Laboratory and field evaluation of *Spodoptera exempta* nucleopolyhedrovirus (SpexNPV) for the control of African Armyworm 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 through the use of synthetic chemical insecticides, however this approach while effective is increasingly 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 2001-2004. Trials demonstrated that both ground and aerial application SpexNPV per to armyworm outbreaks on pasture can initiate rapid outbreaks of NPV disease and population collapses. The SpexNPV was effective when applied at 1 x10^{12} occlusion bodies (OB) per hectare. If applied to outbreaks early when larvae are in 1-III instar mass mortalities appear 3-5 days post treatment. The data from these trials indicate that SpexNPV can have a potential role as a substitute for chemical insecticides in armyworm management programmes.

Keywords

Spodoptera exempta, African armyworm, migratory pest, Baculovirus, Nucleopolyhedrovirus, biological control, IPM, Africa

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 per square metre. 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 in but in some years no serious outbreaks may occur (Scott 1991, Njuki *et al,* 2003). Control of this pest is routinely 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.

It has long been known that the African armyworm had a number of natural enemies and pathogens including viruses, fungi and protozoa but the most important was reported to be a specific baculovirus the Spodoptera exempta nucleopolyhedrovirus (SpexNPV) (Rose *et al,* 2000). This virus had been known since 1965 (Brown and Swayne 1965). 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 this disease is endemic in many parts of east Africa (Odindo 1983) and that this NPV is highly pathogenic to armyworm (Odindo 1981). Cage 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 localised effecting 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 the first physical map of the genome completed (Brown *et al,* 1984). Cross infectivity studies with a range of NPVs have indicated that Armyworm was susceptible only to the SpexNPV (McKinley *et al,* 1977, A C. Cherry pers comms.). 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). This is 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 build up 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 then resumed in 2001 when a follow on study was 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. The use of simple neem extract as a component in pest management had been promoted in East Africa by a number of development agencies and had been reported to show promise against armyworm (Broza *et al,* 1999). This paper reports on the laboratory evaluation and subsequent field trials undertaken to evaluate SpexNPV as a control for armyworm outbreaks in Tanzania. The trials involved evaluating the use of NPV against both the standard chemical insecticides and several neem formulations. 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 the method of choice for many farmers and aerial application, which is the mainstay of national control programme and also used by the largest commercial farmers.

Materials and Method

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 (McKinley *et al*., 1989*,* Hunter-Fuijita *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. After processing the NPV was counted using standard counting protocol (Wigley 1981) then freeze dried and stored at -30°C as a powder. 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 described in Hunter-Fuijita *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 volts and photographed with an MP4 camera. 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.

Laboratory bioassays and trials.

The insects for bioassays in the laboratory were reared on a combination of artificial diet and fresh wheat cuttings at a constant 26 ^{O} C with a 12/12 hour light dark cycle using a long established protocol for armyworm previously published (Smith *et al,*., 1999). The insects came from a wild stock collected in Tanzania in 1997 and subsequently maintained at NRI.

To determine the activity of the SpexNPV produced, the standard assay measures of LD_{50} and LC_{50} were determined. LC_{50} was used for newly hatched, neonate larvae (<1mg), to which it is difficult to give precise doses needed for LD_{50} estimation but for larger (30-50 mg weight) III instars LD_{50} can be determined directly. The LC_{50} values in neonate *S. exempta* larvae were determined using a standard droplet dosing technique (Hughes & Wood 1981). Dose series of the SpexNPV suspension from $5.7x10^{6}$ OB/ml to $9.12x10^{3}$ OB/ml in five fold dilutions were prepared with each dilution being assayed in fifty neonate *S. exempta* larvae and assays were repeated 5 times. 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 seven day mortality data generating LC_{50} values as an indication of virus potency. To determine the LD_{50} 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 as previously described (Shapiro & Evans, 1997, Jones 2000). 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 LD_{50} was determined using the SPSS probit analysis routine.

Trials of SpexNPV applied to wheat seedlings.
The application of SpexNPV to wheat seedlings was carried out in a 20x6 metre polytunnel at NRI following protocol described by Jones (2000)... High volume treatments were water-based and made up of NPV freeze dried 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 at the equivalent of 200 l/ha. Plots of 5.0m x 2.5m were marked out in the polytunnel and trays of seven day old wheat seedlings were placed at regular intervals on a predetermined regularly spaced 3x3 grid pattern throughout the plots. The virus was applied to the plots at application rates equivalent to 5 $x10^{11}$, $1x10^{12}$ and 5 $x10^{12}$ OB ha-1. Spray monitoring to check for coverage was through water sensitive papers placed at three per tray of seedlings. The trial was repeated with two different larval stages of armyworm, late third instar and mid fourth instar. The bioassays of the sprayed trays 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 untreated seedlings so that feeding was not interrupted. After seven days held under standard rearing conditions, by which time larvae had either pupated or succumbed to virus infection, mortality was recorded to check efficacy of each treatment.

The field Trials
The preliminary field trials were carried out during the 1999 then as part of a major follow on project in 2002 and 2004 armyworm seasons at several sites around Arusha in Tanzania. . The 2002 trials were carried out at M’ringa (S 03 20 25 0 E36 37 24 5) and the 1999 and 2004 trials at a site at Tengeru (03`27’S 36`48’E). 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 set up as part of the national armyworm forecasting network. The exact locations were then identified by follow up scouting for larvae (Rose *et al,* 2000). Crop trials were also established in pre-surveyed sites in 2003-2005 but these were not subsequently attacked. The sites around Arusha which is on the equator are high at over 1300 metres so sunlight and UV intensities are generally high. All trials were on pasture land that consisted of mixed grass species though the dominant grasses in quadrats (40%) were star grass (*Cynodon nlemfuensis*) with nutgrass species (*Cyperus* spp.) less common but important. The remainder were a mixture of other species including *Chloris* spp. *Eragrostis* spp. and *Seteria* spp

Ground spray trial 1999

The first site field trial was on grassland around the Tengeru to validate that SpexNPV application could kill larvae under field conditions. Plot sizes at Tengeru were 20m x

10m and the application volume was 200 l ha^{-1} using a standard locally available lever operated knapsack sprayer a “Hardi 15”, were water based and consisted of NPV freeze dried powder mixed with 0.01% v/v Triton. Three treatments were included two low rates of application 1 $x10^{11}$ OB ha^{-1} and $5x10^{11}$ OB ha $^{-1}$ with a control only sprayed with water plus triton. All treatments were replicated four times. At this site larval density was at an average of 51 larvae m^2 at the I-II instar at application. The sprayed plots were monitored daily after application for 7 days post application and the larvae quadrat counted as dead or alive.

Ground spray trial 2002

This ground spray trial in 2002 was again a small plot replicated trial with plots 20x20 metres with three replicates of each treatment .The treatments included NPV used at two application rates 5 $x10^{11}$ and 1 $x10^{12}$ occlusion bodies (OB) ha^{-1} that included 0.1% triton as a dispersant, neem oil at 0.1%, an insecticide check using Sumithion (Fenitrothion) EC 35% at the recommended rate of 200 ml per hectare and a triton-water control. All applications were made using standard lever operated knapsack sprayer “Hardi 15” with applications made at 170 litres per ha and a solid cone 12 bore nozzle and red pressure spring. The pasture on this trial site was mixed species grasslands dominated by the star grass (*Cynodon spp*.) and nutgrass (*Cyperus spp*) and the vegetation was 3 to 15 cm in height. The weather was overcast with wind speeds of > 1.0 metres/second during treatment application and temperatures varying between 18-32 ^{o} C. The applications were carried out between 9-11 am and made at a walking speed of 0.75 m/s with the spray nozzle held 60-80 cm above canopy height. The formulations were applied with a track spacing of 0.8 metres. Heavy infestation with I and II instar larvae was observed prior to commencement of the trials.

Tengeru ground spray trial 2004

This trial was conducted on 0.5 ha plots, one per treatment, on pasture land adjacent to the research station at Tengeru. Following high moth catches in late January an infestation of young larvae (2-3 days old) were found at the paddocks at high densities (>200 larvae per m^2) on the 9th February. Quadrat vegetation counts showed the dominant grasses in quadrats (40%) were star grass (*Cynodon nlemfuensis*) , 25% were mixed Cynodon and others, 10% were nutgrass species (*Cyperus* spp.) the remainder were a mixture of other species including *Chloris* spp. *Eragrostis* spp. and *Seteria* spp. The SpexNPV was applied at 1 $x10^{12}$ OB ha^{-1} ha with 0.1% triton. Other treatments were fresh neem leaf extract at 50% w/v, neem seed extract at 5% at w/v, a chemical insecticide control Diazinon applied at the recommended rate of 1 liter of Diazinon 60%EC per ha and an untreated control. The treatment applications were made by motorised mistblower (Solo 412 aster) with an application rate of 50litres/ha with the nozzle 30-50 cm from the canopy and a swath width of 8 metres was employed. All plots were sprayed on February 12th, starting at 1530h and finished at 1800h. Throughout this period, it was hot (average 26.4 ^{o} C Range 10-33) and sunny with a clear sky and a light breeze (<2m per second). The larvae were approximately 5 days old when the grassland plots were sprayed. No rainfall occurred during the 5 days post spraying. Armyworm counts were made the day before application and at 1, 3, 5, 7 and 9 days post application.

Aerial NPV application trial 2004

The site of the trial was pastureland on the M'ringa estate a mixed farm and coffee plantation some 5 km west of Arusha. Reports from a pheromone had indicated a sudden increase in moth numbers (>200 per night) during the week beginning Monday 8th March. Newly hatched armyworm larvae were found at counts of over 200 per m^2. The pasture was predominantly star grass (*Cynodon spp.*) with some nutgrass (*Cyperus* sp.) and some clumps of non graminacious weeds and was vegetation was 3-10cm in height. 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 for control counts. This area had similar vegetation composition and armyworm counts showed that this control area had armyworm infestation numbers not significantly different to those in the sprayed plot. Aerial spraying was carried out from a Cessna 188 plane fitted with hydraulic Cooper Pegler nozzles set on finest setting. NPV was made up in 165 litres of water with 0.02% triton surfactant to aid dispersion and applied as a 20-metre swath (GPS guided) at 20 litres per ha. Counts of the armyworm were made two days before the application then one, four, six and eight 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 ^{o}C).

Assessment of field trials

Assessment of all the trials was carried out through estimating larval populations within treated plots using 50x50cm quadrat counts the method most commonly used for assessing armyworm control trials (Rose *et al,* 2000). Armyworm counts were carried out 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 larval stage recorded. In small plot trials quadrats were thrown randomly from the centre of the plot. In large plot trials however quadrats were counted at regular intervals along transect lines to ensure 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 non parametric procedures (the median test due to Mood and the Mann-Witney test) were used to analyse count results using the Minitab statistical package. Proportionality data (dead/alive) was analysed for significance using Chi square tests in Minitab.

Comment: Next para useful or should be left out?????

Obtaining trials 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 each field season but many were aborted or produced no data due to natural population collapses from heavy rain or natural NPV. Another problem in collecting data from small plot trials early in the season when grass is short was that larvae in control plots would eat all the grass and migrate out of the plots before the NPV had time to manifest its action.

Results

The results for the laboratory bioassays of the #0045 SpexNPV isolate are presented in table 1. The bioassays mortality time response illustrates that in neonate larvae (Fig 1) mortality is more rapid with 80% mortality by day 3 than in III instar larvae (Fig 2) where mortality only reached 60% by day 5. The time mortality response of III and IV instar larvae exposed to wheat seedlings sprayed with SpexNPV (Figure 3) shows that all the treated rates produced high levels of mortality by day 5 in III instars whereas in IV instars application even at the highest rate equivalent to $5x10^{12}$ OB ha^{-1} failed to produce mortalities exceeding 40% even by day 7. From this laboratory and glasshouse data it may be concluded that to successfully treat outbreaks of armyworm it would be important to apply the SpexNPV to armyworm outbreaks no later than the III instar if control was to be both rapid and effective.

The preliminary ground trials 1999

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 4). Even at the lowest rate used $1x10^{11}$OB ha^{-1}, 96% of larvae were dead after seven days. This result confirms the previous glasshouse trials that low concentrations of SpexNPV are highly effective in killing armyworm even when used as a simple suspension (without formulation) in local sprayers. After the trial, large numbers of virus-killed larvae were observed in the sprayed plots (see Figure 4) 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 results of the ground spray trial in 2002 (Fig 5) illustrate the wide variation in armyworm densities seen in the field with small plot trials. To visualise more clearly the dynamics of control the count data is presented in Table 2 as percentages of the mean plot count on day 0. In the control plots the armyworm counts increased by some 50% 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 three and 10 days post application. Reductions in armyworm numbers were a maximum of 87% in the high-rate SpexNPV treatment and 100% in the insecticide treatment. The insecticide produced an immediate crash in armyworm numbers which then stayed very low throughout the trial though they had risen again by day 10. The higher rate SpexNPV application initially produced no reduction in numbers but afterwards counts fell to some 20% of its initial level by day 10. 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. Day 10 counts for the different treatments were significantly different (chi squared = 64.26, df =4, p = <0.001) with SpexNPV high and insecticide counts significantly different to Control, Neem and SpexNPV low (q = 4.07, Critical value = 3.858). In plots with the lower application rate of SpexNPV no reduction in armyworm was observed during the trial.

The 2004 ground trial shows the control counts were stable over the 9 days of this trial (Fig 6) while in insecticide treated plots counts fell by day 1. Again the data is

presented as percentages of the initial counts in Table 3 to illustrate the relative pattern of the armyworm numbers between the different treatments. In this trial control counts remained similar throughout though falling to 70% initial count by the end. The insecticide again acted rapidly reducing counts to effectively zero by day one and the counts remained low until the end of the trial. The SpexNPV plots gave counts that were significantly lower than controls by day 5 (Chi squared 53.8, df = 4, P = <0.001) and not significantly different to the insecticide treatment by day 10. Counts in both the neem treatments were significantly different to the control by day 7 (chi squared 72.35, df = 4, P = <0.001). On day 9 counts in the neem seed plot were significant lower than in the neem leaf treatment (Chi squared 93.17, df = 4, P=<0.001). Thus of the two neem treatments both saw armyworm counts reduced though the reduction was greater in the neem seed extract plots than the leaf extracts.

In the aerial trials the results of counts of numbers of larvae at both the sprayed and unsprayed sites are shown in Figure 7. It can be seen clearly that the numbers of armyworm population in the NPV and control plot increased after initial scouting as larvae continued to hatch out. By the fourth day after application the number population of live larvae in the NPV plot fell dramatically and then continued to decline until by day 6 the outbreak declined to nearly zero in the sprayed area. In the control plot the numbers population also declined as larvae matured but remained by day 6 at greater than 100 per m^2, a sufficient level to produce heavy damage to the pasture. Counts in the control were significantly higher than for the NPV plot on day 4 (Mood's median test Chi sq. = 48.65, df = 1, P=<0.0001) and day 6 (Mood's median test Chi sq. = 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. Figure 8 shows that by day 4 over 70% of larvae counted in quadrats were dead or dying with clear 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 9). 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 disappear or are eaten by scavengers such as ants that were abundant in these pastures**.**

Discussion

The results for LD_{50} on this strain of SpexNPV are somewhat higher than the LD50 of 48.4 OB per 15 mg larva previously reported by Odindo (1981). It is 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 (Robertson *et al,* 1995) but this data could indicate that the strain studied by Odindo could be 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 genetically different. There have been as yet no studies published of the genetic diversity of SpexNPV though such a study linked to the work reported here is underway. The findings are that there is a wide diversity in genetic makeup of isolates

but as yet none have been found to be more pathogenic in the laboratory than the type tested in the trials reported here. (J S Cory pers. comms.).

The results of the wheat seedling trials showed the speed of kill is much faster when treating larvae at III instar and earlier and indicated the importance of treating armyworm outbreaks as early as possible if SpexNPV was to be most effective. This clearly indicates that SpexNPV is not suitable for treating old outbreaks of late instar larvae that are the most destructive. The need to treat outbreaks early if SpexNPV is to succeed is a potential serious limitation to the use of this agent and will require the national and local forecasting systems to be effective and timely in warning farmers. Training scouts to recognise outbreaks in the earliest stages will also be important. Current efforts to improve these systems to meet this challenge are underway and while the early results are promising (Mushobozi *et al,* 20005) a high and sustained level of effectiveness will be needed if NPV is to play a useful role in armyworm control strategy.

In a series of trials the higher field application rate of 1 $x10^{12}$ OB ha^{-1} did show consistent control of armyworm while the lower rate of SpexNPV applied, 5 $x10^{11}$ OB, per ha 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 $x10^{12}$ OB ha^{-1} was a balance between a rate showing efficacy with the need to keep rates low enough to be economic. The rate of 1 $x10^{12}$ OB per ha 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 $x10^{12}$, (Copping 2004) Helicoverpa *armigera* MNPV 1.5 $x10^{12}$(Cherry *et al,* 1997), *Spodoptera exigua* MNPV 1x 10^{12} (Federici 1999). Rates higher than this are recommended for some other NPVs e.g. Mimestra brassicae NPV at 1 $x10^{13}$ OB ha but these can raise serious cost issues.

The slower speed of action of SpexNPV than the chemical insecticides normally used is an issue. In these trials SpexNPV took between three and six days to produce 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 will remain 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 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 pers. comms.) 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.

These trials described here were carried out on pasture and 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 pers. comms.) 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 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 in trials to apply the NPV 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. 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 after 3pm and peak solar radiation. It was noteworthy that SpexNPV performed given it was applied on an open pasture canopy without additives to improve its UV stability.

In this trial it 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 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, Anon 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 (Anon 2005, D Murray pers. comms). 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 was more likely due to immigration into the plots. This may be a phenomena 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 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 pers. comms).

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 (Lysansky 1997). 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 plants through production in cultured *S.exempta* (Cherry y*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 that of the current chemical insecticides (≥US$10 ha).

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 1.26US$ per ha (Moscardi 1999). This is used to produce some 40 tons 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 NPV (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 NPV 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. Other NPVs have been used in area wide control programmes previously for other major Lepidopteran pests such as *Heliothis* Spp. (Street *et al,* 1997). 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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Figures & Tables

Table 1. Acute toxicity of SpexNPV to different instar of armyworm, LC_{50} and LD_{50} values of SpexNPV in *S. exempta* larvae (n = 5).

Larval Instar	LD/LC_{50} value	95% Fiducial Limits
Neonate LC_{50}	$8.81x10^4$ OB/ml	$6.19x10^4 – 1.19x10^5$
LD_{50} III Instar on artificial diet	$6.50x10^5$ OB/larva	$4.90x10^5 – 1.10x10^6$
LD_{50}III Instar on wheat	$3.16x10^4$ OB/larva	$2.09x10^4 – 5.17x10^4$

Table 2 Effect on armyworm numbers after application of different control agents in 2002 ground trial mean armyworm counts per $0.25m^2$ as a percentage of initial counts before application (n = 4).

	Armyworm counts as percentage of initial count ± S.E.		
Treament	Day 3	Day 6	Day 10
Control	92±22	143±17	149±10
NPV 5 $x10^{11}$	134±36	135±24	110±10
NPV 1 $x10^{12}$	125±14	86±13.5	22±15
Neem	30±30	90±27	119±14
Chemical	0.0±0.0	0.5±0.5	12±4.0

Table 3 Effect on armyworm numbers after application of different control agents in 2004 ground trial mean armyworm counts per 0.25m^2 as a percentage of initial counts before application

Treatment	Day 1	Day 3	Day 5	Day 7	Day 9
Control	102±11.4	92±15.7	104±10.2	135±8.4	71±6.2
Neem leaf	93±12.7	46±6.8	49±5.3	41±3.2	40±2.4
Neem Seed	92±10.3	47±10.4	39±12.3	19±8.9	17±7.2
NPV 1 x10^{12}	66.3±13.2	33±15.5	16±10.8	12±10	9±9.9
Chemical	11±12.7	22±17.6	24±9.4	19±9.1	14±9.5

Figure 1 Cumulative mean mortality of neonate armyworm larvae treated with different concentrations of SpexNPV in laboratory bioassays

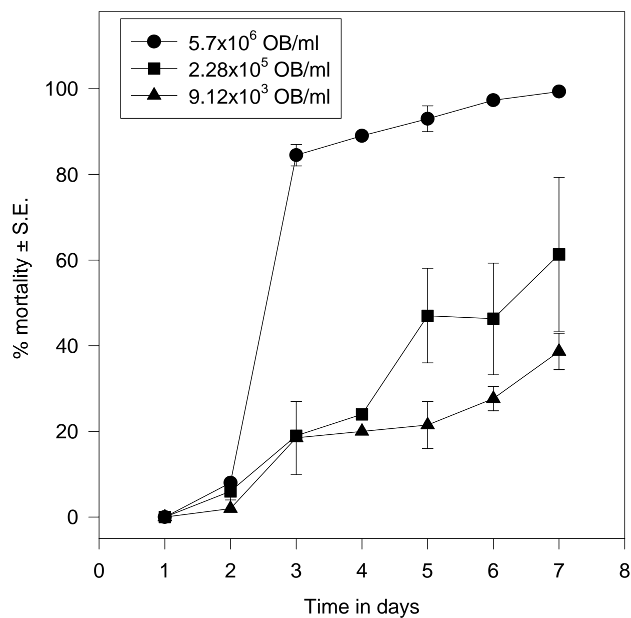

Figure 2 Cumulative mean mortality of III instar armyworm larvae treated with different doses of SpexNPV in laboratory bioassays

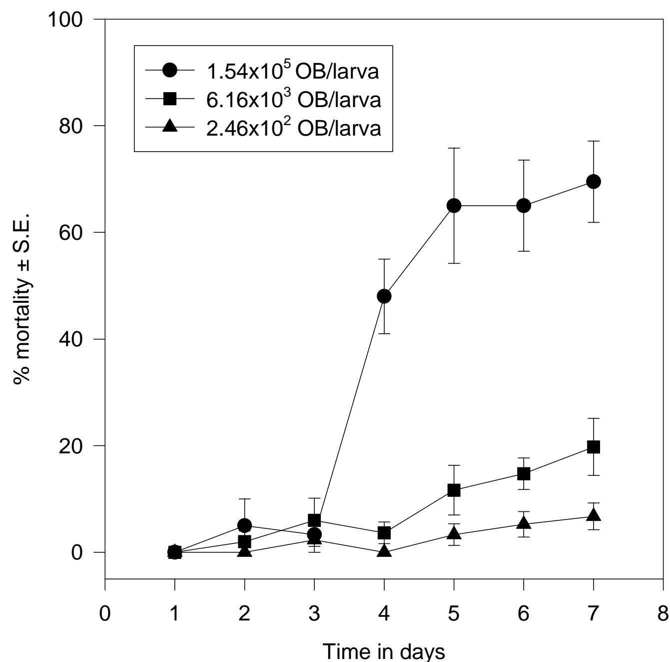

Figure 3 Results of glasshouse trials evaluating different application rates of SpexNPV in OB per hectare on cumulative percent larval mortality of III and IVth instar armyworm larvae on trays of treated wheat seedling.

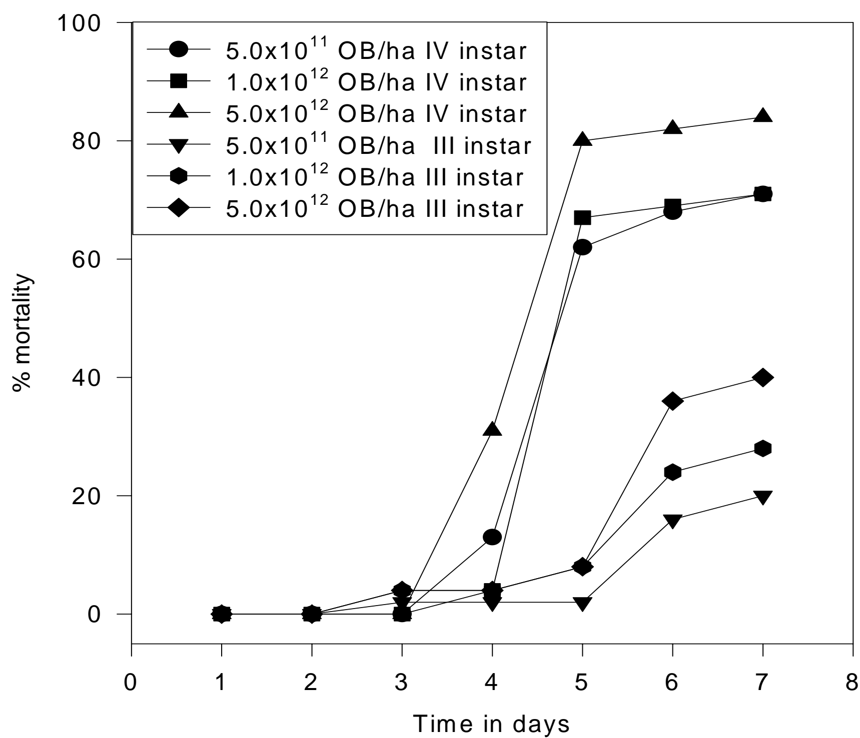

Fig 4 Results of ground spray trial showing mortality of armyworm larvae in small plot trial after application of SpexNPV at two different rates Arusha 1999.

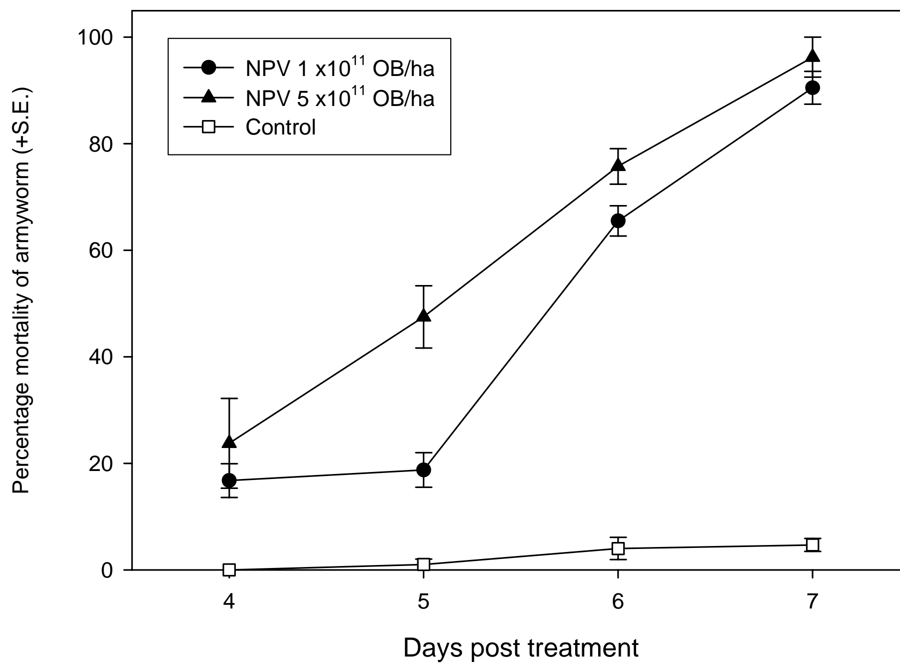

Figure 5 **M'ringa ground spray trials 2002 mean densities of armyworm larvae following treatment with various treatment applications (mean number of larvae per 0.25m^2 quadrat ± s.e.)**

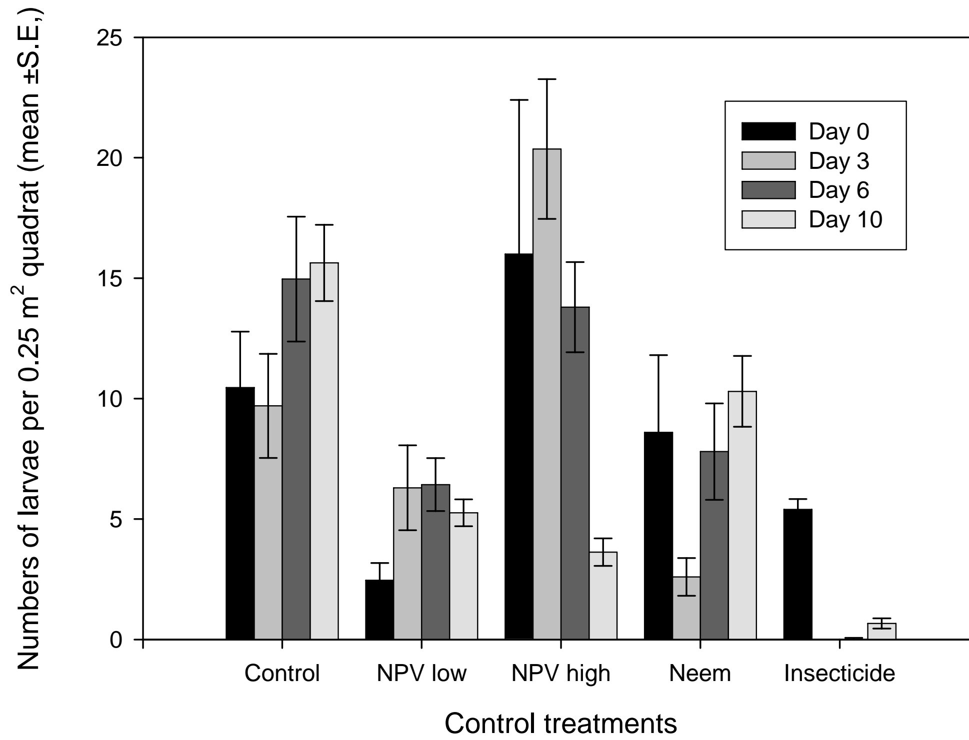

Figure 6: Tengeru ground spray trial 2004 mean densities of armyworm larvae following treatment with various treatment applications (mean number of larvae per 0.25m^2 quadrat $\pm$ s.e.).

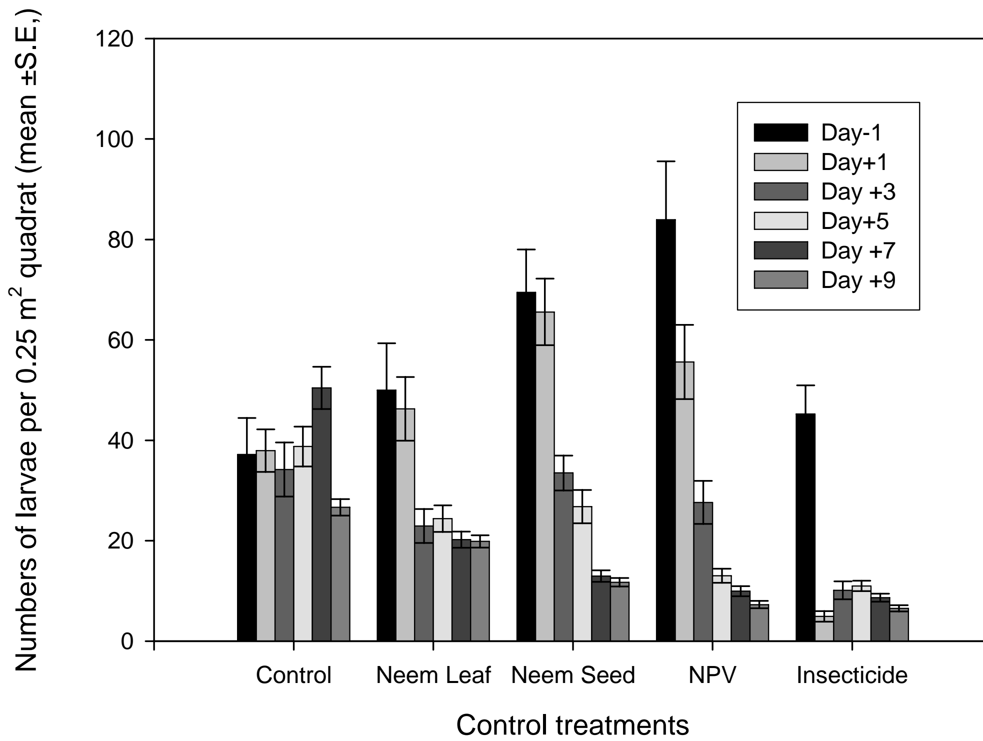

Fig 7 Aerial spray trial M'ringa 2004 effect on armyworm number in NPV treated and control plots after application as mean counts per 0.25m^2

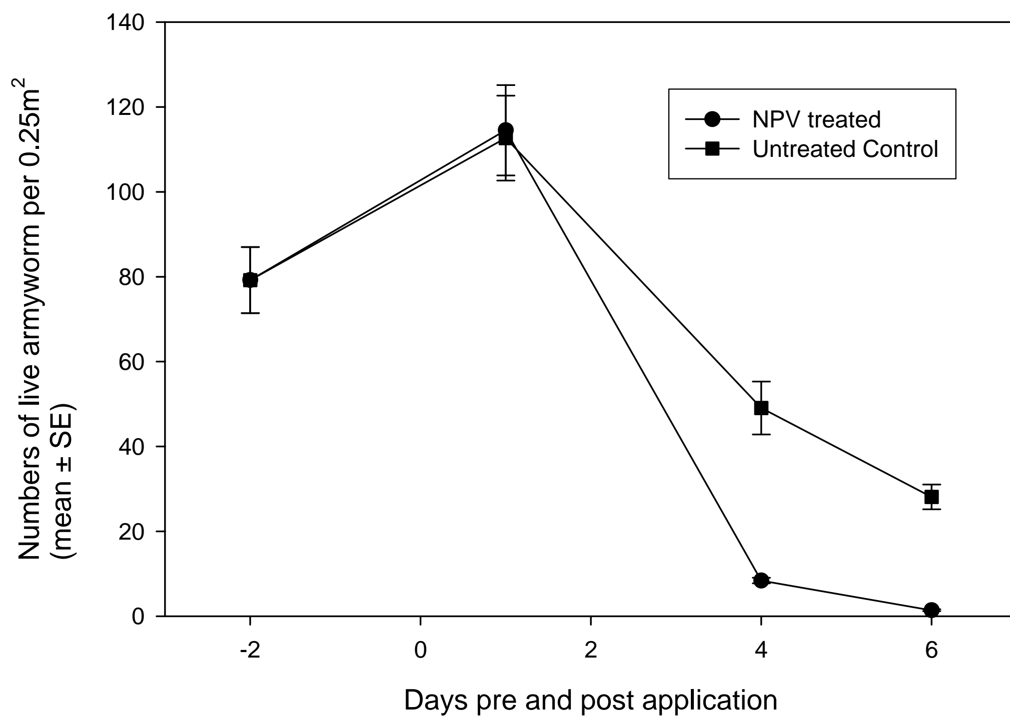

Fig 8 Aerial spray trial 2004 proportion of larvae counted diagnosed as infected or dead of NPV in NPV treated and control plots

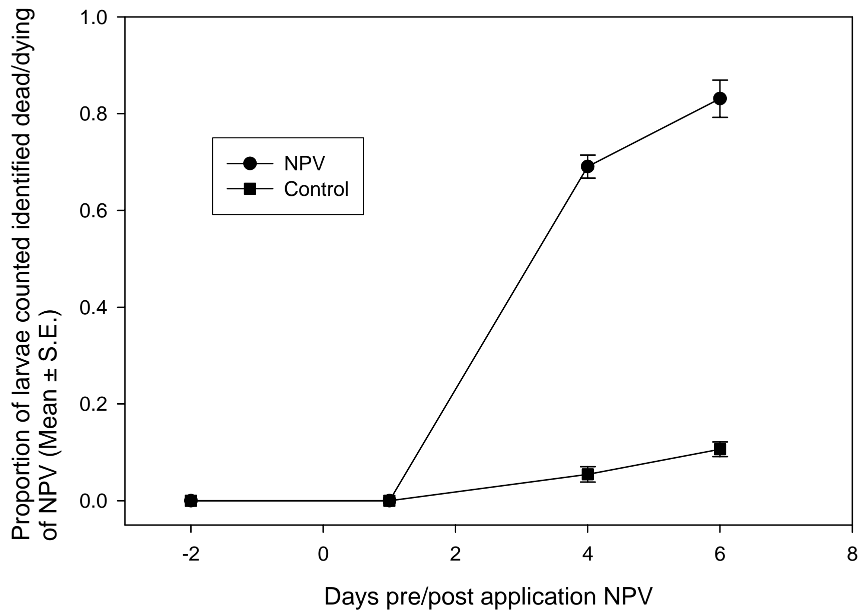

Fig 9 **Aerial spray trial 2004 Effect on numbers of dead larvae per 0.25m^2 counted in NPV treated and control plots**