age for maximum virus replication (*S.exempta*)
2. Grow infected larvae for a time to allow the infection to develop and the virus to replicate.
3. Harvest the larvae and extract the virus.

Larvae from the NRI colony reared on a mixture of artificial diet and wheat seedlings were reared in virus free conditions until they were 7 days old 30-50 mg III-IVth instar then transferred to the virus laboratory for production.

To produce the virus, artificial diet was first poured into 5 cm deep plastic production trays (30 x 60 cm). Enough diet was poured in to feed the larvae to the completion of larval life cycle. This diet was then sprayed with a dose of SpexNPV equivalent to 1×10^6 OB of SpexNPV per larvae then 500 larvae placed on the diet and left for a week at 26°C for the infection to develop and the NPV to multiply in the larvae. The trays were then opened and the successfully infected larvae removed and frozen. On average >90% of larvae became infected with NPV. However some larvae died prior to the harvesting date and as these cadavers became heavily contaminated with bacteria these larvae were not used for virus production. The production efficiency was therefore lower with 55% of the larvae being successfully harvested alive and infected. The colony was able to produce 2,000-6,000 suitably sized larvae per week for virus production.

The NPV produced was processed using standard protocols developed at NRI (McKinley *et al.*, 1989, Hunter-Fujita *et al.*, 1998). The insects were, after storage at -20°C, thawed out and macerated in 0.1% Sodium dodecyl sulphate to release the NPV. The suspension was then filtered through three-layer muslin to remove gross insect debris. The NPV suspension was then concentrated by centrifuging at x2500g in 750 ml buckets at 4° C for 10 minutes. This step was repeated twice more to purify the NPV before suspending in 1% SDS and storing at -20°C to await formulation.

Quality control of NPV

The SpexNPV OB content was quantified by microscopic examination counting in a haemocytometer under phase contrast at x400 (Wigley, 1980). The identity and purity of the progeny virus was confirmed using restriction endonuclease analysis on the viral DNA (Smith & Summers, 1978). The DNA was extracted using an adaptation of the protocol of Smith & Crook (1988) and restriction fragments were obtained using *Pst* I, *Bam* HI, *Hind* III and *Eco* RI enzymes. To visualise the restriction patterns the cut DNA was run overnight on a 0.6% agarose gel at 35 volts and photographed with an MP4 camera.

Bioassay method for determining LC₅₀ values in neonate larvae

To determine the activity of the SpexNPV produced, the standard assay measures of LD₅₀ and LC₅₀ were determined. LC₅₀ is normally used for newly hatched, neonate larvae (<1mg), to which it is difficult to give precise doses needed for LD₅₀ estimation but for larger (30-50 mg weight) III instars LD₅₀ can be determined directly.

For estimating LC₅₀ values in neonate *S. exempta* larvae a standard droplet dosing technique of bioassaying microbial insecticides was used (Hughes & Wood 1981). Dose series of the SpexNPV suspension from 5.7×10^6 OB/ml to 9.12×10^3 OB/ml in five fold dilutions were prepared with each dilution being assayed in fifty neonate *S. exempta* larvae. Treated larvae were held on artificial diet and daily assessments were

carried out for seven days in order to produce mortality over time graphs. Probit analysis using SPSS was performed on day 7 mortality data generating LC₅₀ values as an indication of virus potency.

Bioassay method for determining LD₅₀ values in III instar larvae

To determine the LD₅₀ values of SpexNPV in III instar larvae an artificial diet plug dose bioassay method and a leaf dip bioassay method were used. For both methods, dose series of SpexNPV were prepared and a precise dose delivered to each test *S. exempta* larva via the diet plug or leaf. For each dose tested, 30 III instar larvae were treated and reared on artificial diet for 7 days post dosing. Daily assessments of mortality were made and LD₅₀ values prepared in SPSS. Replicated dose-mortality bioassays were conducted against healthy *S. exempta* larvae to monitor pathogenicity over time and to assist estimation of field dose rates. The assays were carried out on neonate and third instar larvae using standard methods (Shapiro & Evans, 1997). Dose-mortality data was analysed using the SPSS probit analysis routine.

Once the activity of batches of SpexNPV was determined and its identity confirmed specific formulations were produced blending different batches to give the desired standard activity.

Method of SpexNPV formulation for use in field trials on armyworm in Tanzania

A water-based and an oil-based formulation were tested during the field trials. Before formulation could be done, the infected larvae had to be processed to release the SpexNPV produced during the first year of the project and standard procedures as set out in Hunter and Fujita (1998) were followed. Infected larvae were collected in four batches of approximately 12,500 fully infected larvae. Each batch was then macerated to form a homogenate, which was then filtered through butter muslin to remove coarse particles of insect debris. The initial stage of the formulation process was to spin-down virus particles of the homogenate using a standard laboratory centrifuge set at 6000 rpm. The concentrated SpexNPV was then freeze-dried over 24 hours to produce a dry "cake" of virus, which could then be formulated as required.

For the water-based formulation the freeze-dried cake was milled using a dry-powder mill to create a fine, evenly mixed NPV powder. A sub-sample of the powder was then re-suspended in water and counted for NPV concentration before the bulk of NPV powder was aliquoted ready for use in the field trial. To apply the virus the pre-weighed aliquots for each dose were mixed with water and the wetting agent, Triton X100, before being sprayed with a lever-operated knapsack sprayer.

For the oil-based formulation the freeze-dried cake was milled using a wet-mill. The powder was milled with peanut oil in a ratio of 1g of powder to 10mls of oil using a milling time previously calibrated to provide even mixing. NPV content was determined by calculation of NPV content per gram of powder diluted in peanut oil. Aliquots of NPV in oil were then measured ready for use in the field trial. At the time of application the NPV aliquots were mixed with a blend of peanut oil and paraffin oil

in a ratio of 70:30 (peanut:paraffin) and applied using an ultralow volume (ULV) sprayer.

Activity 2.3 Transfer NPV to target country(s).

In early 1997 a full set of safety data on the SpexNPV was presented to the Tanzanian Ministry of Agriculture (MoA) for approval by the MoA was essential before field trials could be conducted. Project personnel made a presentation to the sub-committee of the Plant Protection Advisory Committee in March and permission to import and use the NPV in trials was granted in October 1997.

Output 3 Replicated field trials to assess, demonstrate and promote the efficacy of NPV for armyworm

Activity 3.1 Glasshouse trials of SpexNPV.

It had been intended to start initial work using field cages in Tanzania during the 1998 field season (Jan-May) using artificially-induced outbreaks but despite two visits to Tanzania during the season no outbreaks were located to provide insects for trials that year. It was therefore decided, with the agreement of the CPP, to substitute glasshouse trials of the SpexNPV at NRI to test application systems, formulations and application rates. These were carried out during September-November 1998.

The trial consisted of two application methods delivering varying rates of active ingredient as shown in Table 1. High volume treatments were water-based and made up of NPV dry powder mixed with 0.01% v/v Triton X100 and applied using a hand-held, non-pressure retaining, compression sprayer fitted with a solid cone nozzle. ULV treatments were oil-based and consisted of NPV dry powder blended with a mix of peanut and paraffin oil in a ratio of 30:70 applied using an ULVA Fan battery-operated ULV, spinning-disk sprayer.

Table 1. Details of glasshouse NPV trial treatments

Application method	Formulation & treatment	Application rate (OB/ha)	Application volume equivalent (litres/ha)
High volume hydraulic sprayer	Control	0.00	200
	Water based	5.0×10^{11}	200
	Water based	1.0×10^{12}	200
	Water based	5.0×10^{12}	200
Spinning disc ULV sprayer	Control	0.00	2
	Oil based	5.0×10^{11}	2
	Oil based	1.0×10^{12}	2
	Oil based	5.0×10^{12}	2

OB = viring occlusion bodies

Plots of 5.0m x 2.5m were set up in a 20.0m x 6.0m polytunnel at NRI, and in an attempt to mimic field conditions, trays of 7 day old wheat seedlings were placed strategically throughout the plots. The virus was applied to the plots at each of the application rates for both application methods. Spray monitoring was carried out via bioassay of sprayed wheat seedlings and analysis of droplets falling onto strategically placed water and oil-sensitive paper. The trial was repeated with two different larval stages of armyworm, late third instar and mid fourth instar.

Bioassays were carried out by cutting treated wheat seedlings and placing them into clear plastic boxes on top of filter paper and sterilised vermiculite. Each treatment was kept separate using one box per treatment. Fifty larvae of the appropriate instar were then placed in each box and allowed to feed at will. As wheat was eaten it was replaced by fresh, virus-treated seedlings so that feeding was not interrupted. After seven days, by which time larvae had either pupated or succumbed to virus infection, mortality was recorded to check efficacy of each treatment.

Activity 3.2 Field trials against identified natural outbreaks.

It was planned to conduct two sets of field trials during the armyworm outbreak seasons 1997-98 and 1998-99, however no significant outbreaks occurred in 1997-98 so the first field trials were conducted in 1998-99. In an attempt to get the required second season of trials it was decided, with the agreement of the CPP, to replace the planned final project workshop, the main activity in 1999-2000 with another field trial. However, despite a visit extending into the end of April 2000 there was still no outbreak that year so that the only field trial data came from the two sets of trials run during the 1998-99 season.

It was determined prior to the project that Tanzania is the most likely country to be attacked in Africa with outbreaks occurring in 77% of the years from 1930-88 (Rose *et al.*, 1997). The absence of outbreaks in two of the three years of the project could therefore be a chance result but it is also possible that global climate change has tended to increase the uncertainty in rainfall patterns over eastern Africa which may have altered the frequency and severity of armyworm outbreaks. While in 1997-98 and 1999-2000 no outbreaks occurred, the outbreak in 1998-99 was a major one being the most severe since 1984 (Dewhurst, 1999).

1998-99 Season field trials

On the 22 February 1999, the armyworm-monitoring network of pheromone traps across Tanzania indicated the first armyworm activity of the new season. Arusha Pest Control Services successfully forecast large primary outbreaks would occur in the Kilosa district of Tanzania. Preparations were made to travel immediately from the UK to the area from the UK to carry out field trials with a team of collaborative scientists from NRI, CABI Africa Regional Centre (CABI-ARC), Arusha Pest Control Services (PCS) and the Ilonga Agricultural Research Institute (IARI), Kilosa. On arriving in Kilosa a plan was drawn-up to complete dose rate field trials of SpexNPV against populations of third instar larvae feeding on pasture land. Trials

compared different doses and efficacy of water-based lever-operated knapsack application with oil-based ULV application of SpexNPV on small-scale experimental plots.

Repeated trials of NPV were carried out successfully against the target pest. Infestations occurred in an area of several hundred square kilometres extending westwards from the region of Morogoro to Kilosa and Dodoma in central Tanzania. Subsequent generations of armyworm caused severe outbreaks in many areas of Tanzania on this migration route. This enabled two further trials in the Arusha area north of Kilosa.

Location, crop and layout of trials

Kilosa

Kilosa is approximately 700km (by road) south of Arusha and 250km inland from Dar es Salaam. Five replicates of the trial were attempted at Kilosa and each replicate was carried out on previously untreated pastureland. Each replicated site was divided into suitable plots of land for spraying. Treatments were allocated to plots ensuring suitably sized (10 m) buffer zones between plots and it was made certain that no land down-wind of ULV plots was used to avoid long-drift contamination. Plot size for each treatment was 10m by 10m (100m²) for the lever operated knapsack (LOK) applications and 10m x 20m (200m²) for the ULV applications. ULV plots were larger in order to allow for the cumulative effect of ULV spinning disk application but larvae were sampled from an equal sized area to the LOK plots. The treatments in the trial consisted of ULV (oil-based) application and HV (water-based) methods delivering varying rates of active ingredient.

Listed below are the trial treatments (Table 2). High-volume treatments were water-based and consisted of NPV freeze-dried powder mixed with 0.01% v/v Triton X100 applied using a LOK sprayer. Ultra-low volume treatments were oil-based and consisted of NPV dry powder blended with a mix of peanut oil and paraffin oil in a ratio of 30:70 and applied with a Micro Ulva, spinning disk sprayer. The mixture of carrier oils followed formulation recommendations formally laid down by the (Lutte Biologique contre les locustes et Sauteriaux) LUBILOSA project for application of entomopathogenic fungal formulations. Actual application volumes per plot required for each treatment were 40ml for ULV and 2000ml for the LOK treatments.

Table 2. Treatments included in the Kilosa field trials

METHOD	FORMULATION & TREATMENT	NPV APPLICATION RATE (OB/ha)	APPLICATION VOLUME (Litres/ha)
High volume LOK	Water control	0.00	200
High volume LOK	Water-based low dose	1.0×10^{11}	200
High volume LOK	Water-based medium dose	5.0×10^{11}	200
High volume LOK	Water-based high dose	1.0×10^{12}	200
High volume LOK	Water-based very high dose	5.0×10^{12}	200
Ultra low volume	Oil control	0.00	2
Ultra low volume	Oil-based low dose	1.0×10^{11}	2
Ultra low volume	Oil-based medium dose	5.0×10^{11}	2
Ultra low volume	Oil-based high dose	1.0×10^{12}	2
Ultra low volume	Oil-based very high dose	5.0×10^{12}	2

OB = virus occlusion bodies

Each treatment was applied following good spraying practice guidelines set out by IPARC (Matthews, 1992). Before spraying commenced, all equipment was calibrated to ensure accurate application of NPV and spray procedures followed the standard practice for each type of sprayer. Meteorological conditions were monitored and recorded throughout the spray procedures.

Spray monitoring was carried out through bioassays of treated vegetation and analysis of strategically placed, droplet counting, water and oil-sensitive paper. Outbreaks of suitable size armyworm were identified before the land was marked out and each treatment was allocated to a marked plot. Spraying commenced early in the morning once all plots had been marked and the area was left until late afternoon to allow natural feeding by the larvae. The bioassays were monitored over the next few days until all larvae were dead or had pupated.

For the bioassays, larvae and sprayed vegetation were collected in the late afternoon of spraying and placed into bowls to allow monitoring of mortality. For each treatment, 100 second instar larvae were collected at each trial site and fed on treated vegetation for the following 24 hours. Bowls were covered by muslin, secured by use of elastic bands. For the remainder of the bioassay fresh, unsprayed grass was fed to the larvae. The bioassays were monitored until all larvae were dead or had pupated.

Arusha, Pest Control Services (PCS) station

Trials were conducted in two different areas in the Arusha region. The first site was on grassland around the PCS and meteorological station at Tengeru and was conducted between the 8/4/99 to 15/4/99. From the results of the trial held at Kilosa it was decided to use one single application technique (high volume, water-based LOK) and only two dose rates, low and medium.

Plot sizes at Tengeru were 15m x 10m and 20m x 10m and the application volume was 200 l and four repetitions of each treatment were carried out. The different sized plots were shared equally amongst the two treatments. Heavy rainfall occurred that night after application of treatments therefore all treatments were reapplied the following day. The trial monitoring did not include bioassays for this trial. Instead, larvae were allowed to feed freely on the sprayed plots for the duration of the trial and post-spray monitoring of larval death was by 0.5m x 0.5m quadrat counts of caterpillars. The sprayed plots were monitored for four days, starting from the fourth day after application of NPV as NPV is known to take from 4-7 days to kill.

Arusha, M'ringa Estate Coffee Plantation

This was the second trial site in Arusha and the trial ran from 18/4/99 to 24/04/99. The location identified for the field trials was a private coffee estate, M'ringa Estate, about 8km West of Arusha, which had a crop of barley covering several hectares as well as the coffee crop. The barley was under attack by armyworm so permission to carry out trials on the crop was granted by the Farm Manager, Mr Donough John Mahon. The barley was a commercial crop, therefore an agreement was made that although the decision would be held off for as long as possible, a chemical application would be made if the farm manager thought it was necessary.

The trial was set up as a large-scale trial with plots of 0.5ha for each replicate of the treatments. Three fields of barley, which was about 16cm tall, were identified for the trials and plots were marked out with string. Initial samples of larval densities were made on 16/3/99 using 50cm x 50cm quadrats. The plots were sprayed using LOK sprayers and the water used for all trials was from the farm borehole.

Due to the level of success seen with previous application rates of low (1.0×10^{11} OB/ha) and medium (5.0×10^{11} OB/ha) dose rates, the decision was made to reduce dose levels further, thus finding the dose level at which the system would fail. Volume application rates remained the same as did the simple formulation of freeze-dried NPV powder in water and Triton. Table 3 below gives details of dose rates used.

Table 3. Treatment details of the Arusha large-scale field trial

Actual dose received (OB/ha)	Area treated (hectares)
3.60×10^{10}	0.5
2.50×10^{10}	0.5
4.10×10^{10}	0.5
1.15×10^{11}	0.5
2.00×10^{11}	0.5
2.25×10^{11}	0.5
1.59×10^{11}	0.59

Applications of all reps of the treatments were made on a single day (18 April 1999) and the larvae were left to feed naturally. Rainfall occurred on the evening following treatment application but further spraying the next day was not possible due to NPV stocks having been exhausted.

Monitoring of the trial was carried out in two ways. Bioassays of larvae from each plot were conducted in a similar way to those done at Kilosa, although larvae were left to feed on the sprayed crop for two, three or four days before being collected, and field monitoring of larval numbers was done using 50cm x 50cm quadrats. To improve the accuracy of sampling larvae, as the crop was planted in rows, the number of plants growing within each quadrat were counted, followed by the number of armyworm larvae on those plants. As moths were continually laying eggs on plants, hatching larvae were counted on day 1 and thereafter new hatchlings were ignored, counting only the larger larvae. Plots were monitored and rainfall data collected for six days until 24 April, at which point the farm manager insisted on spraying chemical insecticide. The mortality was assessed in larval collections until the 29 April.

Activity 3.1 Workshop for key personnel from target country

It had been an original objective of the project that the main output of the last year was to hold a workshop in Africa to disseminate the results and promote the new technology during the last year of the project. However this output was, with the agreement of the CPP, dropped in favour of using the last year to organise additional field trials to complete the original field programme the project had not been able to run in 1998 due to the absence of armyworm outbreaks. Also in 1999 the CPP's sponsorship of the "Migrant Pests of agriculture in Southern Africa" conference had provided a more comprehensive forum for disseminating the results and Mr

Mushobozi of PCS and Dr K Jones of NRI attended to present the results of the project activities.

Outputs

Output 1 PCR test developed as diagnostic tool in armyworm virus detection

Polymerase chain reaction (PCR) primers for detecting SpexNPV based upon the structural polyhedrin gene for the virus coat were successfully developed. After testing combinations of upstream and downstream primers, two were chosen flanking a 298 bp sequence on the polyhedrin gene. An annealing temperature of 60°C produced an amplification fragment for all samples known to contain NPV, confirming the presence of the viral genome. The primers were shown to be able to detect active infections in insects from early to late instars before any symptoms of infection were visible.

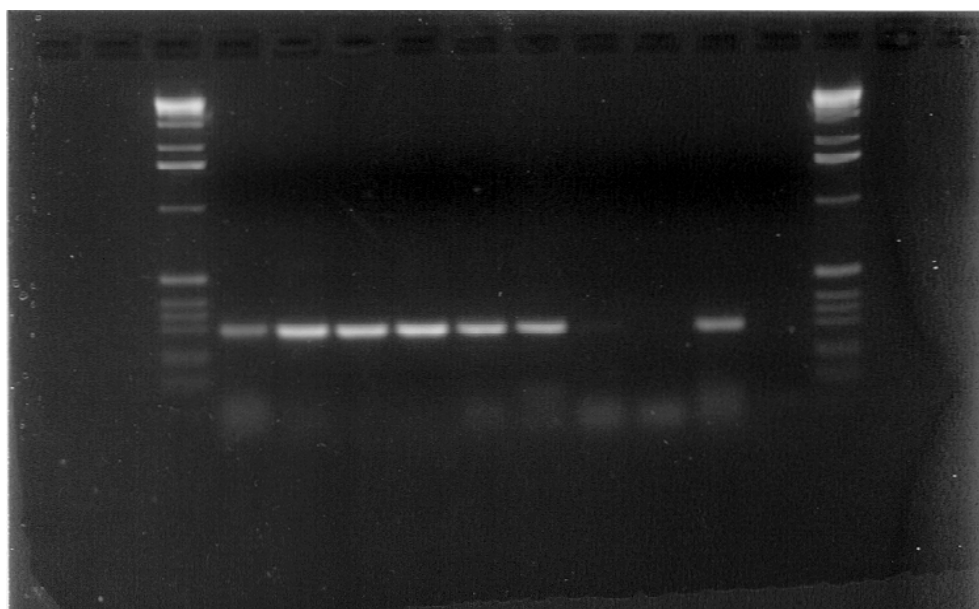


Fig 1 Results of PCR on DNA from different insect viruses. Lanes 1 & 12 Molecular ladder markers. Lane 2 *S.littoralis* NPV, Lane 3 *Helicoverpa.armigera* NPV, Lane 4 *S.exigua* NPV, Lane 5 *S.exempta* NPV, Lane 6 *Autographa californica* NPV, Lane 7 *Bombyx mori* NPV Lane 8 *S.littoralis* GV, Lane 9 *H.armigera* GV, Lane 10 *Pieris brassicae* GV, Lane 11 Control no DNA or marker.

The gel picture in Fig 1 shows the PCR products obtained from DNA extracted from six known species of NPV. Three of the DNA samples were of another closely-related genus of baculoviruses, the granuloviruses (GV), included as negative controls. The marker gives a positive result to all NPVs tested. It can be seen that a positive result was also obtained from the *Pieris brassicae* GV sample. Subsequent examination by microscopy of this pure “GV” samples confirmed that it was indeed contaminated with traces of NPV. This and other gels confirmed that the NPV PCR test is indeed capable of rapidly detecting NPV even in the presence of large amounts

of similar viruses. The test though cannot distinguish NPV specific to *S.exempta* from other NPVs so that to identify virus to strain level restriction enzyme analysis will still be needed.

This PCR test can now be used for rapid diagnosis of NPV in insects so that field samples of sprayed larvae can be screened for successful infection by NPV. In practice though a trained insect pathologist can identify infections in larvae that have died of NPV more easily using traditional microscopy and staining techniques. The advantage of the PCR test is that it can provide useful confirmation for less experienced staff or in cases where the diagnostic viral occlusion bodies are few or absent, e.g. early in infection, in very small larvae or as in the samples of contaminated GV. As such its main role is probably as a diagnostic confirmatory technique or in quality control. It can also be used to screen rapidly, eggs or diet ingredients to test that they are free of SpexNPV infective particles to assist in maintaining clean cultures for experiments, or in bioassays or in virus production where the aim is to produce a specific NPV strain.

Latent NPV detection

It was planned to use this technique to study the occurrence of latent non-symptomatic SpexNPV. It had long been surmised that SpexNPV was capable of existing in the armyworm in a non-symptomatic form that transmitted from generation to generation. The ability to detect latent virus in single larvae or eggs would enable quantitative studies of latent virus to be carried out in the field as well as being a useful tool in monitoring the possible contamination of insect cultures being used for production.

This technique had previously been reported as successfully identifying latent virus infections in *Mamestra brassicae* (Hughes *et al.*, 1993, Hughes *et al.*, 1997). However that study involved pooling large samples of eggs, larvae or adults and extracting and purifying the DNA prior to PCR. The technique would not be feasible to use for screening eggs or single field collected insects such as would be needed for epidemiological studies, so that in this study a single insect rapid DNA extraction technique was used.

When tested against *S.exempta* insect lines showing the characteristics of latent virus infection the PCR technique did not give a positive result for DNA extracted from a single larva. It is possible that the latency mechanism in MbNPV is different to that of SpexNPV so that the Hughes *et al.* (1993) PCR technique is not appropriate for *S.exempta*. More probably the rapid DNA extraction technique, as used here, was inadequate for successful PCR diagnosis of latent NPV infections. Since this work was completed, we have developed improved viral DNA extraction techniques as part of other projects and it is possible that these would be more effective if used with this technique.

It has since been shown by the Baculovirus Ecology group at the NERC Centre for Ecology and Hydrology at Oxford, that a modified technique, reverse transcriptase PCR (RT-PCR), is capable of identifying latent NPV infections by another baculovirus, *Plodia interpunctella* granulovirus (PiGV) in single insects. This may be

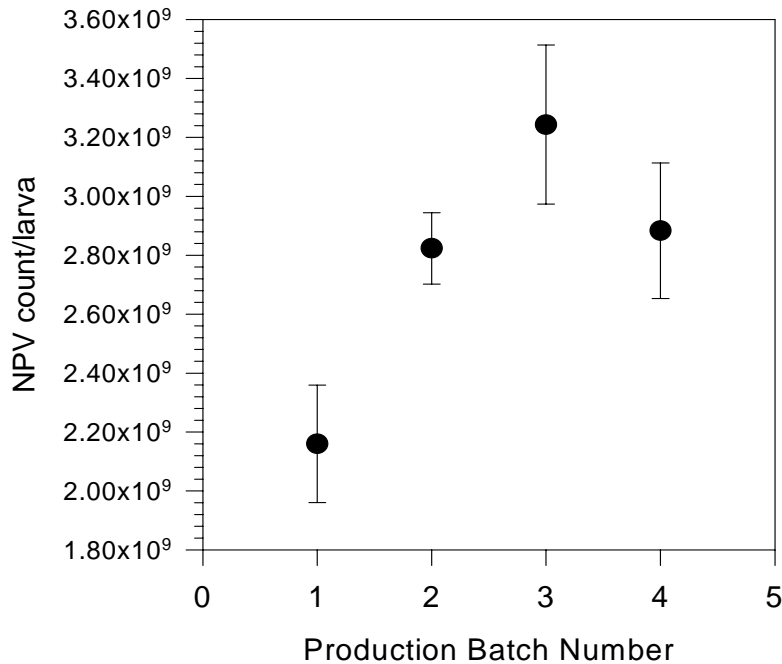
a more promising approach for studying the occurrence of latent SpexNPV. Dr Cory of the Oxford CEH group will be collaborating on the proposed follow-on project to assess the use of both PCR techniques to study SpexNPV latency in armyworm populations in the field in Tanzania.

Output 2 Production and formulation of SpexNPV

A large stock of SpexNPV equivalent to that in 63,000 infected insects was produced at NRI during the first year of the project to provide material sufficient for the planned two years of field trials. To achieve this some 115,000 *S.exempta* larvae were used for production indicating 55% efficiency. The virus productivity overall was at 2.7×10^9 OB per insect. The SpexNPV produced was processed in four batches of approximately 15,000 insects and each batch was analysed separately to quantify production efficiency. From the virus counts of each batch (Fig 2) it can be seen that the production technique improved progressively from the first batch (2.16×10^9 OB/larva) to the second (2.82×10^9 OB/larva), third (3.25×10^9 OB/larva) and fourth (2.88×10^9 OB/larva) batches.

This was better than expected from pre-project research and the production work on SpexNPV was published as a paper (Cherry *et al.*, 1997). The production work which was the main activity in year one showed that it is practicable to produce large amounts of NPV in an *in vivo* production system similar the those already used for commercial biopesticide production in India and Thailand (Grzywacz & Warburton, 1999). The productivity of SpexNPV at 2.7×10^9 OB is similar to that for other commercially produced viruses such as *S.exigua* NPV (SpeiNPV, 2.4×10^9 OB of virus per insect) though lower than that for *S.litura* NPV (SpltNPV, 6.7×10^9 OB of virus per insect). As both of these NPVs are already produced commercially in India or Thailand it is probable that SpexNPV could be produced at a unit cost between that of SpeiNPV and SpltNPV.

Fig 2. Production efficiency of SeNPV in *S. exempta* larvae.



The activity of the SpexNPV evaluated in bioassays is given in Table 4

Table 4. Laboratory bioassays. LC₅₀ and LD₅₀ values of SpexNPV in *S. exempta* larvae.

Larval Instar	LD/LC ₅₀ value	FD Limits
Neonate	8.81x10 ⁴ OB/ml	6.19x10 ⁴ – 1.19x10 ⁵
III Instar on artificial diet	6.50x10 ⁵ OB/larva	4.90x10 ⁵ – 1.10x10 ⁶
III Instar on wheat	3.16x10 ⁴ OB/larva	2.09x10 ⁴ – 5.17x10 ⁴

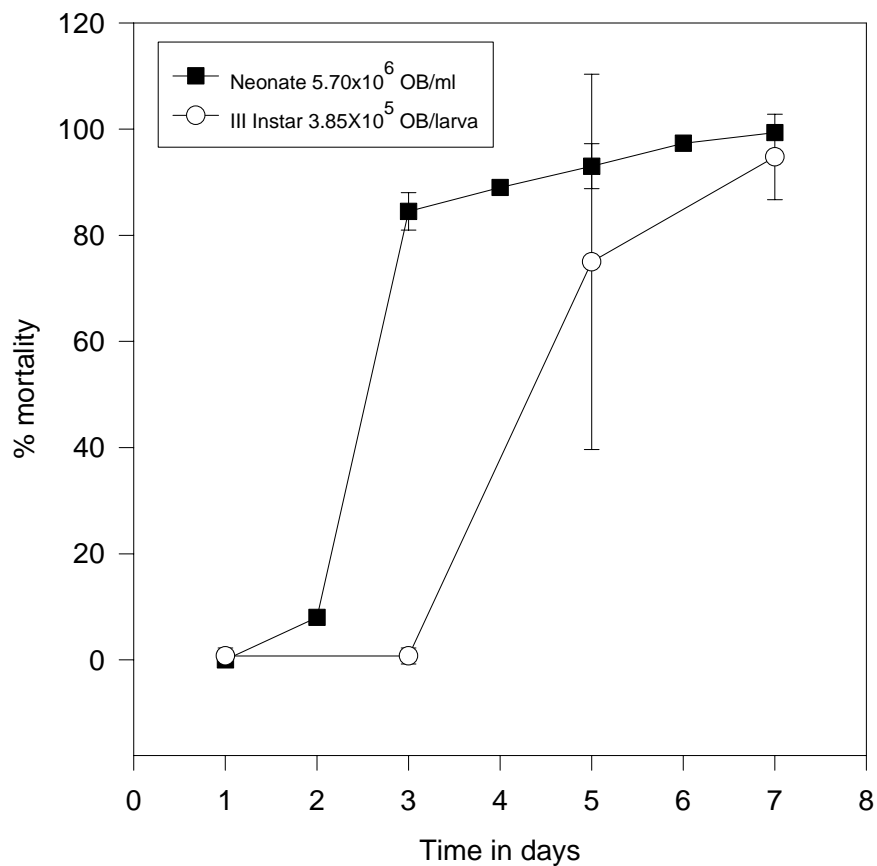
The results for LD₅₀ on this strain of SpexNPV may be compared to those previously obtained by Odindo (1981). Using larvae of 15.8 ± 14.7 mg and a leaf bioassay an LD₅₀ of 48.4 OB per larvae was reported. Although it is notoriously difficult to compare bioassays carried out on the same pathogen/host in different laboratories at different times, and differences of two-three orders of magnitude between different replicate assays and different laboratories are reported (Burges, 1967, Hunter-Fujita *et al.*, 1998) this data does suggest that the strain studied by Odindo could be

significantly more active than the strain used in this project. Unfortunately in the Odindo study no DNA characterisation was included so it is impossible to determine if the two isolates were in fact genetically different. As yet there has been no comprehensive collection of infected larvae from the field to enable studies of the genetic diversity of SpexNPV to be carried out. It is likely however, that much material exists in various insect collections from which viable NPV could be extracted. It has certainly been established in other NPVs e.g. *Heliothis* NPV developed as commercial biopesticides, that wide variation in the genotype exists and that selecting the most effective strain is an important part of developing a successful biopesticide (Lysansky, 1997).

The main use of the LD₅₀ data will be to assess the standard activity of any future batches of SpexNPV produced as part of standard quality control procedures. This LD₅₀ data can also be used in helping to determining the parameters of a successful application when combined with field data on larval age, food consumption and temperature when used as part of the “control window” approach (Evans, 1994). This approach is not yet widely employed in practice except in a few cases where NPV has been used against lepidopteran pests in temperate forests (Entwistle *et al.*, 1990). However it is the intention in the proposed follow-on project to evaluate this approach and determine its viability in the control of *S.exempta* in tropical annual crop and rangeland ecosystems.

These bioassays also showed an interesting effect of larval age on the speed of action. In Figure 3 it can be seen that at the highest dose 80% of mortality in neonate larvae occurred by day three whereas in III instar larvae mortality in treated larvae appeared only after day three and 100% mortality was not reached until seven days after treatment. This may indicate that treating armyworm outbreaks earlier may not only require less NPV but the more rapid action of the NPV on younger larvae may further reduce crop damage.

Fig 3. Laboratory bioassays, mortality over time after dosing with SeNPV. Response to top dose of neonate and III instar *S.exempta* larvae



Output 3 Replicated field trials to assess, demonstrate and promote the efficacy of NPV for armyworm

Output 3.1 Glasshouse trials of SpexNPV

The following figures show the results of mortality against treatment graphically for both larval stages.

Figure 4 Mortality of third instar *S.exempta* larvae sprayed with different rates of SpexNPV using high volume application.

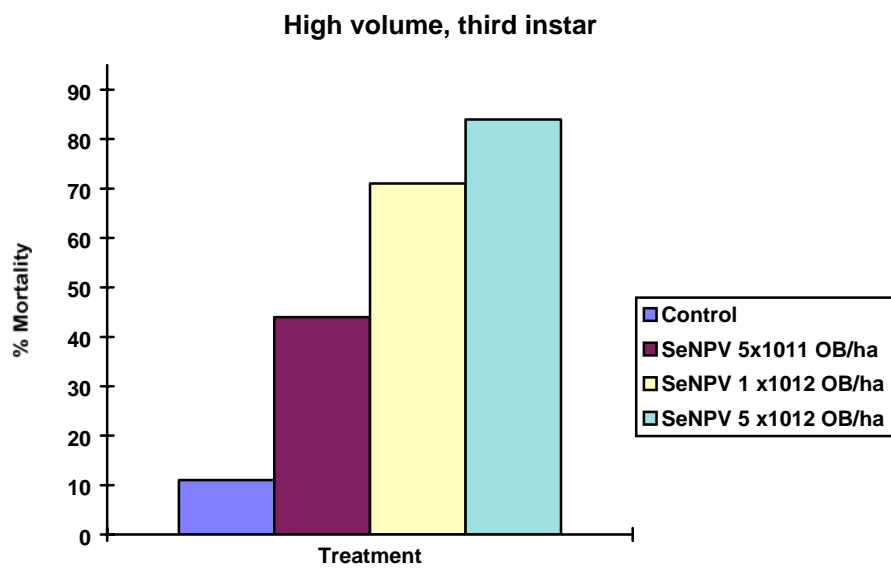
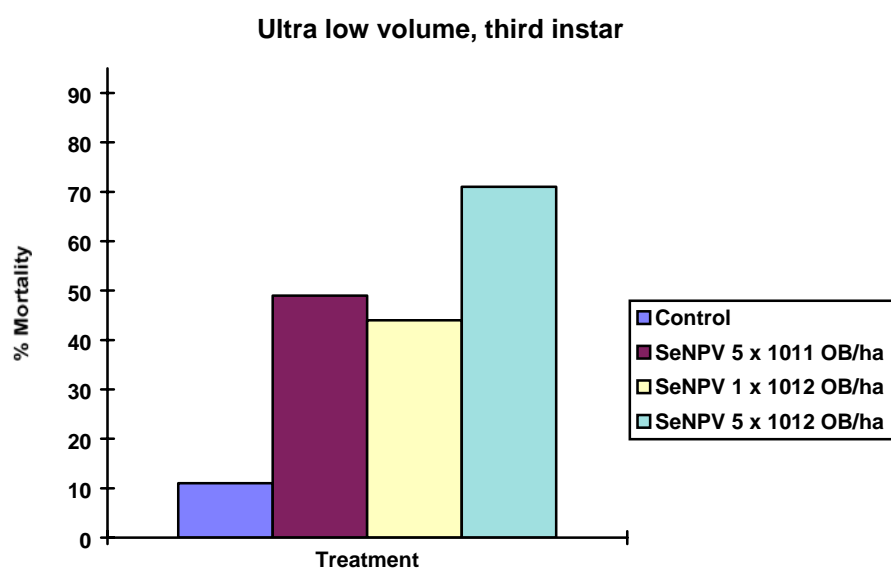


Figure 5 Mortality of third instar *S.exempta* larvae sprayed with different rates of SpexNPV using Ultra low volume application



The data in Figures 4 and 5 show that efficacy of control of SpexNPV against III instar *S.exempta*. The results show that the NPV kills larvae and that for HV the response appears dose dependent.

Control of third instar larvae therefore appears feasible at application rates of 1.0×10^{12} and 5.0×10^{12} OB/ha when using HV application and at 5.0×10^{12} OB/ha when using ULV application.

The finding that HV application is more effective than ULV was a surprise and could be important. Small farmers treating cereal crops use cheap HV backpack sprayers so the finding that SpexNPV is effective delivered as a simple aqueous solution through these is encouraging. However any area wide campaign for strategic control of primary outbreaks would rely on ULV application either vehicle or aircraft mounted. It has been accepted wisdom that ULV sprayers are more effective than HV sprayers at applying microbials and recent results with another NPV found this to be the case in cotton (Parnell *et al.*, 1998).

There is however some reason to suspect that these glasshouse trials under estimated the efficacy of ULV application. This application system relies on wind velocity of at least 1m per second to ensure that the very small droplets they produce (typically microns in diameter) impact onto the crop. In these glasshouse trials there was effectively no wind so that much of the dose applied probably did not impact onto the target crop so that the effective application rate was probably much less than the nominal rate. In comparison with HV application, large droplets are produced and gravity alone is sufficient to ensure good deposition on the crop targets.

In no treatment did mortality exceed 84% and it is a cause for concern that at no rate was there a 100% kill. However it is the intention to spray armyworm outbreaks even earlier than third instar and as younger instars are very much more susceptible, then it is conceivable that higher rates of mortality could be achieved in practice.

Figure 6 Mortality of fourth instar *S.exempta* larvae sprayed with different rates of SpexNPV using high volume application

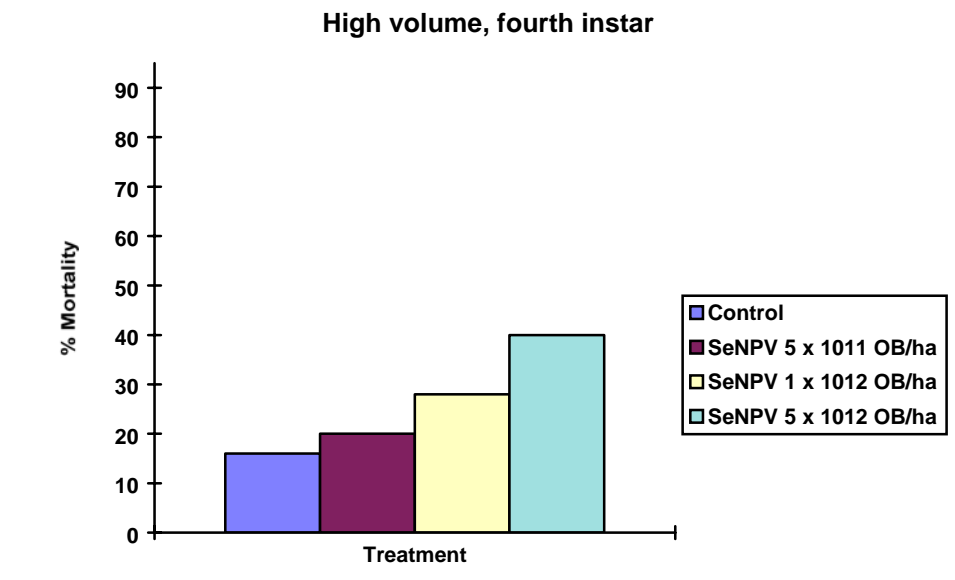
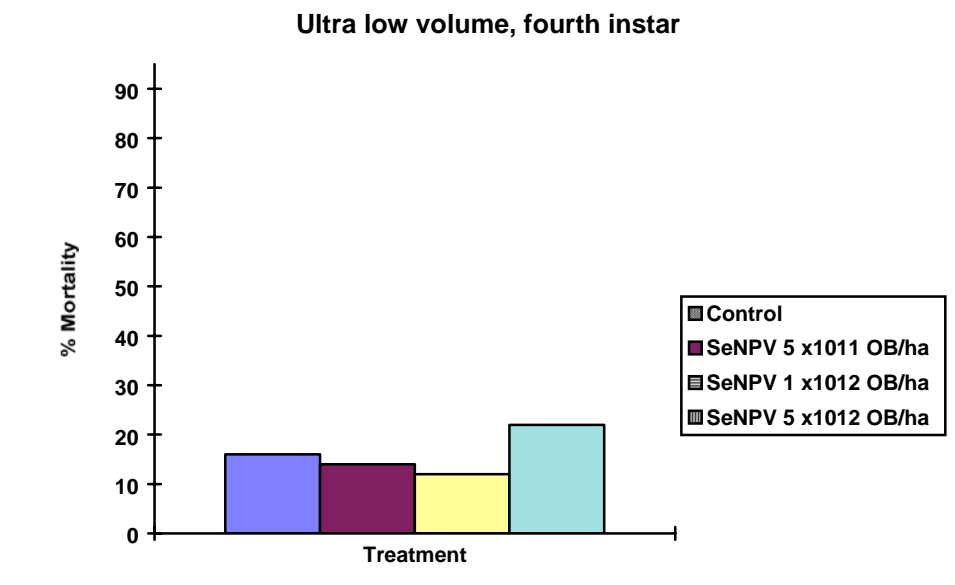


Figure 7 Mortality of fourth instar *S.exempta* larvae sprayed with different rates of SpexNPV using Ultra low volume application



The results in Figure 6 and 7 show that with fourth instar larvae even at the highest dose rate of 5.0×10^{12} OB/ha, a maximum of only 40% larval mortality was achieved in bioassays from the HV treatment. This indicates that control of older fourth instar larvae is unlikely to be effective for either application method. Again with HV application there are indications of a dose related effect but not for the ULV application.

Larval age and instar not only effects the proportion of insects killed at a given application rate of NPV it also influences the speed of kill. The data here (Fig 8) shows that with III instar larvae mortality starts at day four and is complete by day 5 at all spraying rates. When the larger IV instars are sprayed (Fig 9) the NPV is slower to kill with few deaths before day 5 and mortality continuing to rise steadily up to seven days after spraying.

Fig 8. Glasshouse trials of SpexNPV. Mortality against time for III instar *S. exempta* larvae

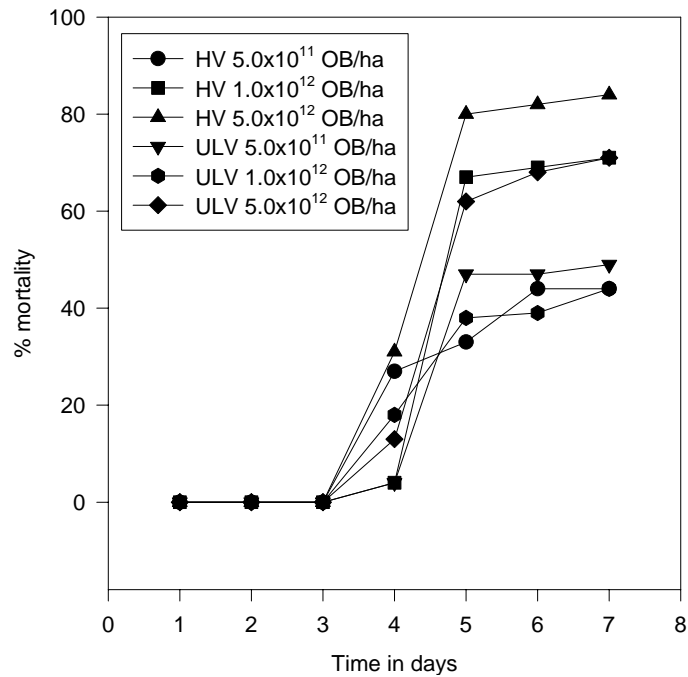
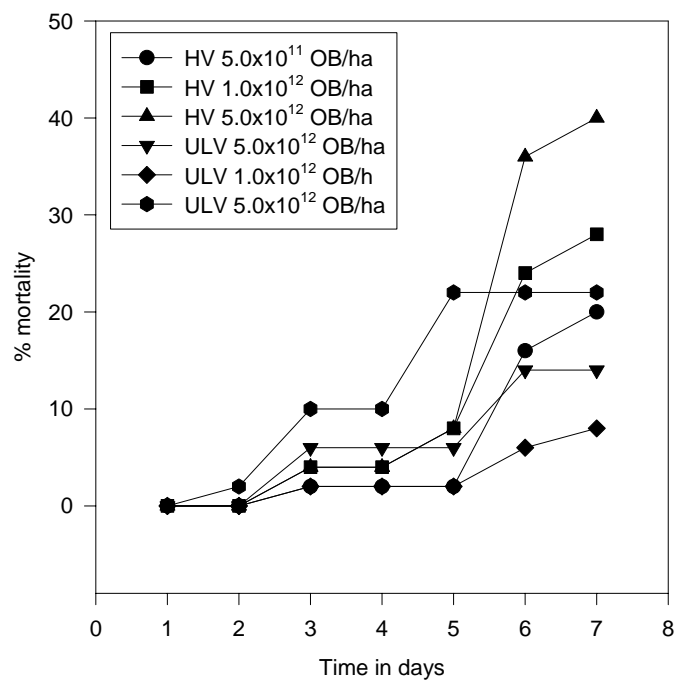


Fig 9. Glasshouse trials of SpexNPV. Mortality against time for IV instar *S. exempta* larvae



The conclusions from these first experiments were that:

- the SpexNPV application needed to target third instars or younger larvae if control was to be effective and not require uneconomically high application rates e.g. greater than 5×10^{12} OB/ha.
- simple HV knapsack sprayers as used by poor farmers are effective at applying NPV.

Output 3.2 Field trials in Tanzania

Kilosa field trials

High numbers of larvae in infested areas characterised the outbreaks of armyworm in Northern Tanzania in 1999. At the Kilosa field site the density of larvae was very high with up to 1160 larvae/m² counted on pastureland (Fig 10). In a nearby rice paddy, counts of larvae per rice plant reached a maximum of 32 fourth or fifth instar larvae per plant. At the Arusha site larval density was lower at an average of 51 larvae/m². Figure 10 illustrates the level of infestation seen at the Kilosa site.

Fig 10. Density of *S.exempta* larvae in the outbreak at Kilosa

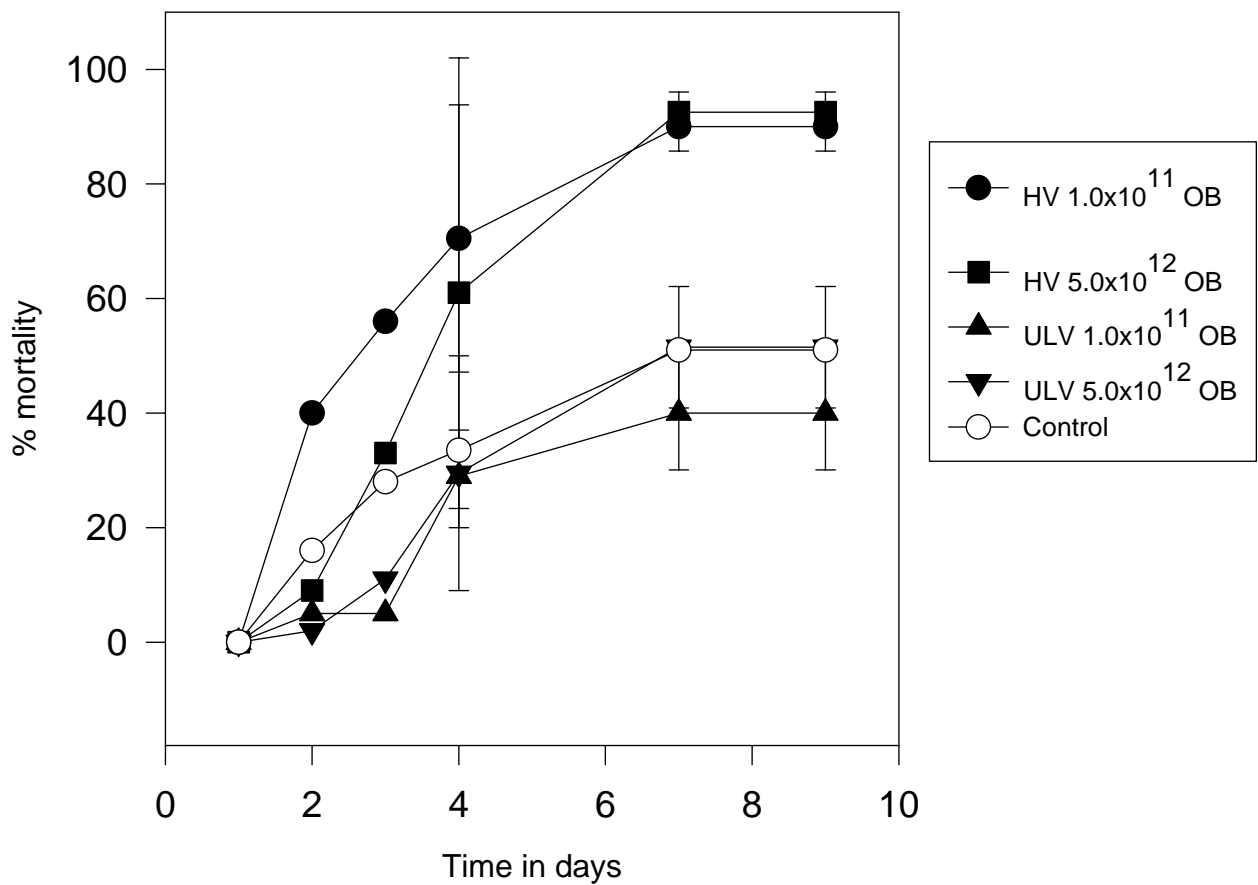


Of the five replicates of the trial carried out at Kilosa, only three provided suitable results for analysis with control mortalities of 15-46%. The other two trials gave between 82-93% mortality in the control treatments (see discussion below) and were

therefore excluded from the analysis. The larvae that died in these two controls were not observed to show any symptoms of NPV infection. Analysis of results from the three successful trials showed that SpexNPV application using the HV water-based method was highly effective in controlling armyworm in the field causing up to 90% mortality in field collected III instar larvae (Fig 11). The results showed that SpexNPV was highly effective even at lower doses than used in glasshouse tests and mortality levels were higher in field tests than in glasshouse trials. This result is very encouraging as it shows even simple suspensions of SpexNPV used in locally available sprayers are effective and could be used by farmers without further improvement.

Oil-based ULV application was less successful on the small-scale plots used in the trial. Many bioassays recorded zero percent larval mortality from ULV treated grass with a maximum of just 56% mortality being achieved in any replicate of the trial.

Fig 11. Field trials of SpexNPV. Mortality against time for III instar *S. exempta* larvae



The poor results observed in ULV treatment bioassays are similar to the results found earlier in the glasshouse trials at NRI. They again might be attributed to poor impact of the applied NPV to the target. In this trial the pasture in the plots was short grassland 6-9cm high and using the ULV sprayers on this could have led to a high level of drift carrying NPV beyond the plot boundaries before it had a chance of impacting on the low lying vegetation. One observation was that the best ULV results were from a replicate on pasture plots where the grass was taller (10-30 cm) than in the other replicates. It may be that it is inappropriate to use ULV oil-based sprays on low pasture. ULV may still however be useful on taller crops such as maize, barley and wheat. However this system has been successfully used to apply oil-based fungal bioinsecticides onto pastureland for locust control (Bateman *et al.*, 1993). Indeed given the large areas that would need to be treated for strategic control of armyworm in Tanzania some form of ULV is probably the only viable application system for pastureland. Clearly further work is needed to identify the problem with ULV application and to determine whether it can be successfully adapted to deliver NPV onto pastureland and crops if area-wide control is to be possible.

Following these poor initial results from ULV the ULV treatment was removed from subsequent Arusha trials to allow the trial plan to allow the trials to focus on the more promising HV water-based treatments. Control mortalities were high in some replicates. Some plots of rangeland contained a high proportion of the grass *Cynodon dactylon*. This is a common grass species in Tanzanian rangeland and a common host plant of *S. exempta* (Rose *et al.*, 1997) but grazed or damaged plants produce high levels of cyanide in their tissues (Geordis & McNaughton, 1988). Normally armyworm can feed on these peripatetically, constantly moving onto fresh grass, to avoid this build up of cyanide but in the bioassays larvae were fed on cut grass and did not have this option. Even in the successful trials some of the relatively high control mortalities could be due to the presence of some *C.dactylon* in the grass fed to bioassay larvae. Also, it was believed that feeding larvae with wet grass, it being a very rainy period, created stressful conditions within the bioassay bowls contributing to non-virus related deaths. It was noticed that when monitoring the bioassays that many larvae were very reluctant to feed and even after seven days in the bowls, larvae remained small and underweight. Thus larvae in these bioassays probably ingested a lower dose of NPV than they would have done if they had fed normally on standing vegetation.

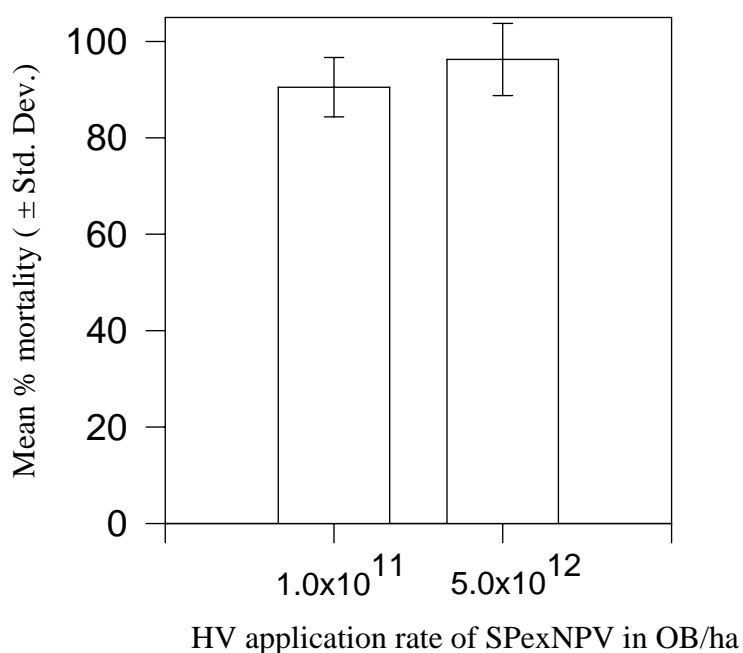
These problems with high control mortality in this trial led to us abandoning this bioassay procedure for the trial held in Arusha. In that trial assessment was through direct quadrat counts of larvae in the sprayed plots themselves. The following trials carried out on barley at the M'ringa Coffee Estate utilised both methods and results showed again that there were problems with that bioassay technique under field conditions.

Arusha, Pest Control Services trials

The results of the trials at the PCS site confirmed that HV treatments gave highly effective control of armyworm with mortalities in sprayed sites ranging from 96-100% (Fig 12). Even at the lowest rate used 1×10^{11} OB ha⁻¹, 96% of larvae were dead

after seven days. This rate is equivalent to the NPV content of 50 infected larvae and for comparison is between a fifth and a tenth of the rates recommended for other already commercial baculovirus insecticides such as *H.zea* NPV (5×10^{11} OB ha⁻¹), *H.armigera* NPV (1×10^{12} OB ha⁻¹), *S.litura* NPV (1×10^{12} OB ha⁻¹) and *S.exigua* NPV (1.5×10^{12} OB ha⁻¹).

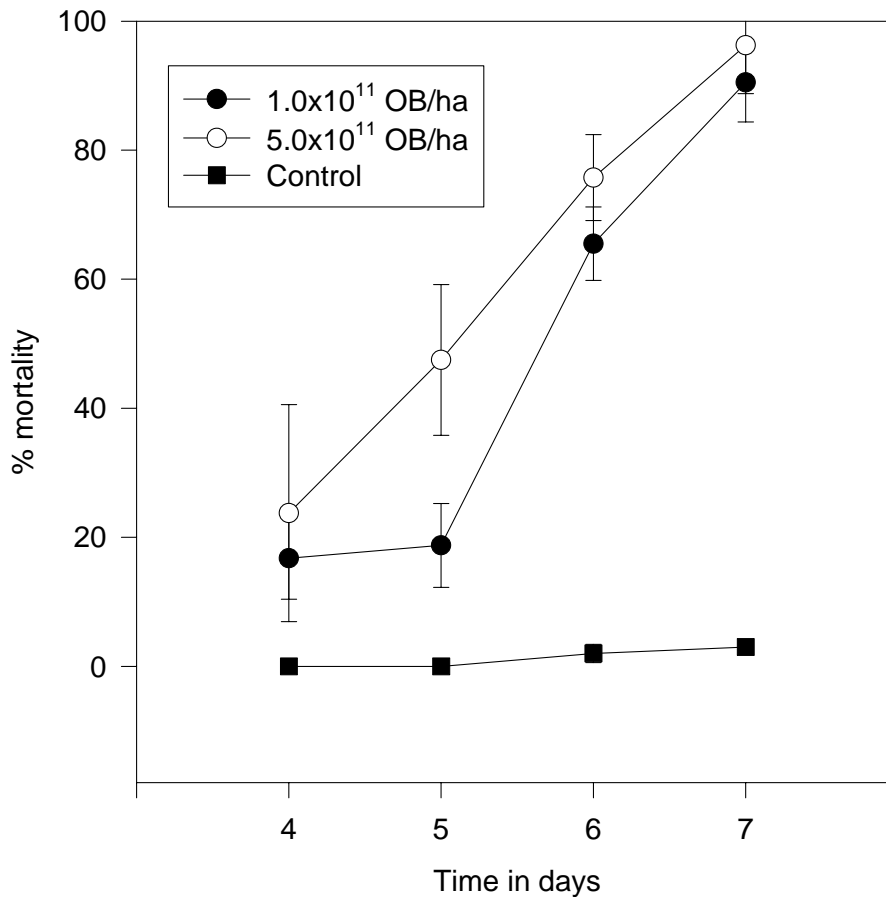
Fig 12. Graph of mortality for different application rates of SpexNPV in trials at PCS Offices, Arusha, April 1999



This result confirms the previous field result that low concentrations of SpexNPV are highly effective in killing armyworm even when used as a simple suspension (without formulation) in local sprayers. The results are so encouraging that the next stage is clearly to take this approach directly onto farms for farmer participatory trials on target cereal crops. These results also excited the local farmers and pest control officers and created very strong support for the project amongst counterparts and extension workers..

One very interesting observation is that the SpexNPV was effective at much lower doses in the field than in the glasshouse. This is very much the opposite of the experience with chemical pesticides where application rates in glasshouse trials tend to be 10-150 times lower than those needed in the field (Krahmer, 1994). This may reflect the advantage of using a natural pathogen like SpexNPV in the environment in which it evolved to target armyworm under natural conditions in the field. It may therefore be more effective in the field than in bioassays and glasshouse trials where the standardised conditions remove many of the fluctuations in extraneous factors. These fluctuations and other factors clearly tend to reduce the efficacy of chemical insecticides but may improve the performance of complex biological agents that have evolved under these dynamic conditions.

Fig 13. Field trials of SpexNPV. Average mortality against time in quadrat counts of the Arusha field trial



The data in Figure 13, above, shows that the speed with which the NPV acted in the field was comparable with that in earlier results obtained in the laboratory and glasshouse. With no mortality before five days the NPV is clearly taking much longer than the few hours needed for traditional broad-spectrum chemicals or even the one-two days for a toxin-based biological insecticide such as *Bacillus thuringiensis*. This may be a problem with using SpexNPV on crops such as cereals where damage thresholds may be low and this may well have to be taken into consideration and trials carried out on such crops. However it may not be an insuperable problem as NPV-infected insects cease to feed some time prior to death. Also a 5-day period prior to onset of mortality is not dissimilar to the 3-5 day period seen with the insect growth regulator insecticides already widely used in the control of migratory locusts.

After the trial, large numbers of virus-killed larvae were observed in the sprayed plots (see Figure 14) and in subsequent visits up to 2 months after the application, *S.exempta* numbers on the plots were low and virus killed larvae could still be recovered. The large number of intact virus-killed larvae seen observed after spraying has important implications for SpexNPV production. In resource poor countries such as Tanzania setting up factories to produce NPV from cultured insects, like those established in America, France, India, Thailand and China is conceivable,

but at a cost equivalent to those for chemical pesticides. However if the intense natural outbreaks of armyworm that occur in Tanzania could be sprayed with NPV and the infected insects harvested to provide new NPV for control of subsequent outbreaks, a much more cost-effective solution to producing NPV in a poor country such as Tanzania would be possible.

Fig 14. SpexNPV infected armyworm (circled) at the Arusha field site 7 days after application.



The high densities of natural outbreaks of armyworm make in-field production potentially both practicable and more economic than producing the NPV in a custom-built plant. Selected outbreaks of armyworm on low value pasture land could be left for the larvae to develop to a large size then sprayed deliberately late to optimise the amount of virus produced in the infected insects. The infected larvae would then be collected as raw material for treating the next outbreak.

The persistence of NPV once sprayed onto grass is unlikely to exceed a week so that the virus kills seen weeks or months after spraying can only represent the effect of natural recycling where virus produced in larvae infected by spraying replenishes the stock of NPV in the plots. Active recycling of NPV is important for long-term protection and to prevent the re-infestation of treated areas and was clearly happening with SpexNPV in these trials.

Although the results showed that effects in the field were superior to those observed in the glasshouse, the close correspondence between the results for HV field-applied NPV and those obtained in the glasshouse are useful as they confirm that glasshouse trials can be used to time application rates and formulation improvements.

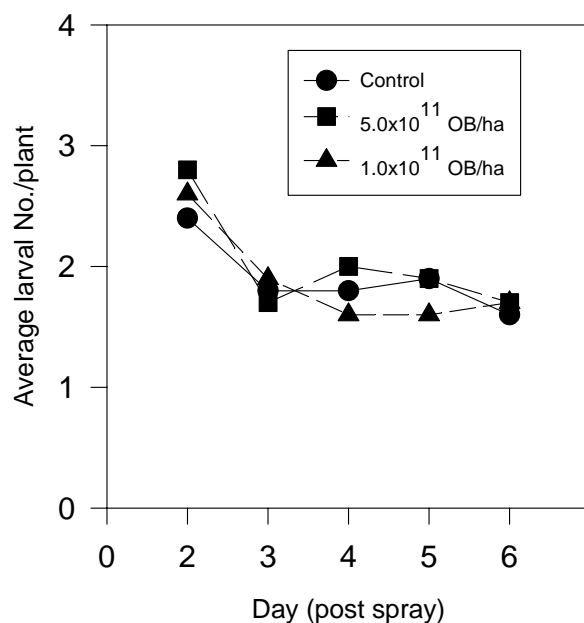
Arusha, M'ringa Estate Coffee Plantation (MECP) trials

After the PCS trial, where even the lowest application rate gave effective control, this next trial employed a series of application rates that went even lower in order to try to determine the threshold for lowest effective NPV application. The other changes were that the scale of the trials was increased to 0.5 ha plots and the target crop was barley rather than pastureland as in earlier trials.

These trials failed to meet their objective as all concentrations tested, even those previously killing 96%, were ineffective in controlling the armyworm outbreak with the maximum level of mortality in bioassays on treated barley reaching only 24%. The field monitoring indicated the maximum level of mortality in sprayed plots was less than 5% of larvae present in quadrat counts. There was no significant difference in average number of larvae per plant between treated and control plots at any time in the six days after application (Figure 15). After this the Estate management insisted on spraying the trials with insecticide to prevent further damage to the crop.

The trial was clearly a failure both as a control for armyworm and as a scientific trial to determine the lowest threshold rate for NPV control of armyworm. The reasons for this failure need to be carefully studied and may provide some useful information. Here both post-spray monitoring through quadrat counts (Fig 15) and bioassays (Table 5) of sprayed barley indicate that the NPV was not killing the armyworm.

Fig 15. Field trials of SpexNPV. *S. exempta* larval counts for M'ringa coffee estate NPV field trial



The validity of the bioassays were not in doubt in this trial as the complications of cyanogenesis in cut grass that occurs in pasture trials are absent in barley.

Table 5 Results of bioassays to assess mortality in plots at M’ringa field trials

Treatment	% Mortality
SpexNPV 1 $\times 10^{11}$ OB ha-1	36
SpexNPV 3 $\times 10^{10}$ OB ha-1	41
Control	17

The most obvious explanation for this lack of effect lies in the rain that fell on the trial site during the evening after application. It is likely that this washed the NPV off the barley crop. Wash off by rain is also a problem encountered in insecticide applications. NPV once it has dried onto crops has, even without specific formulation additives, excellent persistence even when rain is heavy. Pure NPV adheres well to leaves but the unpurified formulation used here, with its associated insect derived proteins, has especially good persistence. However the application in this trial was only completed as night fell a couple of hours before it rained. Given the humid conditions the formulation had probably not had time to dry onto the leaves before the ensuing heavy rain commenced and if the NPV droplets had not dried the OBs would have been readily removed.

Thus there is good reason to suspect this trial was not a valid measure of the effectiveness of NPV when used on barley or on larger-scale plots. The problem it raises though of NPV application during periods where heavy rain occurs is one that cannot be dismissed lightly. It is during the rainy season that outbreaks of armyworm start and indeed it is the new plant growth they produce that trigger the outbreaks. Thus it is quite likely that it will be necessary to apply NPV during periods when heavy rain is an almost daily occurrence. If NPV is to be a viable control technology then it will need to be applied in formulations that can dry rapidly to ensure natural adhesion or contain stickers to ensure it is not washed off when still wet.

Output Summary

The achievements of the project are:

- The production and successful field testing of SpexNPV as a control tool for African armyworm.
- Demonstration that this technique can successfully control armyworm in the field.
- That SpexNPV can be used in the widely available local sprayers as a simple suspension.

This work should now move into on-farm participatory trials to validate it and begin active farmer demonstration and promotion. However it has not been able, due to the

absence of an outbreak in 1998 and 2000, to conduct enough trials to confirm that this approach can work on large scale (>100 ha) area control trials. If this approach is to be feasible an effective ULV formulation of SpexNPV will need to be developed for use with vehicle mounted ground sprayers or aircraft. In the proposed follow on project this will be a major objective.

It will be necessary to determine the economic viability of using SpexNPV or its feasibility within the constraints of the current East African migratory pest control institutions. While the evidence appears good that outbreaks can be controlled if sprayed early enough with NPV it will need to be determined that existing forecasting systems and pest control institutions are effective enough to ensure that this can be done on a large scale.

The NPV work has been enthusiastically supported by the Tanzanian organisations charged with both migratory pest control and crop protection services at the district level. They see it as a cost-effective safe technique that would enable them to reduce the use of chemical pesticides. Local low cost production of SpexNPV would enable Tanzania to adequately provide for farmer's needs in a way they are unable to do using imported chemical pesticides.

Contribution of outputs

This project has shown that NPV can be used to destroy armyworm infestations in the field using locally available equipment. This now needs to be evaluated in practice by farmers on food crops. It also needs testing on a larger scale and key economic data gathered from which to assess its feasibility on a national and regional level.

The successful conclusion of this work in developing locally sustainable methods for controlling armyworm should result in an increase in the production of cereals, thus increasing the nutritional status of the rural and urban poor, and stabilise poor farm family incomes. The beneficiaries are intended to be all farmers whose food crops are attacked by armyworm but particularly poorer subsistence cereal growers in East Africa who do not have access to chemical pesticides. To impact on them it will be necessary to identify low-cost local production and effective distribution mechanisms for NPV.

Establishing local production of NPV will increase available supplies of pest control products to resource poor farmers. Currently about 70% of farmers cannot afford the imported chemical pesticides that are their only method of controlling armyworm. The MoA normally supplies these chemicals during outbreaks but due to cost and importation difficulties their supplies are equal to only 10-30% of the demand during major outbreak years. Local production of NPV will also create jobs and increase the incomes of farmers involved with in-field NPV production. By reducing the need for imported chemical pesticides it will reduce foreign exchange expenditure by Tanzania.

The environmental impact of replacing the current use of broad spectrum toxic chemicals with a specific and safe biocontrol agent is likely to be positive but should

be quantified as part of any evaluation before deciding to implement a major NPV control programme. The success of the fieldwork has advanced the progress in non-chemical control of armyworm and increased enthusiasm and understanding of the technology in government authorities responsible for armyworm control.

Follow up Indicated/Planned

A proposal for a follow-on project to complete development and evaluation of SpexNPV has been requested by the CPP and the concept note has been approved. Following this a full project memorandum has since been prepared and recently submitted. The new phase involves not only the original collaborators NRI, CABI and PCS Tanzania but in addition Dr Cory of the NERC Centre for Ecology and Hydrology, an expert on baculovirus ecology and latency. Also involved will be Dr K Wilson of University of Stirling who has a NERC-funded fellowship to study pathogen-host interactions and the *S.exempta*/SpexNPV system in particular.

In addition a proposal for a study “Persistence of pathogens in Migratory Pests” led by Dr Wilson but including the other project collaborators has been submitted to NERC for funding to study *S.exempta*/SpexNPV as a model system to identify the mechanisms by which pathogens persist in migratory pests. While this is fundamental research on host-pathogen relationships it is expected to generate much additional data important in determining the likely success of a strategic control programme for *S.exempta* with SpexNPV (see Annex 1). After these proposed three-year research projects are completed in 2003 enough data should be produced to realistically evaluate the technical and economic case for developing national and regional control programmes for armyworm based upon SpexNPV.

Promotion Pathway

In Tanzania the immediate agency for promotion should be the Pest Control Services as the mandated organisation for migrant pest control and the Ministry of Agriculture through its network of district and village crop protection services. These are already organised as a mechanism for nationally promoting IPM of all pests. This system through its volunteer village-level representatives reaches 60-70% of villages in the affected districts. They currently adopt a chemical control policy towards armyworm but have indicated they would be very keen to adopt NPV control approach to armyworm outbreaks. The MoA already fund the extensive purchase and distribution of chemicals to farmers but would be willing to use alternative technologies if available, especially if these could be locally procured at lower cost than the existing chemical pesticide importation programme.

There will be a need to promote regionally any successful armyworm control programme to other African countries effected by armyworm and here two routes might be utilised, either through DLCO-EA or direct to national ministries responsible for migrant pest control. While in the past DLCO –EA would have been the obvious candidate its current financial difficulties have impaired its effectiveness and these options will need to be evaluated in detail in due course before a regional promotion strategy can be decided.

Further stages needed

Several further stages in research are needed before the decision to implement a major NPV control programme based upon NPV could be made.

- Complete the technical development and economic evaluation of NPV for armyworm control.
- Evaluate the ability of the Tanzanian national pest organisations to implement an effective NPV based control in Tanzania.
- Determine feasibility of using NPV in for the strategic control of NPV.
- Develop low cost field production of NPV in Tanzania.

These needs will be addressed as follows

The proposed follow-on CPP project already submitted will provide the data to complete the technical development of SpexNPV. This project will also collect data on the cost effectiveness of NPV control of armyworm to form the economic basis of economic cost-benefit analyses.

While it has been shown that NPV can be used effectively to control armyworm if applied in time, the feasibility of the technique will rest on the speed at which outbreaks are identified and control measures initiated. There will be a need to determine that existing systems in place in Tanzania and Kenya are capable of forecasting and reacting to outbreaks. To help answer this a specific study of migrant pest control infrastructure is planned as part of the socio-economic studies included in the new proposal.

It is likely that any NPV-mediated armyworm control will operate in two distinctive ways. In controlling armyworm outbreaks on cereal crops such as maize, wheat barley etc SpexNPV would be locally produced and supplied to farmers. They would then apply it to control outbreaks on their own farms. NPV could either be procured by the MoA and supplied free to the poorest farmers as at present or locally produced and sold. If local field production could be established using the techniques used in the Brazilian *Anticarsia* NPV model then costs per ha of around 1.50-2.30 US\$ might be expected and this would be cheaper than conventional chemical pesticides (> 6 US\$).

The second approach would be strategic control where the primary outbreaks in pastureland are sprayed with Spex NPV to infect the armyworm and cause population collapses before they multiply and migrate onto croplands. These low value rangelands do not justify active control by the owners or users so that some strategic authority (national ministries or regional) DLCO-EA would need to initiate action. Previous studies have suggested that such an approach even using chemicals with control costs(including organisation and application) of 16-33 US\$ per ha would be

justified (Cheke & Tucker 1995). However regional and donor funding for strategic operations has not been forthcoming and this approach has not been adopted. However using a self-replicating biological control agent such as NPV could be both much more effective and therefore lower in cost. Once applied to developing populations it could spread and travel with the migrating moths to infect following generations. At least two recent reports of strategic control of *H.armigera* in the US (see below) and Australia (Anon 2000) have shown evidence this spraying of one generation with NPV can significantly suppress subsequent generations.

The feasibility of strategic area-wide control programmes using NPV would need proper evaluation. The basic ecological data on the SpexNPV and its role in host population dynamics would be generated by the proposed follow on projects submitted to CPP and NERC. Such a programme would aim to speed up the natural spread of SpexNPV. This virus currently is very rare in early season outbreaks of armyworm but appears later in the season after which it spreads rapidly and infects up to 98% of insects causing populations to crash but usually too late to avoid major crop damage (Rose *et al.*, 1997).

A strategic programme has already been developed and implemented successfully in the southern USA for controlling another noctuid moth *Heliothis virescens* using *H.zea* NPV (HzNPV). The project treated early season alternate wild hosts of *H.zea* to reduce the later migration of this pest into the cotton crop. The NPV was produced in laboratories and 8 million bollworms were reared to produce NPV each year. The HzNPV was sprayed at an equivalent of 88 infected larvae per ha and in 1994-97 216,000 acres were treated each year at a cost per ha of 6 US\$. Applications were estimated to kill between 70-100% of pests in the target areas and reduced adult emergence by >70% (Street *et al.*, 1998). However it will be necessary to be evaluate if institutional structures, facilities and resources to successfully operate such a system are available or could be established in East Africa.

There needs to be an economic study to determine how NPV might be produced locally. The high densities of natural outbreaks of armyworm make in-field production potentially both practicable and more economic than producing the NPV in a custom-built plant. Selected outbreaks of armyworm on low-value pasture land will be left for the larvae to develop to a large size then sprayed deliberately late to optimise the amount of virus produced in the infected insects. The infected larvae will then be collected as raw material for treating the next outbreak. Given that outbreaks average at least 90 third instar larvae per square metre (often >200), the yield per larva is 2×10^9 NPV occlusion bodies (OB) and estimated application rates for SpexNPV against third instar larvae is not more than 1×10^{11} OB per ha. Then with an infection rate of 80% (already achieved in trials) an insect recovery rate of only 10% then 1 ha of pasture could be managed to produce enough NPV to treat a minimum of 1430 ha at very low cost.

It will be necessary to identify techniques for cheaply collecting and processing the insects, then storing the NPV in a stable form for up to five years. Some techniques have already been developed in Australia by CSIRO for harvesting *Helicoverpa armigera* NPV (A Richards CSIRO per comms). In Brazil the programme to control

Anticarsia gemmatalis and *Spodoptera frugiperda* uses field production to produce enough NPV to treat over 2 million ha per year. This system is very cost-effective with costs to the farmer for formulated NPV of US\$ 1.20-1.50 per ha (Moscardi, 1999). In comparison insecticides in Tanzania cost currently about US\$ 10.00 per ha. The Brazilian NPV production system uses simple low cost technology and is probably the most appropriate model for use in a developing country such as Tanzania where manufacturing skills and production infrastructure are very limited. It is proposed in follow on work that project staff will visit Dr Moscardi's institute EMBRAPA in Brazil to study the low cost NPV harvesting and storage techniques and these will be adapted for use in Tanzania.

Funding of future stages

It has been agreed that the CPP will fund a further three year research project to develop and evaluate NPV for armyworm control. In addition a proposal for equivalent funding to conduct underpinning studies on the ecology of SpexNPV has been submitted to NERC (Annex 1).

Further funding after these two research projects (post 2003) to implement an NPV-based control system would probably require a major investment to develop either a national armyworm control programme in Tanzania or a regional strategic programme for East Africa as a whole. Possible routes might be through DFID bilateral programme or the International Biopesticide Consortium for Development (IBDC). The IBCD is already engaging with prospective donors including the World Bank, DANIDA, FINIDA, CDC, DFID and other prospective funding agencies to procure funding for biopesticides projects in developing countries. It intends to generate two million pounds per annum from 2002 onwards to fund biopesticide production projects including public-private production initiatives. Key collaborators in IBCD have been and are still involved in the LUBILOSA project and this would be a model of how to organise future development of biological pesticides such as NPV.

Publications

Refereed publications

CHERRY, A. J., PARNELL, M., GRZYWACZ, D., BROWN, M., and JONES, K.A., (1997) The optimisation of *in vivo* nuclear polyhedrosis virus production of *Spodoptera exempta* (Walker) and *Spodoptera exigua* (Hubner). *Journal of Invertebrate Pathology*, **70**, 50-58. (Annex 5)

Conference presentations

PARNELL, M.P., MUSHOBOZI, W., GRZYWACZ, D., and DEWHURST, C., (2000) Advances in the use of *Spodoptera exempta* nucleopolyhedrovirus (SpexNPV) to control the larvae of *Spodoptera exempta* (East African Armyworm) in Tanzania. *33rd Annual Meeting of Society of Invertebrate Pathology*, Guanajuato, Mexico, August 13-18 2000.[Poster] (Annex 2)

Reports

Parnell, M. & Dewhurst, C., (1999) Entomopathogenic baculoviruses for control of East African armyworm *Spodoptera exempta*, in Tanzania: field trials of *Spodoptera exempta* nucleopolyhedrovirus carried out against armyworm outbreaks on rangeland in Tanzania., NRI Report No. 2457, Natural resources Institute, Chatham, pp 32. (Annex 3)

Other dissemination

GRZYWACZ D., (2000) Pathogens:viruses, The ecology and importance of *Spodoptera exempta* NPV to African armyworm. In: *The African armyworm handbook*. Rose,D., Dewhurst C and Page W (Eds), [Science] (Publication summer 2000), NRI Chatham, pp 165. (See Annex 4).

Annexes

Annex 1: Text of NERC grant proposal for £324,000 three year project submitted 01/06/2000 "Persistence of Pathogens in migratory insect pests!". Wilson K A, and Cory J S.

Annex 2: Presentation. Advances in the use of *Spodoptera exempta* nucleopolyhedrovirus (SpexNPV) to control the larvae of *Spodoptera exempta* (East African Armyworm) in Tanzania.

Annex 3: NRI Report. Entomopathogenic baculoviruses for control of East African armyworm, *Spodoptera exempta*, in Tanzania: Field trials of *Spodoptera exempta* nucleopolyhedrovirus carried out against armyworm outbreaks on rangeland in Tanzania.

Annex 4: Section on Armyworm NPV for Armyworm Handbook.

Annex 5: Publication. Cherry, A. J., Parnell, M., Grzywacz, D., Brown, M. & Jones, K.A. (1997) The optimisation of *in vivo* nuclear polyhedrosis virus production of *Spodoptera exempta* (Walker) and *Spodoptera exigua* (Hubner). *Journal of Invertebrate Pathology*, **70**, 50-58

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Appendix 5 - Inventory Control Form

NRIL Contract Number: ZA. 0148

DFID Contract Number: R6746.....

Project Title: Entomopathogenic viruses for control of African armyworm *Spodoptera Exempta* in Tanzania

Project Leader: David Grzywacz

[List all single equipment items with a purchase value higher than £500 and items with a purchase value lower than £500 but deemed to be of an attractive nature (i.e. cameras, motorcycles, etc.) purchased during the quarter.]

Please fill in ALL the information requested in the table below for each item

Item	Make and Model	Serial No.	Date received	Purchase price	Location	Disposal		
						To	Date	Authorised
None								