

Morphological and genetic characterization of a South African *Plutella xylostella* granulovirus (*PlyxGV*) isolate

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Plutella xylostella (L.) (Lepidoptera: Plutellidae), also known as diamondback moth, is a destructive insect pest of cruciferous crops (Talekar & Shelton 1993; Shelton 2004). The pest occurs wherever its host plants are cultivated and the global annual cost of damage and control is estimated to be US\$4–5 billion (Zalucki *et al.* 2012). The extensive use of synthetic pesticides for control combined with the high fecundity of *P. xylostella* has resulted in the pest developing resistance to nearly all classes of insecticides (Grzywacz *et al.* 2009). Moreover, these chemicals have negative environmental implications and may affect non-target species, some of which are natural enemies of the pest.

The family Baculoviridae consists of four genera of insect viruses: *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus* and *Deltabaculovirus* (Jehle *et al.* 2006; Carstens & Ball 2009). Viruses from two of these genera are used in biological control of insect pests, namely *Alphabaculovirus* (nucleopolyhedroviruses isolated from Lepidoptera), *Betabaculovirus* (granuloviruses isolated from Lepidoptera). Currently, six baculovirus biopesticides namely, Cryptogran™, Cryptex™ (for control of false codling moth, *Phaenatomotibia leucotreta*) (Moore & Hattingh 2012), Helicovir™, Bolldex™ (for control of bollworm, *Helicoverpa armigera*) (Moore & Kirkman 2010), Madex™ and Carpovirusine™ (for control of codling moth, *Cydia pomonella*) (Eberle *et al.* 2012) have been registered in South Africa. The use of baculoviruses as biocontrol agents in integrated pest management programmes is gaining increasing popularity in many countries due to their ability to control pest species that are resistant to chemical pesticides. Moreover, these viruses are host specific and not known to have adverse effects on beneficial insects or the environment (Groner 1990). Baculoviruses typically infect the larval stages of host insects upon ingestion of contaminated plant material, enter the midgut and fuse with the

microvilli on the columnar epithelial cells where genome replication takes place. Subsequently, progeny virions are produced that initiate a secondary infection resulting in the formation of numerous occlusion bodies (Friesen 2007). In the case of a *P. xylostella* infection, symptoms include swelling of the larval integument, a change in colour from bright green to pale yellow accompanied by the presence of a turbid white exudate and death of the insect host (Asayama & Osaki 1970; Federic 1997; Parnell *et al.* 2002). *P. xylostella* granulovirus (*PlyxGV*) has been isolated from infected insects in Japan (Asayama & Osaki 1970), India (Rabindra 1997), Taiwan (Kadir *et al.* 1999) and Kenya (Parnell *et al.* 2002; Muthamia *et al.* 2011). This is a first report of the recovery and genetic characterization of a South African *PlyxGV* isolate from a laboratory-reared *P. xylostella* colony.

Insects (pupae and asymptomatic larvae) were collected from a cabbage field at a single site on Varnam Farm in the Belmont Valley outside Grahamstown (33°18'S 26°32'E) in the Eastern Cape, South Africa and maintained in rearing chambers in a controlled environment room at 25 °C. Cotton wool soaked in sugar solution was provided as a source of nutrition for the adults, and eggs were allowed to hatch on canola seedlings before transfer to cabbage leaves in a separate larval rearing chamber. Larvae were transferred to new cabbage leaves at two-day intervals and pupae were returned to the adult rearing chamber. Virus manifestation occurred as the laboratory colony grew, leading to death of the colony. Symptomatic larvae were collected daily and stored at –20 °C for subsequent analysis.

Occlusion bodies (OBs) were purified using a protocol described by Hunter-Fujita *et al.* (1998) and Moore (2002) using approximately 1.8 g of insect larvae. For transmission electron microscopy (TEM), approximately 5 µl of purified OBs was placed onto a carbon-coated grid, dried and

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