The development of endemic baculoviruses of *Plutella xylostella* (diamondback moth, DBM) for control of DBM in East Africa

David Grzywacz\(^1\), Mark Parnell\(^1\), Gilbert Kibata\(^2\), George Oduor\(^3\), Walter Ogutu\(^3\), Douglas Miano\(^2\) & Doreen Winstanley\(^4\)

\(^1\)Sustainable Agriculture Group, Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK

\(^2\)Kenya Agricultural Research Institute, Waiyaki Way, PO Box 14733, Nairobi, Kenya

\(^3\)CAB International, Africa Regional Centre, PO Box 633, Village Market, Nairobi, Kenya

\(^4\)Horticultural Research International, Wellesbourne, Warwickshire, CV35 9EF, UK

Corresponding author: d.grzywacz@gre.ac.uk

**Abstract**

A project to develop non-chemical methods of DBM control on *Brassica* crops in Kenya has been exploring the use of endemic pathogens as potential control agents. Initial surveys for endemic pathogens identified *P. xylostella* granulovirus (*PlxyGV*) on farms in Kenya. Subsequently 14 genetically distinguishable isolates were identified from field collected material. These were purified and ranging bioassays showed these isolates were pathogenic to Kenyan strains of DBM with LC\(_{50}\)s varying from 2.36 x 10\(^6\) to 3.95 x 10\(^7\) occlusion bodies (OB) per ml for II instar DBM. One isolate (Nya-01) was selected and subsequently used for field trials in Kenya. The trials showed that unformulated *PlxyGV* applied at weekly intervals at a rate of 3.0 x 10\(^13\) OB/ha could control DBM on kale more effectively than available chemical insecticides. After application, infection rates in DBM can reach 90%. Further field trials are currently underway to determine the lowest effective dose rate for this virus when applied as a formulation. Initial virus production studies using *in vivo* propagation in II instar DBM reared on cabbage showed an initial productivity of 4.0 ± 0.44 x 10\(^10\) OB/larva.

**Keywords**

*Brassica*, granulovirus, biocontrol, Kenya

**Introduction**

Larvae of the diamondback moth (DBM) *Plutella xylostella*, feed only on plants from the family Brassicaceae and are a major pest of *Brassica* vegetables (kale, cabbage, rapeseed, etc.) throughout Kenya (Michalik 1994). Presently, conventional chemical insecticides are heavily relied upon to control them (Kibata 1997). It is well known that DBM has become resistant to chemical insecticides in many countries throughout the world (Roush 1997) and current programs underway in Kenya have indicated that chemical resistance in DBM is also occurring there (Kibata 1997). The chemical insecticides currently recommended for control are expensive, damaging to the environment and in some areas simply not available to the small-scale farmers who account for a high percentage of the *Brassica* vegetable production of Kenya (Kibata 1997).

To address this issue, a collaborative project between the Natural Resources Institute (NRI), the Kenya Agricultural Research Institute (KARI) and CAB International, Africa Regional Centre (CABI-ARC) was set up to investigate alternative methods of DBM control. One component of the project investigated the possible use of endemic baculoviruses.

Before this study GVs of *P. xylostella* had been reported from Japan (Asayama & Osaki 1970), Taiwan (Wang & Rose 1978, Abdul Kadir 1986), China (Abdul Kadir et al. 1999) and India (Rabindra et al. 1997), but there were no previous published records from Africa. A number of other NPVs, some uncharacterised (Padamvathamma & Veeresh 1989), have been reported as infecting DBM, but a review of the potential of DBM pathogens concluded that only the GV showed promising levels of pathogenicity (Wilding 1986). More recently an NPV has been identified from *P. xylostella* in China. This was characterised as being genetically similar to, though genetically distinct from, *Autographa californica* MNPV and *A. falcifera* MNPV (Kariuki & McIntosh 1999).
Materials and methods

Pathogen survey and identification
To collect baculoviruses, a survey of Brassica farms was conducted around Nairobi. In total, 27 farms were surveyed within a 170 km radius of Nairobi. In field sampling, larvae showing signs of baculovirus infection, puffy appearance and pale-yellow to white colouration (Asayama & Osaki 1970) were collected and individually stored for later examination. Standard, unstained wet mounts of infected larvae were examined using a microscope and dark-field contrast at x400 magnification to detect the presence of baculoviruses. Each candidate GV isolate was propagated in vivo in 15 II instar DBM following methods described by Parnell (1999). Restriction endonuclease analysis (REN) of the baculovirus isolates was performed on each of the GV isolates individually following the protocol of Smith and Summers (1978) as modified by Rabindra et al. (1997).

Bioassay of pathogen strains
The pathogenicity of the different isolates was determined by means of two bioassay methods. Comparative bioassays using single discriminate doses were performed on nine GV isolates displaying different DNA profiles. Subsequently, in order to obtain LC_{50} values, dose series bioassays were carried out on three of those eight isolates and the PlxyGV-Tw isolate. The concentration of GV was determined by counting using a 0.02 mm depth bacterial spore-counting chamber viewed under dark phase illumination at x200 magnification. Discriminate dose bioassays and dose response bioassays were carried out as per Parnell (1999). Bioassay data were corrected using Abbot's correction for control mortality and dose series data analysed using a probit analysis with the SPSS data analysis package.

Field trials
To evaluate the potential of the Kenyan PlxyGV to control crop loss caused by DBM, isolate Nya-01 was selected for mass production and use in small-plot field trials. This isolate was selected because it had been indicated as the most pathogenic strain in the laboratory bioassays. The virus was applied as a simple unformulated suspension using standard farmer equipment. Volume application rate for all treatments was 800 litres/ha. The first field trial was carried out on the research farm at Jomo Kenyatta University of Agricultural Technology (JKUAT) 25 km outside Nairobi and ran for 12 weeks in late1998. This was a randomised-block design trial carried out on small plots of 5 m x 5 m with a one metre gap between plots and a plant/row spacing of 60 cm. The test crop was kale (var. Thousand headed). This trial compared two virus treatments, a weekly application of high application rate of 3.0 x 10^{14} (occlusion bodies {OB}) and a medium rate of 3.0 x 10^{13} OB/ha. There was a no treatment control and a standard farmer insecticide treatment schedule based upon weekly application of the local standard pyrethroid insecticide (Karate®- lambda-cyhalothrin).

A second field trial was carried out at the National Agricultural Research Laboratory (NARL) farm on the outskirts of Nairobi in 2000. In this trial there were five treatments arranged in randomised replicated plot design. The treatments were three virus application rates (3 x 10^{12}, 3 x 10^{13} and 3 x 10^{12} OB/ha) a no treatment control and a standard insecticide treatment with Karate® as before. The plots were 5 x 5 m with a one metre gap between plots and a plant spacing of 60 x 60 cm.

In both trials, 10 random plants in the central area of the plot were sampled weekly for numbers of DBM larvae, numbers showing symptoms of GV infection and damage caused by DBM. In addition, in the second trial yield data were also collected. To assess more precisely the disease incidence in the plots, after three weeks of the trial 45 larvae of each instar were collected from each treatment and reared individually in the laboratory and the disease occurrence recorded. The yield data were analysed using 2 way ANOVA on the SigmaStat statistical package (SPSS Inc., USA).

PlxyGV productivity
In order to estimate the productivity of the PlxyGV when produced in vivo, two hundred II and III instar larvae were inoculated with a range of concentrations of the strain Nya-01 and reared under standard conditions until death. Progeny virus was collected, counted and its identity confirmed using REN.

Results
During the field survey, 127 larvae with disease symptoms were collected from eight of the 27 farms included in the survey. Microscopic examination confirmed that 95 larvae collected from four of the eight...
farms were suffering from GV infection. The areas in which GV-infected larvae were found were Nyathuna (84 larvae-two farms), South Kinangop (9 larvae) and Naivasha (2 larvae).

The REN analysis of the 95 PlxyGV isolates showed that 14 had fragment profiles that could be distinguished from any other with both EcoR1 and PstI cuts (Figure 1). Comparison of these 14 Kenyan PlxyGV isolates to an isolate of PlxyGV from Taiwan (PlxyGV-Tw) revealed that, although the profiles had many similarities, there were major band differences between all isolates. Both the PstI and EcoR1 digests revealed between 2 and 6 major band differences between isolates, even in those collected from the same location (Figure 1).

Figure 1. Comparison of PlxyGV isolates. DNA of each isolate was digested with PstI restriction endonuclease, fragments were separated on 0.6% agarose gel. Track 1 (far left of page), 1kb molecular size standard; tracks 2-16, Kenyan PlxyGV isolates from Nyathuna (Nya-01, Nya-02, Nya-03, Nya-06, Nya-07, Nya-14, Nya-15, Nya-25, Nya-27, Nya-29, Nya-35, Nya-37, Nya-40, Nya-42, Nya-52 respectively), track 17, PlxyGV isolate from South Kinangop (SK-01); track 18, Taiwanese PlxyGV; Track 19, λ 19-Mix molecular size standard.

Results from the discriminate dose assay showed every Kenyan isolate to be significantly more potent than the PlxyGV-Tw with average % mortality ranging from 26.2% to 40.3% compared with 5.2% for the PlxyGV-Tw (Figure 2). However in the dose response bioassays, no significant differences in LC50 values between Kenyan isolates and the PlxyGV-Tw isolate were observed. Average LC50 values for II instar DBM larvae varied from $2.36 \times 10^6$ OB/ml for Nya-01 PlxyGV to $3.95 \times 10^7$ OB/ml for Nya-40 PlxyGV. In comparison, the LC50 for the PlxyGV-Tw was $1.55 \times 10^7$ OB/ml.
The management of diamondback moth and other crucifer pests

Proceedings of the 4th International Workshop, Nov. 2001, Melbourne, Australia

Figure 2. Average percent mortality in discriminate dose bioassays of Kenyan *PlxyGV* and Taiwanese *PlxyGV*.

The field trials carried out at JKUAT showed that the *PlxyGV* when sprayed using standard farmer application equipment was highly infectious to DBM, spreading rapidly in trial plots and infecting 80-90% of larvae within two to three weeks of application (Figure 3). Very little occurrence of infected insects was recorded from the control or insecticide treated plots. Both the high dose rate of $3.0 \times 10^{14}$ OB/ha and the lower dose of $3.0 \times 10^{13}$ OB/ha reduced DBM damage to crops to below that seen in either unsprayed controls or insecticide treated plots (Figure 4).

In the second trial at NARL, the yield data (Figure 5) showed that the highest application rate dose gave significantly higher yield than the no treatment control (37% higher, $P<0.001$ df=4 and 28, $F=6.25$) or the insecticide treatment (17% higher, $P<0.001$ df=4 and 28, $F=6.25$). The average DBM numbers in each treatment showed an application-rate effect with the lowest numbers occurring in the highest virus rate treatment (Figure 6). In the second trial, average observed DBM infection rates in virus treated plots also showed a clear application-rate trend with the highest dose producing an average of 40% (Figure 7). In this trial there was some infection observed in the control and insecticide plots. From insects sampled from the *PlxyGV* application-rate plots, the true infection rate was much higher than that observed in the field and Table 1 shows the percent virus mortality recorded from insects taken from the plot treated at $3 \times 10^{13}$ OB/ha.
Figure 3. The level of *PlxyGV* infection observed in treatments from the first field trial in Kenya.

Figure 4. The level of crop damage observed in treatments from the first field trial in Kenya.
Figure 5. Average kale yield per hectare for each of the treatments from the NARL field site.

Figure 6. Average DBM population per hectare for each of the treatments at the NARL field site.
The management of diamondback moth and other crucifer pests

Figure 7. Average PlxyGV infection-rate observed in DBM larvae for each of the treatments at the NARL site.

Table 1. Percentage GV infections that developed in laboratory-reared larvae sampled from virus plots sprayed with $3 \times 10^{13}$ OB/ha

<table>
<thead>
<tr>
<th>Larval instar</th>
<th>DBM Infection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>90</td>
</tr>
<tr>
<td>II</td>
<td>82</td>
</tr>
<tr>
<td>III</td>
<td>64</td>
</tr>
<tr>
<td>IV</td>
<td>60</td>
</tr>
</tbody>
</table>

The maximum productivity of the $Plxy$GV was found to be $4.0 \pm 0.44 \times 10^{10}$ OB/larva obtained from II instars inoculated with $2.0 \times 10^6$ OB/ml.

Discussion

The pathogen survey revealed that the GV of DBM occurred on 50% of the farms surveyed, though, in all cases, with a relatively low incidence. On no farm were widespread epizootics observed or reported by local farmers questioned. The discovery of so many different genetic isolates (14) in the small number of infected larvae collected is therefore striking. Previously reported work (Abdul Kadir et al. 1999) has characterised only two genetically distinct isolates one from China and one from Taiwan. Other studies of DBM pathogens have also only reported finding a single genetically distinct isolate from India (Rabindra et al. 1997) and Japan (Yamada & Yamaguchi 1985).

The GV isolates from Kenya are genetically similar to, though genetically distinct from, the previously reported Taiwanese isolate. This isolate we now know is itself similar to and closely related to the Chinese isolate (Abdul Kadir et al. 1999). The two isolates studied differed from each other by one to three major bands in the $Eco\text{R}1$, $Bam\text{H}1$ and $Hind\text{III}$ profiles. The differences in the Kenyan isolates studied here were greater at two to six bands with only two profiles $Eco\text{R}1$ and $Pst\text{l}$, even amongst isolates collected from the same farm. This genetic diversity amongst isolates of $Plxy$GV from Kenya could be extremely useful as a diverse genetic resource that could be exploited in the development of a GV for DBM control. The high level of variation in the $Plxy$GV isolates could indicate a long association between $Plxy$GV and DBM in the region and could have a bearing on the debate concerning the origin of DBM. This was generally considered to be somewhere in Mediterranean Europe having evolved on cultivated brassicas also believed to have
European origin (Hardy 1938). Recently however, the Mediterranean origin of DBM has been brought into question by Kfir (1998) who hypothesised a southern African origin for DBM on the basis of the diversity of wild hosts and endemic parasitoids found in South Africa. The genetic variation in PlxyGV isolates discovered in Kenya during the present study and apparent lack of diversity in isolates from other regions of the world might be interpreted as providing additional support to the theory that the origin of DBM lies in Sub-Saharan Africa.

The initial discriminate single dose bioassay results showed all the Kenyan isolates to be significantly more pathogenic than the Taiwanese isolate. However the LC₅₀ data from the subsequent dose response assays showed no significant differences, even though the mean LC₅₀ for Taiwanese isolate was 6.5 times higher that of the most active Kenyan isolate (Nya-01). This result reflects the high variability in response seen with some Kenyan isolates including Nya-01. These were originally in vivo propagated, but not cloned, which might have reduced this variability. These isolates have since been cloned and the assays are currently being repeated on these cloned isolates.

The productivity of the Kenyan isolates is high at 4.0 ± 0.44 x 10¹⁰ OB/larva, equivalent to 8.0 x 10⁹ OB/mg. This may be compared with between 1.9 x 10¹⁰ and 4.5 x 10⁹/larva reported with other GVs produced in Lepidoptera (Evans 1986). High productivity is a valuable asset in a potential biopesticide as it reduces the number of insects needed to produce the desired application rate. At this rate of production, the highest application rate used in these trials, 3.0 x 10¹⁴ OB/ha would be equivalent to 7,500 infected larvae/ha. In comparison, most existing commercial baculovirus products are applied at rates of between 50-500 larval equivalents/ha (Moscardi 1999).

The first field trial showed that application of PlxyGV at 3 x 10¹³ OB/ha could reduce DBM damage much better than either the use of the standard chemical insecticide or the no treatment control. The very limited effectiveness of the standard insecticide, lambda-cyhalothrin, suggests that significant resistance has developed in DBM. Resistance has since been confirmed by other work in Kenya (Cooper 2001) and lambda-cyhalothrin is now no longer recommended for DBM control.

The speed with which weekly sprays of PlxyGV initiated infection rates of 90% could indicate that one or two applications of PlxyGV at the start of the season might be sufficient to start an epizootic infection in resident DBM populations. However whether augmentative approach alone would be sufficient to produce control of DBM numbers and damage, would need testing under field conditions. While collection of a high percentage of infected insects in virus treated plots suggests that recycling of PlxyGV is very important, its precise contribution to control remains to be quantified.

In the second trial, the yield results showed that again the PlxyGV performed significantly better than the chemical insecticide at the highest application rate used 3 x 10¹⁴ OB/ha. A similar result in terms of controlling DBM numbers has been reported by Su (1989) using a Taiwanese isolate applied as here at seven day intervals. However direct comparisons are difficult, as in Su's (1989) trial, the PlxyGV was quantified in terms of larval equivalents per litre and no direct enumeration of the GV was carried out.

Glasshouse trials again have showed that application of the Taiwanese isolate can reduce DBM numbers and that there is a dose response over the range 9 x 10¹¹ to 9 x 10¹³ and at the highest dose the PlxyGV reduced damage as effectively as application of Bacillus thuringiensis (Abdul Kadir 1992). In addition it was shown that the addition of molasses to a formulation could increase the viruses efficacy by a factor of ten and allow for a consequent reduction in the application rate of PlxyGV. This finding closely mirrors that of Ballard et al. (2000) who found that addition of 10% molasses produced a similar 10 fold increase in efficacy with the codling moth (Cydia pomonella) granulovirus (CpGV) on apples.

The two granuloviruses that have been commercialised to date, CpGV and Adoxophyes orana granulovirus, are both sold for application at rates of 1 x 10¹³ OB/ha. In comparison, the rate of PlxyGV used here which produced a significant increase in yield is 3 x 10¹⁴ OB/ha. Even given that the Kenyan PlxyGV seems to be more productive than other GVs, this suggests a need to reduce the application rate by a factor of ten if its use is to be commercially attractive.

The trials reported here did not include formulation ingredients and field trials of such a formulation are underway now in Kenya to evaluate the efficacy of reduced rate formulated PlxyGV. Formulation might also address the short persistence time on field crops seen with GVs. Abdul Kadir (1986) reported that with
PlxyGV-Tw exposure of unformulated virus to seven hours of sunlight in Malaysia was sufficient to reduce virus efficacy by 50%. Although the persistence of the Kenya PlxyGV has yet to be quantified it is unlikely to be longer.

In conclusion, while the results of these trials of PlxyGV are promising it has yet to be determined that PlxyGV can be effective or reliable enough for consistent control of DBM.

References


Abdul Kadir HB, Payne CC, Crook NE & Winstanley D. 1999. Characterization and cross-transmission of baculoviruses infective to the diamondback moth, Plutella xylostella, and some other Lepidopterous pests of brassica crops. Biocontrol Science and Technology 9, 227-238.


Ballard J, Ellis DJ & Payne CC. 2000. The role of formulation additives in increasing the potency of Cydia pomonella granulovirus for codling moth larvae in laboratory and field experiments. Biocontrol Science and Technology 10, 627-640.


The management of diamondback moth and other crucifer pests

1985, Tainan, Taiwan, The Asian Vegetable Research and Development Center, Shanhua, Taiwan, AVRDC Publication No. 86-248, pp. 219-232.
