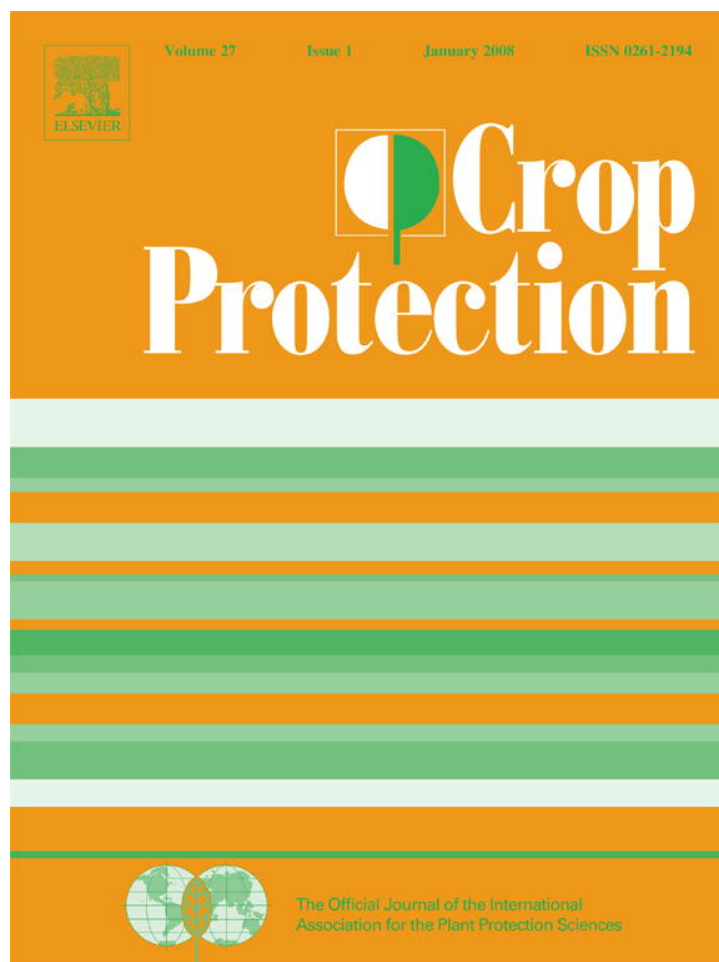


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# Evaluation of *Spodoptera exempta* nucleopolyhedrovirus (SpexNPV) for the field control of African armyworm (*Spodoptera exempta*) in Tanzania

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## Abstract

The African armyworm *Spodoptera exempta* is a major episodic migratory crop pest over much of Eastern and Southern Africa. Control of this pest has been reliant on the use of synthetic chemical insecticides. However, this approach fails to protect poor farmers and is becoming unacceptable for environmental and cost reasons. A programme of field trials was conducted in Tanzania to evaluate the endemic baculovirus, the *S. exempta* nucleopolyhedrovirus (SpexNPV), as an alternative control. Field trials demonstrated that both ground and aerial application of SpexNPV to armyworm outbreaks on pasture can initiate outbreaks of NPV disease and population collapses. The SpexNPV was effective when applied at  $1 \times 10^{12}$  occlusion bodies (OB) per hectare if applied to outbreaks early, when larvae are in I–III instar—mass mortalities appear 3–10 days post treatment. The data from these trials indicate that SpexNPV can have a potential role as a substitute for chemical insecticides in strategic armyworm management programmes.

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**Keywords:** *Spodoptera exempta*; African armyworm; Migratory pest; Baculovirus; Nucleopolyhedrovirus; Biological control; IPM; Africa

## 1. Introduction

The African armyworm (*Spodoptera exempta*) is a serious pest of rangeland and cereals that erupts in episodic plagues across sub-Saharan Africa. These outbreaks vary from year to year but have a major impact on cereal production and livestock in the outbreak countries (Scott, 1991). The annual outbreaks of armyworm most often start in the identified primary outbreak areas of Tanzania and Kenya in January following the main rains (Haggis, 1987). Adults from these outbreaks then migrate to new areas following the seasonal rainfall patterns, to start new outbreaks in other parts of Eastern and Central Africa, and this sequence may continue through until June spreading North, East and South from the primary areas

(Rose et al., 2000). Outbreaks may extend over many square kilometres with larval densities in excess of 1000 m<sup>2</sup>. Outbreaks are annual but intensity varies greatly from year to year. In Tanzania alone, during bad outbreak years many hundreds of thousands of hectares of crops may be attacked, but in some years no serious outbreaks may occur (Scott, 1991; Njuki et al., 2004). Control of this pest is routinely done through application of synthetic chemical insecticides. While these are technically effective, there is increasing concern over the environmental impact of these chemicals applied over wide areas. In addition, the cost of chemical insecticide is beyond the resources of farmers or national control agencies so that in many years only 30% of outbreaks are treated, with considerable loss of crops and damage to rangeland (Njuki et al., 2004). These shortcomings have stimulated the search for other more specific biological control options that would be safer and more sustainable.

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It has long been known that the African armyworm has a number of natural enemies and pathogens, including viruses, fungi and protozoa, but the most important was reported to be a specific baculovirus, the *S. exempta* nucleopolyhedrovirus (SpexNPV) (Rose et al., 2000). This virus had been known since 1965 (Brown and Swaine, 1965) and its potential as a control agent has been highlighted on a number of occasions (Tinsley, 1979), but little progress to develop the NPV was made, probably because cheap and effective broad spectrum chemicals were available and considered acceptable. Research studies have confirmed that this disease is endemic in many parts of east Africa (Odindo, 1983) and that this NPV is highly pathogenic to armyworm (Odindo, 1981). Case studies on armyworm outbreaks confirmed that natural NPV can be a major cause of mortality in armyworm outbreaks (Persson, 1981). However, the NPV is rarely apparent in primary outbreaks of the pest, only appearing later in the season, and even then it can be highly localized, affecting only small parts of the outbreak area (McKinley, 1975). Trials to use crude macerated suspensions of infected larvae containing the NPV as an insecticide against armyworm had showed that this approach had promise (Brown, 1966). Subsequently, the virus was characterised (Harrap et al., 1977) and cross infectivity studies with a range of NPVs have indicated that the armyworm was susceptible only to the SpexNPV (McKinley et al., 1977, A.C. Cherry personal communication). The SpexNPV has been safety tested following FAO/WHO recommended protocols and no evidence of toxicity to mammals or non-target hosts was found (Harris, 1973) in agreement with a major recent safety review of Baculoviruses, which showed no evidence of adverse environmental impact from any use of baculoviruses as crop protection agents (OECD, 2002).

A new study to evaluate SpexNPV as an alternative to existing chemical and other control options began with a laboratory programme to optimise the mass production techniques for SpexNPV (Cherry et al., 1997) and the build up of a stock of SpexNPV for laboratory evaluation prior to field trials in Tanzania. Initial fieldwork began in 1999 at the end of the first phase, and then resumed in 2001 when a follow on study had begun. The field work in Tanzania was aimed at assessing SpexNPV in comparison to the existing chemical insecticide based control and also the use of local neem formulations, which had been reported to show promise against armyworm (Broza et al., 1999).

This paper reports on the field trials undertaken to evaluate SpexNPV as a control for armyworm outbreaks in Tanzania. The field trials reported here were carried out on pasture land, as it is commonly on pasture that eggs are laid and in which armyworm pass the early instars before migrating onto nearby land to attack cereal crops. In evaluating SpexNPV it was important to determine its viability when applied by lever operated knapsack systems used by smallholders, motorised mist blower, which is the method of choice for many farmers, and aerial application,

which is the mainstay of the national control programme and also used by the largest commercial farmers.

## 2. Materials and method

### 2.1. The virus

The SpexNPV was a multiply enveloped NPV isolate (#0045), one of a number collected originally from wild *S. exempta* in Tanzania and Kenya in 1974. The virus was mass produced in third instar larvae using methods previously reported (Cherry et al., 1997). The NPV produced was processed using standard protocols developed at NRI (Hunter-Fujita et al., 1998). The insects were, after storage at  $-20^{\circ}\text{C}$ , thawed out and macerated in distilled water to release the NPV. The suspension was then filtered through three-layer muslin to remove gross insect debris. After processing, the NPV samples were counted using standard counting protocol (Wigley, 1980), then freeze dried and stored at  $-30^{\circ}\text{C}$  as a powder. The identity and purity of the progeny virus were confirmed using restriction endonuclease analysis on the viral DNA (Smith and Summers, 1978). The DNA was extracted using an adaptation of the protocol described in Hunter-Fujita et al. (1998) and restriction fragments were obtained using *Pst* 1, *Bam* H1, *Hind* III, and *Eco* R1 enzymes. To visualise the restriction patterns the cut DNA was run overnight on a 0.6% agarose gel at 35 V and photographed with an MP4 camera. Once the activity of batches of SpexNPV had been determined, and their identity confirmed, specific formulations were produced blending different batches to give the desired standard activity.

### 2.2. The field trials

The preliminary field trials were carried out during 1999, then as part of a major follow on project in the 2002 and 2004 armyworm seasons at several sites around Arusha in Tanzania. Details of the trial sites, treatments and application details are given in Table 1. No successful trials were conducted in 2001 and 2003 due to the limited nature of armyworm outbreaks in those years (Njuki et al., 2004). All of the trial sites were on pasture on farms or research stations at sites identified initially from moth catches in pheromone traps, then identified by follow up scouting for larvae (Rose et al., 2000). The sites around Arusha, which is on the equator, are high at over 1300 m, so sunlight and UV intensities are generally high. All trials were on pasture land that consisted of mixed grass species, though the dominant grasses were star grass (*Cynodon nlemfuensis*) with nutgrass species (*Cyperus* spp.) less common but important. The remainder was a mixture of other species including *Chloris* spp., *Eragrostis* spp., and *Seteria* spp.

The 1999 ground spray trial was the first field test of the SpexNPV and used two rates of application, both of which had been shown to be effective in earlier polytunnel trials in

Table 1  
Details of armyworm SpexNPV field trials

	Ground spray trial 1999	Ground spray trial 2002	Ground spray trial 2004	Aerial spray trial 2004
Location	Tengeru (03°27'S 36°48'E)	M'ringa (S 03 20 25 0 E36 37 24 5)	Tengeru (03°27'S 36°48'E)	Tengeru (03°27'S 36°48'E)
Treatments	(1) $5 \times 10^{11}$ OB (2) $5 \times 10^{12}$ OB	(1) $5 \times 10^{11}$ OB (2) $1 \times 10^{12}$ OB	(1) $1 \times 10^{12}$ OB	(1) $1 \times 10^{12}$ OB
SpexNPV application rates OB (ha <sup>-1</sup> )				
Insecticide control (g/l)	None	Fenitrothion EC 35% (7g/ha)	Diazinon EC 60% (60g/ha)	None
Other treatments	Control	(1) Fresh neem leaf 40% w/v (2) Neem seed 5% w/v Control	Control	Control
Sprayer	Lever operated backpack Hardi 15	Lever operated backpack Hardi 15	Motorised mist blower Solo 412 aster	CP aerial hydraulic
Nozzel	Hardi hollow cone red	Hardi hollow cone red	Air shear	CP-03
Pressure (kPa)	310	310	50	310
Application volume (litres per hectare)	200l water 0.01% Triton	50l water 0.01% Triton	50l water 0.01% Triton	165l water 0.02% Triton
Plot size	20 × 10 m	20 × 20 m	0.5 ha	5 ha
Replicates	4	3	1	1
Travel speed	Walking	Walking	Walking	Flying
Application height above canopy	60–80 cm	60–80 cm	30–50 cm	10 m
Count samples per plot	30	30	30	30

the UK (Grzywacz, 1999). Sprayed plots were monitored daily after application for 7 days post application and the larvae quadrat-counted as dead or alive. The 2002 ground spray trial was again a small plot replicated trial with two application rates of  $5 \times 10^{11}$  and  $1 \times 10^{12}$  occlusion bodies (OB) ha<sup>-1</sup>, a local botanical insecticide neem, a chemical insecticide control, used at the recommended rate for armyworm, and an untreated control. The 2004 ground spray trial was conducted on 0.5 ha plots. The five treatments were SpexNPV, fresh aqueous neem leaf extract at 50% w/v, neem seed aqueous extract at 5% w/v, a chemical insecticide control, Diazinon applied at the recommended rate for armyworm, and an untreated control. Armyworm counts were made the day before application and at 1, 3, 5, 7 and 9 days post application. In all trials, the control treatment plots were sprayed with water plus Triton as a comparable rate to other plots.

The aerial NPV trial site was pastureland with newly hatched I–II instar armyworm larvae which were found at counts of over 200 per m<sup>2</sup>. The SpexNPV treatment was applied to a 5 ha block of the pasture. An adjacent upwind block of pasture of 3 ha on the western border of the sprayed site was used as a control. Counts of the armyworm were made two days before the application then 1, 4, 6 and 8 days afterwards using standard quadrat counts replicated 30 times on each plot. Ambient conditions during the trial were overcast to sunny with an average temperature of 23 °C (range 14–31 °C).

### 2.3. Assessment of field trials

Assessment of all the trials was carried out through estimating larval populations within treated plots using 50 × 50 cm quadrat counts, the method most commonly used for assessing armyworm control trials (Rose et al., 2000). Armyworm counts were performed prior to trials,

on the day of application, then at predetermined intervals for up to 14 days post application. Armyworm counts were not made after 10–14 days as by this time larvae in control treatment plots would invariably have consumed all suitable vegetation and then migrated away from the plots. The counting technique consisted of placing quadrats onto the ground in treated areas and the larvae in each square were counted and the larval stage recorded. In small plot trials, quadrats were placed randomly but no counts were made within 2 m of the edge of the plot to avoid edge effects. In large plot trials, quadrats were counted at regular intervals along transect lines to ensure that the sampling over these larger areas was representative. For each replicate, 30 separate quadrat counts were made on each assessment day. Prior to trials, a pre-spray assessment was made to provide base-line data, after which further assessments were made on selected days post application. The numbers of insects infected with SpexNPV were also estimated visually based on clear symptoms of SpexNPV infection (immobility, darkening of cuticle, loss of turgor, obvious lesions).

Plots of armyworm counts indicated that the distributions of counts were of positive skew and in many cases included outliers, so the median test due to Mood (Gibbons and Chakraborti, 2003) was performed, using the Minitab statistical package, to test the null hypothesis that the median counts in populations of armyworm given the treatments under study were equal. This test consists of a chi-square test of association on a contingency table with  $k$  rows (one for each of  $k$  treatments) and 2 columns, showing in one column the number of observations less than the median of the combined samples, and in the other column the number of observations greater than or equal to this median. We preferred the median test to the Kruskal–Wallis test (for more than two treatments) or the Mann–Whitney (for two treatments) as it is more robust

against outliers. However, when there are highly significant differences between treatments these alternative tests would also give very small  $p$ -values. When significant differences between medians were found, a multiple comparisons test for medians (test statistic denoted by  $q$ ) was performed by hand (Levy, 1979).

Obtaining trial data on episodic migratory pests such as armyworm, especially with slower acting microbial or chemical pesticides, can be difficult. This is due to the uncertainty of outbreaks, the variable population density, and the mobility of the pests, and is a problem recognised in other migratory pest species (Inglis et al., 2000). In this study several trials were set up in each field season but many were aborted or produced no data due to natural population collapses from heavy rain or natural NPV.

### 3. Results

The results of the preliminary ground trials at the PCS site (Fig. 1) confirmed that knapsack sprayer applied SpexNPV gave effective control of armyworm, even at the lowest SpexNPV rate used,  $5 \times 10^{11}$  OB ha<sup>-1</sup>; 96% of larvae were dead after seven days.

The results of the ground spray trial in 2002 (Fig. 2) illustrate the wide variation in armyworm densities seen in the field with small plot trials. In the control plots, the armyworm counts increased after treatment as insects hatched out during the course of the experiment. Overall, of the five treatments, the high-rate SpexNPV and insecticide both produced marked reductions in armyworm counts over initial counts between 3 and 10 days post application. Reductions in armyworm numbers were a maximum of 77% in the high-rate SpexNPV treatment and 100% in the insecticide treatment. Day 10 counts for the different treatments were significantly different ( $\chi^2 = 64.26$

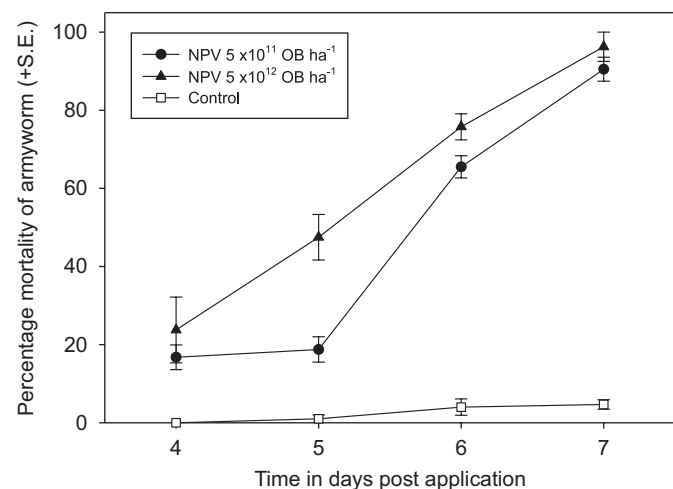


Fig. 1. Results of ground spray trial showing mortality of armyworm larvae in small plot trial after application of SpexNPV at two different rates Arusha 1999.

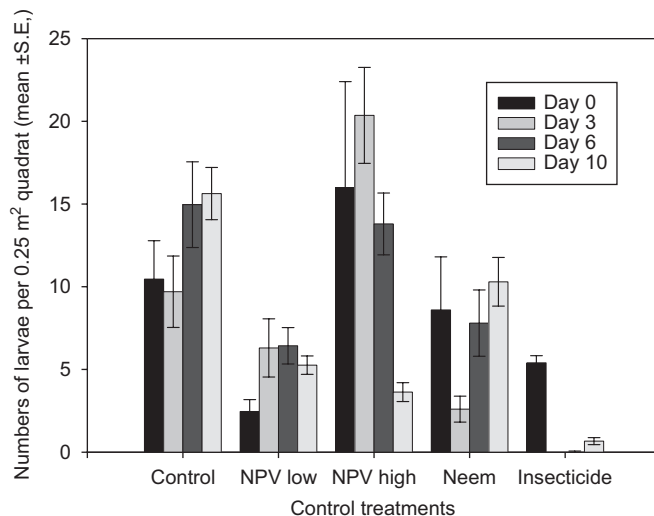


Fig. 2. M'ringa ground spray trials 2002, mean densities of armyworm larvae following treatment with various treatment applications (mean number of larvae per 0.25 m<sup>2</sup> quadrat  $\pm$  s.e.).

for the Mood median test,  $df = 4$ ,  $p < 0.001$ ). The multiple comparisons test for medians at day 10 (Levy, 1979), using a 5% level of significance (critical value = 3.858, Zar, 1984, Table B.5), showed that the SpexNPV high median count was significantly different from the control median count (test statistic  $q = 8.006$ ) and that the insecticide and control median counts were significantly different from each other ( $q = 9.826$ ), and the neem and SpexNPV low median counts were not significantly different from each other ( $q = 1.820$ ). In plots with the lower application rate of SpexNPV no reduction in armyworm was observed during the trial. The marked difference was that while the insecticide acted quickly in the armyworm counts in the SpexNPV high rate plots were only reduced after 6 days. The neem treatment produced a substantial fall 70% in counts by day 3 but the counts increased thereafter to above the pre-trial level by day 10.

The results of the 2004 ground trial are presented in Fig. 3. The control mean counts were stable over most of the trial, though falling to 72% of the initial mean count by day 9. The insecticide again acted rapidly, reducing counts to <10% by day one, and thereafter the counts remained low at 8–12% initial level until the end of the trial. There were highly significant differences ( $df = 4$ ,  $p < 0.001$ ) between the medians of all five treatments on days 5 (from Mood's median test = 53.8), 7 ( $\chi^2 = 72.35$ ), and 9 ( $\chi^2 = 93.17$ ). In day 5 SpexNPV median counts were significantly lower than the control counts (test statistic  $q = 7.278$ ) but were not significantly different to the insecticide counts. The neem leaf treatment was also significantly different to the controls ( $q = 4.367$ ) and insecticide ( $q = 5.095$ ) on day 5, and neem seed was significantly different to insecticide ( $q = 6.186$ ) and to NPV ( $q = 4.003$ ). By day 7 neem seed was significantly different to neem leaf ( $q = 4.003$ ). On day 9, the median count in the



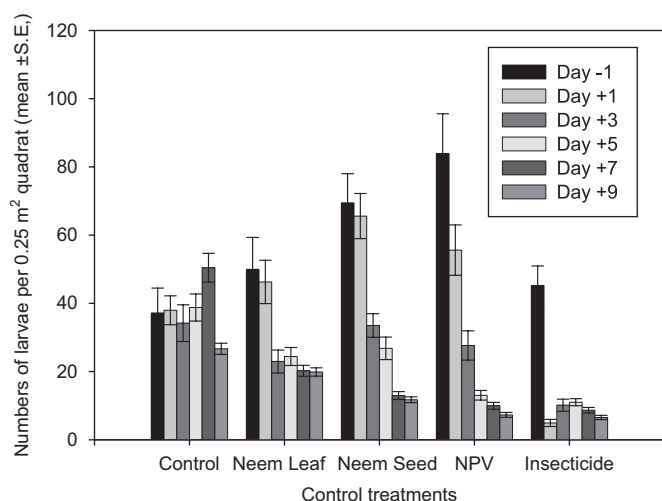


Fig. 3. Tengeru ground spray trial 2004 mean densities of armyworm larvae following treatment with various treatment applications (mean number of larvae per 0.25 m<sup>2</sup> quadrat ± s.e.).

neem seed plot was significantly lower than in the neem leaf treatment ( $q = 5.458$ ). Thus of the two neem treatments both reduced armyworm counts though the reduction in days 7 and 9 was greater in the neem seed extract plots than the leaf extracts.

The results of the 2004 aerial trials are shown in Fig. 4 as counts of numbers of larvae at both the sprayed and unsprayed sites. It can be seen clearly that the numbers of armyworms in the NPV and the control plot increased after initial scouting as larvae continued to hatch out. By the fourth day after application, the number of live larvae in the NPV plot had reduced dramatically and then continued to decline until by day 6 the outbreak had declined to nearly zero in the sprayed area. In the control plot, the numbers also declined as larvae matured, but remained by day 6 at greater than 100 per m<sup>2</sup>, a sufficient level to produce heavy damage to the pasture. Counts in the control plot were significantly higher than for the NPV plot on day 4 (Mood's median test  $\chi^2 = 48.65$ ,  $df = 1$ ,  $p < 0.001$ ) and day 6 (Mood's median test  $\chi^2 = 60$ ,  $df = 1$ ,  $p < 0.0001$ ). In the NPV sprayed plot the decline in live armyworm count was accompanied by the appearance of many NPV infected and killed larvae hanging from grass stems and on the ground. Fig. 5 shows that by day 4 over 70% of larvae counted in quadrats were dead or dying with symptoms of NPV infection and this rose to more than 80% by day 6. In the control areas NPV killed larvae were relatively few, at 5% by day 4 and only 11% by day 6. The numbers of dead larvae seen in the NPV plot were high, accounting for about half the numbers of the population initially present (Fig. 6). The failure to find an exact correspondence between larval reduction and the number of corpses seen is not surprising as many of these larvae dying were still very small (II–III instar) and quickly disappeared or were eaten by scavengers such as ants that were abundant in these pastures.

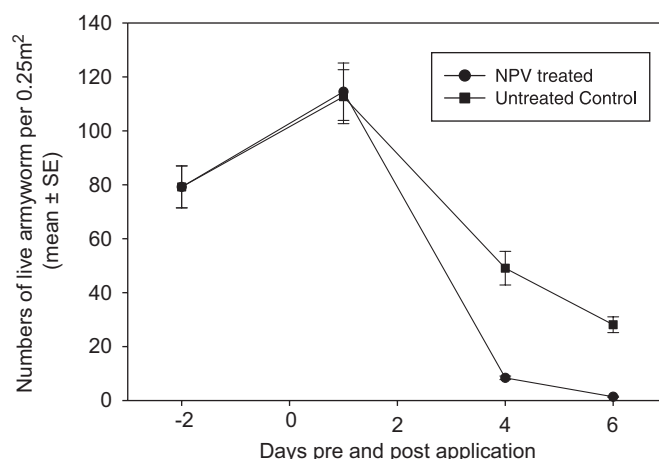


Fig. 4. Aerial spray trial M'ringa 2004 effect on armyworm number in SpexNPV treated and control plots after application as mean counts per 0.25 m<sup>2</sup>.

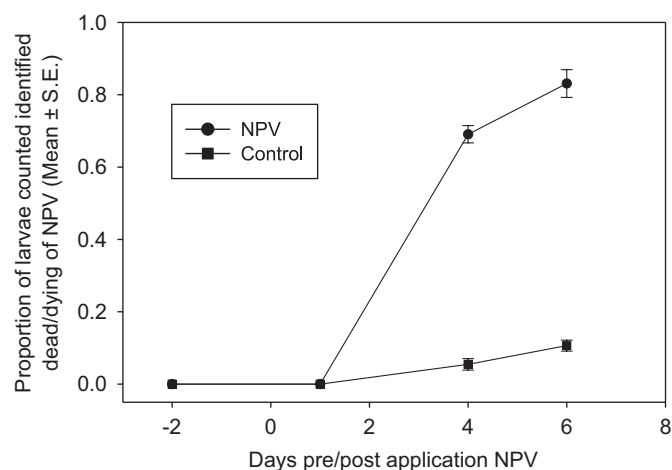


Fig. 5. Aerial spray trial 2004 proportion of larvae counted diagnosed as infected or dead of NPV in NPV treated and control plots.

#### 4. Discussion

In this series of trials the higher field application rate of  $1 \times 10^{12}$  OB ha<sup>-1</sup> showed consistent control of armyworm, while the lower rate of SpexNPV applied,  $5 \times 10^{11}$  OB ha<sup>-1</sup> did not reduce outbreak numbers in the 2002 field trial, although it had previously shown promise in glasshouse and preliminary field trials. The selection of the higher rate of  $1 \times 10^{12}$  OB ha<sup>-1</sup> was a balance between rates showing efficacy with the need to keep rates low enough to be economic. The rate of  $1 \times 10^{12}$  OB ha<sup>-1</sup> is similar to the recommended application rates for a number of other Lepidopteran NPVs already in commercial use. e.g., *Heliothis zea* NPV  $1.5 \times 10^{12}$  (Copping, 2004), *Helicoverpa armigera* MNPV  $1.5 \times 10^{12}$  (Cherry et al., 1997), *Spodoptera exigua* MNPV  $1 \times 10^{12}$  (Federici, 1999). Rates higher than this are recommended for some other NPVs e.g. *Mamestra brassicae* NPV at  $1 \times 10^{13}$  OB ha<sup>-1</sup> but these can raise serious cost issues.

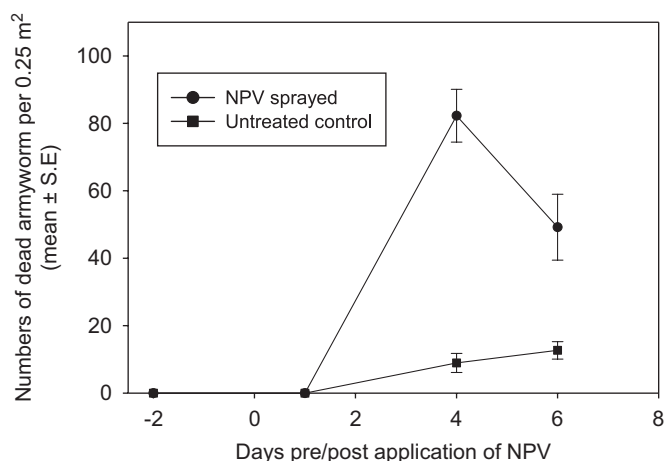


Fig. 6. Aerial spray trial 2004 Effect on numbers of dead larvae per 0.25 m<sup>2</sup> counted in NPV treated and control plots.

The slower speed of action of SpexNPV than the chemical insecticides normally used is an issue that could limit the utility of this approach. In these trials SpexNPV took between three and ten days to produce full mortality, while the insecticides killed within a day. The shortest lethal periods were when application was to larvae that were mainly I–II instar. It is unlikely that SpexNPV would, however expeditiously applied, produce major kills in less than three days, a period determined by the virus biology and replication time. It remains to be seen if this slower killing time will be acceptable to users. It must also be remembered that although death may take 5–7 days in older larvae, feeding itself ceases earlier, so post application damage may be less than what might be expected. It should be noted that a number of insecticides used for migratory pest control based upon insect growth regulators have similar lethal times (J.F.C. Cooper, personal communication) so it may not be an insuperable limitation to adoption. However, the slower action of SpexNPV may restrict the use of NPV to strategic national control operations, in pasture land, at least until its use in crops has been validated. The greater sensitivity of early instars to NPV is recognised and earlier work in the laboratory confirmed this was so for SpexNPV (Cherry et al., 1997; Grzywacz, 1999). This makes early identification of outbreaks through pheromone trap based forecasting a crucial part of any programme that might use SpexNPV. To this end, the current national system of traps is being extended to community run trap networks in villages. These are proving more effective, but whether they will provide early enough warning to enable the SpexNPV to be targeted on outbreaks at the sensitive I–III instar stage has yet to be determined.

The trials described here were carried out on pasture, but trials have not yet been carried out on crops such as wheat, maize and rice, which would be primary targets in a campaign to prevent direct crop losses. Laboratory trials have indicated SpexNPV is no less effective on these crops

(J. S. Cory, personal communication) but the slower action of SpexNPV may make its use unacceptable where farmers are not able to identify and apply it quickly to outbreaks. Trials to assess its suitability on crops in the field must be high priority in the full evaluation of this agent's utility in crop protection.

The 2004 aerial trial shows that SpexNPV can be very effective when aerially applied, which is important as aerial application is the primary tool in national control of armyworm in most countries. In the aerial trial, no attempt was made to apply the SpexNPV at dusk in order to minimise solar inactivation as is often recommended for other pests (Rabindra et al., 1989). This was because this recommendation was felt to be impractical for both farmers and government pest control services given the need for rapid application to large areas during major outbreaks. It may be noted that NPV acted more rapidly in the aerial trial in 2004 than in the ground trial that year, where spraying was carried out at 3 pm and after peak solar radiation. It is noteworthy that SpexNPV performed well given that it was applied on an open pasture canopy without additives to improve its UV stability.

In this trial, SpexNPV was sprayed at midday under clear conditions at a location almost on the equator at an altitude where UV levels would be high. In the tropics NPVs can be rapidly degraded by UV on crops where deposition sites are not protected by canopy architecture from UV (Jones et al., 1993). This has led to the belief that without the addition of UV stabilisers NPV is likely to be of limited effectiveness in the tropics. It has often been a recommendation by IPM practitioners that NPV is best applied at dusk to reduce UV inactivation (Rabindra et al., 1989; Reynolds, 2001; Jayaraj, 2001). The explanation for the effectiveness of SpexNPV in these trials may lie in the rapidity with which it is acquired by armyworm hosts. Work in Australia on *Heliothis* NPV has suggested that 80% of larvae acquire the NPV within one hour of spraying (D. Murray, personal communication). African armyworm feeds voraciously during daylight hours after they warm up. There may be a strong argument that timing application to match when larvae are feeding most actively is a better strategy for some NPV/host systems than trying to get farmers to spray late in the day when UV degradation is reduced. Most farmers for practical reasons are resistant to do evening spraying and it may be that applying NPV during the cooler nights when some species feed less actively may confer little advantage.

Chemical insecticides produce a very quick population crash with armyworm, but as results here show, some re-appearance in insecticide treated plots does occur. This may possibly be due to new hatching, but given the ovicidal action of insecticides it is more likely due to immigration into the plots. This may be a phenomenon associated with small plot trials and not a problem where whole outbreaks are dealt with in area wide treatment with insecticide. However, it may illustrate that the current generation of insecticides approved for armyworm control are not highly

persistent and may quickly disappear due to volatilisation or chemical breakdown. NPV itself can often be observed to disappear from treated foliage within a few days of application in the tropics (Cherry et al., 1997), but its capacity to replicate in infected insects enables NPV to be recycled and thus persist in sprayed areas for considerable periods after initial application. After trials in 1999 and 2002 infected insects were still being recovered from sprayed areas two months after application (W. Mushobozi, personal communication).

The availability and cost of SpexNPV will be crucial to its viability and sustainability as an armyworm control in East Africa. A significant constraint to the adoption of NPVs has been their generally higher cost. In Tanzania, chemical control costs are about 10US\$ per ha for insecticide but the country cannot afford enough insecticide in most years to treat more than 30% of outbreaks (Njuki et al., 2004). SpexNPV can be produced in dedicated NPV production units through production in cultured *S. exempta* (Cherry et al., 1997), but the cost is likely to be at least comparable to or higher than existing chemical insecticides, a cost Tanzania already cannot afford. In order to be a really viable option SpexNPV would need to be produced at a cost lower than the cost ( $\geq$ US\$10 per ha) of the current chemical insecticides.

An alternative to producing NPV in dedicated production plants is to use field production. Field production works by infecting host outbreaks in the field and harvesting the dying infected insects as a source of NPV. Given the propensity for armyworm to produce outbreaks with very high densities of larvae ( $> 500$  per  $m^2$ ) this species would seem very promising as a candidate for field production. One such field production system has been developed by the EMBRAPA research institute in Brazil for producing *Anticarsia gemmatalis* NPV (AgMNPV) at a cost of US\$1.26 per ha (Moscardi, 1999). This is used to produce some 40 tonne of infected insects annually that are processed into a biological insecticide used now on some 2 million hectares of soy crop each year. Trials in Tanzania in 2004 have shown that it is feasible to collect SpexNPV in quantity from armyworm outbreaks previously sprayed with SpexNPV (Mushobozi et al., 2005). A programme is now underway to adopt the EMBRAPA approach and its low cost clay formulations to the mass production of SpexNPV.

It has in the past been proposed that it would be a cost effective option to conduct the strategic control of armyworm by spraying primary outbreaks even in non-crop areas (Cheke and Tucker, 1995). However, as many of these outbreak areas occur in grazing habitats or national parks whose biodiversity is high and whose economic value is low this has never been considered environmentally acceptable or economically feasible. However, the use of a self-propagating biological agent such as SpexNPV, which is highly specific (OECD, 2002), would have no adverse environmental impacts. Indeed, given the slower action of a biological agent like SpexNPV its use in the strategic

control of outbreaks prior to their move into croplands may be the most appropriate role this agent could play in armyworm control. If the SpexNPV could be produced cheaply, using a combination of field production and a cheap formulation, then the strategic control of armyworm using SpexNPV could become a viable option. The benefits of preventing armyworm plagues spreading across Africa by treating the starting points in the primary outbreak sites in Tanzania and Kenya would be considerable.

In conclusion, although the work described here is at an early stage, it has demonstrated that SpexNPV is a promising viable alternative to the use of chemicals as part of a strategy for armyworm control and its further evaluation by the Tanzanian Ministry of Agriculture and Food Security is underway.

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### References

- Brown, E.S., 1966. Experiments on field application of virus to control armyworm. In: Minutes of 12th Meeting Specialist Entomologists and Insecticides Committee, 28–30 September 1966, Mombasa, Kenya (Appendix 16).
- Brown, E.S., Swaine, G., 1965. Virus disease of the African armyworm, *Spodoptera exempta* (Walker). Bull. Entomol. Res. 56, 671–684.
- Broza, M., Brownbridge, M., Shavit, A., Maniana, N.K., Sneh, B., 1999. Control of African armyworm *Spodoptera exempta* and the Egyptian cotton leafworm *S. littoralis* with *Bacillus thuringiensis* var aizawai, neem and a combination of both control agents. In: Abstract International Symposium on Biological Control Agents in Crop and Animal Protection, 24–28th August 1999, University of Swansea.
- Cheke, R.A., Tucker, M.N., 1995. An evaluation of the potential economic returns from the strategic control approach to the management of African armyworm *Spodoptera exempta* populations in East Africa. Crop Prot. 12 (2), 91–103.
- Cherry, A.J., Parnell, M., Grzywacz, D., Brown, M., Jones, K.A., 1997. The optimization of *in vivo* nuclear polyhedrosis virus production of *Spodoptera exempta* (Walker) and *Spodoptera exigua* (Hubner). J. Invertebr. Pathol. 70, 50–58.
- Copping, L.G., 2004. Manual of Biocontrol Agents, third ed. British Crop protection Council, Alton, UK, p. 702. ISBN 1 901396 35 5.
- Federici, B.A., 1999. Naturally occurring baculoviruses for insect control. In: Hall, F.R., Menn, J.J. (Eds.), Biopesticides Use and Delivery, Methods in Biotechnology, vol. 5. Humana Press, Totowa, USA, pp. 301–320.
- Gibbons, J.D., Chakraborti, C., 2003. Nonparametric Statistical Inference, fourth ed. (revised and expanded). Dekker, New York.
- Grzywacz, D., 1999. Final Technical Report of Crop Protection Programme Project R6746 Entomopathogenic Viruses for Control of African Armyworm *Spodoptera Exempta* in Tanzania, Natural Resources Institute, Chatham, 34pp.
- Haggis, M., 1987. Distribution, frequency of attack and seasonal incidence of the African armyworm *Spodoptera exempta* (Walk.) (Lep. Noctuidae), with particular reference to Africa and south-western Arabia. NRI Bull. L69.



- Harrap, K.A., Payne, C.C., Robertson, J.S., 1977. The properties of three baculoviruses from closely related hosts. *Virology* 78, 92–116.
- Harris, R.J.C., 1973. Safety testing of NPV of *Spodoptera exempta*. Centre For Overseas Pest Research London Technical Report 531 Annex 2, pp. 44–48.
- Hunter-Fujita, F.R., Entwistle, P.F., Evans, H.F., Crook, N.E., 1998. Insect Viruses and Pest Management. Wiley, New York, pp. 390–454.
- Inglis, G.D., Goettel, M.S., Erlandson, M.A., Weaver, D.K., 2000. Grasshoppers and locusts. In: Lacey, L.A., Kaya, H.K. (Eds.), *Field Manual of Techniques in Invertebrate Pathology*. Kluwer Academic Publishers, Dordrecht, pp. 651–680.
- Jayaraj, S., 2001. Microbial pesticides and integrated pest management for sustainable crop production. In: Ignacimuthu, S., Sen, A. (Eds.), *Microbials in Insect Pest management*. Science Publishers Inc., Enfield, USA, pp. 87–99 ISBN 1-57808-171-8.
- Jones, K.A., Moawad, G., McKinley, D.J., Grzywacz, D., 1993. The effect of natural sunlight on *Spodoptera littoralis* nuclear polyhedrosis-virus. *Biocontrol Sci. Technol.* 3, 189–197.
- Levy, K.J., 1979. Pairwise comparisons associated with the  $K$  independent sample median test. *Am. Stat.* 33 (3), 138–139.
- McKinley, D.J., 1975. NPV in the control of some lepidopterous pests of tropical agriculture current work and thoughts on strategy. *Meded. Fac. Landouwwet. Rijksuniv. Ghent* 40, 261–265.
- McKinley, D.J., Brown, D.A., Payne, C.C., Harrap, K.A., 1977. Cross infectivity and activation studies with four baculoviruses. *Entomophaga* 26, 79–90.
- Moscardi, F., 1999. Assessment of the application of baculoviruses for the control of Lepidoptera. *Annu. Rev. Entomol.* 44, 257–289.
- Mushobozi, W., Grzywacz, D., Wilson, K., Musebe, K., Kimani, M., 2005. New approach to improve livelihoods for poor farmers and pastoralists in Tanzania through monitoring and control of African armyworm, *Spodoptera exempta*. In: Harris, D., Richards, J.I., Silverside, P., Ward, A.F., Witcombe, J.R. (Eds.), *Aspects of Applied Biology* 75, Pathways out of poverty, pp. 37–45.
- Njuki, J., Mushobozi, W., Day, R., 2004. Improving armyworm forecasting and control in Tanzania: a socio-economic survey. CAB International Africa Regional Centre Nairobi, Unpublished Report, 49pp.
- Odindo, M.O., 1981. Dosage-mortality and time-mortality responses of the armyworm *Spodoptera exempta* to a nuclear polyhedrosis virus. *J. Invertebr. Pathol.* 38, 251–255.
- Odindo, M.O., 1983. Epizootiological observations on a nuclear polyhedrosis of the African armyworm *Spodoptera exempta* (Walk.). *Insect Sci. Appl.* 4, 291–298.
- OECD, 2002. Consensus document on information used in assessment of environmental applications involving baculoviruses. Series on Harmonisation of Regulatory Oversight in Biotechnology, vol. 20. ENV/JM/MONO(2002)1 OECD.
- Persson, B., 1981. Population fluctuations of the African armyworm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), in outdoor cages in Kenya. *Bull. Entomol. Res.* 71, 289–297.
- Rabindra, R.J., Muthuswami, M., Jayaraj, S., 1989. Controlled droplet application of nuclearpolyhedrosis virus with adjuvants and UV protectants for the control of *Heliothis armigera* on chickpea. *J. Biol. Control* 3, 37–39.
- Reynolds, S., 2001. From darkness into light. *New Agriculturalist* 23, 5 <[www.new-agri.co.uk/01-5/develop/dev02.html](http://www.new-agri.co.uk/01-5/develop/dev02.html)>.
- Rose, D.J.W., Dewhurst, C.F., Page, W.W., 2000. *The African Armyworm Handbook*, second ed. Natural Resources Institute, Greenwich, UK, 304pp., ISBN 0 85954 523 7.
- Scott, P.J., 1991. A general review of the evidence on the economic importance of the African Armyworm. Desert Locust Control Organisation East Africa Technical Report 100, 46pp.
- Smith, G.E., Summers, M.D., 1978. Analysis of baculovirus genome with restriction endonuclease. *Virology* 89, 517–527.
- Tinsley, T.W., 1979. The potential of insect pathogenic viruses as pesticidal agents. *Annu. Rev. Entomol.* 24, 63–87.
- Wigley, P.J., 1980. Counting insect viruses. In: Kalamakov, J., Longworth, J.F. (Eds.), *Microbial Control of Insect Pests*. New Zealand DSIR Bulletin No 228. Wellington.
- Zar, J.H., 1984. *Biostatistical Analysis*, second ed. Prentice-Hall, New York.