HpNPV technology for Biocontrol of Teak Defoliator - Hyblaea puera

HpNPV technology

T V Sajeev | V V Sudheendrakumar

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Government of India

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An institution of Kerala State Council for Science Technology & Environment

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Cover photograph | Teak defoliator moth which has just emerged from the pupal stage. This is the most mortality prone stage of the insect since it cannot fly. Insect blood, called **haemolymph** is being pumped into the erstwhile folded wings. It takes 2 hours to get the wings functional | T.V.Sajeev |

भारत सरकार
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सत्यमेव जयते

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 agricultural systems since the former is more diverse than the later. The teak defoliator (Hyblaea puera) has been recignised as the most devastating pest in teak plantations in Kerala. It is estimated that about 44 percent of the potential volume increment is lost due to teak defoliator attack. It is also estimated that by controlling the teak defoliator an additional teak wood of 3 cubic meter/ha/annum can be realized from teak plantations which is a substantial gain considering the cost of about Rs.30,000-40,000 for one cubic meter of high quality teak. It is in this context that the research on the most serious pest of teak- the teak defoliator (Hyblaea puera) assumes importance.

The Department of Biotechnology, Government of India with its task force on Biopesticides and Crop management started supporting research on teak defoliator management in 1992 in a research project entitled Management of the teak defoliator using Nuclear Polyhedrosis Virus. The project for the first time field tested a crude suspension and found it effectively controlling the teak defoliator population. Studies where 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 teak 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 Biotechnolgy, Thiruvananthapuram entitled Tracing the origin and spread of teak defoliator outbreaks through a molecular approach. Using an innovative methodology combining the disciplines of molecular biology and insect population dynamics, this study proved that controlling the small epicenter populations of the insect, which occur during the early months, could prevent large-scale pest outbreaks.

[illegible] the teak defoliator research team of KFRI has come to a stage where they have standardized HpNPV technology for use in the field, have applied for patent for the baculovirus product and have already started transferring the technology to the major stake holder- the State Forest Department. It is with immense pleasure that I recollect the productive association between DBT and KFRI in tackling a major pest problem of the country.

[illegible] documents yet another success story of DBT in supporting R & D efforts in the country. I am sure that the dedicated research team [illegible] this will strive to solve many a problems, small and big, in its continued journey towards non-hazardous pest management.

New Delhi
[illegible] September [illegible]

Seema Wahab
Advisor, Department of Biotechnology, Government of India

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An institution of Kerala State Council for Science Technology & Environment
Peechi - 680 653 | Kerala | India

The teak defoliator, Hyblaea puera has been recognized as the most devastating pest in teak 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 teak defoliator attack. It is also estimated that by controlling the teak defoliator an additional teak wood of 3 cubic meter/ha/annum can be realized from teak plantations. Recognizing the importance of this pest in plantation forestry, KFRI initiated studies in right earnest for developing a suitable management strategy against this pest almost three decades back. The pioneering efforts by a team of KFRI Scientists in Forest Entomology yielded significant results of applied value for managing the teak defoliator problem through biological methods which groppled the foresters and entomologists equally since this pest was discovered in 1898. The team of scientists working on the problem have not only made the biological control of this pest a reality but also its commercialization through mass production of the biocontrol agent, an insect virus, HpNPV which is highly specific to the teak defoliator.

The publication of this Handbook is very timely and I am hopeful that it will help to disseminate the technology and make foresters and scientists aware of its potential for field application.

I also congratulate the team of scientists for their achievement especially Drs. V.V.Sudheendrakumar, T.V.Sajeev and R.V.Varma whose concerted efforts have made it possible to bring out this excellent publication on teak defoliator management technology.

Peechi
21 March 2006

J.K.Sharma)
Director | Kerala Forest Research Institute

Defoliated plantation | Sooraj V Kolleri

"A group of atleast two million Hyblaea puera moths descended on about 20,000 newly flushed teak trees in a 30-hectare patch with in 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.

AcMNPV	Autographa californica multiple nuclear polyhedrosis virus
DNA	Deoxyribo Nucleic acid
DBT	Department of Biotechnology
ECV	Extra cellular virus
HpNPV	Hyblaea puera Nuclear polyhedrosis virus
IMI	International Mycological Institute
KFRI	Kerala Forest Research Institute
PIB	Polyhedral Inclusion Body
RGCB	Rajiv Gandhi Centre for Biotechnology

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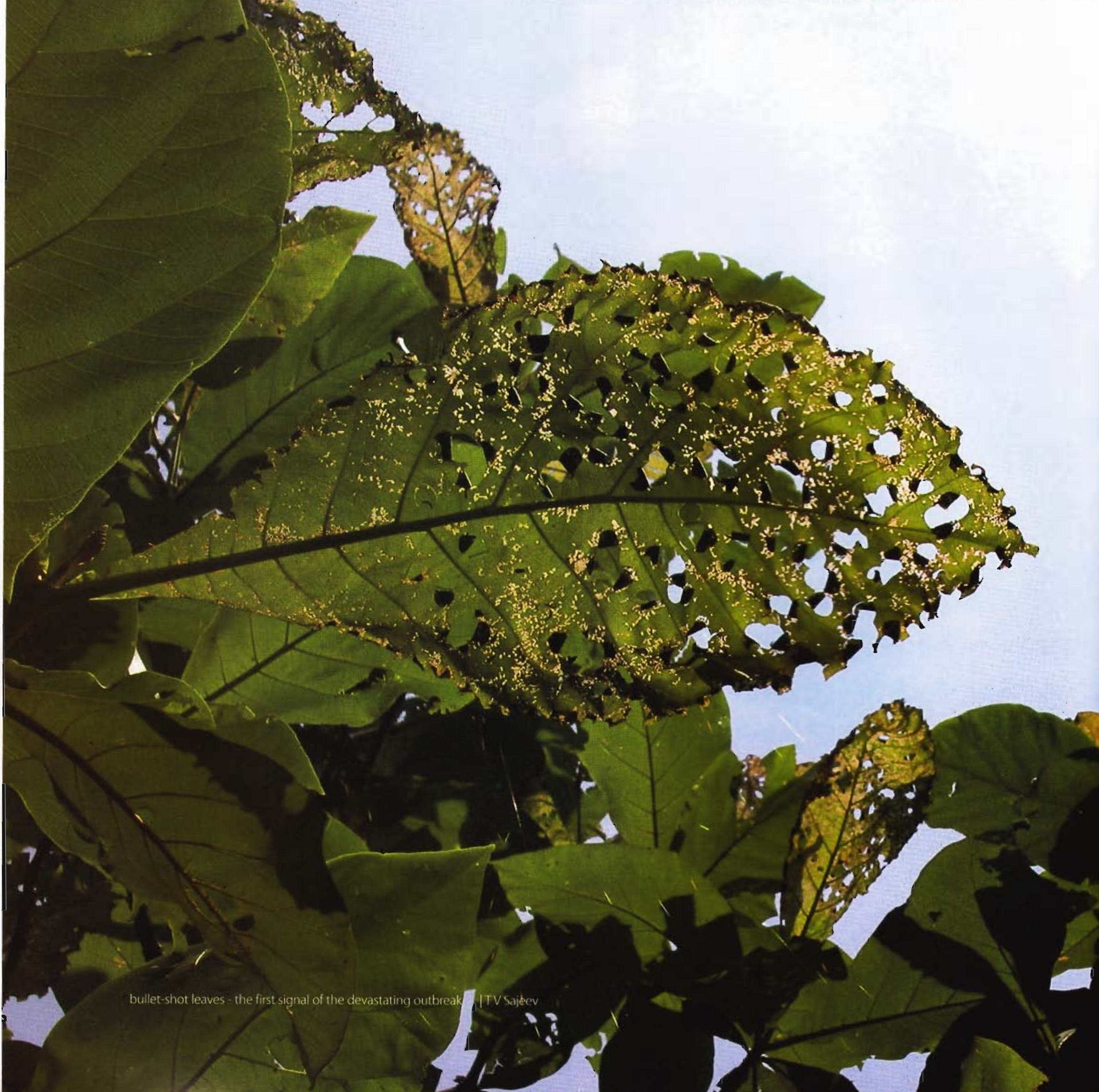


bullet-shot leaves - the first signal of the devastating outbreak | T V Sajeev

Teak defoliator has been recognized as a serious pest of teak for more than a century. A package of silvicultural cum 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 an year all through the century, researchers continued to claim that the problem 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 ariel 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.

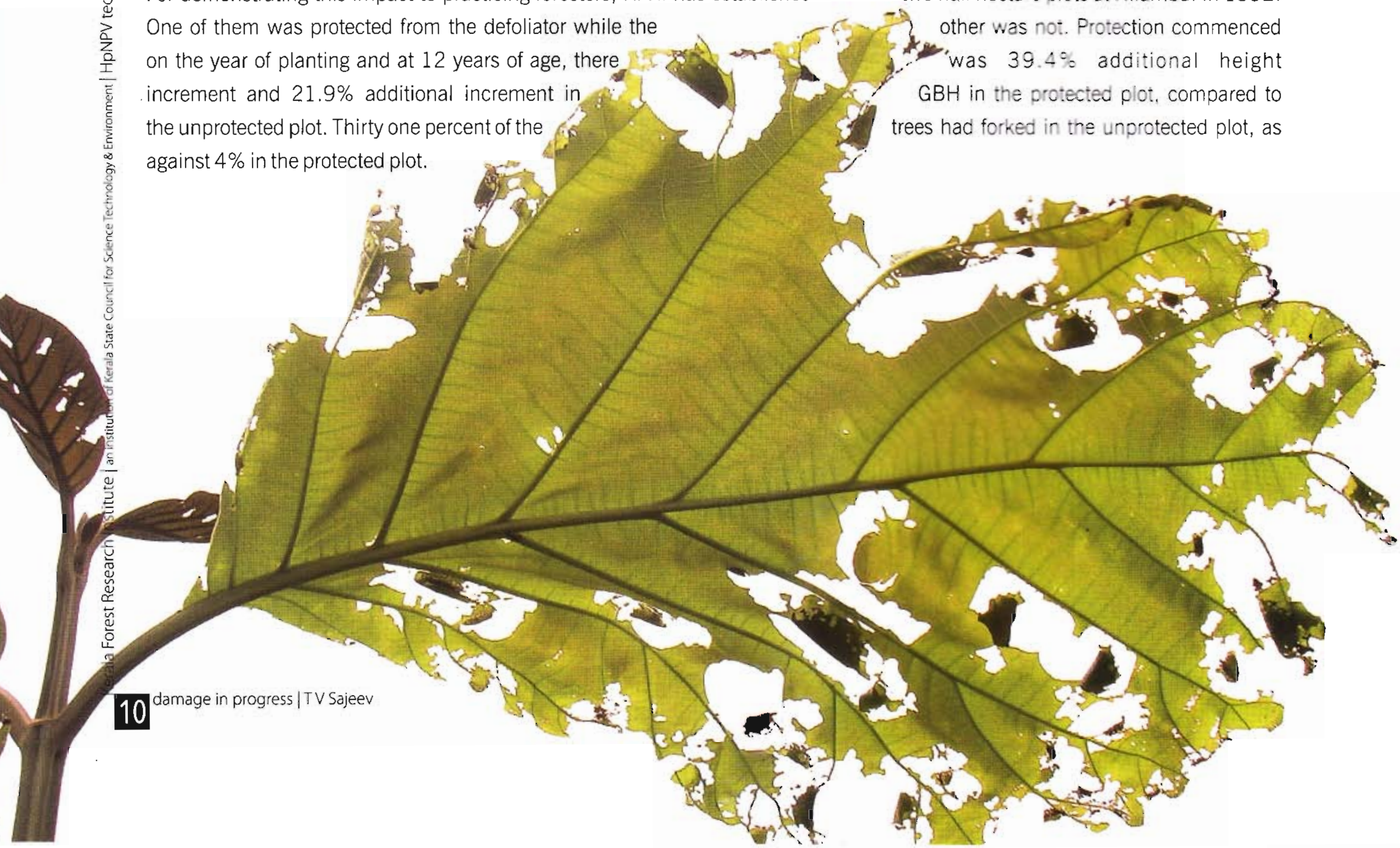
The success is the story, you are going to read.

Economic impact

Teak defoliator larvae strips 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 K F R I showed that defoliation by H.puera caused an average loss of 44% of the potential volume increment in 4-9-year-old plantation. Although it was not possible 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 26years 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 39.4% additional height increment and 21.9% additional increment in GBH in the protected plot, compared to the unprotected plot. Thirty one percent of the trees had forked in the unprotected plot, as against 4% in the protected plot.



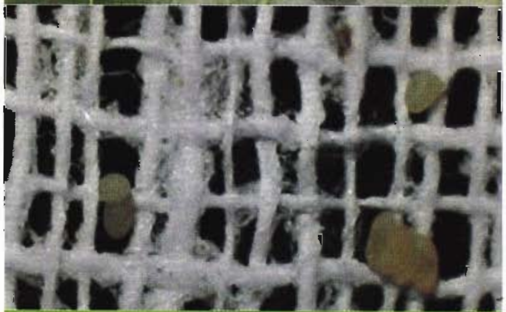
Moth

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. Egss are laid over a week-long period starting the third or fourth day after emergence, the longest recorded oviposition period being 12 days.


Moth | Sooraj V Kolleri

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.


Eggs on cloth | TV Sajeev

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 ocherous band and lateral white lines. The dark and light forms occur together in the same population, with the darker forms predominating during epidemics.


Fifth instar larva | TV Sajeev

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.

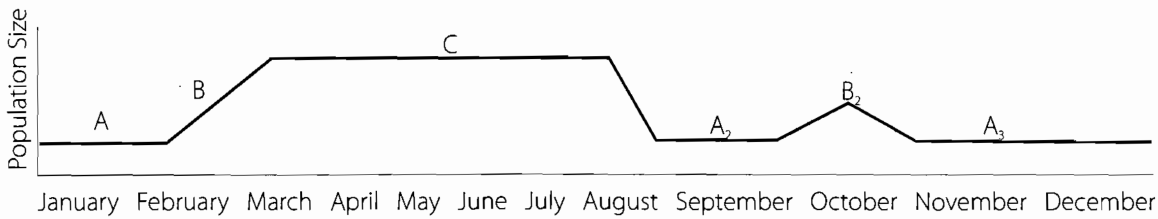

Pupae | Sooraj V Kolleri

Nair, K.S.S. (1988) The teak defoliator in Kerala, India. In Forest Insects: Principles and practice of population management Ed. Berryman A.A. pp 267-289. Plenum Press New York.

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 consistant 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.

Nair KSS et. al (1989) Search for natural resistance to the insect pest Hyblaea puera in teak. KFRI Research Report # 62



The teak defoliator is present the 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 occurs 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_2). In some years, there will be fresh outbreaks during the month of October(B_2). From then on until the next year, the population remains at the endemic level (A_3).

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

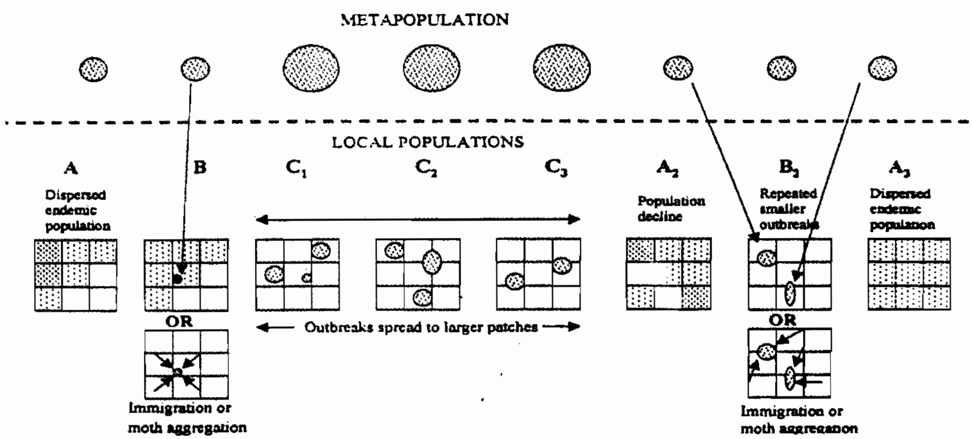
Population type	Month of occurrence	Description	Insect composition
Endemic	October/November/ December	sprase, low density population	uneven age
Epicentre	late February/early March	localised. high density tree top infestations	even aged larvae
Epidemic	April/May/June/July/August	high density. large area infestations	even aged larvae

Sajeev TV (2000) Spatial dynamics of teak defoliator (Hyblaea puera Cramer) outbreaks: patterns and causes. PhD thesis. Cochin University of Science and Technology.

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_1,C_2, C_3). In this phase a series of infestations occur in large teak plantation areas. After the epidemic phase, the population enters an endemic phase (A_2) followed in some years by repeated outbreaks in small areas (B_2) or more usually the endemic phase (A_3).

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.



Schematic diagram showing the spatial dynamics of teak defoliator outbreaks

Nair KSS and Sudheendrakumar VV (1986) The defoliator, Hyblaea puera: defoliation dynamics and evidences for short range migration of moths. In Proceedings of Indian Academy of Sciences (Animal Sciences) 95(1); 7-21.
Sajeev TV (2000) Spatial dynamics of teak defoliator (Hyblaea puera Cramer) outbreaks: patterns and causes. PhD thesis. Cochin University of Science and Technology.

Parasites

In our studies on natural enemies of teak defoliator at Nilambur teak plantations, five parasites were found: the tachinid Palexorista solennis, an eulophid Sympiesis hyblaeae, the chalcidid Brachymeria lasus and two unidentified ichneumonids. Overall parasitism by all species was about 9%, the tachinind Palexorista 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 Palexorista.

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 S. hyblaeae is not a suitable candidate for use in the biological control programme against the teak defoliator.

Palexorista solennis is an endoparasite which infests the third and fourth instar larvae of H puera. 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.

Sudheendrakumar , V.V. (1986) Studies on the natural enemies of the teak pests, Hyblaea puera and Pyrausta machaeralis, KFRI Res. Rep. # 38.
Sudheendrakumar , V.V.(1997) Evaluation of parasitoids for biological control of the teak defoliator KFRI Res.Rep. # 129.

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 Bonette macaque are known to comprise the predator complex of Hyblaea puera. 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 with in and between teak defoliator populations.

 predatory wasp hunting teak defoliator larva | TV Sajeev

Zacharias VJ and Mohandas K (1990) Bird predators of the teak defoliator Hyblaea puera. Indian Journal of Forestry.13(2):122-127.

Pathogens

Microbes represent a major fraction of the natural enemy complex of Hyblaea puera. In surveys conducted at teak plantations of Nilmbur 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 (KFRI 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 of use 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 synnematous 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).

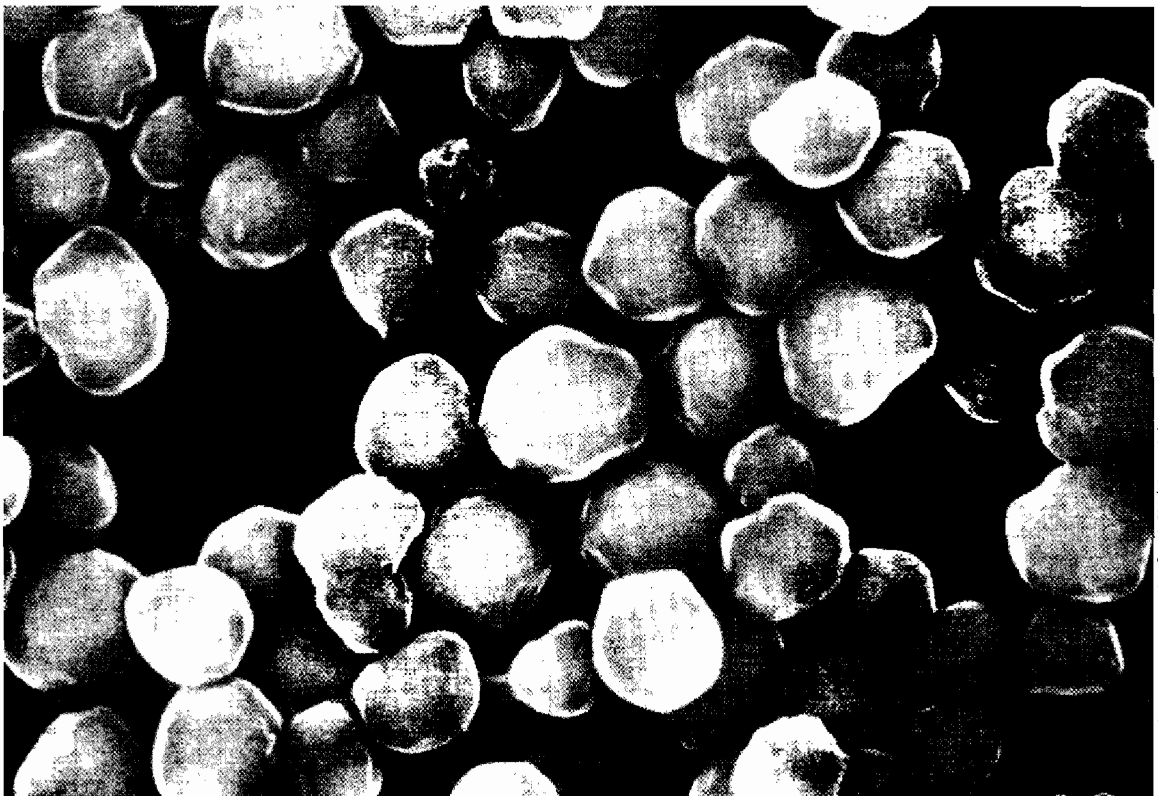
The major advantage of HpNPV is that it is absolutely target specific.

Mohammed Ali (1991)Evaluation of microbial pathogens for biocontrol against important pests of Ailanthus and Teak. KFRI Research Report # 72.

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 aquous 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.



scanning Electron Micrograph of HpNPV | Jean Adams

Sudheendrakumar et al (1988) Nuclear Polyhedrosis Virus of the teak defoliator Hyblaea puera. Journal of Invertebrate pathology 61 (3): 307-308.

teak defoliator larvae dead due to HpNPV infection | VV Sudheendrakumar

The NPV which enters the insect gut, lyses in the alkaline environment of the midgut, releasing virions. Virions invade the columnar cells of midgut epithelium and releases the DNA into the nucleus of midgut cells. At this point a virogenic stroma can be observed in the host cell nucleus, during which the viral DNA takes over control of the cellular machinery to reproduce itself. The progenies which are released to the haemocoel from the midgut cells are not the PIB, but what is called the ECV. They are a thousand times more infectious than the PIB and mediate disease spread within the insect body. A rapid spread of infection in the insect body leads immediately to cessation of feeding and later on, to death. However, this route of entry and spread of infectious particles can be bypassed as in the case of AcMNPV, where some of the parental virions pass through the plasma membrane reticular system of midgut cells and directly infect the hemocytes.

In the case of Hyblaea, we observed that within 4 hours of ingesting PIBs, infectious particles are present in the haemolymph. This suggests that the source of infection could be the parental nucleocapsids that pass directly into the hemolymph through the midgut. The time required for viral replication and production of PIBs is less than 4 hours in the case of HpNPV infection in Hyblaea puera. Even in the fully mature larvae of teak defoliator, HpNPV can cause a kill in 60-72 hours, making it one of the fastest acting insect viruses.

Baculoviruses can be produced only in live host cells. For producing adequate quantity of HpNPV for field application, healthy Hyblaea 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 polypropelene has been specially designed for the rearing and incubation of teak defoliator larvae. The following are the statistics of mass production:

Ideal stage of the insect	early fourth instar
Ideal weight of the insect	0.027 - 0.036 g
Inoculation method	spraying on to artificial diet in rearing tubes
Input dose of HpNPV	1 x 10^5 PIB / larvae
Incubation period	72 h
HpNPV yield	3.3 x 10^9 PIB / larvae

diet tubes for mature larvae + young larvae on teak leaves | T V Sajeev

Nair et al (1989) Search for natural resistance to the insect pest Hyblaea puera in teak. KFRI Research Report # 62

HpNPV suspension awaiting formulation | VV Sudheendrakumar

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.

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 x Di

where,

CE = 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

= (1×10^{10}) LAI x 1/(s x fr) droplets per ha

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

fr = feeding rate of the target instar

and

Di = Initial dose expressed as PIB/mm^2

= d x a

where,

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

a = estimated activity loss of virus after application

where,

N = Number of droplets emitted by atomiser per litre /Volume Median Diameter (VMD),

Theoretical minimum volume = CE/N litres per ha

Dose per ha = CE x Di expressed in PIB/ha

Dose per litre = N x 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= 4×10^{10} droplets per ha (assuming fr= 4 mm^2, LAI= 8, s = 50%)

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

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

Then,

Theoretical minimum volume	= $(4 \times 10^{10}/1.53 \times 10^{10})$	= 2.6 litres
Dosage per ha	= $(4 \times 10^{10} \times 1000)$	= 4×10^{13} PIBs per ha
Dosage per litre	= $(1.53 \times 10^{10} \times 1000)$	= 1.53×10^{13} PIBs per litre

Crude suspension in water / High volume / 1993

At Kariem Muriem 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.

Semipurified suspension in oil / Ultra low volume / 1998

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 2×10^{11} PIBs per ha. This represents nearly 1000 larval equivalents, *ie*, 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 2×10^{4} 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.

Nair *et al* (1996) Field efficacy of nuclear polyhedrosis virus for protection of teak against the defoliator, Hyblaea puera Cramer (Lepidoptera:Hyblaeidae). Journal of Biological control. 10: 79-85.
Nair *et al* (2001) Use of baculovirus control agents with in an integrated management strategy against the teak defoliator, Hyblaea puera in India. KFRI Research Report #203

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 *Achaea janata*, *Atteva fabriciella*, *Catopsilla crocale*, *Eligma narcissus*, *Eutechtona machaeralis* and *Bombyx 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 Mynah (*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 device a variety of spray schedules- from lattice spraying to strip spraying.

Vertical Transmission

By way of trans-ovum (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- ethal 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 contain 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.

training programme in session | T V Sajeev

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 untimely 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
Champion estimated a loss of Rs. 130 per acre.
1936 **Zenry** 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 unattacked 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
Sudheendrakumar reported 15 parasites from **Nilambur**
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
Vaisampayan reported temporal separation of activity of defoliator and skeletoniser at Madhyapradesh. Defoliator during July, August and skeletoniser during September
Nair proposed the epicentre concept
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
Mohanadas developed life tables for field populations of Hyblaea puera
1996 **Nair** and **Mohandas** 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

research team

Nair K.S.S.	Population dynamics | Outbreak causation | Ecology.
Varma R.V.	Microbial pathogens | Demonstration plots.
Sudheendrakumar V.V.	Parasites | Pathogens | NPV- standardization | Extension.
Mohandas K.	Life tables | Predators.
Sajeev T.V.	Spatio-temporal dynamics | Field application | Modelling.
Evans H.F	Dosage parameterization.

research scholars

Biji. C.P	Genetic characterization | Dosage-mortality relationships.
Mahiba Helen	Formulation | Safety testing.
Shanto Mathew	Mass production | Training.

Field trial using formulated HpNPV | VV Sudheendrakumar

"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

Department of Biotechnology
Government of India

Kerala Forest Research Institute
An institution of Kerala State Council for Science Technology & Environment