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Research Note

Mass production of nucleopolyhedrovirus of the teak defoliator, *Hyblaea puera* Cramer using host population in teak plantations

V. V. SUDHEENDRAKUMAR,* T. V. SAJEEV and R. V. VARMA
Division of Entomology
Kerala Forest Research Institute
Peechi 680653, Kerala, India
Email* sudhi@kfri.org

ABSTRACT: **The nucleopolyhedrovirus of the teak defoliator, *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae) (*Hp*NPV) is a potential biocontrol agent of the pest. This paper describes a method to mass multiply *Hp*NPV *in situ* in the field population of *H. puera*. The method is based on application of the virus suspension on young teak plantation infested with *H. puera*. An average yield of 4.8816 x 10^8 Polyhedral Occlusion Bodies (POBs) per fifth instar larva was obtained with this method. The advantage of this method of *Hp*NPV production is that it is less expensive as it does not depend on host larval culture maintained in the laboratory.**

KEY WORDS: *Hyblaea puera*, *Hp*NPV, mass production, nucleopolyhedrovirus, teak defoliator

The management of the teak defoliator (*Hyblaea puera* Cramer) (Hyblaeidae; Lepidoptera), an important forest insect pest has received considerable attention in the recent years. Studies have indicated the prospects of using a baculovirus (*Hp*NPV) for managing this pest (Sudheendrakumar *et al.*, 1988; Nair *et al.*, 1996). Large-scale availability of the pathogen is a primary requisite in the biocontrol programme. In general, baculoviruses are mass-produced through *in vivo* replication in the homologous host maintained on artificial diet. Various methods of *Hp*NPV production have been attempted in the past. Nair *et al.* (1998) reported *Hp*NPV production by feeding host larvae collected from teak plantation with virus contaminated teak leaves in the laboratory. The disadvantage of this method was the high bacterial contamination of the harvested virus. Subsequently, larvae fed with virus treated leaves were reared individually maintained on artificial diet. Though an average yield of 1.9 x 10^8 POBs per larva could be obtained in this method, the cost of production was very high.

This paper reports the methodology and economics of *Hp*NPV mass production by infecting the teak defoliator larvae with *Hp*NPV *in situ* in teak plantations.

The study was carried out in a 7-year old teak plantation at Nilambur in the month of May. The experimental plot measured 0.04ha in area with about 600 trees planted at a spacing of 2x2m. The trees measured 2-4 meters in height. All the trees in the plot had teak defoliator infestation (about 15-20 third-fourth instar larvae/leaf).

The virus inoculum was prepared from a stock suspension of *Hp*NPV containing $1x10^7$ Polyhedral Occlusion Bodies (POBs)/ml. One litre of spray solution containing $1x10^{10}$ POBs in distilled water was sprayed in the plot using an ultra low volume hand held MICRON Ulva + disk atomiser sprayer (Micron Sparyers Ltd, UK). To minimise the effect of ultra violet radiations on the virus applied on leaves, spraying was carried out during the evening hours. Dead larvae were collected from the trees starting at 72 hours post inoculation. Further collection was made at 90-96 hours and 111-120 hours. Only fifth instar larvae were included in the sampling for dead larvae. The larvae were stored at –20°C until processed for virus extraction.

In order to estimate the virus count from the retrieved larvae, 100 fifth instar larvae which were harvested between 90-96 hour post inoculation were randomly selected from the lot and weighed. The larvae were macerated with distilled water using a pestle and mortar and then filtered through muslin cloth. The filtrate was centrifuged at 8000 rpm for 20 minutes, and the supernatant was removed. The pellets were resuspended in distilled water and allowed to settle for 5 minutes. The supernatant was collected and the number of POBs estimated using haemocytometer. From the count, the total virus produced against the initial inoculum applied in the field, larval equivalent and virus per mg of body weight were estimated.

A total of 7278 dead fifth instar larvae were harvested from the virus-applied plot between 72–120 hours post inoculation. Highest numbers of larvae were recovered during 90-120 hour post inoculation. The cause of larval death was confirmed to be due to *Hp*NPV infection.

The virus yield from one hundred randomly selected fifth instar larvae weighing 22.5g was 4.8816×10^{10} POBs, the larval equivalent being 4.8816×10^8 POBs. The virus produced per unit body weight was 2.1653×10^9 POBs /mg. Total yield of virus produced from the study plot was estimated as 3.5528×10^{12} POBs which is about 335 times more than the virus applied in the field.

A total of five man-days were required for harvesting 7278 larvae from the field, which incurred labour cost of Rs.500. The quantity of 3.5528×10^{12} POBs produced from 0.04ha area is sufficient to cover about 22 ha of teak plantation (ultra low volume spraying) with a dosage of $1.63x10^{11}$ POBs/ha targeting third instar larvae (Sudheendrakumar *et al.*, 2001)). The cost of production depends on various factors including the density of host population surviving at the time of harvest. The optimum harvest time is a crucial factor influencing the percentage of larval harvest. Beyond the optimum incubation period, collapse of the larvae would result in decreased larval harvest.

One of the advantages of using the field population is that the larvae are healthier compared to the larvae in the laboratory culture. The virus productivity is also on the higher side under this method. While the yield obtained in the laboratory was $1.9x10^8$ POBs (Sudhendrakumar *et al.*, 2001) the yield obtained in the field method is 4.8816×10^8 POBs. The major advantage of the field method is that it does not incur expenditure for maintaining a host culture.

A large variation in the yield of virus depending on the sites of replication, the type of virus, and body weight of larva used and time of harvesting has been reported by various workers. Ignoffo (1966) estimated that at least 6×10^9 POBs were produced per larvae in late instars of *Heliothis zea*. Evans (1986) reported that within the family Noctuidae, NPV yield between $5x10^8$ and $5x10^9$ POBs per larva could be expected from the final instar larvae. The mean larval equivalent of *Hp*NPV estimated in the present study ($4.8816X10^8$POBs) confirms the virus yield predicted for the noctuids in general.

In the present study, the virus yield from the sample in terms of POBs per unit body weight was $2.1653x10^9$. Cherry *et al.* (1997) estimated the POBs/mg body weight of *Spodoptera exempta* and *S. exigua* as $1.7x10^7$ and $1.1x10^7$, respectively. Based on the above figures and data on nine lepidopterans taken from literature they suggested the mean

POBs/mg body weight in lepidopterans as 1.01± $0.17x10^7$. The figure generated in the case of *H. puera* is rather on higher side.

The population of *H. puera* in teak plantations can be used for *Hp*NPV production only at the initial pest build-up period, namely, during April-May when the larvae are fairly healthy and free from parasitisation. With increase in pest population there is a corresponding increase in parasitism making the filed collected larvae unsuitable for virus production. Also *Hp*NPV production method described here depends on availability of the host larvae in young plantations of reachable height. This method may not be advantageous during rainy season as heavy loss of larvae can be expected. However, further studies are required to optimise the virus productivity to make this method more cost-effective.

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