HpNPV technology for Biocontrol of Teak Detractor - Hyblaea puera

HpNPV technology

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Currently, the use of natural enemies for the management of pest insects is not just an option but the only way considering the adverse environmental and health impact of chemical pesticides. This is more important in the forest ecosystem than in agricultural systems since the former is more diverse than the latter. The tea defoliator (Hylobius abietis) has been recognized as the most devastating pest in tea plantations in Kerala. It is estimated that about 40% of the potential volume increment is lost due to tea defoliator attack. It is also estimated that by controlling the tea defoliator an additional tea wood of 3 cubic meter/ha/annum can be utilized from tea plantations which is a substantial gain considering the cost of about Rs.30,000-40,000 for one cubic meter of high-quality tea. It is in this context that the research on the most serious pest of tea— the tea defoliator (Hylobius abietis) assumes importance.

The Department of Biotechnology, Government of India, with its task force on Bioprocesses and Crop management, started supporting research on tea defoliator management in 1992 in a research project entitled Management of the tea defoliator using Nuclear Polyhedrosis Virus. The project for the first time tested a crude suspension and found it effective controlling the tea defoliator population. Studies were also carried out on the population dynamics of the pest insect. In the next stage DBT supported KFRI to establish a pilot scale NPV production unit under the project entitled Demonstration of mass production, formulation, and application of a baculovirus for management of the tea defoliator. It was under this project that a mass production system for HpNPV was standardized and field application procedures developed. While this project was ongoing, DBT supported a collaborative programme between KFRI and Rajiv Gandhi Centre for Biotechnology, Trivandrum, to develop a new strain of NPV and establish an in vitro mass production system. It is envisaged that this will refine the technology developed so far and pave the way for the large-scale production of the NPV.

The project documents yet another success story of DBT in supporting R&D efforts in the country. I am sure that the dedicated scientists at KFRI will continue to solve many problems, small and big, in its continued journey towards non-hazardous pest management.
The tea defoliator, *Hyphantria cunea* has been recognized as the most devastating pest in tea plantations in Kerala. In a study carried out in Kerala, it is estimated that about 44 per cent of the potential volume increment is lost due to tea defoliator attack. It is also estimated that by controlling the tea defoliator, an additional 10000 tons of tea crops could be realized from tea plantations. Recognizing the importance of this pest in plantation forestry, KFRI initiated studies in 1988 with the view for developing a suitable management strategy against this pest. The pioneering efforts by a team of KFRI Scientists in Forest Entomology yielded significant results of applied value for managing the tea defoliator problem through biological methods which proved the pest is not as damaging as earlier thought. Since this pest was discovered in 1998, the team of scientists working on this problem have not only made the biological control of this pest a reality but has also improved the post-harvest management through mass production of the biocontrol agent, an insect-pathovirus, HpNPV which is highly specific to the tea defoliator.

The publication of this Handbook is very timely and I am hopeful that it will help disseminate the technology among foresters and scientists to make this a reality.

I also congratulate the team of scientists for their achievement, especially Drs. V.V. Suseendran and T.V. Saju and S. V. V. and C. V. S. V. whose concerted efforts have made it possible to bring out this excellent publication on tea-defoliator management technology.

Peechi
21 March 2006

(A.K. Sharma)
Director, Kerala Forest Research Institute
A group of at least two million *Hyblaea puera* moths descended on about 20,000 newly flushed teak trees in a 30-hectare patch within the plantation. Together they deposited between 50 to 100 eggs per leaf on most of the tender leaves in the top canopy, and by morning, all the moths had disappeared. Within a fortnight, the trees were stripped clean by the feeding caterpillars, and the falling frass sounded like mild rainfall on the dry leaves. Other nearby plantations remained untouched. "No one knows where the moths came from or where they went."

Nair, 1988.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AcMNPV</td>
<td>Autographa californica multiple nuclear polyhedrosis virus</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DBT</td>
<td>Department of Biotechnology</td>
</tr>
<tr>
<td>ECV</td>
<td>Extracellular virus</td>
</tr>
<tr>
<td>HpNPV</td>
<td>Hybrid passerine nuclear polyhedrosis virus</td>
</tr>
<tr>
<td>IMI</td>
<td>International Mycological Institute</td>
</tr>
<tr>
<td>KFRI</td>
<td>Kerala Forest Research Institute</td>
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<tr>
<td>PIB</td>
<td>Polyhedral Inclusion Body</td>
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<tr>
<td>RGCB</td>
<td>Rajiv Gandhi Centre for Biotechnology</td>
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</table>

Teak defoliator has been recognized as a serious pest of teak for more than a century. A package of silvicultural and biological control methods was advocated in 1934 by forest entomologists to manage this pest; however, these recommendations were never implemented in the field. While the area under teak continued to expand and while thousands of hectares of teak stands suffered teak defoliator outbreaks many times a year all through the century, researchers continued to claim that the situation would have been solved if the foresters had implemented the package.

Establishment of KFRI in 1975 and its initiative in teak defoliator research marked the end of this long period of complacency. From focused research at Nilambur, the land of teak- we knew that the 1934 recommendations against the teak defoliator, will not work. We also argued against the aerial spraying of chemical pesticides in teak plantations of Madhya Pradesh and Kerala. It was thus at the right time and ambience that KFRI took up the challenge of developing an economically viable and environmentally safe management strategy for teak defoliator.

The success is the story, you are going to read.
Economic Impact

Teak defoliator larvae strip the trees of foliage. The trees respond to this loss by producing new foliage. Availability of new flush makes the trees once again susceptible and the pest outbreak repeats. In teak plantations of Nilambur, this can happen six times an year, averaging three. This series of defoliations apart from the natural defoliation of this deciduous tree species, reduces the potential volume increment. The tree spends its resources to put up new foliage rather than to increase its body size. Moreover, when the pest population is high, the terminal buds are eaten off, leading to epicormic shoots and eventually forking. Forking of the stem depreciates the timber quality by reducing the length of main bole available and also by reducing the growth rate.

Defoliation does not kill teak trees, but it has been assumed to cause heavy loss in increment. During the 1930's rough estimates based on several assumptions placed the loss at 6-65% of the potential volume increment of teak plantations, but a 1941 estimate of 13% loss, based on fewer assumptions, was generally accepted and quoted extensively in subsequent years. Recent studies at the KFRI showed that defoliation by H. puerca caused an average loss of 44% of the potential volume increment in 4-9-year-old plantation. Although it was not possible to quantify the benefit in terms of volume gain over the entire rotation, it was estimated that protected trees can yield the same volume of wood in 26 years as unprotected trees would yield in 60 years.

For demonstrating this impact to practicing foresters, KFRI has established two half hectare plots at Nilambur in 1992. One of them was protected from the defoliator while the other was not. Protection commenced on the year of planting and at 12 years of age, there was an additional increment and 21.9% additional increment in the unprotected plot. Thirty one percent of the trees had forked in the unprotected plot, as against 4% in the protected plot.
Moth
The moths are comparatively small, with a wing span of 3-4cm, and have a characteristic resting posture that conceals the black and orange-yellow hindwings under the grayish-brown fore-wings. Newly emerged moths can sometimes be found resting on the surface of leaves of teak coppice or other shrubs. They are inactive during the day but, when disturbed, fly briskly to adjacent shrubs. Males and females emerge more or less simultaneously, and mating takes place within a couple of days. Eggs are laid over a week-long period starting the third or fourth day after emergence, the longest recorded oviposition period being 12 days.

Egg
Eggs are laid on tender new leaves, placed singly near the veins, and usually on the undersurface. They are oval, flat, and white and measure about 1mm in length. About 500 eggs are laid per female with a recorded maximum of 1000. Larvae hatch in about 2 days.

Larva
There are five larval instars. The neonate larva eats a shallow depression on the surface of the tender leaf and protects itself with strands of silk. The first and second instars feed mainly on the leaf surface. Starting with the third instar, the larva cuts out a leaf flap, usually at the edge of the leaf, folds it over, fastens it with silk, and feeds from within. Fourth and fifth instar larvae also feed from within the shelter of leaf folds. The entire leaf, excluding the major veins of tender leaves, is eaten, but more veins are left in older leaves. The early instars cannot feed successfully on old tough leaves and fail to establish when they are given no other food. Under optimal conditions, the larval period lasts 10-12 days. The full-grown larva measures about 3.5-4.5 cm, and there is considerable colour variation in the fourth and fifth instars; the body may be either wholly black or dark grayish to black, with longitudinal colored bands that may include a dorsal orange or ochreous band and lateral white lines. The dark and light forms occur together in the same population, with the darker forms predominating during epidemics.

Pupa
Following heavy defoliation, the mature larvae descend to the ground on silken threads and pupate under a thin layer of leaf litter or soil, within a loosely built cocoon made of dry or decayed leaves, or soil particles held together with silk. Pupation may sometimes occur within green leaves of other plants in the undergrowth, folded or juxtaposed with silk. The average pupal period lasts 6-8 days under optimal conditions. There is no evidence of hibernation or aestivation of pupae.

Are some teak trees resistant to defoliator attack?

This question was raised because within large plantation areas with severe defoliator attack, some trees are found uninfested by the teak defoliator. We made a search in Kerala for teak clones resistant to attack from the teak defoliator. Extensive areas of plantations, natural forests, and three seed orchards representing 31 plus trees were examined during periods of defoliator attack. It was found that many isolated trees were left distinctly unattacked amid totally defoliated trees. Detailed investigations revealed that this is not due to genetic resistance but due to, what may be called, phenological resistance. Tender foliage is essential for the initial establishment and survival of the teak defoliator. Phenological resistance is resistance to attack due to asynchrony between the flushing time of the tree and insect population cycles. Early flushers had a greater chance of escape from defoliation but the escape is circumstantial and not consistent over years so that it is of little practical utility. No instance of genetic resistance to the defoliator was discovered in teak clones of Kerala.
The teak defoliator is present year-round in teak plantations, but in varying population densities. During the period of natural defoliation of teak (November, December, January), the pest density is very low (A). Every year, at Nilambur, high intensity outbreaks of teak defoliator occur immediately after the premonsoon showers in late February or early March (B). These epicentres are highly localised outbreaks which represent the transitional stage between very sparse endemic population (A) and high density outbreak populations (C). The months of April, May, June and July witness a series of large outbreaks. During late July or September, the population declines to the endemic level (A). In some years, there will be fresh outbreaks during the month of October (B). Then from now onwards, the population remains at the endemic level (A).

Based on the population density, three distinct types of teak defoliator populations have been described as follows:

<table>
<thead>
<tr>
<th>Population type</th>
<th>Month of occurrence</th>
<th>Description</th>
<th>Insect composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endemic</td>
<td>October/November/December</td>
<td>sparse, low density population</td>
<td>uneven-aged</td>
</tr>
<tr>
<td>Epicentre</td>
<td>late February/early March</td>
<td>localised, high density tree-top infestations</td>
<td>even-aged larvae</td>
</tr>
<tr>
<td>Epidemic</td>
<td>April/May/June/July/August</td>
<td>high density, large area infestations</td>
<td>even-aged larvae</td>
</tr>
</tbody>
</table>
Spatial dynamics

At the end of the endemic period (A), teak defoliator outbreaks originate in small patches termed as epicentres (B). These epicentres will be 0.5 to 1.5 ha in area and are characterised by heavy tree top infestation. The larval stages will be almost uniformly aged. The moths which emerge from this epicentres will always take up short range migration and cause infestation in a larger area. This is the start of the epidemic phase of outbreaks (C, C, C). In this phase a series of infestations occur in large teak plantation areas. After the epidemic phase, the population enters an endemic phase (A) followed in some years by repeated outbreaks in small areas (B) or more usually the endemic phase (A).

However, these chain of outbreaks are not a local phenomenon. The teak defoliator moth can travel long distances. Immigration and emigration of moths have been observed in plantations. Explaining the local scenario of outbreaks require an understanding of this long distance migration of moths.

Metapopulation is the assemblage of local populations between which individuals can move. The metapopulation of Hyblaea puera may extend to the entire teak growing areas in India and possibly the neighbouring countries also. While the local populations exhibit drastic shift in population densities and may even go extinct, the metapopulation will be relatively stable. In other words, the ability to migrate provides the teak defoliator the advantage of making use of resources in large landscapes according to the variation in time of flushing of teak.

Diagram showing the spatial dynamics of teak defoliator outbreaks.

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Parasites

In our studies on natural enemies of teak defoliator at Nilambur teak plantations, five parasites were found: the tachinid Palearista solennis, an eulophid Sympiesis hyblaeae, the chalcid Brachymetria larus and two unidentified ichneumonids. Overall parasitism by all species was about 9%, the tachinid Palearista solennis accounting for nearly 6%. Parasites were either absent or rare at the beginning of the epidemic, but their numbers increased subsequently particularly in the case of Palearista.

Sympiesis hyblaeae preferred to lay eggs on first or second instar host larvae. The larvae selected for oviposition was paralysed and a single egg was deposited on the lateral side of the body in the intersegmental region. A single parasitoid laid an average of 15 eggs during its lifespan. Continuous multiplication of the larvae in the laboratory was not possible as the parasitoid entered diapause in the pupal stage during the months February-May. As the diapause period coincides with the early phase of pest build-up, the parasitoid population is not able to numerically respond to the increasing pest population. The scope for mass multiplying the parasitoid for release in the field during the critical period of pest incidence thus appears to be limited. It is concluded that Sympiesis hyblaeae is not a suitable candidate for use in the biological control programme against the teak defoliator.

Palearista solennis is an endoparasite which infests the third and fourth instar larvae of T. puer. The female lays an average of 43 eggs during its lifespan. Host larva is not paralysed prior to oviposition. Normally a single parasitoid larva developed within a host. Laboratory studies established the feasibility of continuous rearing of the parasitoid on host larvae. An agar based artificial diet was developed and method of rearing P. solennis tested. The feasibility of mass multiplication suggests that this species can be produced in large numbers. However, further refinement of the methods of multiplication is needed before using this species for practical control.

predators

One of the major adverse impact of using chemical pesticides against the teak defoliator is that on the natural predators of the insect. Predatory insects including wasps, spiders, birds and the Bonnet macaque are known to comprise the predator complex of *Hyptia indica*. Forty eight species of birds have been recorded as feeding on teak defoliator larvae during large scale outbreaks. Recent studies have indicated that birds also function as dispersal agents of HpNPV within and between teak defoliator populations.
Pathogens
Microbes represent a major fraction of the natural enemy complex of *Hyblaea puera*. In surveys conducted at teak plantations of Nilambur and Peechi and also screening of dead larvae in the laboratory culture to identify prospective biocontrol agents, the following pathogens were found to cause mortality in teak defoliator larvae.

Bacteria
Short rod, gram negative, non-sporulating bacterium *Enterobacter aerogenes* (Kurse) (IMI B.10740)
Endospore forming, gram positive *Bacillus thuringiensis* var thuringiensis (KFR1 1294)
Two gram negative, non-sporulating bacteria *Pseudomonas aeruginosa* (Schnoeter) (IMI B. 10976) and *Serratia marcescens* Bizio (IMI B. 11386)

All the four bacteria identified as causing mortality to the teak defoliator is also pathogenic to many other insects, including beneficial ones. This broad spectrum pathogenicity makes them unsafe to be used in controlling teak defoliator populations since the application has to be done in the forest ecosystem which hoards high diversity of insects.

Fungus
A new species of synnematus fungi *Hirsutella* (IMI 328626)
The fungi belonging to the genus *Hirsutella* is highly sensitive to ambient conditions. This makes it difficult to be used during the summer months in the teak plantations when the temperatures will be extremely high. It can still be used by using oil instead of water as the carrier but that entails high cost of application.

Virus
A new record of virus with refractile polyhedral inclusion bodies, staining blue in Giemsa and thick blue in Buffalo black. Identified as the *Hyblaea puera* nuclear polyhedrosis virus (HpNPV). The major advantage of HpNPV is that it is absolutely target specific.
Nuclear polyhedrosis virus

Large-scale deaths of teak defoliator larvae characterised by cessation of feeding, flaccidity, and subsequent liquefaction of body tissues has been reported by Stebbing as early as 1903. However, the discovery of the causative pathogen had to wait until a systematic screening of microbial pathogens of teak defoliator was undertaken in the Nilambur teak plantations by KFRI. In 1985, Sudheendrakumar and others detected several dead insects with the characteristic symptoms as observed by Stebbing. Microscopic observation of tissues revealed the presence of refractile polyhedral inclusion bodies, which stained blue in Giemsa, and measuring 0.9-2.4 micrometers in diameter in the scanning electron micrograph taken by Jean Adams at USDA, confirming its identity as NPV.

NPV extracted from the diseased larvae was used for pathogenicity tests. Healthy, laboratory reared teak defoliator larvae were fed with teak leaves sprayed with an aqueous suspension of NPV. The feeding rate of the larvae declined on the second day, and the larvae stopped feeding by the third day. The larvae became sluggish with flaccid bodies and died within 4-5 days.
residual actinic vesicles.

In the case of HRP incorporation into the microtubules in the absence of light, the vesicles were observed to be present within 4 hours of exposure. This is in contrast to the case of photoreceptors, where no vesicles were observed even after 4 hours of exposure. The absence of vesicles in the photoreceptors suggests that the incorporation of HRP into the microtubules is a result of the actinic vesicles, which are present in the photoreceptors but not in the photoreceptors in the absence of light.

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In the case of HRP incorporation into the microtubules in the absence of light and the presence of actinic vesicles, the vesicles were observed to be present within 2 hours of exposure. This is in contrast to the case of photoreceptors, where no vesicles were observed even after 4 hours of exposure. The presence of vesicles in the photoreceptors suggests that the incorporation of HRP into the microtubules is a result of the actinic vesicles, which are present in the photoreceptors but not in the photoreceptors in the absence of light.

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Baculoviruses can be produced only in live host cells. For producing adequate quantity of HpNPV for field application, healthy *Hygaea puera* larvae reared in the laboratory or collected from the field are fed with low dose of HpNPV and the virus produced in the insect is harvested. The steps involved in the process are as follows:

1. Collection of early fifth instar larvae from the field population / laboratory culture.
2. Screening of larvae to select larvae within the weight range of 80 -110 mg.
3. Spraying of purified HpNPV to diet tubes using chromatographic sprayer / atomizer.
4. Transfer of selected insects to HpNPV sprayed diet tubes.
5. Incubation at 20-25°C.
6. Retrieval of larvae at 96 hours post inoculation.
7. Homogenization of larvae in homogeniser with 0.1% Sodium dodecyl sulphate.
8. Filtration using muslin cloth.
9. Centrifugation at 130 g for 5 minutes. Supernatant retained.
10. Centrifugation at 6360 g for 25 minutes. Pellet retained.
11. Re-suspension in de-ionized water, thrice.
12. Storage at 4°C until formulation.

The above protocol have been standardised by KFRI in its effort to maximize HpNPV production. A three piece rearing tube made of polypropylene has been specially designed for the rearing and incubation of teak defoliator larvae. The following are the statistics of mass production:

<table>
<thead>
<tr>
<th>Ideal stage of the insect</th>
<th>early fourth instar</th>
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<tbody>
<tr>
<td>Ideal weight of the insect</td>
<td>0.027 - 0.036 g</td>
</tr>
<tr>
<td>Inoculation method</td>
<td>spraying on to artificial diet in rearing tubes</td>
</tr>
<tr>
<td>Input dose of HpNPV</td>
<td>1 x 10^6 PIB / larvae</td>
</tr>
<tr>
<td>Incubation period</td>
<td>72 h</td>
</tr>
<tr>
<td>HpNPV yield</td>
<td>3.3 x 10^6 PIB / larvae</td>
</tr>
</tbody>
</table>
There are more reasons than one for converting the crude virus into a formulated product. Formulation prevents replication of any contaminant microorganisms during the storage period, and improves shelf life by providing protection against extreme temperatures and incident ultraviolet radiation. The biological activity of the virus is better retained when formulated. The formulation can also contain additives like stickers, spreaders, wetters, thickeners and protectants which provide hassle free application of the virus which can persist long at the target site.

The HpNPV is now a formulated product. Seven types of formulations have been synthesized, five being wettable powders, one flowable concentrate and one micro-encapsulated product. Laboratory bioassays indicated that the wettable powder synthesized using freeze drying procedure provided the best retention of biological activity of HpNPV. This formulation was field tested during natural outbreak of teak defoliator in a 12 year old teak plantation. It provided 18.5% additional foliage protection than the unformulated virus.
dosage arithmetic

Calculations of tank mix for field application of HpNPV is made using simple relationships between virus, insect, host tree, and droplet parameters. The arithmetic of calculating HpNPV needed per hectare of the plantation is as follows:

Dose per ha ($D_{ha}$) = $CE \times Di$

where,

$CE = \text{Capture Efficiency defined by the number of droplets required to ensure at least one droplet per feeding area, expressed in terms of droplets per unit ground area}$

= ($1 \times 10^{10}$) $LAI \times 1/(s \times fr)$ droplets per ha

where,

$1 \times 10^{10}$ = area of 1 ha in mm$^2$

$LAI = \text{Leaf Area Index, a multiplier to express surface area of leaves in units of ground area}$

$s = \text{loss of spray fluid to non-target area}$

$fr = \text{feeding rate of the target instar}$

and

$Di = \text{Initial dose expressed as PIB/m}^2$

= $d \times a$

where,

$d = LD_{95}$ (dose required to kill 95% of the population)

$a = \text{estimated activity loss of virus after application}$

where,

$N = \text{Number of droplets emitted by atomiser per litre/Volume Median Diameter (VMD)}$

Theoretical minimum volume = $CE/N$ litres per ha

Dose per ha = $CE \times Di$ expressed in PIB/ha

Dose per litre = $N \times Di$ expressed in PIB/litre

The principle of tank mix calculation can be illustrated as follows:

For the initial dosage ($Di$) of 1000 PIBs,

$CE = 4 \times 10^{10}$ droplets per ha (assuming $fr = 4$ mm$^2$, $LAI = 8$, $s = 50\%$)

Let $N = 1.53 \times 10^{10}$ droplets per litre (assuming 50 mm VMD)

Let $Di = 1000$ (assuming $d = 500$, $a = 0.5$ (50% loss))

Then,

Theoretical minimum volume = ($4 \times 10^{10}$)$ \times (1.53 \times 10^{10})$ = 2.6 litres

Dosage per ha = ($4 \times 10^{10}$)$ \times ($1000$) = 4 $\times 10^{10}$ PIBs per ha

Dosage per litre = ($1.53 \times 10^{10}$)$ \times ($1000$) = 1.53 $\times 10^{10}$ PIBs per litre
Crude suspension in water / High volume / 1993

At Kariem Muniram teak plantations, Nilambur, a 100 tree plot was protected from teak defoliator using crude suspension of HpNPV. The plot was attacked five times by the teak defoliator during the year 1993. Therefore, five sprays were needed. The protection afforded was estimated based on leaf damage, larval mortality and growth increment as compared to an untreated plot of comparable stand composition. The study showed that 70-76% of the leaf damage by teak defoliator can be prevented by timely, one time application of HpNPV during each outbreak. The protected trees registered 39% higher basal area increment than the unprotected trees.


At Valluvassery teak plantations, Nilambur, semipurified HpNPV was used against teak defoliator at five different dosages with five replicates. A randomized complete block design was used. HpNPV was suspended in coconut oil emulsion and applied using Ulva + sprayer. Efficacy of HpNPV spray was estimated by adopting a destructive sampling procedure in which all larvae, classified as live or dead, were collected at random per row per plot. Sampling was done at 0, 48, 72 and 96 hours post spray. The study showed that 80% of larval mortality could be achieved with dosages over 2 x 10^7 PIBs per ha. This represents nearly 1000 larval equivalents, i.e., we need virus produced from 1000 larvae to carry out pest control in one hectare area.

Formulated product in water / High Volume / 2002

The freeze dried formulation of HpNPV was field tested in the Valluvassery teak plantations, Nilambur. The formulation was mixed in water. The dosage of spray was 2 x 10^6 PIBs/ml. Each tree within the treatment plot was individually sprayed using a motorized high volume sprayer (Birla Yamaha). Results indicated that the formulated product could afford 18.47% additional foliage protection than the unformulated HpNPV.

advantages

Target Specific

HpNPV possess the target specificity demanded by a pesticide to be used in the teak forest ecosystem. Cross infectivity studies on insects like Achara Janata, Atteva fabricella, Catopsilia crocale, Elytra nascissus, Eutechona mahaeralis and Bombby mori were all tested negative. It has been proved to cause no cytotoxic effect on Spodoptera frugiperda ovarian cell lines.

Safe

Baculoviruses does not cause infection in any of the vertebrate species. We studied the cytotoxic effects of formulated HpNPV on human- larynx (Hep-2) cell line and African Green Monkey kidney cell line (Vero) and found no effect on the cells. It was also found safe against the Indian Myna (Acridotheres tristis) during our in vivo studies.

Horizontal Transmission

With in a large population, if a few larvae are infected by the virus, they die within 2-3 days and a large amount of virus is released in the field. This secondary inoculum spreads the disease to healthy insects within the population. Thus, when we use HpNPV, unlike the inert chemical pesticides, we get a magnified effect. Horizontal transmission helps us to devise a variety of spray schedules— from lattice spraying to strip spraying.

Vertical Transmission

By way of trans-oovum (egg surface contamination) and trans-ovarian (presence of virus with in the egg) modes, HpNPV can transmit from one generation to the next. This happens when the late larval instars imbibe sub-lethal dose of HpNPV. The larvae does not die but lives on, infected. The virus particles will either be in the inert phase or in the sub-letal infection phase while the larvae matures to pupae and then to adult. If the virus was in the inert mode, they get transmitted from the female adult to the eggs by trans ovarian transmission and if in the sub-lethal infection mode, it will be transferred to the next generation by egg surface contamination.

Magnification

Giving a hundred viral particles to the teak defoliator will cause infection, and by the time it dies, there will be 1300000000 PIBs within it. Once dead, the virus will be released which would cause infection in other healthy insects. Thus, unlike other pesticides, more HpNPV work to suppress the insect population than we apply. This amplification is the major factor which makes the HpNPV able to control large scale epidemics.

Fast Kill

HpNPV kills the host insect faster than any other known baculoviruses. While most of the baculoviruses take more than 100 hours to kill the host insect, HpNPV does it in 60-70 hours depending on the larval age.

Ease

HpNPV can be applied using a variety of spraying equipments ranging from high volume, low volume and ultra low volume applicators.
Extension

The methods of mass production, formulation and application of HpNPV have been standardised. The technology developed is being transferred to the Kerala Forest Department. This process started in the year 2004 during which the methods for detecting teak defoliator outbreaks early enough so as to help mount control measures was demonstrated to the field staff of the Kerala Forest Department. In the year 2005, the techniques for application of HpNPV was transferred to spraying crews identified in each Forest Range. With the transfer of the technology for mass production of HpNPV which is envisaged for the next year, the century long problem is beginning to be tackled.

Research

As the story has unfolded, the HpNPV is much more than a pesticide. Current research is focussing on pathways to integrate the transmission characteristics of HpNPV with our precise understanding of the population dynamics of the pest to develop a landscape level management strategy for the teak defoliator. This research programme supported by DBT will attempt sublethal dosing of HpNPV in epicentre populations of the teak defoliator. The application procedure and dosage will be modelled in such a way that the epicentre population survives the HpNPV, but become infected. The vertical transmission parameters will be set so as to get the next generation killed due to HpNPV. While the procedure will increase the viral inoculum load in the ecosystem, it also aims to tap the specific biological characteristics of this biopesticide, rather than using it as a conventional pesticide.

Prospects

Teak which naturally occur only in India, Myanmar, Laos and Thailand, is now grown in 64 countries across globe, along the tropics and subtropics. The teak defoliator has begun to expand its distribution along with its host tree as indicated by the recent outbreak in Brazil. For many teak growing countries, the option now exists to check the population build up of teak defoliator using HpNPV technology.
milestones

1777 Species description and naming of the insect
1793 Formation of genus *Hyblaea* by Fabricius
1898 First description of *Hyblaea puera* as a pest of teak by Bourdillon
1903 Description of *puera & constellata* species by Stebbing and the nigra variety
1904 Comment by Hole that it is unlikely to call nigra a variety, while accepting *puera* and *constellata*
1908 Distribution, morphology and life history description by Stebbing. Also reported the nature of damage and natural enemies.
1921 Mackenzie estimated the economic loss as 1.5 lakhs annually for teak plantations in Burma
1926 Atkinson reported types of defoliation in teak by teak defoliator, skeletoniser and curculionids
1928 Beeson commented that the shift from endemic to epidemic phase is the time to adopt control measures
1931 Beeson estimated 8.2% loss in volume increment per growth season
1934 Beeson recommended silvicultural cum biological control methods
1936 Champion estimated a loss of Rs. 130 per acre.
1936 Zenery and Meir classified *Hyblaea* under the family Hyblaeidae
1941 Comparative life span data from Nilambur, Coorg, Bombay, Hoshangabad and Dehradun by Beeson
1951 Kadambi found that un attacked teak trees amidst an outbreak are early sprouters
1955 Description of larval and pupal stages
1974 Report of 30 species of parasitoids as natural enemies of teak defoliator
1980 Nair cautioned the use of chemical pesticides against teak defoliator
1985 Nair et al came up with the first empirical estimate of timber loss due to teak defoliator- 44% of potential volume increment per hectare per year
1983 Vaisampayan identified July to August as the outbreak period at Jabalpur
1984 Agarwal and Rajak reported *Beauveria bassiana* infection in teak defoliator larvae
1986 Sudheendrakumar reported 15 parasites from Nilambur
1986 Nair postulated migration as a mechanism of parasite evasion
1986 Nair and Sudheendrakumar reported evidence for short range migration of newly emerged moths
1987 Vaisampayan detected relationship between south west monsoon and defoliator outbreaks
1988 Nair reported migration instead of diapause as a cause of absence of teak defoliator activity during part of the year
1988 Vaisampayan reported temporal separation of activity of defoliator and skeletoniser at Madhyapradesh. Defoliator during July, August and skeletoniser during September
1988 Nair proposed the epicentre concept
1990 Sudheendrakumar et al discovered NPV as a mortality agent
1990 Mathew et al developed an artificial diet for rearing teak defoliator
1995 Nair et al reported that parasites cannot be used for controlling defoliator outbreaks
1996 Mohanasas developed life tables for field populations of *Hyblaea puera*
1996 Nair and Mohanasas reported that the defoliator population build up is of the eruptive type
1997 Search for teak defoliator resistant trees. Nair et al found that genetic resistance seldom exist
1998 Traced the epicentres of teak defoliator outbreaks at the landscape level
2000 Sajeev explained spatio-temporal dynamics of teak defoliator populations in the light of metapopulation theory
2001 Sudheendrakumar et al proved that control of epicentre populations can prevent large scale outbreaks
2002 Using molecular methods KFRI & RGCB proved that epicentres are caused by immigration of moths and not by assemblage of endemic population
2003 First HpNPV formulation developed by Mahiba et al at Entomology Laboratory Nilambur
2004 Biji et al identified and characterised multiple strains of HpNPV
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<td>Mohandas K.</td>
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<td>Sajeev T.V.</td>
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<td>Biji C.P.</td>
<td>Genetic characterization</td>
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"what the executive forest officer needs are not learned treatises containing suggested remedies and lifehistories of insects, but tested remedies and death histories of insects."

Beeson, 1934