CHARACTERIZATION OF *PLUTELLA XYLOSTELLA* GRANULOVIRUS (PLXYGV) ISOLATES FOR THE MANAGEMENT OF DIAMONDBACK MOTH IN KENYA

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ABSTRACT

Baculoviruses (Family Baculoviridae) have been used to control Lepidopteran pests in China, Japan and India. Several studies on baculoviruses have been done in Kenya but there has been no commercial use. Collection of local isolates and characterization is recommended to identify and select the most potent isolate for use in mass production. Two Kenyan isolates of *Plutella xylostella* granulovirus, from field-collected and insectary larvae respectively, were characterized by electron microscopy and bioassays. The isolates were found to show the typical morphological features of Baculovirus in the genus granulovirus. The results of surface contamination bioassays for both isolates exhibited the characteristic dose-mortality response curve, with LD_{50} values that were not significantly different from each other. The median survival time of 2nd instar larvae at an average temperature of 25°C ranged from 5.5 to 7.5 days depending on the virus concentration to which the larvae were exposed. Further investigation is recommended.

Key words: Biological control, Baculovirus, Granulovirus, *Putella xylostella*.

Introduction

Plutella xylostella (L) **(**Lepidoptera: Plutellidae), commonly known as the diamondback moth (DBM), is a major pest of cruciferous crops worldwide, particularly of cabbages. DBM is oligophagous and feeds on crops containing mustard glucosides (Thorsteinson, 1953). In Kenya, damage of up to 100% has been observed even with frequent application of pesticides (Michalik, 1994) consequently it is considered the most limiting factor of successful production of cruciferous vegetables. In tropical countries, this pest with a short life cycle of 16-23 days, may have 10 or more generations per year (Thompson, 1992) , resulting in rapid population growth in the field. Growers in Kenya rely primarily on an intensive schedule of insecticide applications for control of DBM. Frequent use of synthetic chemical insecticides has led to resistance (Gathui *et al.*, 1994). This observation underscores the need for development of a rational system for management of this pest (ICIPE, 1997).

Insect viruses, notably members of the family Baculoviridae, are attractive as biological control agents and could be feasible alternatives to chemical insecticides (Sun and Peng, 2007). Baculovirus [*P. xylostella* granulovirus (PlxyGV)] infection of DBM was first reported in Japan (Asayana and Asaki, 1969). Since then several workers have reported that granulovirus shows promise as a control agent for DBM (Nakahara *et al.*, 1986).

Granuloviruses consist of a virus particle embedded in an ovicylindrical protecting, paracrystalline protein body (granule) of approximately 150 x 350 nm. The virus infection cycle starts when a larva ingests virus-contaminated foliage. Viral granules dissolve under the alkaline mid-gut conditions and virus particles are released, pass through the peritrophic membrane and enter columnar midgut epithelium cells. Virus

replication takes place and progeny virus particles bud through the cellular membrane into the haemolymph. Secondary infections are then initiated throughout the host body, where new granules are synthesized in massive numbers (Federici, 1997). After death of the caterpillar its cuticle ruptures and virus granules are released onto the leaf surface. The time period for the virus to kill the host depends on the virus: host combination and the environmental conditions, particularly ambient temperature (van Beek *et al*., 1998; 2000). This study sought to characterize local Kenyan isolates of baculoviruses and identify potent isolates for mass production and use in integrated pest management.

Materials and Methods

Insects and virus isolates

P. xylostella larvae used for virus multiplication and bioassays were reared in styrofoam boxes on artificial diet (Multi species diet, Southland Products Inc., Lake Village, Arkansas) to which 7.5 ml of linseed oil and 4 g of dried, pulverized kale leaf per litre was added. Two granulovirus isolates were studied, one collected in the field near Nanyuki (designated F4) and the other isolated from a spontaneous virus outbreak in Kenya Biologics' insectary (designated I5). The virus isolates were extracted from larvae exhibiting the typical symptoms of baculovirus infection (moribund, whitish and flaccid when dead).

For virus propagation 2nd instar DBM larvae were inoculated through contamination of the diet with diluted samples of both isolates. After a suitable incubation period, larval cadavers were harvested, homogenized and purified using differential centrifugation (Hames *et al*., 1989), and stored under refrigeration (-20˚C) until use in subsequent experiments.

Morphological characterization

Pellets of PlxyGV were fixated in 3% glutaraldehyde and 2% paraformaldehyde, treated with osmiumtetroxide, followed by dehydration in increasing concentrations (50, 70, 80 and 95%) of alcohol, and finally embedded in London Resin (LR) White. Sectioning was done on a Leica Ultracut E microtome and the Ultrathin sections of approximately 60nm of the pellets were then stained with 2% uranylacetate, contrasted with lead citrate and photographed under a Jeol electron microscope (model JEM 1011).

Biological characterization

The two isolates of PlxyGV were used to inoculate 2nd instar larvae of DBM applied on diet surface at five concentrations: 0, 99, 494, 2468, 12342, and 61711 granules/mm^2 for isolate I5 and 0, 67, 335, 1675, 8375, and 41875 for isolate F4, respectively. Each assay was carried out using forty larvae per treatment replicated once. Mortality was recorded daily after the first four days for a period of 7 days. The data was subjected to probit analysis (Biostat, 2008) to determine the median lethal dose (LD_{50}).

Results and Discussion

Morphological characterization

The transmission electromicrographs showed a homogeneous collection of granule-shaped particles sectioned at different angles, and possessing all the features typical of a granulovirus. They were ovicylindrical capsules surrounded by an envelope enclosing each one membrane-bound virion (**Plate 1**). The capsules measured approximately 150 by 350 nm. The I5 sample was indistinguishable from F4 in morphology.

Biological characterization

Bioassay results indicated a clear dose-response relation for both the field and insectary isolates (**Fig. 1**). The shape of the

curves is sigmoid-like as may be expected for a larval population with normally distributed susceptibility to the virus. Probit analysis of the mortality data showed median lethal dose estimates of 7,109 granules/mm^2 for the field isolate and 7,649 granules/mm^2 for the insectary isolate (**Table 1**). Although the LD_{50} of the insectary isolate was higher than that of the field isolate, their confident limits overlap hence the isolates are not different in terms of virulence.

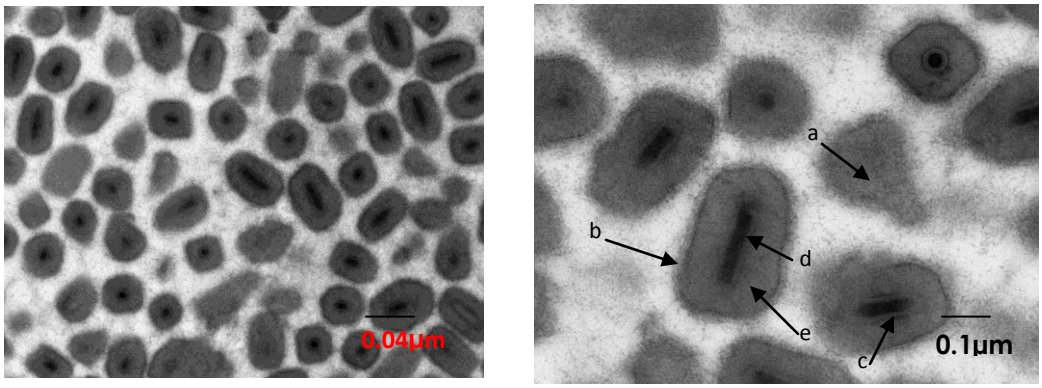

Plate1. Electromicrographs of PlxyGV-F4 at a magnification of 10KV (left panel) and 25KV (right panel). Arrows indicate: (a) particle section outside the plane of the virus particle; (b) granular envelope; (c) virion envelope; (d) nucleocapsid; (e) paracrystal protein matrix (granulin).

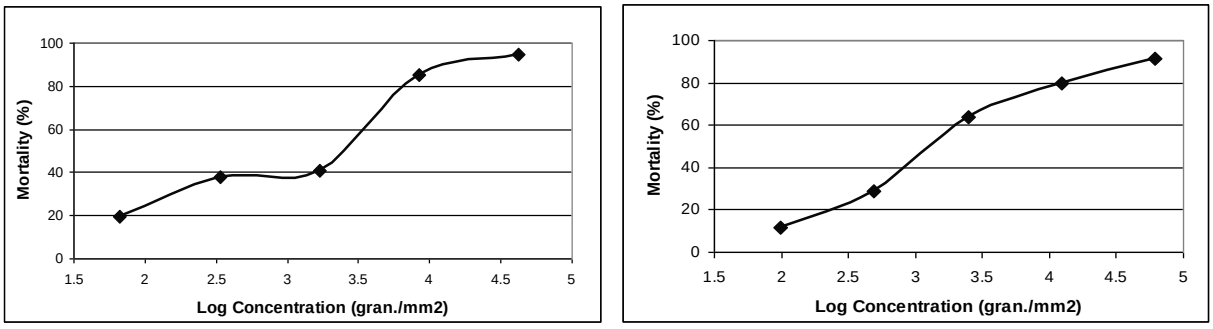

Fig.1. Larval mortality scored at different application rates of granulovirus on the diet surface. Left panel, F4 isolate; right panel, I5 isolate.

Table 1. Estimates of the median lethal dose (LD_{50}) of PlxyGV isolates F4 & I5, Standard errors and 95% confident limits as obtained from Probit analysis

Virus isolate	LD_{50} (granules/mm2	Standard error	95% (confidence limit)
PlxyGV-F4	7,109	1,535	4,085-10,133
PlxyGV-I5	7,649	2,358	3,012-12,286

The survival time of 2nd instar larvae inoculated at different doses and kept at an average temperature of 25°C (range 23.2-27.1) showed that higher concentrations lead to faster mortality (**Fig. 2**). The medium survival time of the larvae that died was estimated to range from 5.5 to 7.5 days for the F4 and 6.3 to 7 days for the I5 sample, respectively.

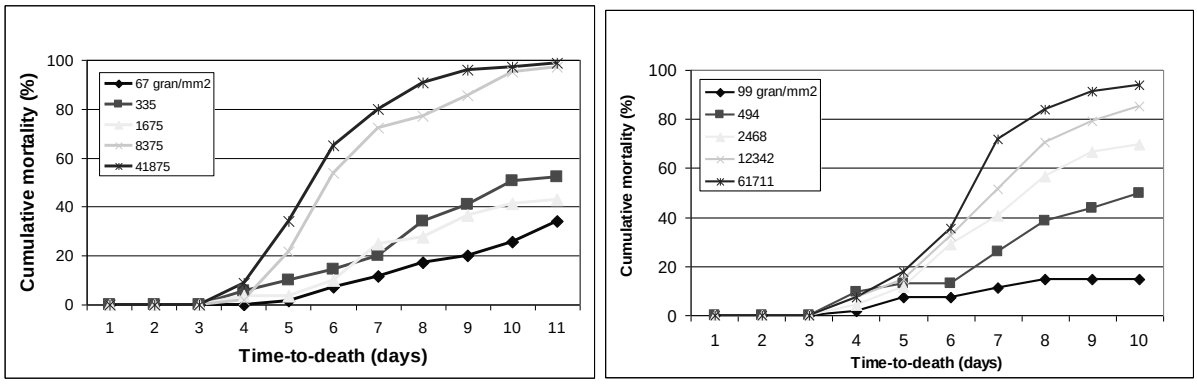

Fig. 2. Graphical representation of survival time of 2nd instars P. xylostella exposed to five different concentrations of PlxyGV. Left panel, PlxyGV-F4; right panel PlxyGV-I5.

The results obtained have demonstrated that both isolates show typical appearance of granulovirus. It is likely that the isolates represent *P. xylostella* granulovirus, in view of the extreme specificity of the genus. However, restriction endonuclease profiling will provide definitive proof that they are *P. xylostella* granulovirus isolates.

The bioassay method used, surface-contamination assay, does not allow for quantification of the dose ingested by the larvae, moreover, it is notably imprecise. The dose ingested by individual larvae varies due to different feeding behavior and uneven virus distribution over the diet surface. Nevertheless, the results of the bioassays conform to expectations of dose-responses in the form of sigmoid-shaped curves seen, and as cumulative mortality plots over time showed that increasing doses is directly proportional to the time to die of the larvae.

The level of imprecision of the method can be seen from the standard errors, which at 22 and 31% of the LD_{50} estimates for F4 and I5, respectively, are higher than desirable (10%). The confidence intervals of the LD_{50} overlap; hence the potency of the isolates cannot be distinguished with the bioassay method used. The small, early instar *P. xylostella* larvae are difficult to assay with more precise assay methods, such as the droplet feeding assay (Hughes *et al*., 1986), or diet-plug assays (Harrison and Bonning, 2001).

In conclusion, the natural occurrence of *P. xylostella* granulovirus in Kenya has been demonstrated. The potency of two isolates was also demonstrated, a method which could be used to select the best isolate for mass production as a promising viable alternative strategy to the use of chemicals for management of DBM. However, further evaluation for field efficacy of the isolates is recommended.

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