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USE OF BACULOVIRUS CONTROL AGENTS WITHIN AN INTEGRATED PEST MANAGEMENT STRATEGY AGAINST TEAK DEFOLIATOR, *HYBLAEA PUERA*, IN INDIA

K.S.S. Nair
R.V. Varma
V.V. Sudheendrakumar
K Mohanadas
T.V. Sajeev
Division of Entomology

Kerala Forest Research Institute
Peechi- 680 653, Kerala, India

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K.S.S. Nair
R.V. Varma
V.V. Sudheendrakumar
K Mohanadas
T.V. Sajeev

Kerala Forest Research Institute
Peechi- 680 653, Kerala, India

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ABSTRACT OF THE PROJECT PROPOSAL

1. Project No	:	KFRI 245/95
2. Title of the Project	:	Use of baculovirus control agents within an integrated pest management strategy against teak defoliator, *Hyblaea puera*, in India
3. Objectives	:	To develop an IPM strategy for management of *H. puera* populations, based primarily on improved monitoring and prediction of pest outbreaks and on the use of the baculovirus, *HpNPV*, as a pest control agent
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Project Personnel (KFRI)		
Principal Investigator	:	Dr. K.S.S. Nair
Investigators	:	Dr. R.V. Varma Dr. V. V. Sudheendrakumar Dr. K. Mohanadas Mr. T. V. Sajeev (since February 1997)
Research Fellows	:	Mr. T.V. Sajeev (upto January 1997) Mr. K.V. Sathyakumar (since January 1996) Mr. P.K. Gireesh (since June 1997) Mr. Byju Hameed (upto December 1995)
Collaborating Scientist (Forestry Research, UK)	:	Dr. H.F. Evans
Collaborating Institute	:	Forest Research (an Agency of the Forestry Commission) Alice Holt Lodge, Wrecclesham Farnham, Surrey, GU10 4LH United Kingdom

ABSTRACT

The main consideration in this study was to use the *HPNPV* (*Hyblaea puera* nuclear polyhedrosis virus) economically and effectively for controlling the teak defoliator. The optimal use of the virus was examined considering various parameters within the Control Window conceptual model. The information quantified included aspects of virus-host interaction, virus production methods, virus yield and the effect of environmental factors like ultra violet light and rainfall on virus persistence. The efficiency of different spraying systems was also evaluated based on the quantified information on droplet emission and field capture rates. The parameters predicted by the model was field tested to calculate the optimal field dosage rates.

Analysis of the relationship between virus dosage and host mortality revealed that response of the five larval instars varied with larval age and weight. Young larvae were more susceptible than older larvae such that the LD_{50}s were 17, 70.2, 72.7, 3932 and 20125 Polyhedral Inclusion Bodies (PIBs) for instars I to V respectively. This indicated that dosages for late larval instars will be too high for field application and therefore, it is good to target the control at the third instar stage.

Under virus production, tests with individual larvae indicated that up to $7x10^8$ PIBs could be produced from a single fifth instar larva. Various methods of mass production of *Hp*NPV were examined and finally a combination of individual feeding on virus contaminated leaf, followed by feeding on semi-synthetic diet gave a better yield with minimal bacterial contamination. The mean yield achieved was approximately $2x10^8$ PIBs per larva for semi-purified virus.

Studies on comparison of virus persistence and activity in direct sunlight and in darkness indicated that when exposed to full sunlight, mortality of third instar larvae fed with known quantities of virus dropped from 97% to 70% over a six-hour period. This rate of decay continued up to about 24 hours, after which it was stabilised at 13% mortality up to 12 days. When subjected to various levels of artificial rainfall (up to 200 mm per hour) there was rapid loss of virus activity and the mortality of the test larvae dropped from 90% to15%.

For field application of the virus, ultra low volume (ulv) controlled droplet application (cda) was employed. Three sprayers namely, Micron Sprayers Ulva+ rotating disk atomiser: Micron Sprayers Ulva Fan rotating disk atomiser and Stihl SR400 motorised mistblower with Micronaire AU8000 rotating cage atomiser were evaluated. While the ulva + and ulva fan

models were useful for spraying small trees of about 3.5 meters height, Stihl SR400 was found to be useful for spraying trees up to 14 meters.

Tests were also carried out to find out a potential carrier fluid to provide stability of the formulated virus preparation which will not evaporate quickly under the high temperatures. The formulation developed was a stable emulsion with 94% coconut oil, 5% water and 1% emulsifier (Laboleine or Tween 80).

Field trial employing four virus dosages (5 $x10^{10}$, $1x10^{11}$, $2x10^{11}$ and $4x10^{11}$ PIBs per ha) gave 90% infection at the highest dose compared to 70% infection at the lowest dose. Assessment of damage was carried out by visual observation of percentage loss and by fresh weights of leaves taken at random from the sprayed trees. Analysis revealed significant differences in leaf damage and leaf weight between treated plots and untreated control plots. The trend in foliage weight indicated a direct response between virus dosage and leaf weight in relation to time of post spray, the maximum weight gain being achieved at the highest virus dose.

The study revealed the scope of using *HpNPV* in the most efficient way against H. puera, by considering various parameters within the Control Window concept.

1. INTRODUCTION

Teak defoliator, *Hyblaea puera*, (Lepidoptera: Hyblaeidae) is the most significant insect pest of teak (*Tectona grandis*) through most of its range in the tropics and sub-tropics. Studies carried out in Kerala have indicated losses averaging 44% of potential growth increment over a five year study on trees of four to eight years old (Nair *et al.*, 1985; Nair *et al.*, 1996).

The pest management options considered in the past included the use of natural enemies-parasitoids, predators and diseases (Nair *et al.*, 1995, Sudheendrakumar 1986; 1997, Mohamed Ali *et al.*, 1992), silvicultural management through exploitation of relative susceptibility at the tree level (Nair *et al.*, 1997) and studies on the dynamics of *H. puera* outbreaks (Nair, 1988; Mohanadas, 1997). Among these, the most promising control agent was found to be a baculovirus isolated from *H. puera* larvae (Sudheendrakumar *et al.*, 1988). Baculoviruses are double stranded DNA viruses that have been isolated only from arthropods. They have been used extensively in pest management programmes world-wide and have been shown to be highly specific, with no recorded effects on non-target organisms outside the arthropods. The *H. puera* baculovirus was identified to be a nucleopolyhedrovirus (NPV) (*HpNPV*) which affects the nuclei of infected cells and in which the virions (virus particles) are occluded within a crystalline protein coat called polyhedral inclusion body (PIB). NPVs of Lepidoptera affect most of the organs in the insect body and multiplies. The PIBs can survive outside the host, within and between larval generations.

Initial work at KFRI indicated that *HpNPV* could be multiplied in *H. puera* larvae in the laboratory (Mohamed-Ali *et al.*, 1992). Under field conditions, a high volume application of a crude preparation of *HpNPV* gave reduction in leaf damage up to 76% (Nair *et al.*, 1998). However, further improvement of the virus mass production method, standardisation of virus application method, determination of precise field dosage etc., was necessary for developing a an effective biocontrol strategy against the teak defoliator. It was also recognised that the methods used to evaluate *HpNPV* in both laboratory and field needs to be improved. With these views in mind, a collaborative project was developed in collaboration with the Forestry Commission Research Agency, U.K. (FCRA).

The project was based on the *Control Window* concept (Evans, 1994) which was successfully employed for control of pine beauty moth, *Panolis flammea* (Lepidoptera: Noctuidae) and pine sawfly, *Neodiprion sertifer* (Hymenoptera: Diprionidae) in Scotland using baculovirus (Entwistle *et al.*, 1990). Evans (1998) developed this concept further to provide a framework for determining optimal dosages of microbial insecticides to reduce or eliminate the need for *ad hoc* experimentation in the field. The essential components are provision of quantitative measures of host susceptibility, host age/size distribution, host feeding rates, host feeding sites on the tree, rates of attrition of virus in the field and detailed knowledge of droplet emission and field capture rates for given spray applicators. There was little or no prior information on any of these components and, therefore, the current programme was initiated with a fresh look at using *HpNPV*, as a control agent. However, the basic information already generated by KFRI on the dynamics and distribution of *H. puera* in teak forests helped a lot in developing strategies for timing the application of *HpNPV*.

OBJECTIVES

The objective of the project was to develop an IPM strategy for management of *H. puera* populations, based primarily on improved monitoring and prediction of pest outbreaks and on the use of the baculovirus, *HpNPV*, as a major component in the control programme. Quantification of the following aspects was also carried out to achieve the main objective.

- Insect biology: Monitoring systems for adult moths (moth behaviour, aggregation pheromones, spatial dynamics, choice of host tree and oviposition site), larval biology (feeding sites, feeding rates, within and between plant distribution, development rate), pupal biology (choice of pupation site, duration).
- Virus biology: Dosage-mortality and virus productivity data for all larval stages, linked to larval weight, rate of decay of virus in the field.
- Plant factors: Tree age, leaf phenology, relationships to other host plants, physical and chemical characteristics of leaves of different ages.
- Environment: Ultra-violet light, temperature, humidity, rainfall, pre- and post-monsoon effects on adult dispersal.
- Application: Equipment (Low Volume (LV) and Ultra Low Volume (ULV)), formulation for stability and flowability, optimisation of droplet production and deposition in the field.

2. MATERIALS AND METHODS

The whole programme was based on the control window concept. A visual representation of the factors that have been studied in this programme is represented. (Figure 1). It centres around five primary variables- the *Host (Hyblaea puera)*, the *Virus (HpNPV)*, *Environmental Factors*, *host tree (Teak)* and *Spray technology*. The *Control Window* is a conceptual model that provides a means of bringing these variables together quantitatively.

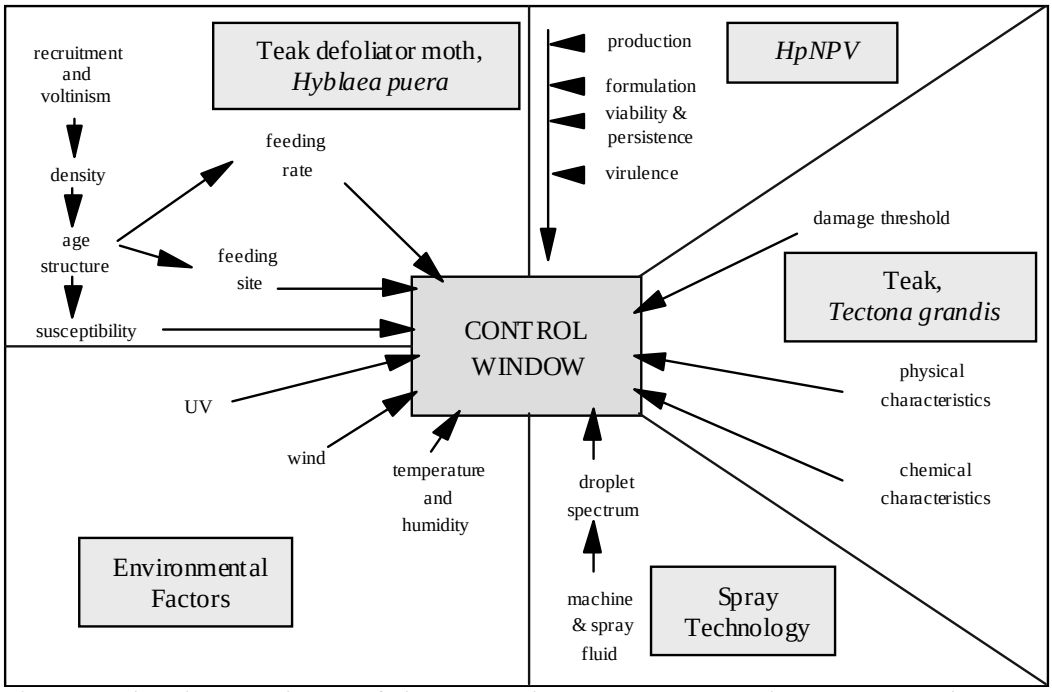

Fig. 1. The interactions of host, pathogen, crop, environment and spray technology in defining a Control Window for use of baculovirus in IPM (adapted from Evans 1994).

2.1 MOTH POPULATION MONITORING

2.1.1 Light trap assessments

A solar powered light trap (Nair *et al.*, 1998) was installed on the top of a hillock at Ambalakkunnu in Kariem-Muriem, Nilambur to monitor *H. puera* moth population so as to plan the field experiments. The number of moths caught and their sex were recorded each day.

2.1.2 Visual assessment through ground surveying

The pest incidence in about 1000 ha teak plantations at Kariem-Muriem (Nilambur North Forest Division) was visually monitored and mapped during

1995-1997. The study area was divided into twenty Observation Units Two trained field observers were deployed to detect the defoliation and record the observations. One round of observation was completed within a fortnight. Data on pest incidence, phenology, intensity and spread of infestation etc.,were entered on a database and used to generate maps of the infestations over time.

2.2 BACULOVIRUS PRODUCTION AND FIELD APPLICATION

The objectives under these components were:

- developing appropriate methods for baculovirus production, quality control and a formulated product for large-scale use.
- developing methods to apply baculovirus, by wide-area spray application and to standardise an appropriate technology to apply the *HpNPV*.

More specifically, the work was divided into different components which provided quantitative data on most of the elements shown in Figure 1.

2.2.1 The host (*Hyblaea puera*)

Information on biology of the teak defoliator, distribution pattern of larvae on tree, feeding rate etc., were generated to determine dosage rates and other useful data for standardising spraying procedures under field conditions.

2.2.1.1 Growth rates and weight relationships

Data on larval weight, head capsule width and duration of each instar was reccorded. The larval weight, is directly linked to both susceptibility and to virus productivity.

2.2.1.2 Larval distribution on the host tree

The following aspects of the dispersion of immature stages of *H. puera* within the teak tree were considered (i) within-shoot dispersion of eggs and larvae, (ii) age structure of populations of immature stages and (iii) distribution of different instars within the tree crown. The methodology followed was as suggested by Mohanadas (1997). The information on the larval distribution on host trees is required in directing the spray cloud during *HpNPV* application in the field.

2.2.1.3 Larval feeding rates over time

Experiments were carried out to measure the surface area of teak leaf eaten by each larval instar within a six hour period as the upper limit of time for the larvae to encounter and consume a lethal dose of virus.

2.2.2 The Virus

2.2.2.1 *Dosage-mortality relationships for larval stages*

This aspect was studied under 3 heads as follows:

i. Developing methods to carry out quantitative bioassays.

The data from the experiments in section 2.2.1.3 were used to determine the amount of teak leaf that each instar could consume in six hours and, hence, the leaf surface area that could be employed to feed a precise dose of *HpNPV* PIBs to each instar during that time. Experiments were confined to leaf disks of uniform size on which known quantity of virus was placed using a precision micro-pipette.

ii. Ranging assays to determine approximate limits of dosage-mortality relationships

Within each instar, 10 larvae within a narrow weight range were each fed with a precise dose of *HpNPV* and left for 6 hours, after which they were transferred to individual tubes containing semi-synthetic diet. Larvae were allowed to feed and were observed twice daily until death or pupation. In these ranging assays the dosages were spaced at logarithmic intervals in order to ensure that the full range of responses from 0 to 100% would be accommodated. The LD_{50} was then calculated for each instar (using probit analysis) and the value used as the central dose in the detailed assays described below.

iii. Detailed bioassays to quantify the precise relationships between dosage and mortality.

For detailed bioassay experiments, the dose range employed (at least five dilutions) was much narrower (approximating 0.2 logarithm steps) and at least 30 larvae were employed per dose. Thirty larvae taken at random from the same set of larvae were used to provide untreated controls to determine natural mortality rates. All data were subjected to probit analysis, using a maximum likelihood routine to calculate the slope and associated statistics of the relationships.

2.2.2.2 Virus productivity relationship for different larval stages

Third and fourth instar larvae were fed with a known lethal dose of *HpNPV* and observed until their death. They were then macerated individually with a known volume of distilled water. Polyhedral Inclusion Bodies (PIBs) were counted using either a haemocytometer or more accurately, by a dry counting procedure after staining the the PIBs with Buffalo Black (Wigley, 1980; Hunter-Fujita *et al.*, 1998). The data were used to determine optimal virus yields per mature larva.

2.2.2.3 Methods for mass production of HpNPV

Under mass production trials, the ideal time for harvesting was determined and efficient method of virus extraction was studied. The use of non-ionic surfactants such as sodium dodecyl sulphate (SDS) was also investigated to reduce or prevent clumping of the final virus suspension. Storage of the virus suspension at various temperatures was also evaluated.

2.2.3 Environmental effects

2.2.3.1 Effects of sunlight (ultra-violet (uv) light) on viability of HpNPV on foliage

Known quantities of virus were applied to precisely defined areas on the upper leaf surfaces of potted plants which were either left in direct sunlight or in shaded conditions. The leaves were removed at specified intervals and the leaf area containing the known dose of virus was fed to individual *H. puera* larvae.

In the trial carried out in 1996,the virus treated leaves were removed at 3 hour intervals (i.e., daylight hours) for the first 12 hour duration and thereafter at 24 hour intervals. The total duration of the experiment was 12 days. In the 1997 trial, the total experimental period was 4 days and the leaf removal was done at 3 hour interval for the first 2 days and at 24 hour interval thereafter.

2.2.3.2 Effects of simulated rainfall on persistence of HpNPV

H. puera infestations are associated with monsoon rains. Experiments were carried out to assess the rainfastness of known quantities (LD_{95} dosage for third instar larvae) of *HpNPV* applied to lower and upper the surfaces of leaves and exposed to artificial rain equivalent at intervals up to a maximum of the highest average rainfall recorded in Kerala State (210 mm in 1 hour). The virus was then assayed using third instar larvae and the results analysed using probit analysis.

2.2.3.3 Assessment of the potential effect of stage of the leaf and chemistry on viability and persistence of HpNPV

There are variations in the chemistry and texture between tender and mature teak leaves. This difference has a clear impact on the ability of larvae to utilise the leaves. First and second instars are able to feed only on tender leaves. On other plants, such as cotton, leaves have been shown to exude alkaline dew which has a direct inactivating effect on baculoviruses (Elleman & Entwistle, 1985). Experiments were, therefore, carried out to test the effect of leaf chemicals on virus viability.

The following experiment was set up just before dusk, in order to remove possible UV effects.

PIBs applied directly on leaves :

1. PIBs of *HpNPV* were applied directly to the surface of tender leaves.
2. PIBs were applied directly to the surface of mature leaves.

PIBs applied on the inner surface of a polypropylene cover and then,

1. Tender leaves were covered with the virus treated cover but avoiding contact between the leaf and the virus
2. Mature leaves covered with the virus treated cover, again avoiding direct contact between leaf and virus.
3. Virus was applied to a polypropylene cover and exposed to the air.

This experimental set up was left overnight and, after 12 hours, virus PIBs were removed by washing with a known volume of 0.1% SDS, and then counted using a haemocytometer. A total of 1000 PIBs from each sample was dispensed onto individual leaf disks and bioassays conducted using third instar larvae.

2.2.4 Spray technology

During the past 20 years there has been considerable advances in the technology of spray application for both chemical and microbial insecticides (Matthews, 1992; Picot & Kristmanson, 1997). Foremost in this technology is ultra low volume (ulv) controlled droplet application (cda). The principles are based on producing large numbers of small droplets (usually in the range 50 μm to 150 μm diameter) while, most importantly, keeping the range of droplet size around the average as small as possible (known as the span of the droplet distribution). The very small droplets employed in ulv cda applications have a high surface to volume ratio and, if they are based on aqueous formulations, are prone to rapid evaporation and loss of droplets, especially at high temperatures. In such situations, anti-evaporant carrier fluids such as mineral or vegetable oils could be employed.

All carrier fluids must also be tested under the local conditions of temperature and humidity, concentrating on flow rates through the sprayers and on actual field performance in terms of droplet deposition on targets or directly on leaves. Droplet spectra for the sprayers employed during this project were analysed on laser droplet equipment available in the International Pesticide Application Centre (IPARC), Silwood Park, U.K.

The following three sprayers were employed for experimental and field use (Fig. 2).

Micron Sprayers Ulva + rotating disk atomiser: This relies on natural wind and turbulence to project droplets into the canopy.

Micron Sprayers UlvaFan rotating disk atomiser: This employs both natural wind and fan assisted airflow to direct the droplets to the target.

Stihl SR400 motorised mist blower with Micronaire AU8000 rotating cage atomiser This sprayer uses petrol driven forced air to project droplets up to 14 m from the spray head. This can be used to spray tall trees and in still air conditions.

2.2.4.1 Development of anti-evaporant carriers for spray application

A range of vegetable oils was tested for flowability through the gravity fed sprayers -Micron Sprayers Ulva + and UlvaFan spinning disk atomisers. Tests were also carried out using the Stihl SR400 mist blower in which a pressurised pump delivered the fluid, rather than gravity feed. The virus extracted from infected larvae was re-suspended in distilled water and, therefore, it was necessary to produce an emulsion to enable the virus to be dispersed evenly through the oil-based carrier fluid. Tests were carried out to determine the precise mixtures and surfactants necessary to produce a stable emulsion that did not result in rapid settling and/or aggregation of the viral PIBs.

2.2.4.2 Calibration of spray equipment, including flow rates, rpm, etc.

Flow rates of spray fluid (water, oil or emulsion), rpm rates (free rotation or under load) using a range of flow restrictors were tested for all the spray equipments used.

While calibrating the Stihl SR400 using various spray formulations, the time taken for release of the spray was noted and the volume of liquid sprayed was determined by subtracting the dead volume of liquid remaining in the tank from the initial volume added. The flow rates were assessed using various restrictors that determined the maximum potential flow. Flow rates were assessed for gravity feed and also during use of a circulation pump that produced a constant pressure, forcing the spray liquid through the spray head. The volume of liquid delivered was measured for 60 seconds and converted to generate the flow rate per minute.

2.2.4.3 Assessment of droplet distribution during field application of spray fluid, using the Stihl 400 mistblower with Micronaire AU8000 spinning cage atomiser.

The Stihl SR400 sprayer with AU8000 atomiser was used for application of the virus in the plantation. Walking speed (0.5 m/sec.) with sprayer on the back was determined before the actual spray was carried out. The average tree height was 5.40 meters. Keeping the spray head at 45^{o} angle, spray was applied to the trees perpendicular to the direction walked. Leaf samples were taken from 8 trees located in the middle of the walked distance (20m) in two rows, from two levels,

i.e. top and middle. One leaf from both the front and back of each tree was collected, i.e. 4 leaves per tree, and observed for droplet distribution. The spray fluid was water mixed with lumogen (a water mixable fluorescent dye). The droplet distri-bution on the leaves was observed in a dark room in the presence of fluorescent light. The following scale was used to record the number of droplets per cm^2.

No droplets	= 0
< 5 droplets	= 1
< 10 droplets	= 2
> 10 droplets	= 3

2.2.4.4 Assessment of droplet spectra for selected spray equipment

Droplet spectra were assessed using laser droplet analysis equipment at IPARC, Silwood Park, UK. All spectra were produced on a Malvern laser analyser which produced computer printouts of size distributions in μm and also calculated volume median diameter (D[v,0.5]), span and various other parameters.

2.2.4.5 Assessment of droplet distribution during field application of spray fluid

Ultra low volume droplets generated by the spray equipment are too small to be seen directly on leaf surfaces. Hence a fluorescent particulate dye (Lumogen suspension in oil) was added to the oil emulsion at a concentration of 2.5% (vol: vol). Spraying was carried out on individual trees and also in a simulation of full incremental spraying at 2 m intervals across the prevailing wind. Leaves were removed from sprayed trees at three heights and from front to back of the tree relative to the spray head. Numbers of droplets were counted under UV light to see the fluorescent particles within the droplets (bright yellow under UV). In the case of tests on incremental sprays, samples were taken from trees at intervals downwind from the spray path. Examination of leaves *in situ* at several heights up the tree and throughout the plots was made at night using portable UV lamps.

2.2.4.6 Estimation of spray coverage during incremental spraying.

The layout of the plot in which the estimation of the spray coverage was made is shown in Figure 3.

2.2.4.7 Assessment of spray height achieved using the Stihl SR400 with Micronaire AU8000 spray head

In order to assess the spray height capacity of the Stihl AU8000 sprayer with spray head, a trial was carried out at Nilambur, using water with lumogen (water-soluble fluorescent dye). Of the five restrictors of the sprayer Restrictor No. 5 was used during this spraying trial (VMD=120 μm) (Restrictor controls the flow rate).

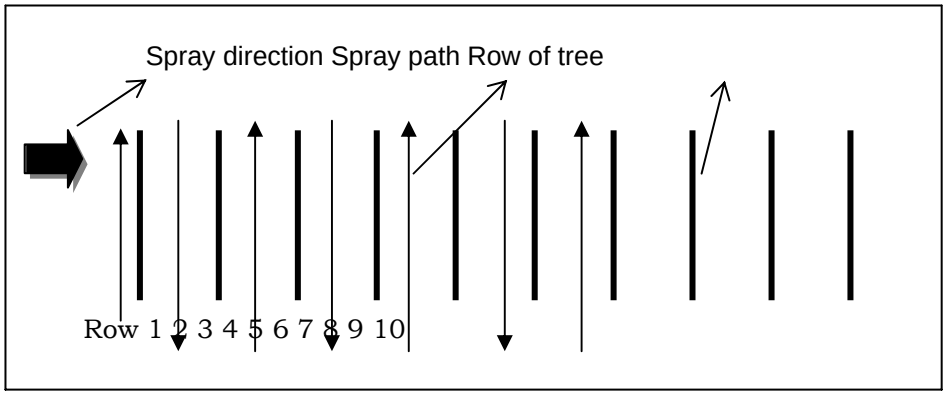

Fig. 3. Layout of incremental spray test plots for spraying with Ulva+sprayer

Two trees of 12 m and 14.45 m height were sprayed using Restrictor No. 5 for 30 seconds and 45 seconds respectively, standing 2 m away from the base of the tree base. Spraying was carried out in the morning under still air conditions. The height to which the spray droplets reached was noted visually and, after completion of spraying, leaves were collected from various tree heights. Three pairs of leaves were taken from the bottom, middle and top sub divisions, taking into account the front and back of the tree and dorsal and ventral surfaces of the leaves. These leaves were observed under fluorescent light in a dark room. The number of droplets per cm^2 on both the ventral and dorsal sides were counted and recorded. The scoring was done using the following scale.

No spray droplets	= 0
< 5 droplets/cm^2	= 1
< 10 droplets/cm^2 area	= 2
> 10 droplets /m^2 area	= 3

2.2.5 Calculation of field dosage rates

Data from the separate experiments were generated to provide the individual quantitative elements for the *Control Window* so that a theoretical dosage per ha could be calculated. The sequence of calculations is shown below.

2.2.5.1 Determining the theoretical dosage rate per ha to achieve >95% mortality of target larvae

Calculations of tank mix can be made using simple relationships between virus, host and droplet parameters (Evans, 1998). Predictions from the following equations provide a reliable approximation to the required field dosage:

N = Number of droplets emitted by atomiser per litre: for convenience use Volume Median Diameter (VMD) (if span is small)

CE = Capture Efficiency defined by the number of droplets required to ensure at least one droplet per host feeding area, expressed in terms of droplets per unit ground area, based on Leaf Area Index (LAI), loss to ground, etc.

Determine feeding rate (*fr*), LD_{95} (*d*) and virus activity loss (*a*) to give initial dose D_i expressed as PIB/mm^2.

Dose per ha and final concentration of the tank mix is determined by:

$$CE = (1 \times 10^{10}) \text{ LAI } \frac{1}{s \times fr} \text{ droplets per ha} \quad \text{(i)}$$

where 1×10^{10} = area of 1 ha in mm^2

LAI = Leaf Area Index, a multiplier to express surface area of leaves in units of ground area

s = Loss of spray fluid to non-target area

Theoretical minimum volume, $V = \frac{CE}{N}$ litres per ha (ii)

Dose per ha, $D_{ha} = CE \times D_i$ expressed in PIB/ha (iii)

Dose per litre $D_l = N \times D_i$ expressed in PIB/litre (iv)

The principle of tank mix calculation can be illustrated by use of hypothetical data for the above equations. In this case the initial dosage, D_i is 1000 PIBs.

Using equation (ii), *CE* = 4 x 10^{10} droplets per ha(assume *fr* = 4 mm^2, LAI = 8, loss to non-target area = 50%)

Let N = 1.53 x 10^{10} droplets per litre (assume 50 μm VMD)

Let D_i = 1000 (assume d = 500, a = 0.5 (50% loss))

Using equation (iii), V = (4 x 10^{10}/1.53 x 10^{10})= 2.6 litres

Using equation (iv), D_{ha} = (4 x 10^{10} x 1000)= 4 x 10^{13} PIBs per ha

Using equation (v), D_l = (1.53 x 10^{10} x 1000)= 1.53 x 10^{13} PIBs per litre

Equation (v) determines the actual tank concentration of PIBs that relates to total volume of spray fluid in the tank, including any volume attributed to formulation products.

2.2.5.2 Assessing the practical dosage per ha in terms of cost vs projected mortality

The equations in 2.2.5.1 provide guidance on the theoretical dosage to achieve a given mortality (usually aiming at >95%) by considering the calculated

parameters. However, the dosage calculated is usually much higher than the actual requirement and therefore, a compromise is required in setting the dose parameters. Aspects, such as mitigating factors that reduce effect of UV on virus in the field, knowledge of potential impacts of secondary inoculum arising from early mortality from the original applied dose, etc. can help to reduce the required dose. This process can only be carried out after all the data are available for calculating the theoretical tank mix.

2.2.6 Trials of HpNPV against field populations of *H. puera*

2.2.6.1 Small scale trial at Nedungayam

Depending on the Initial data on some of the parameters, such as ranging bio-assays, preliminary assessment of UV degradation and droplet capture estimates, a test trial was carried out at Nedungayam teak plantation in October 1996. Virus was applied using the Ulva + sprayer on an incremental spray pattern in plots of 7 x 5 trees, each occupying an area of 140 m^2. The spray head was held at head height and spraying commenced on the downwind side of the plot (Figure 4). Details of the spray trial are given in Table1. Average tree height was 2.25 m.

Fig. 4. Field application of virus using Ulva + sparyer

Table 1. Virus dosage details -Field trial at Nedungayam, October 1996

Dose per plot (PIBs)	Equivalent dose per ha (PIBs)	Number of replicates
1.19×10^7	8.5×10^8	3
9.04×10^7	6.46×10^8	3
6.15×10^8	4.39×10^{10}	2
Untreated control	No virus	1

Larvae were collected from leaf samples taken at random from the centre 5 x 5 trees within the experimental plots and classified according to instar. Dead and live larvae were separated. Dead larvae were smeared for the presence of virus. The larval samples represented total larval counts per tree. However, high levels of predation by a wasp reduced the number of larvae obtained in some of the plots.

2.2.6.2 Preliminary trial at Valluvassery, Nilambur

A large-scale trial using the parameters mentioned under *Control Window* concept, was carried out at Valluvassery, Nilambur during June 1997. Although originally planned as a complete randomised block design, the terrain and distribution of larval infestations made the final design a complete randomised layout. Although this reduced the capacity to allow for blocking of the design across the study area, the distribution of plots was such that all doses were represented in all sections of the plantation, but the number of replicates in each section differed. Statistical analysis was, therefore, based on one way analysis of variance without blocking.

Parameters mentioned under *Control Window* were based on targeting the third instar larvae, using dosages that included a theoretical 90% kill rate. The plots were based on a 7 x 7 tree layout and virus was applied using an Ulva+ sprayer for incremental spraying across the wind, commencing on the downwind edge of the sprayed area. Dosage and replication parameters are shown in Table 2. Sampling was carried out at 0, 48, 72 and 96 hours post spray. A sequential sampling regime was employed to collect 30 live larvae per tree. The larvae were collected at random from within each row of each plot, taken from the central 5 x 5 tree area. Live larvae were grouped instar-wise and then smeared for presence of *HpNPV*. All dead larvae found on the leaves during sampling were collected and also assessed for presence of virus.

Table 2. Preliminary field trial at Valluvassery, June 1997

Dose per ha (PIBs)	Number of replicates
Control (untreated)	5
5×10^{10}	5
1×10^{11}	5
2×10^{11}	5
4×10^{11}	5
8×10^{11}	5

2.2.6.3 Final trial at Valluvassery, Nilambur

The trial was carried out during mid-June 1998. The experimental site was the same where the preliminary trial was carried out in 1997. A randomised complete block design was used, employing the same dosages as given in Table 2, excluding the highest dose. PIBs were suspended in coconut oil emulsion prepared with 94% oil, 5% aqueous virus stock and 1% Tween 80 (emulsifier). Spraying was carried out across wind on an incremental pattern using the Ulva+ attached to a bamboo pole, to take the spray head to the top of the canopy. A destructive sampling was adopted and all larvae, classified as live or dead, were

collected from a single tree taken at random per row per plot. Samples were carried out at 0, 48, 72 and 96 hours post spray. Larvae were stored at –20^{o}C until they were diagnosed, by smearing, for the presence of virus. Due to shortage of time and resources only 25% of all live larvae collected were smeared. A visual assessment of damage, expressed as percentage loss of leaf surface and measurement of leaf loss by leaf weight over time post-spray were taken to assess the potential damage reduction due to application of the virus.

3. RESULTS AND DISCUSSION

3.1 MOTH POPULATION MONITORING

3.1.1 Light trap assessments

Preliminary data indicated that light trap catches of moths did not always coincide with the start of new infestations in plantations. Analysis revealed that light trap catches were more efficient in reflecting the emergence of moths from already infested areas than in detecting incoming populations.

3.1.2 Visual monitoring

There were three widespread infestations during each year surveyed. Even though the infestations were widely distributed, the populations were numerically too low.

Infestation pattern in 1995

The sequence of defoliator infestation during 1995 is shown in Figure 5. The first visible defoliation occurred at a small patch in the second fortnight of February. Subsequent small patch infestations occurred during March (first fortnight) and April (second fortnight). During the first fortnight of May, there was a major outbreak covering the entire area. After this, only two small patches were defoliated, one during the second fortnight of May and the other during the first fortnight of June. The outbreak, which occurred during the second half of June, was widespread. A small area was infested in July and in September the third widespread outbreak occurred in almost all the northern Observation Units in the study area. Thus, there were nine distinct infestations during the year, of which three were widespread.

Infestation pattern in 1996

The first infestations during the year were found in six small patches in the first fortnight of April (Figure 6). Following this, the entire area was infested during May and June. In July, the infestation was widespread. The last infestations of the year were the four small patches, which occurred during August. Overall there were five distinct infestations, of which three were widespread.

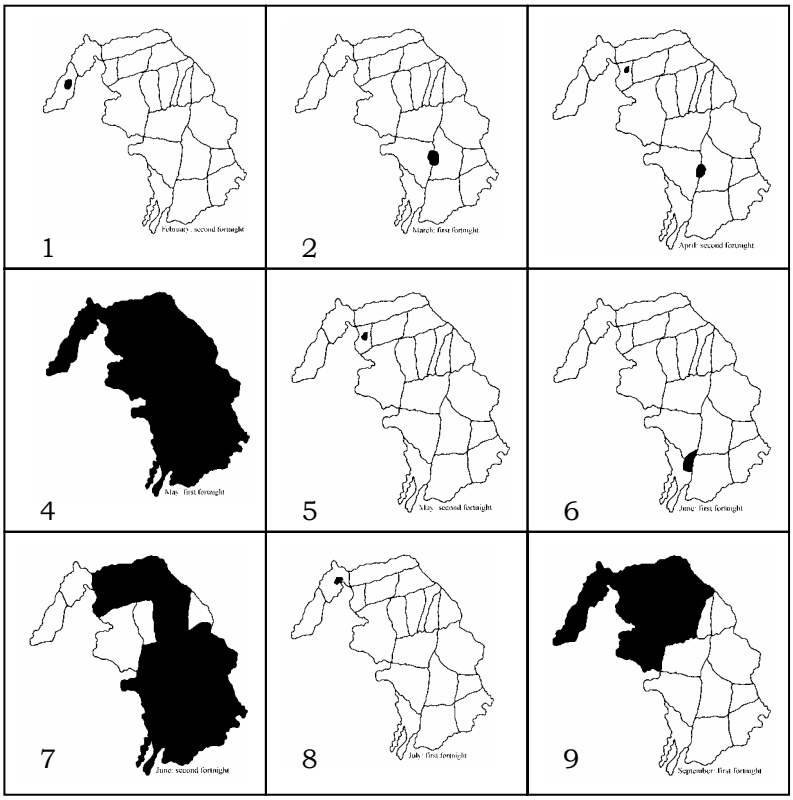

Fig. 5. Sequence of teak defoliator outbreaks at Kariem Muriem in 1995 (Area under outbreak is shaded in black)

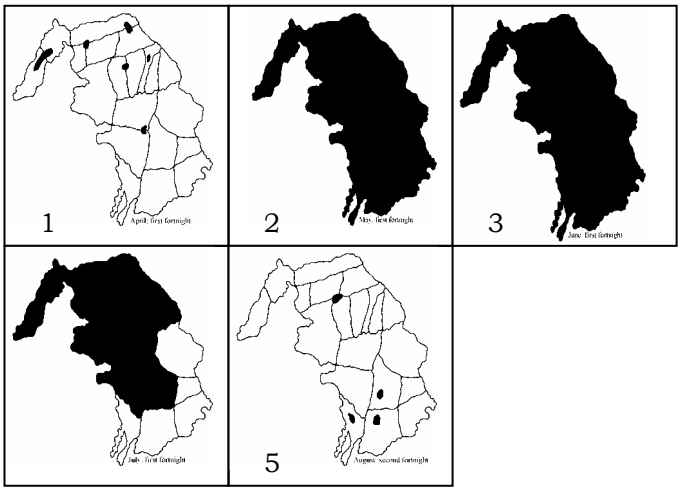

Fig. 6. Sequence of teak defoliator outbreaks at Kariem Muriem in 1996 (Area under outbreak is shaded in black)

Infestation pattern in 1997

In contrast to the early two years in which the initial infestations were confined to small patches, in 1997, the very first outbreak was widespread (Figure 7). There were two distinct patches, which occurred during the first fortnight of May. The second and third infestations, which occurred during May and June, were more widespread, covering almost the entire area. A major outbreak, confined to the southern Observation Units, occurred during July. During August and October, the infestations were confined to smaller areas. In summary, there were six infestations, of which four were widespread.

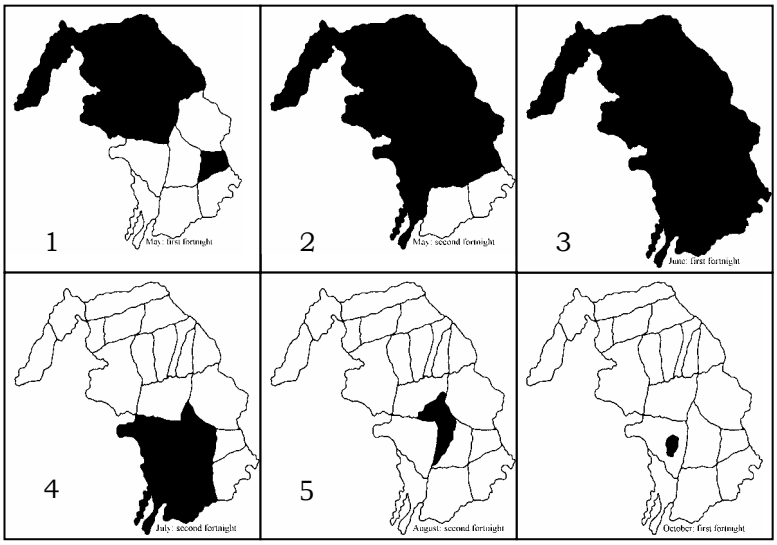

Fig. 7. Sequence of teak defoliator outbreaks at Kariem Muriem in 1997 (Area under outbreak is shaded in black)

The pattern of infestations described above shows that, although majority of them were distributed over small areas, there were several widespread infestations during the period May–September.

Visual detection of outbreaks is possible only when the leaf folds are visible on teak trees. Larvae in the late second instar stage and above make leaf folds and feed within them. Earlier studies have established correlation between the occurrence of the first pre-monsoon showers and the appearance of early epicentres of *H. puera* and the appearance of early epicentres of *H.puera* infestation (Nair *et al.*,1985). Thus, the pre-monsoon showers signal the time when field observations for detection of outbreaks should begin Thereafter, there must be close co-ordination between the field observers and the scientists, so that early decisions and immediate action can be ensured, if populations are deemed to be high enough to justify application of virus.

Field monitoring by visual observation for the early signs of attack was found to be effective, although the cost in terms of manpower was high. This method could be rather affordable in intensively managed young teak plantations under private sector where resource availability and commitment are more than in public sector plantations.

3.2 BACULOVIRUS PRODUCTION AND FIELD APPLICATION

3.2.1 The host (*Hyblaea puera*)

3.2.1.1 Larval growth rate and weight relationships

H. puera has five larval instars, each of which feeds on the leaves of teak. Instars I and II are able to eat only newly expanded, tender leaves whereas the remaining instars are capable of feeding on leaves of all stages. The larval period of the first and second instar larvae is 2 days and the instars, 3-5 require 3 days to complete the larval period. Average weights and head capsule widths (an accurate indicator of larval stage) are given in Table 3. Mean weights are used for assessment of the relationships of lethal dosage to larval development.

Table 3. Mean weight and head capsule width of *H. puera* larvae

Instar	Mean weight	Head capsule width +/- SD (mm)
I	0.1 mg	0.334 +/- 0.018
II	2.0 mg	0.590 +/- 0.032
III	9.0 - 12.0 mg	1.007 +/- 0.006
IV	27.0 - 36.0 mg	1.673 +/- 0.106
V	80.0 - 110.0 mg	2.622 +/- 0.170

Although weight varied considerably within each instar, the width of the chitinous head capsule remained fixed until the next moult and was, therefore, a useful measure to identify the instar.

3.2.1.2 Larval distribution on the host tree

In general, eggs are laid only on tender leaves, and the larvae feed preferentially on the 1st to 3rd pairs of leaves. Neonate (newly hatched) larvae do not survive on older leaves. High-density infestations are characterised by the dominance of one developmental stage at any given time and there is no overlap of generations. However, low-density populations have a mixed age structure. Presence of all instars are noted at all levels of the crown.

3.2.1.3 Larval feeding rates over time

The average leaf area consumption by each instar during a six-hour period is presented in Table 4. This information was used to assess the required coverage per unit area of droplet applications for use in the *Control Window*.

Table 4. Leaf area consumed within 6 hours by *H. puera* larvae

Instar	Leaf area consumed (mm^2)
I	2.0
II	12.0
III	18.0
IV	200
V	300

3.2.2 The Virus

3.2.2.1 Dosage-mortality relationships for larval stages

Following a series of ranging bioassays that were used to standardise a methodology and to determine the approximate LD_{50} and slopes of the relationships, detailed assays were carried out for all five larval instars. The data were processed using Probit analysis and are presented (Table 5 and Figure 8).

Table 5. LD_{50} values and associated statistics for instars I to V of *H. puera.*

Instar	LD_{50} (PIBs)	95% fiducial limits (PIBs)	Slope	Intercept
I	17	*	1.09	-.83
II	70.2	*	1.07	-1.48
III	72.7	25-271	1.10	-1.55
IV	3932	2810-6157	1.17	-3.71
V	20125	9206-34488	1.26	-4.94

It was difficult to apply the virus inoculum to first and second instar larvae, because of their small size and high sensitivity to the virus. Thus the variability was too high to calculate 95% fiducial limits. However, because the slopes were not significantly different (chi-square 1.31, df = 4) it was possible to calculate LD_{50} and associated statistics for the common slope, using all data combined. The results are shown in Table 6. Here the effect has been to increase the limits for instars III to V, but has also enabled to calculate the fiducial limits for instars I and II, in all cases without substantially altering the calculated LD_{50}s.

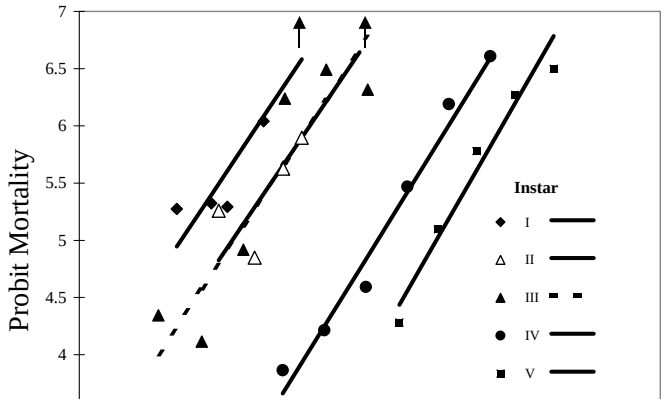

Fig. 8. The relationship between Log dosage of nucleopolyhedrosis PIBs and mortality (in probits) of the five larval instars of *H. puera*.

Table 6. LD_{50} and associated statistics for instars I to V of *H. puera* exposed to *HpNPV* based on a common slope for all instars

Instar	LD_{50} (PIBs)	95% fiducial limits (PIBs)	Common slope value
I	17.7	8.5-43.0	1.14
II	74.3	39.3-130.7	
III	73.3	39.0-135.6	
IV	3936	2168-7165	
V	18673	8323-39398	

Figure 8 illustrates how the response of larvae to dosage of virus decrease with larval age and emphasises that the difference in response are a function of age rather than any major change in the type of response. In other words, the parallel slopes indicate that the population as a whole responds in a similar way to virus dosage but the absolute dosage is determined by some factor related to larval age. In fact, the principal factor is larval weight, as has been found in many other lepidopteran- virus-host interactions (Evans, 1986). This is illustrated in Figure 9 in which both LD_{50} and LD_{50}/mg body weight are plotted against body weight. Although the surprising similarity of dosage between the second and third instar larvae introduces variability, it is clear that weight accounts for the majority of the variation in observed LD_{50} with larval age. With regard to targeting of larvae in the field for control, the identical response of second and third instar larvae to the *HpNPV* was helpful in determining field dosage. The third instar was easy to target than the first or second instars due

to a number of reasons - it eats more foliage and, thus, has a greater likelihood of taking in the whole a virus dose given; it is more open in its feeding habits and can feed on older foliage also. Field dosage rates were, therefore, based on Control Window parameters for the third instar, although some preliminary trials first instar population were carried out.

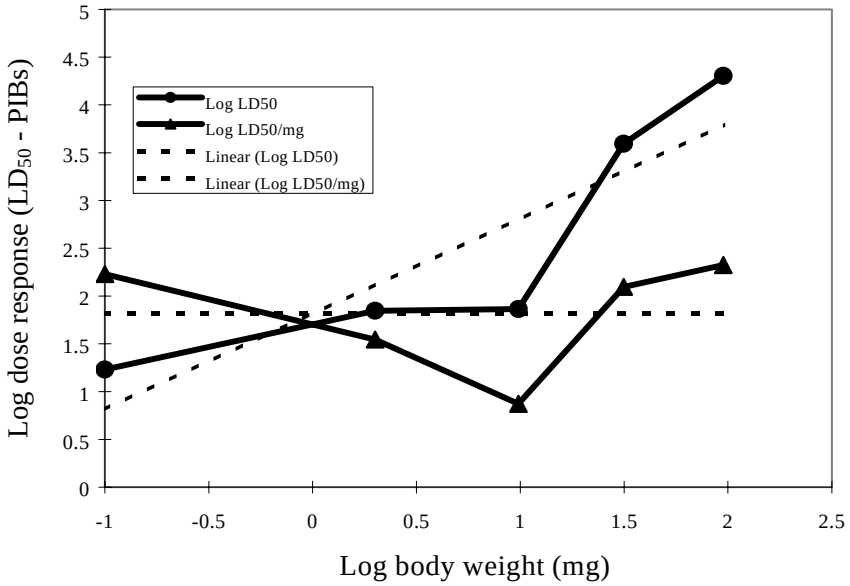

Fig. 9. The relationship between Log LD_{50} and Log LD_{50}/mg and body weight of *H. puera* larvae in relation to infection by *HpNPV*

Response of the fourth instar to HpNPV was extremely unusual. The data presented above included response up to 72 hours post infection, a period sufficient for the great majority of larvae to get infected and die. Up to this period, the population response was almost identical with other instars (parallel slopes for the dosage-mortality regression lines). However, if the fourth instar larvae were left for longer periods, (beyond 72hours), mortality at lower dosages became significant, finally resulting in high mortality even at the lowest dose tested. Contamination of the larvae was ruled out by the demonstration of no virus mortality in the untreated control larvae taken from the same stock of insects. Dosages and dilutions were also checked carefully to eliminate the possibility of incorrect administration of PIBs. The results over time are illustrated in Figure 10.

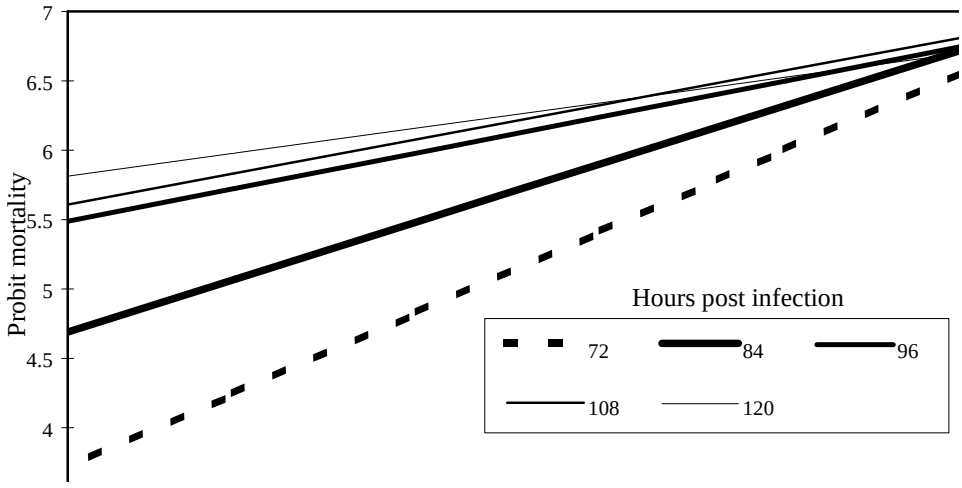

Fig. 10. Change in response of fourth instar *H. puera* larvae with time post Infection Note the progressive flattening of the slope as infection increases even with low dosage.

The above response on fourth instar was repeatedly observed in further trials. Although this result could be attributed to the theory that only a single virus particle is required for infection, it is, nevertheless, unusual to have more mature larvae dying at such low dosages. However, assuming that the same response applies in the field, it does imply that very low dosages of virus will be sufficient to kill a significant proportion of fourth instar larvae remaining on the trees.

3.2.2.2 Virus productivity relationships for larval stages

Data on virus productivity per larva are presented in Table 7. The increase in yield per larva (larval equivalent – LE) was encouraging and was confirmed in experiments in which individual larvae were assessed to determine maximum yield with a view to increase the productivity. It was observed that up to 7×10^8 PIBs could be produced. Based on these observations it was estimated that an yield of 3×10^8 PIBs per larva would be a realistic target for mass production.

Table 7. Virus yields obtained during preliminary trial spray

	Trial 1	Trial 2	Trial 3
No. of dead larvae retrieved	19206	42151	35600
Approx. larval equivalent of NPV (PIBs)	6.2×10^5	5.7×10^4	1.1×10^8
Approx. available quantity of NPV (PIBs)	1.2×10^{10}	2.4×10^9	3.91×10^{12}

3.2.2.3 Methods for mass production of HpNPV

Arising from the results above, a method for mass production of *HpNPV* was developed and standardised during the study period, although there is still room for further refinement and scaling up.

Materials required

HpNPV stock

H.puera larvae

Tender leaves of teak

Semi-synthetic larval diet (Mathew *et al.*, 1990)

Plastic tubes (10cm x 2.5 cm) with perforated cap
Micropipettes (0.5 -10µl),
Fine brushes, Scissors, Glass plate (60 cm x 60 cm).

Larval source

Teak defoliator larvae reared either on artificial dietin the laboratory or healthy larvae collected from the field were used. Fifth instar larvae were preferred for virus production as they gave maximum virus yield in relation to its body weight.

Inoculum feed

Two ml of semi-synthetic larval diet is dispensed in plastic tubes, atleast 3-4 hours before inoculation. Leaf discs (2 cm x 2 cm) cut from tender teak leaves placed on a sterilized glass plate with sufficient space in between to prevent touching of the leaves. Using a micropipette, 2µl of NPV stock at a concentration of 5×10^8 PIBs/ml is spotted over each leaf disc, to obtain a deposit of 1×10^6 PIBs/leaf disc. The leaf disc is left for some time until the virus deposit is dry. Individual leaf discs are then transferred to the rearing tube and placed over the

diet. Individual early fifth instar larvae are then introduced into each plastic tube. The larva first feeds on the leaf disc containing the virus and after completely consuming the leaf, switch over to the diet.

Incubation and retrieval of the dead larvae

Larvae that have died within 48 hours of inoculation are discarded, as the death could be mainly due to bacterial infection. Larvae that die between 48 and 96 hours post inoculation are collected and transferred to a deepfreeze where they are maintained at –20°C. After 108 hours, all remaining larvae both dead and live are retrieved and transferred to the deep freeze.

Harvest of virus

The frozen larvae are macerated in a pestle and mortar with chilled 0.1% SDS solution. After maceration, the virus suspension is filtered using a muslin cloth to remove coarse insect debris. The filtrate is again filtered, this time using muslin cloth folded into three layers. The virus suspension is centrifuged at 8000 rpm for 20 minutes to produce a pellet, composed mainly of virus PIBs. The supernatant is removed and discarded. The pellet is again suspended in chilled distilled water, and centrifuged at 1000 rpm for 5 minutes. The pellet containing any remaining impurities is removed and the supernatant, containing virus PIBs, retrieved and stored.

Purification of virus

The semi-purified virus stock is further purified by ultra centrifugation in a sucrose gradient comprising 48%(w/w), 51%(w/w), 54%(w/w), 57%(w/w) and 60%(w/w) of sucrose solutions layered (6ml from each stock) into a Sorvall centrifuge tube using a disposable syringe (5ml). The prepared sucrose gradient is kept at 4°C overnight to stabilise the sucrose across the range of concentrations. Three ml of the virus preparation is layered on top of the gradient centrifuged in AH627 swinging bucket rotors in a Sorvall ultra centrifuge at 25000 rpm for 40 minutes. The *HpNPV* forms a band in the region of 52 to 54% of sucrose concentration. The band is collected with a syringe and needle. The PIBs suspended in sucrose solution are diluted in distilled water and pelleted at 8000 rpm to remove the sucrose. The pellet of PIBs can be re-suspended in distilled water. This procedure is repeated once more to remove any remaining residues of sucrose.

Virus yield

The virus yield, based on semi-purified stock, from 5 trials carried out as per the above method is given in Table 8. In the method tried in the 1995-'96 trials,

fourth instar larvae of field origin were kept together in a yard and fed with whole leaves treated with virus. This method was abandoned due to high risk of contamination and wastage of virus at the time of inoculation and difficulties in retrieving the dead larvae.

Table 8. HpNPV yield using the standard virus production protocols

Trial No.	Dosage	No. of larvae used	Instar	No. of virus infected larvae retrieved +	Total yield	Larval equivalent
1	$5x10^5$	330	V	311	1.28×10^{11}	4.115×10^8
2	$1x10^6$	1558	V	1340	2.128×10^{11}	1.559×10^8
3	$1x10^6$	822	IV	659	1.344×10^{11}	2.0394×10^8
4	$1x10^6$	769	IV, V	628	8.096×10^{10}	1.289×10^8
5	$5x10^5$	228	V	198	1.6×10^{10}	8.08×10^7
					Mean larval equivalent	$1.9621x10^8$

+ larvae dead before the required incubation period discarded.

Merits and demerits

The merits of this method is that freezing of the dead larvae minimises bacterial contamination of the virus suspension. Elimination of dead larvae within 48 hours of inoculation prevents bacterial contamination in the virus. The drawback of this method is that since the larvae are not allowed to putrefy, the PIBs are not always completely released from the nuclear groups within the infected cells, resulting in reduced yield of free virus. Clumping of the inclusion bodies is high.

Comparison with early methods

In the 1995-'96 trials, group rearing of field-collected fourth instar larvae were fed with whole leaves treated with virus in a yard. This method was abandoned due to high risk of contamination and wastage of virus at the time of inoculation and difficulties in retrieving the dead larvae.

In the 1996 -97 trials, larvae were kept individually and first fed on virus treated leaf disc and then transferred to artificial diet until death. The process of inoculation under this method involved high risk of contamination. The final method discussed above is a modified version of the previous method. Here the

larva is kept in a tube containing the virus treated leaf disc and the diet together which helps inoculation fast and easy. This method is less labour intensive and with minimum risk of contamination.

3.2.3 Environmental effects

The key elements that are known to lead to degradation of virus in the field are UV light, rainfall and possible effects from the host plant (Evans & Harrap, 1982). Information on these aspects is important for determining loss of virus during the vital first six hours after spray application in the field.

3.2.3.1 Effect of sunlight (ultra-violet (UV) light) on viability of HpNPV on foliage

The results of the two sets of experiments, carried out in 1996 and in 1997, show the rate of decay of virus when exposed to UV and when protected in virtual darkness (Figure 11). The patterns of decay are similar for both sets of experiments, showing that sunlight has a dramatic early effect on virus survival so that mortality dropped from an initial 97% to around 70% (probit 5.5) in six hours.

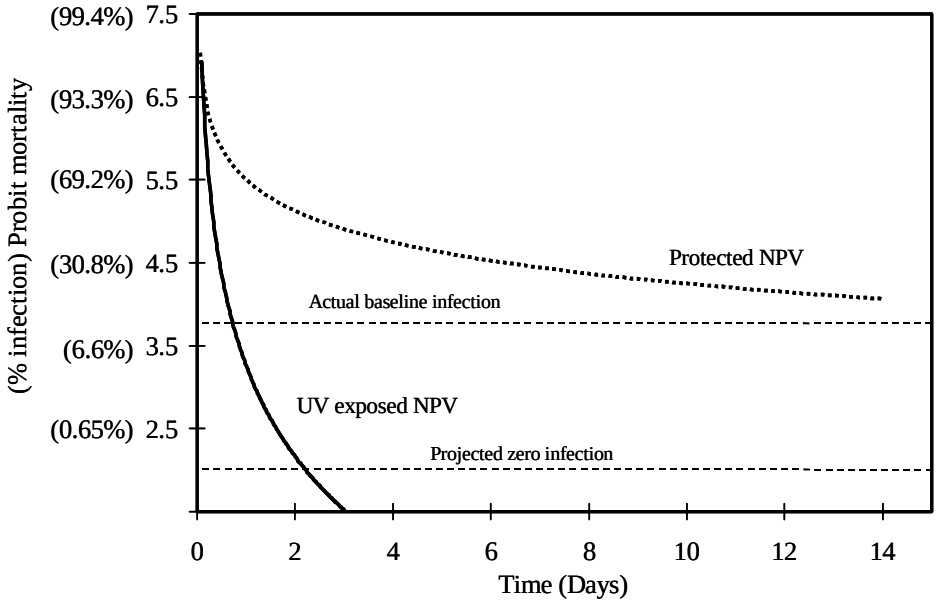

Fig. 11. Effect of exposure to ultra violet light on activity of HpNPV

The rate of decay continued on a predicted line as shown in Figure 11 but, unexpectedly, stabilised at approximately 13% mortality (probit 3.9) for the remaining period of the experiment. This was repeated in both years. There are

several possible explanations for this result: although it was not possible to carry out experiments to verify the following hypotheses:

- Presence of a small amount of insect debris must have afforded some protection against UV. However, the fact that the virus was virtually free of debris tends to rule out this explanation.
- The period of UV exposure is restricted to around 12 hours each day and, therefore, the virus is not continually exposed to harmful wavelengths of light.
- It is known that baculovirus DNA can repair itself after exposure to UV light and it is possible that the 12 hours of darkness in each 24-hour period may enable the virus to retain a base-line activity level.
- Some characteristics of teak leaves may provide protection against loss of virus activity, although this was not proved based on results presented in section 3.2.3.3.

Retention of activity over an 11-day period under direct exposure to sunlight was unexpected and further study is required to confirm this finding. However, the finding indicates that *HpNPV* has the remarkable capacity to remain viable under adverse conditions, which makes it a promising candidate for effective management of the pest.

3.2.3.2 Effect of simulated rainfall on persistence of HpNPV

The linking of *H. puera* population dynamics to the presence of monsoon rains poses the obvious question of whether *HpNPV* can remain on the leaves during periods of high rainfall. Results from experiments to test this question are presented in Figure 12, where proportionate mortality has been transformed to probits.

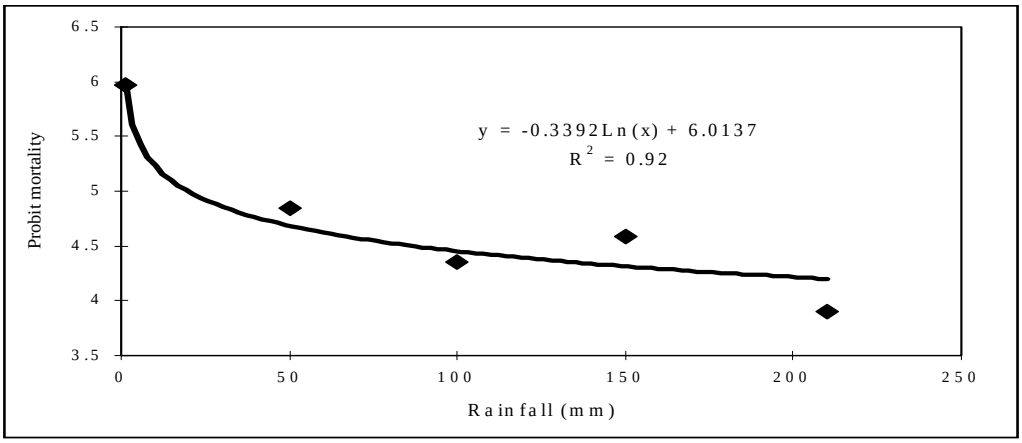

Fig. 12. The effesct of simulated rainfall on persistence of *HpNPV* on teak leaves

The highest rainfall results in a decline from over 90% mortality to approximately 15% mortality, indicating that under extremes of rainfall, losses of virus can be dramatic. However, these data represent the situation when virus is fully exposed on the upper surfaces of leaves. In nature, virus infected larvae tend to be found on the underside of leaves and are, at least to some extent, protected from the direct effect of monsoon rains. Nevertheless, there is likely to be some loss of virus, if heavy rainfall occurs within six hours of spray application, a factor that must be considered while timing spray application.

3.2.3.3 Assessment of the potential effects of stage of the leaf and chemistry on viability and persistence of HpNPV

The results presented Table 9 indicates that there is no effect of either leaf surface or leaf volatiles on *HpNPV*. This possibility can, therefore, be reviewed while determining field persistence of virus applied for control of teak defoliator.

Table 9. Effect of leaf surface and leaf volatiles on persistence of *HpNPV*

Type of leaf	Virus applied on leaf surface			Virus exposed to leaf volatiles on inert surface			Control		
	larvae	dead	% mortality	Larvae	dead	% mortality	larvae	dead	% mortality
Mature leaves	47	46	97.9%	53	52	98.1%	50	3	6.0%
Tender leaves	50	48	96.0%	50	46	92.0%	65	5	7.7%

Virus applied to inert surface of polypropylene cover					
Virus treated			Control		
Larvae	dead	% mortality	Larvae	dead	% mortality
39	32	82.1%	45	2	4.4%

3.2.4 Spray technology

3.2.4.1 Development of anti-evaporant carriers for spray application

Vegetable oils locally available in Kerala were tested as a carrier in relation to flow rates and ability to form stable emulsions with water and an emulsifier. It was found, by trial and error, that stable emulsions could be formed with proportions of 94% oil, 5% water and 1% emulsifier. Both Labolein, a

commercial detergent preparation, and Tween80, a non-ionic detergent, proved to be suitable emulsifiers. Flow rates of oil alone and oil emulsions were greater using coconut oil compared to sunflower oil. Coconut oil, therefore was chosen for all field spray applications.

3.2.4.2 Calibration of spray equipment, including flow rates, rpm, etc.

Many tests were carried out to assess flow rates for both Ulva+ and Stihl SR400 sprayers, the former under gravity feed and the latter under both gravity and pump feed. Average RPM rates varied with the power applied to the respective sprayers. For the Ulva+ the revolution rates varied with voltage as per the manufacturer's specifications. However, the trials were carried out with the maximum 12 v setting, delivering 11000 rpm (see section 0). The Stihl SR400 airflow through the Micronaire AU8000 spray head delivered up to 13000 rpm at maximum throttle settings.

Experiments to assess flow rates under both gravity feed and pump-assisted conditions were carried out for the SR400 sprayer and are presented in Table 10.

As expected, the flow rates are considerably greater when assisted by the pressure pump within the SR400. Flow for oil alone or the oil emulsion was slower than for water alone, a result that is compatible with other data on gravity feed for the Ulva+ sprayer.

3.2.4.3 Assessment of droplet spectra for selected spray equipment

The majority of field trials were carried out with the Ulva+ sprayer and, therefore, data on droplet spectra obtained at IPARC, Silwood Park, using the Malvern laser droplet analysis equipment (Table 11). Results indicate that droplet diameter drops with increases in rpm. The maximum rpm rate for the Ulva+ produces droplets with volume median diameter of 55 μm, providing large numbers of droplets per litre of spray fluid and improvements in droplet coverage on the foliage.

3.2.4.4 Assessment of droplet distribution during field application of spray fluid

Table 12 shows the spray coverage in a young teak plantation (average tree height 5.40 m) using a Stihl SR400 sprayer with Micronaire AU8000 rotating cage atomiser.

Table 10. Calibration of Stihl SR400 sprayer under a range of conditions and carrier fluids

Restrictor No.	Water alone				Coconut oil alone				Emulsion			
	Engine				Engine				Engine			
	Off		On		Off		On		Off		On	
	Ml/min	Time for 1 litre flow out Min. Sec	Time for 1 litre flow out Min. Sec	Ml/min	Ml/min	Time for 1 litre flow out Min. Sec	Time for 1 litre flow out Min. Sec	Ml/min	Ml/min	Time for 1 litre flow out Min. Sec	Time for 1 litre flow out Min. Sec	Ml/min
1	32.20	36.25	6.20	157.90	4.00	252.70	9.40	110.29	6.50	167.80	9.43	102.00
2	67.00	17.45	2.20	491.80	12.50	84.50	5.70	195.44	14.00	76.10	4.34	219.00
3	145.00	7.48	1.21	740.70	40.00	25.50	4.10	249.94	46.00	21.50	3.10	331.49
4	244.00	5.10	1.12	833.30	49.80	21.70	3.20	329.67	40.00	25.80	2.70	472.40
5	337.00	4.47	1.50	923.10	54.00	19.30	1.30	645.16	64.00	17.90	1.15	798.00

3.2.4.3 Assessment of droplet spectra for selected spray equipment

The majority of field trials were carried out with the Ulva+ sprayer and, therefore, data on droplet spectra obtained at IPARC, Silwood Park, using the Malvern laser droplet analysis equipment (Table 11). Results indicate that droplet diameter drops with increases in rpm. The maximum rpm rate for the Ulva+ produces droplets with volume median diameter of 55 μm, providing large numbers of droplets per litre of spray fluid and improvements in droplet coverage on the foliage.

Table 11. The relationship between rpm and volume median diameter for Ulva + sprayer with oil emulsion carrier

Revolution rate (rpm)	Droplet volume median diameter (μm)
3000	116.49
4000	104.42
5000	92.74
6000	81.96
7000	74.35
8000	71.82
9000	62.01
10000	60.01
11000	55.00

3.2.4.4 Assessment of droplet distribution during field application of spray fluid

Table 12 shows the spray coverage in a young teak plantation (average tree height 5.40 m) using a Stihl SR400 sprayer with Micronaire AU8000 rotating cage atomiser.

Table 12. Droplet distribution (score) for Stihl SR400 with Micronaire AU8000 atomiser

Row No. 1.				
Tree No.	Tree level			
	Top		Middle	
	Front	Back	Front	Back
1	1	1	3	3
2	1	0	3	0
3	0	0	0	0
4	0	0	0	0
Row No. 2				
1	1	1	1	1
2	0	1	3	3
3	1	0	1	1
4	0	1	1	0

High foliar coverage (score 3; > 10 droplets/cm^2) was achieved in the middle layers of trees in the first and second rows from the spray head, but few droplets penetrated horizontally to rows 3 and 4. Therefore, despite the velocity applied to the droplets, it would still be necessary to spray at least every other row in a full-scale application.

Incremental spraying with the Ulva+ sprayer

The results of the incremental spray trials are shown in Table 13 where the numbers of droplets/cm^2 on leaves in various positions on the tree are recorded.

Table 13. Number of droplets captured during incremental spraying with the Ulva+ sprayer

Row No.	Level	Droplets/ cm^2	Droplets/ cm^2
1	Top	21.65	7.95
	Middle	2.22	
	Bottom	0.00	
2	Top	0.27	16.95
	Middle	34.20	
	Bottom	16.39	
3	Top	0.00	20.11
	Middle	37.90	
	Bottom	22.45	
4	Top	3.01	10.29
	Middle	4.61	
	Bottom	23.25	
5	Top	3.81	19.63
	Middle	17.19	
	Bottom	37.90	
6	Top	17.64	31.14
	Middle	37.90	
	Bottom	37.90	
7	Top	23.25	22.98
	Middle	23.25	
	Bottom	22.45	
8	Top	6.21	18.79
	Middle	23.25	
	Bottom	26.91	
9	Top	2.46	7.40
	Middle	13.53	
	Bottom	6.21	
10	Top	2.22	8.54
	Middle	19.59	
	Bottom	3.81	

The results indicate that average coverage is good, the incremental spraying building up from rows 1 to 3, after which it remains remarkably constant until the final two rows, neither of which was sprayed, which is a standard practice in incremental spraying.

The results, therefore, indicate that good coverage of foliage can be obtained using the Ulva+ sprayer and coconut oil emulsion as the carrier. In fact, the coverage obtained was greater than required to ensure that third instar lar vae obtained the required number of droplets during the six hour feeding period, as defined in the *Control Window*.

3.2.4.5 Assessment of droplet distribution during field application of spray fluid at Nilambur to assess the height reached by the spray droplets.

These tests were carried out using the Stihl SR400 mistblower which has a manufacturer's recommended maximum spray height of approximately 12 m. The trials were carried out using the booster pump to ensure even flow of spray fluid through the AU8000 spray head. The first trial was carried out on trees with 12 m height, as shown below (Table 14). The second trial was on trees with 14.45 m height (Table 15).

Table 14. Number of droplets (score) captured at different tree heights when using a SR400/AU8000 spray system to spray trees 12 m tall (Restrictor 5, Tme, 30 seconds)

Height of tree	Front		Back	
	Sides of the leaves observed			
	Ventral	Dorsal	Ventral	Dorsal
11.5 m	0	0	1	0
10.0 m	0	0	1	0
9.0 m	3	3	1	1
8.0 m	1	0	2	0

Table 15. Number of droplets (score) captured at different tree heights when using a SR400/AU8000 spray system when used to spray trees 14.45 m tall (Restrictor 5, Time 45 seconds)

Height of tree	Front		Back	
	Sides of the leaves observed			
	Ventral	Dorsal	Ventral	Dorsal
14.15 m	1	0	0	1
13.00 m	0	0	0	0
12.00 m	1	0	2	2
9.00 m	3	2	1	1

Note: Scores used to record the droplet numbers per cm^2 area.

No. Droplet = 0 : < 5 droplets = 1 : < 10 droplets = 2 : > 10 droplets = 3.

<u>Spray coverage obtained with the Ulva+ sprayer</u>

Estimation of spray coverage on individual trees.

Date of spray	11-04-1996
Place of spraying	Kariem Muriem
Duration of spray	5 seconds

Wind speed	9.6 km/hour
Height of spray head from ground	9 metres
Sprayer	Ulva+ (with 8 cells)
RPM	11000
Nozzle	Pink
Spray fluid	Coconut oil emulsion with suspended fluorescent dye

The spray coverage obtained on three trees, which were at three different levels from the spray head, was estimated. The details are given in Table 16, 17 and 18.

Table 16. Droplet capture on trees, 9 m from the spray head, using the Ulva+ spray system

Height of the sampled shoot	Position of the sampled shoot	Leaf pair	Surface	Mean droplets/cm^2
8.5 metres	Front	1	upper	2.00
			lower	15.40
		2	upper	5.85
			lower	4.35
		3	upper	10.05
			lower	2.45
	Back	1	upper	0.00
			lower	0.00
		2	upper	0.15
			lower	1.15
		3	upper	2.00
			lower	0.30
5.5 metres	Front	1	upper	0.75
			lower	10.20
		2	upper	4.95
			lower	0.30
	Back	1	upper	0.80
			lower	4.20
		2	upper	5.95
			lower	1.90
		3	upper	2.25
			lower	4.50
2.5 metres	Front	1	upper	0.10
			lower	0.00
		2	upper	0.00
			lower	0.05
	Back	1	upper	0.00

			lower	0.00
		2	upper	0.02
			lower	0.00
		3	upper	1.80
			lower	0.00

Table 17. Droplet capture on trees, 15 m from the spray head, using the Ulva + spray system

Height of the sampled shoot	Position of the sampled shoot	Leaf pair	Surface	Mean droplets/cm^2
7.5 metres	Front	1	upper	0.00
			lower	0.00
		2	upper	0.45
			lower	0.05
		3	upper	0.00
			lower	0.00
	Back	1	upper	0.10
			lower	0.00
		2	upper	0.00
			lower	0.00
		3	upper	0.10
			lower	0.00
		4	upper	0.00
			lower	0.00
4 metres	Front	1	upper	0.45
			lower	0.05
		2	upper	0.40
			lower	0.00
		3	upper	0.00
			lower	0.00
	Back	1	upper	0.20
			lower	0.00
		2	upper	0.60
			lower	0.00
		3	upper	0.00
			lower	0.00

Table 18. Droplet capture on trees, 21 m from the spray head, using the

Ulva+spray system

Height of the sampled shoot	Position of the sampled shoot	Leaf pair	Mean droplets/cm^2
8.5 metres	Front	1	0.000
		2	0.000
		3	0.000
	Back	1	0.075
		2	0.025
		3	0.000
5.5 metres	Front	1	0.000
		2	0.075
	Back	1	0.000
		2	0.000
		3	0.000
2.5 metres	Front	1	0.000
		2	0.500
	Back	1	0.000
		2	0.000
		3	0.000

Results indicated that adequate coverage was obtained only on the trees closest to the sprayer, confirming that incremental spraying is necessary to achieve full coverage.

3.2.5 Calculation of field dosage rates

3.2.5.1 Determining the theoretical dosage rate per ha to achieve >95% mortality of target larvae

Using the equations described in section 2.2.5 incorporated into an Excel spreadsheet, it was possible to calculate a number of potential dosage rates per ha, depending on assumptions of larval target stage, rates of attrition, feeding rates, etc. A typical outcome is shown in Table 19 which includes calculated values for third instar larvae, assuming spraying in mid to late afternoon (to reduce UV attrition) and young teak trees (2-4 m tall) with relatively low leaf area indices. The result indicates that, in this case, a theoretical 90% kill of target larvae should be achievable using a dose equivalent to 5.49 x 10^{11} PIBs per ha.

Table 19. Spreadsheet printout of equations to calculate projected dosage per ha

for *HpNPV* applied against third instar *H. puera* larvae in teak plantations

Equations to calculate theoretical dosage per ha using pre-determined parameters			
	Target stage	Third instar	
Capture efficiency = (area)*(LAI*(1/(s*fr))	CE	2.47E+09	
Number of droplets per litre:	N	1.53E+10	
Initial dose (PIB/mm^2) = LD_{90}*a	D_i	222	
Theoretical Volume = CE/N litres per ha	V	0.16	
Dose per litre	D_l	3.40E+12	
Dose per ha	D_{ha}	**5.49E+11**	
Area of ha in mm^2	area	1.00E+10	
Calculation of CE	Calculate CE	2.47E+09	required droplets per ha
Feeding rate of larvae (mm^2)	fr	18	
Leaf Area Index (ratio to ground area)	LAI	4	
Loss of spray fluid to ground area (1/propn.)	s	0.9	
Virus attrition rate (propn of original)	a	0.9	
LD_{90} for target larval stage	LD_{90}	200	

3.2.5.2 Assessing the practical dosage per ha in terms of cost vs projected mortality

Although the projected dosage for 90% was within the range tested in preliminary trials described below, there are a number of potential options for reducing the quantity of virus to be used while spraying under field conditions. Bearing in mind that the maximum larval equivalent achieved during mass production of *HpNPV* has, so far, been around 2 x 10^8 PIBs (Table 8), the projected dosage calculated in Table 19 represents approximately 2700 larval equivalents per ha. Clearly, any

measure that can reduce the number of larvae required for virus production will reduce costs and logistics. Thus, it may be possible to accept a lower initial level of mortality, allowing for the potential release of secondary inoculum arising from initial deaths.

Further gains can be obtained by:

- developing a suitable formulation to reduce losses from UV light degradation,
- making allowance for the anomalous dosage response of the fourth instar stage and
- accounting for the massive production of secondary inoculum arising from early deaths of larvae attributable to the applied field dose.

As indicated in the results of the field trials, it would appear that both fourth instar response and production of secondary inoculum may have had an influence on the high responses obtained using relatively low dosages in the field.

3.2.6 Trials of HpNPV against field populations of *H. puera*

3.2.6.1 Small scale trial at Nedungayam

Data on assessment of larvae for the presence of virus infection from field collected larvae are presented in Table 20.

Table 20. Rate of infection in the small-scale trial at Nedungayam

Dose (PIBs/ha)	Hours post spray	Number of larvae infected	Total larvae sampled	Percentage of larvae infected
0	0	0	19	0.00
	24	0	23	0.00
	48	0	7	0.00
	60	0	18	0.00
	72	0	2	0.00
8.5×10^8	0	0	39	0.00
	48	40	52	76.92
	60	66	68	97.06
	72	27	32	84.38
6.46×10^9	48	16	18	88.89
	60	45	50	90.00
	72	4	5	80.00
4.39×10^{10}	48	12	21	57.14
	60	28	33	84.85
	72	2	4	50.00

The time gap between the collection of samples was shorter compared subsequent trials and, therefore, it was difficult to make allowance for the very rapid mortality of infected larvae. Data are presented as instantaneous infections, but with the provision that samples at 60 and 72 hours post spray are underestimates of total population mortality. This preliminary trial targeted predominantly on first instar larvae (Figure13) and this is reflected in the calculated dosage per ha which is considerably lower than for later trials in which third instar larvae were targeted. The data also provided useful results showing high mortality while using ulv technology. However, a large number of larvae were predated by wasps in the experimental site, which affected the data on virus infection for the 72-hour sample at the highest dose.

Infection rates were remarkably high, even in the lowest dosage (equivalent to 8.5 x 10^8 PIBs per ha) and there was no significant dosage response with increasing dose per ha. This shows the potential value of targeting the first instar stage. However, the difficulty in obtaining sufficiently early warning of impending infestations makes it impractical to consider targeting the first instar for routine field application. If it is possible to target the third instar in the field through monitoring, the quantity of virus to be applied will be much less as shown in Figure 13.

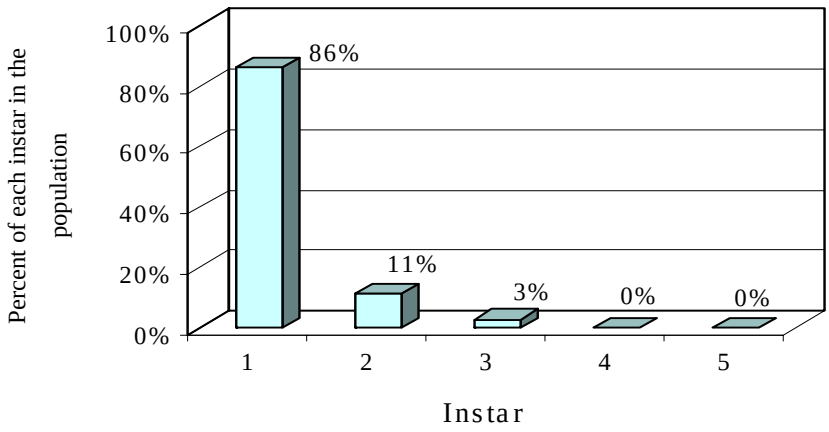

Fig. 13. Distribution of the larval instars of H. puera in the small scalefield trial plots at Nedungayam

3.2.6.2 Preliminary trial at Valluvassery, Nilambur

Assessment of the effects of virus application was confined to a standardised sample of 30 larvae per tree and collection of all dead larvae seen during the sampling process. Due to problems in laying out field trials in a randomised block design, it was not possible to use blocking to allow for potential differences in population sizes, tree sizes, etc. across the experimental plots. Nevertheless, the data were collected in a consistent manner and provided a measure of infection that can be compared across treatments.

A factor that also had to be taken into account was the very rapid rate of mortality once larvae were infected, as measured by larval smearing. In effect, larvae that were diagnosed with definite presence of baculovirus PIBs in one sample would have died by the next sampling, 24 hours later. This reflects the observations made in laboratory assays, at temperatures similar to those observed in the field, that larvae died within 24 hours showing overt symptoms of infection, which was confirmed though light microscope diagnosis. Based on this, two measures of infection were recognised in assessing field results; (1) presence of PIBs was recorded in each larva as an "instantaneous" measure of infection in the existing population of insects at the time of sampling, (2) on the assumption that larvae in the previous sample would have died by the time of the next "instantaneous" sampling, the percentage infection was expressed as a proportion of the population that had survived from previous sampling. This measure was then added to the previous percentage to provide an accumulated total percent infection. At the same time the number of larvae found dead and diagnosed positively due to virus infection were also assessed, thus providing an independent measure of accumulated mortality in the sampled populations.

A summary of the data gathered in the trial is shown in Table 21. The percentage infection data have not been transformed, but statistical analysis of the data employed probit transformation to determine standard deviations.

Table 21. Result of small-scale field trial at Nilambur, 1997

Dose	Repli-cate	Hours	No. larvae	No. infected	Percent-age infected	Accumu-lated percentage	NPV dead	Accumu-lated dead larvae
0	1	0	6	0	0.00		0	
0	1	48	29	2	6.90		0	
0	1	72	30	0	0.00		0	
0	1	96	20	1	5.00		11	11
1	All	48	149	52	34.90		0	
1	All	72	116	50	43.10		16	
1	All	96	112	31	27.68	73.21	89	105
2	All	48	149	51	34.23		3	
2	All	72	149	68	45.64		76	
2	All	96	93	24	25.81	73.47	64	143
3	All	48	136	37	27.21		0	
3	All	72	149	60	40.27		60	
3	All	96	138	44	31.88	70.38	83	143
4	All	48	147	54	36.73		0	
4	All	72	144	85	59.03		73	
4	All	96	111	61	54.95	88.32	113	186
5	All	48	145	46	31.72		0	
5	All	72	149	95	63.76		106	
5	All	96	123	53	43.09	85.92	127	233

The key features of these data are the confirmation of the flat dose response curves observed in laboratory experiments. This has resulted in high infection and mortality rates across all treatments but, as predicted by the laboratory results, did not reach 100% infection in any of the plots. The data are summarised in Figure 14 in which both probit and percentage scales are indicated. Variability was high for the highest dosages tested, showing no significant difference in the mean percentage infections achieved. However, application of probit analysis to the data showed a significant regression line at the 95% probability level. The slope of this line (0.521) was considerably flatter than that observed for the third instar in laboratory bioassays (slope = 1.10). However, the potential effects of secondary inoculum should also be accounted due to the death of the larvae infected from the initial spray application, and also of the anomalous dose response for the fourth instar observed in the laboratory.

The key features of these data are the confirmation of the flat dose response curves observed in laboratory experiments. This has resulted in high infection and mortality rates across all treatments but, as predicted by the laboratory results, did not reach 100% infection in any of the plots. The data are summarised in Figure 14 in which both probit and percentage scales are indicated. Variability was high for the highest dosages tested, showing no significant difference in the mean percentage infections achieved. However, application of probit analysis to the data showed a significant regression line at the 95% probability level. The slope of this line (0.521) was considerably flatter than that observed for the third instar in

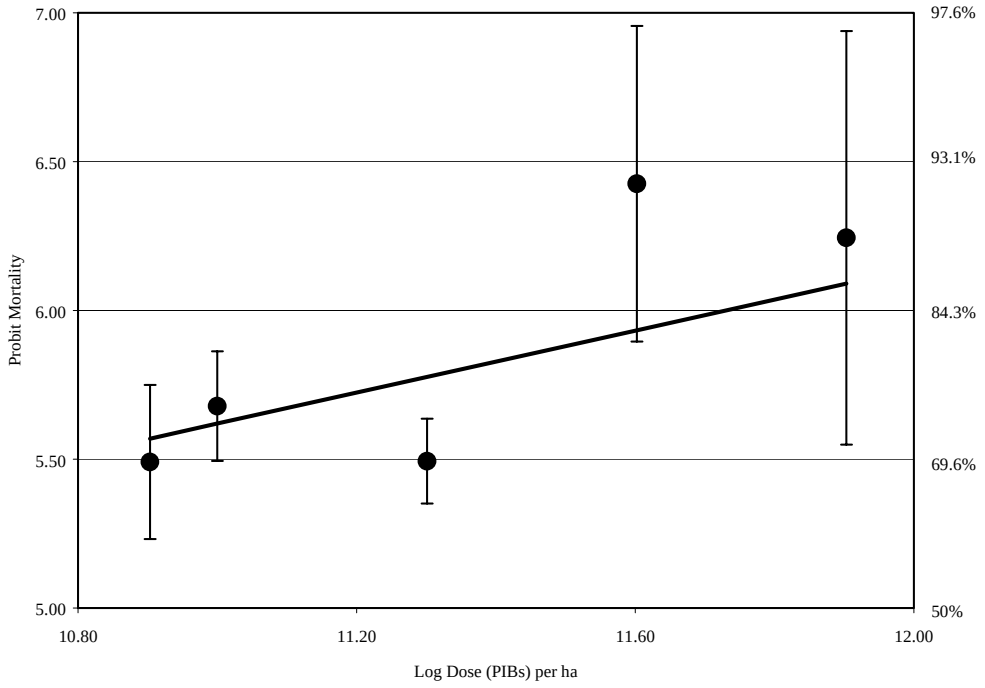

Fig. 14. Per cent infection (probit transformed) in relation to dosage applied in the preliminary field trial at Nilambur

laboratory bioassays (slope = 1.10). However, the potential effects of secondary inoculum should also be accounted due to the death of the larvae infected from the initial spray application, and also of the anomalous dose response for the fourth instar observed in the laboratory.

Nevertheless the trends are similar and confirm that the infection data observed in live larvae do A further trend emerges when the data on accumulated number of virus-killed larvae in the plots are analysed. In this case the data represent all larvae in this category found on leaves while collecting live larvae and, thus, is a quantitative measure of accumulating mortality over time post infection. The data are presented in Figure 15 showing a clear linear relationship between dosage and accumulated host mortality. However, because the methods of collection are not directly comparable, it is not possible to make a direct correlation between the two measures of the impact of virus on *H. puera* populations. result in rapid, accumulated mortality on foliage. The latter measure is undoubtedly an underestimate of actual mortality because of factors such as monsoon rainfall, wind abrasion, disintegration of the cadavers, etc. that will reduce the number of bodies remaining on foliage between sample intervals.

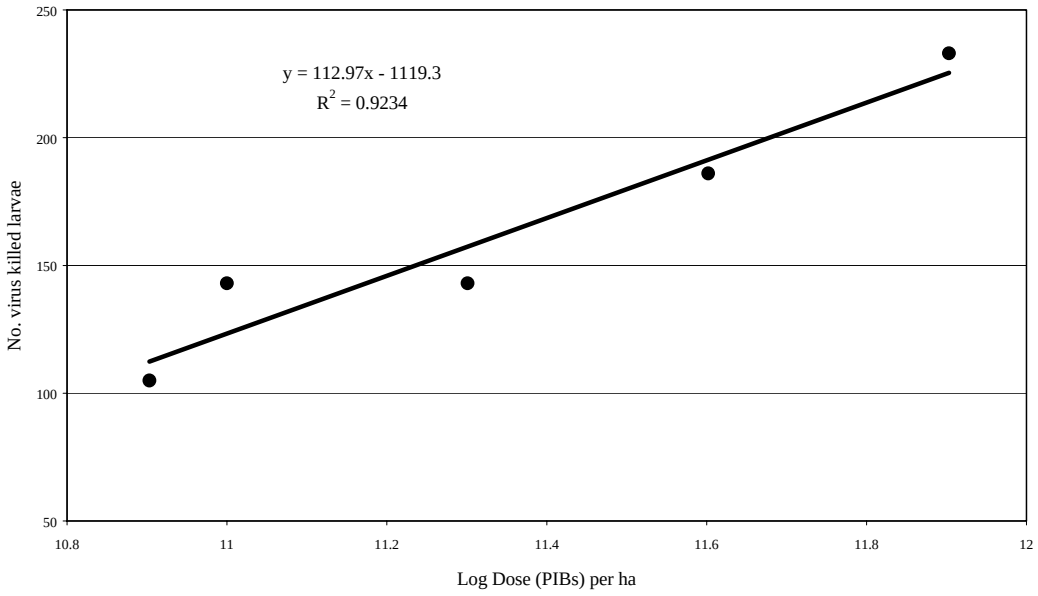

Fig. 15. Mortality in relation to dosage of baculovirus applied in the1997 trial at Nilambur

The relationship between the virus dosage and mortality as shown in Figure 14 can be used to predict the dosage required to kill a particular proportion of the host population. For example, the dosage required to kill 95% of larvae is predicted to be 1.05 x 10^{13} PIBs per ha. Such a quantity of virus represents the productivity

from approximately 50000 infected larvae (larval equivalents) and is unrealistic. However, the flat slope of the regression also indicates that significant mortality can be induced by low doses also; for example 80% mortality requires a predicted dose of 2.53 x 10^{11} PIBs per ha, which is over 40 times lower than the 95% dosage.

3.2.6.3 Final trial at Valluvassery, Nilambur

Using the same procedures in section 2.2.6.2, accumulated percentage infection was calculated for the four dosages tested (note that the highest dose in this case was 4 x 10^{11}, which is 50% lower than the highest dose used in the 1997 trial). The data were subjected to probit analysis to provide the dosage mortality relationship (Figure 16).

The slope (0.538) is very similar to that obtained in the preliminary trial in 1997 (slope = 0.521) indicating very similar responses to the dosages applied. However, there is less variability in the 1998 trial. It appears, therefore, that over 80% mortality can be achieved with dosages over 2 x 10^{11} PIBs per ha. This represents approximately 1000 larval equivalents, based on the data in Table 8.

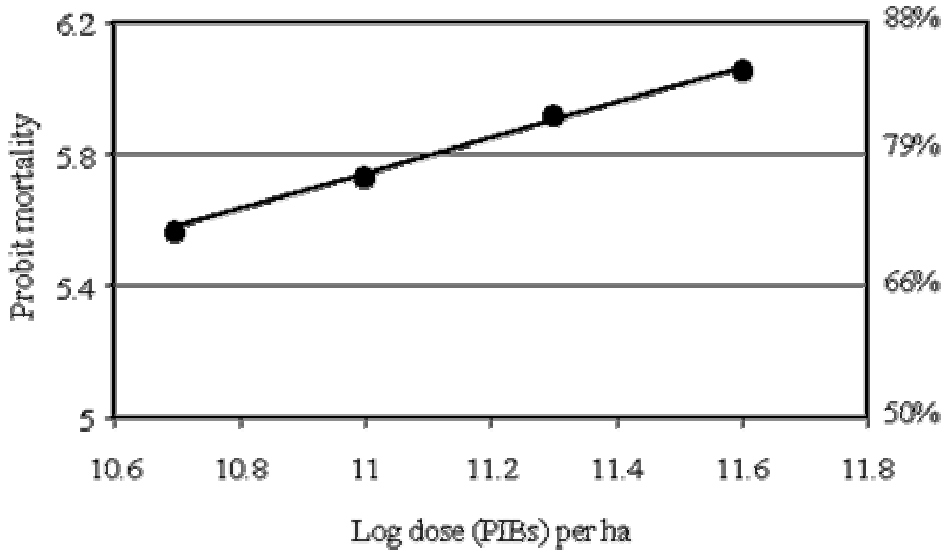

Fig. 16. Larval mortality (probit transformed) in relation to dosage applied during the final trial at Nilambur

The slope (0.538) is very similar to that obtained in the preliminary trial in 1997 (slope = 0.521) indicating very similar responses to the dosages applied. However, there is less variability in the 1998 trial. It appears, therefore, that over 80% mortality can be achieved with dosages over 2 x 10^{11} PIBs per ha. This represents approximately 1000 larval equivalents, based on the data in Table 8.

A further measure of the success because of virus application, is the extent of reduction in leaf damage following larval mortality. This was measured in two ways - visual assessment of percentage defoliation and by taking the fresh weight. The latter measure clearly had a high degree of variability because of the different sizes of leaves, but was still felt to be a useful measure on the basis that sufficient leaves were weighed to provide a comparable mean weight for all leaves taken. The trial was targeted at the third instar stage, and hence some defoliation due to larval feeding had already occurred by the time the virus was applied. This was therefore measured as a baseline against which further defoliation was scored.

Table 22 demonstrates that, when compared separately against the untreated controls, all treatments provided a significant reduction in damage (t-tests, $p<.05$), but there was no significant difference between virus treatments. However, the variability in the percentage values was high and, using Duncan's Multiple Range Test for the final percentage damage recorded, the only significant difference was between the highest dose and the untreated control ($p=0.05$). This trend was confirmed when the mean weights of the leaves were compared.

Table 22. Percentage damage (leaf loss) in the final trial at Valluvassery, 1998

Dose per ha	Percentage damage (loss of foliage)		
	Initial (in %0	Final (in %)	Difference (in %)
Control	37.74	81.68	43.94
5×10^{10}	42.28	73.44	31.16
1×10^{11}	33.56	61.12	27.56
2×10^{11}	35.52	67.08	31.56
4×10^{11}	24.24	50.36	26.12

Figure 17 shows the trend of mean weights against time for each dose tested (no measurements were taken for the highest dose at 96 hours). During the first 48 hours, the mean weights of leaves (with the exception of the highest dose) dropped, reflecting the feeding of larvae on leaves prior to any noticeable effect of the virus on insect mortality. The only exception was the highest dose in which larvae were killed more rapidly than lower doses. After 48 hours, mean weight of leaves in the virus treated trees increased again, indicating recruitment of new leaves (with little or no damage) to the sampled cohort and also the increasing effects of virus on the larvae. By contrast, the mean weights of leaves in the untreated controls continued

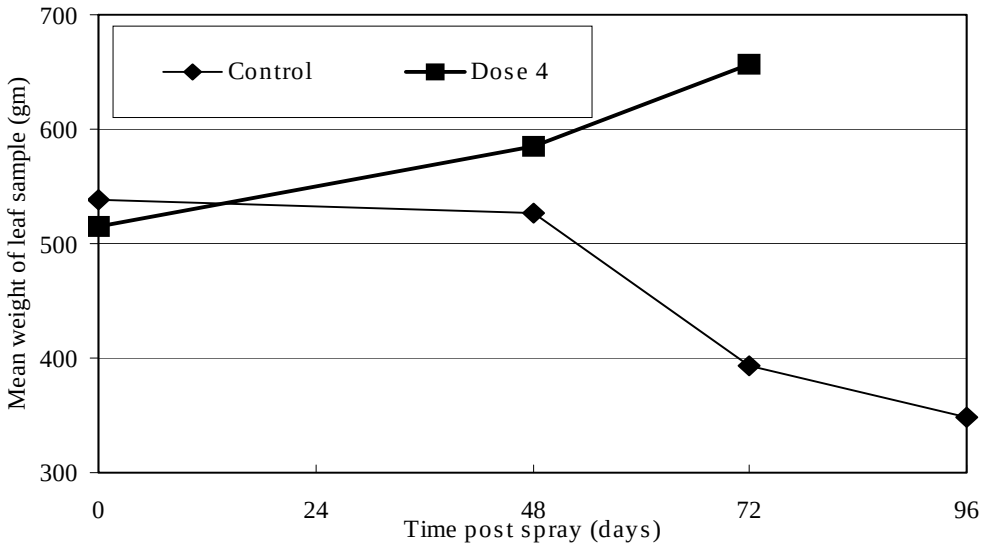

Fig. 17. Mean weight of leaf samples collected from the final trial at Valluvassery, 1998

to decline, despite recruitment of fresh leaves to the sampled cohort. These data indicate that there was a significant decrease in the quantity of foliage consumed, reflected both in visual assessments and in the fresh weights of leaves sampled. It was not possible to look at other parameters such as tree diameter in order to assess effects on growth increment. However, it is likely that the greater photosynthetic area on the treated trees would have resulted in increased growth, at least at the highest dose tested.

4. CONCLUSIONS

The major consideration in this study was the effective use of *HP*NPV in the field for controlling the teak defoliator. The optimal use of the virus was examined considering various parameters under the Control Window concept which included the host characteristics like the host age, distribution, feeding rate as well as the virus characteristics, like the rates of attrition in the field due to environmental factors. The efficiency of different sprayer systems was also studied wherein the information on droplet emission and field capture rates were considered. As part of the programme protocol for monitoring the pest population was also developed.

The bioassay indicated that *H. puera* is susceptible to *Hp*NPV, but control needs to be focussed on the third or fourth instars as older larvae are more resistant to the virus. This emphasises the need for detecting the outbreak at the earliest possible to se the virus in time.

The studies on the plant chemistry revealed that the virus is not deteriorated by any plant chemicals. The studies on effect of UV light and rain on the field applied virus though encouraging, needs further confirmation.

Studies on the virus application system indicated that ground based ULV sprayer Stihl SR 400 is effective for trees up to 14m height. This result is crucial to the adoption of this technique as in the teak forest system it is unlikely that aerial application will be a viable option for various reasons including environmental and cost issues.

The field dosage predicted considering various parameters within the control window concept showed that to achieve a 90% kill, application of the virus at the rate of 5.4×10^{11} per ha is required. One of the reasons for the such a high dosage may be due to the fact that the Control Window is not taking into consideration other important factors such as the secondary inoculum build-up. Hence further refinement of the model is required.

Field trial carried out indicated that 80% of the mortality could be achieved by virus application at the rate of 2×10^{11} equivalent to 1000 larvae per ha. However, the data on the effect of virus application on protection of foliage is insufficient.

Further refinement of the dosage, field application system and validation trials are required before the virus is taken to the field for practical purpose.

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