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MANAGEMENT OF THE TEAK DEFOLIATOR (HYBLAEA PUREA) USING NUCLEAR POLYHEDROSIS VIRUS (NPV)

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ABSTRACT

This study was carried cut during the period January 1'992 to December 1995 to evaluate the usefulness of a naturally occurring a Nuclear Polyhedrosis Vis of the teak defoliator *Hyblaea purea* (HpNPV) and develop suitable field application methods. Various aspects pertaining to this central issue were investigated in this study,

The HpNPV was confirmed to be a DNA virus belonging to the family Baculoviridae. Electrophoretic analysis of the viral DNA using resitriction endonucleases, and SDS-PAGE analysis of the polyhedrin protein showed its similarity to the NPV of other insects. Cross infectivity studies revealedthe HpNPV to be highly specific to its host and safe for field application.

Epizootiological studies showed that HpNPV has little environmental persistence and does not persist even for a week when applied onto teak foliage.

Disease transmissionstudies indicated intra-host persistence of the virus and transovarian transmission from parental generation upto the second filial generationunder controlled laboratory conditions. These results indicated that it would not be possible to induce disease edzootics under field conditions by enhancing the inoculum load m the envioronment artificially. It was therefore concluded that inundative spray of polyhedra during the course of an infestation is the only control option.

Under the field trial progmmme in 1993, repeated infestations of the teak defoliator in a selected 100-tree plot were Controlled by timely application of HpNPV during each pest outbreakIt was shown that 70-76% of the foliage loss caused by the pest was prevented by application of a high volume spray of 0.75 to 1.75 litres of the spray fluid per tree, containing $1 x 10^5$ PIB/ml.

A unique method for mass production of HpNPV was developed which makes use of the cheap availability of large number of larvae during the natural pest outbreaks in teak plantations. Methods were also developed for NPV production in the laboratory using larvae reared in an artificial diet. Methods were standadised for timely application of NPv using a pest monitoring system involving moth catches employing a solar powered light trap designed as part of this study.

1. INTRODUCTION

The teak defoliator, *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae) is a well known pest of teak plantations in India. The seriousness of the pest problem has been recognized for long and attempts have been made to standardize both biological (Beeson, 1941) and chemical (Basu Chowdhury, 1971: Singh, 1980) methods of control. No accurate estimate of the loss in wood volume was available until recently, but Nair *et al.* (1985), based on a 5 year study, in a 4-9 year old teak plantation demonstrated that 44% of the potential volume growth is lost due to defoliation caused by *H. puera.* It was estimated that the gain due to rotection amounted to 3m3 of wood per hectare per year. Thus substantial economic gain can be obtained by controlling the teak defoliator. With over 1.4 million ha of teak plantations in the country and an average value of Rs.20,000 for one m^3 of teak wood, the economic worth of the wood that can be saved is enormous.

The silvicultural cum biological control methods advocated earlier (Beeson, 1941), necessitated retention of natural forest belts in between plantations to encourage survival of natural enemies of the teak defoliator and silvicultural manipulation of understorey to promote desirable plant species which supported the alternative hosts of parasites. However, these recommendations were neither tested to demonstrate effectiveness nor adopted in practice. More recent studies carried out at KFRI (Nair and Sudheendrakumar, 1986) showed that native parasitoids can have little impact on outbreak populations of *H. puera* because of the highly mobile nature of such populations. The KFRI team of researchers, (Nair *et al.,* 1989) also looked for possible resistance in teak against the teak defoliator, but no genetic resistance was found. Since application of chemical insecticides entails recumng cost and long-term environmental damage, it was not considered an acceptable option.

Several years of observations on the population dynamics of the teak defoliator in Kerala showed that the outbreak populations usually collapsed, after two or three waves of defoliation, possibly due to a naturally occuring disease. This was identified as a Nuclear Polyhedrosis Virus (NPV) disease (Sudheendrakumar *et al.* 1988). Laboratory studies confirmed the pathogenicity of the virus against the teak defoliator and its potential as a biocontrol agent (Mohammed Ali *et al.* 1991). The specificity of baculoviruses and their safety to other biota make them very useful, particularly under the forest environment. Several of them have been successfully used to control forest pests in the Western countries (Entwistle, 1976 ; Hunter *et al.* 1984).

The present study was therefore undertaken to gather additional scientific data necessary to utilize the *Hyblaea puera* NPV (HpNPV)for practical control of the teak defoliator and to field-test its efficacy. The aspects studied included further characterization of the NPV its natural incidence in field populations and epizootiology, persistence of the virus and transmission between generations and methods of mass production, in addition to field efficacy. Since successful application of the NPV for control of the teak defoliator would depend upon our ability to apply it at the right time, studies were also conducted to standardise pest monitoring methods using light traps and other field observations.

2. GENERAL METHODS

2.1. STUDY AREA

The field studies were carried out during the year 1992-95 at Nlilambur (Fig, 2.1), in about 10,000 ha of teak plantations, distributed over 500 km^2 of geographicalarea Sampling for epizcotiological studies were made in plantations at Nedumgayam Aravallikkavu, Panayangod, Chaliyamukku,Mayilady and Kariem Muriem (Fig 2.2). Population monitoring light trap studies and weather data recording were made at Kariem-Muriem - a 1000 ha area of teak plantations (Fig. 2.3) located between Latitude 11° 22.7 and 11°25.7 and longitude 76°16.44' and 76°18.47 in the Vazhikkadavu Forest Range of Nilambur North Forest Division.

2.2. MONITORING OF PEST INCIDENCE

Monitoring of pest indence had three major objectives:

1. To facilitate epizootiological studies, 2. To faclitate field trialsof the NPV to test effectiveness to suppress the populations, and 3. To Develop a monitoring protocol for timely detection of infestations.

A brief descripton of the biology of H.purea is given here as background information.

The moth (Fig. 2.4a) lays eggs on the ventralside of tender teak leaves. The neonate larva feeds at the site ofemergence and the second instar moves to the leaf edge where it cuts out a small fold and feeds from within. Bigger leaf folds are made as the larva grows. The mature fifth instar larva (Fig.2.4b)descends to the groud and pupates in the soil The life span from egg to adult is about 21-24 days.

General observations on host population build up was made throughout the teak plantaions in Nilambur to gather information on the initiation and progress of outbreaks.

Detailed population monitoring was carried out atKariem- Muriem It started in June 1992 and continued until the end of the project in December 1995. The study site was Kariem-Muriem The study area of about 1000 ha wasdividedinto20grids demarcated by natural boundaries like streams and footpaths. At fortnightly intervals, observations were made by trained field observers,walking through the grid in a previously defined zig-zag path Visual estimates were made on the followingparameters and recorded on data sheets.

a) Percentage area withtender foliage
b) Level of infestation (nil/low/high/very high)
c) Percentage area with defoliatorinfestation

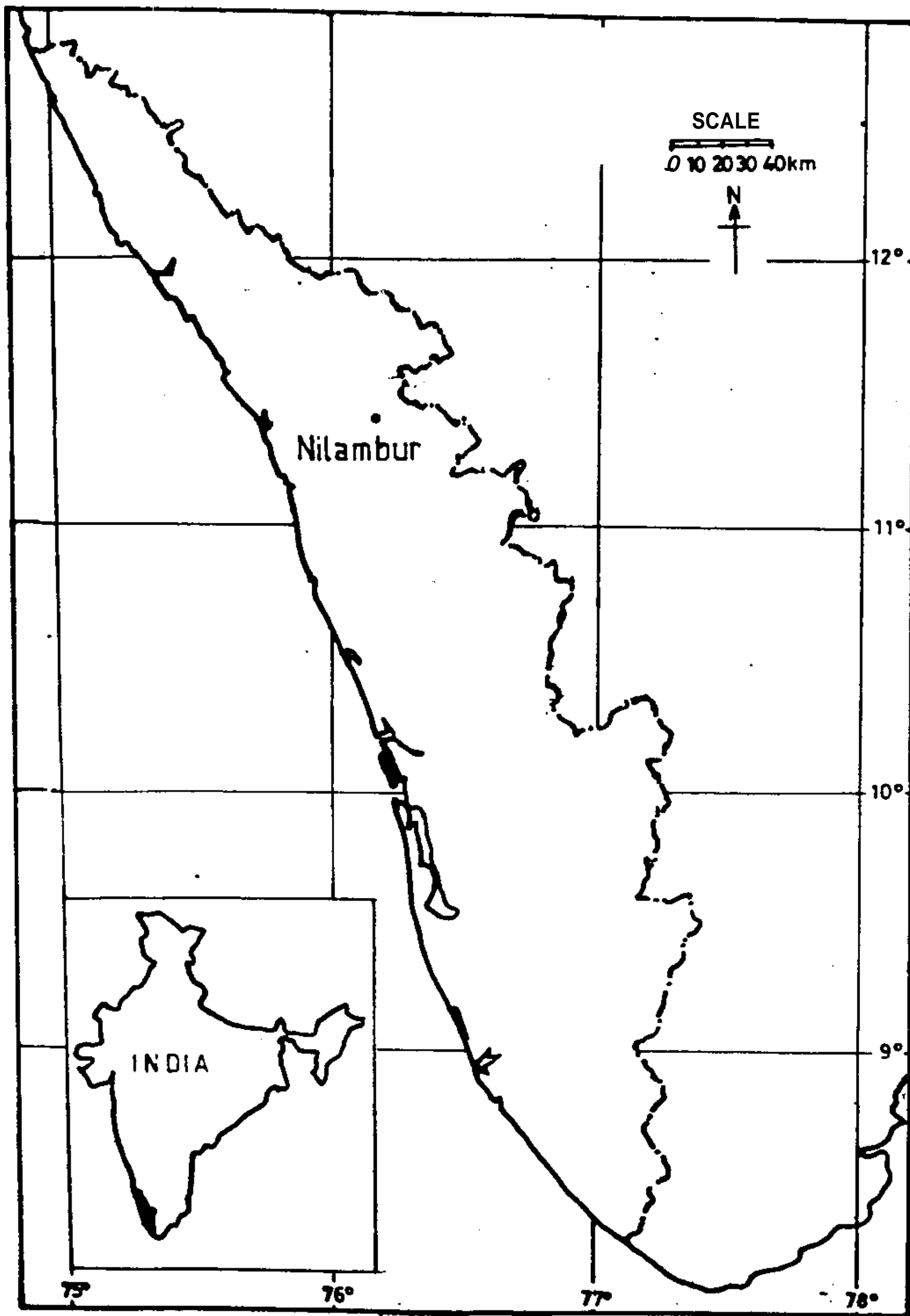


Fig.2.1. Map of Kerala showing the location of the study area

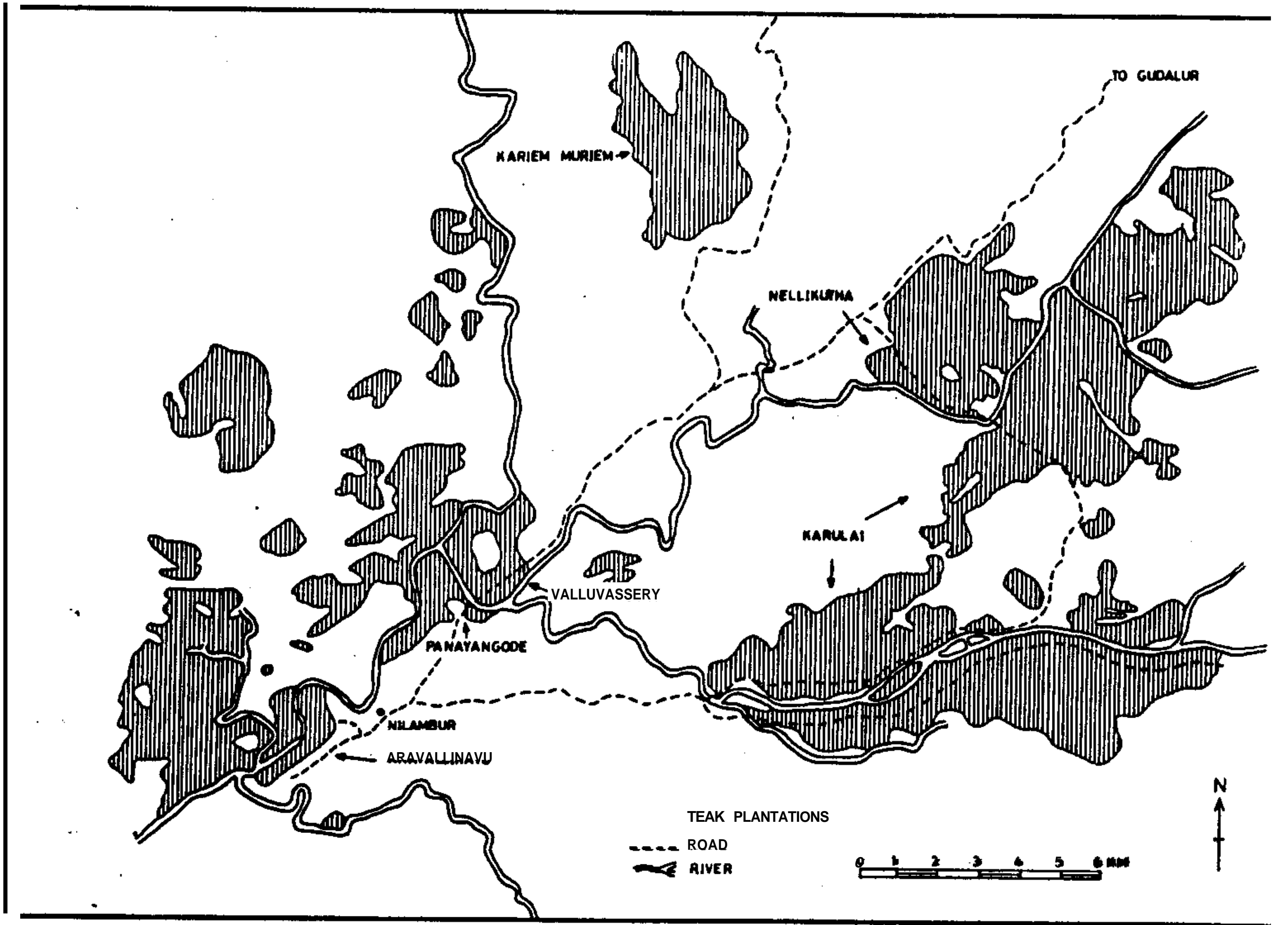


Fig.2.2. Map of Nilambur showing the teak plantations

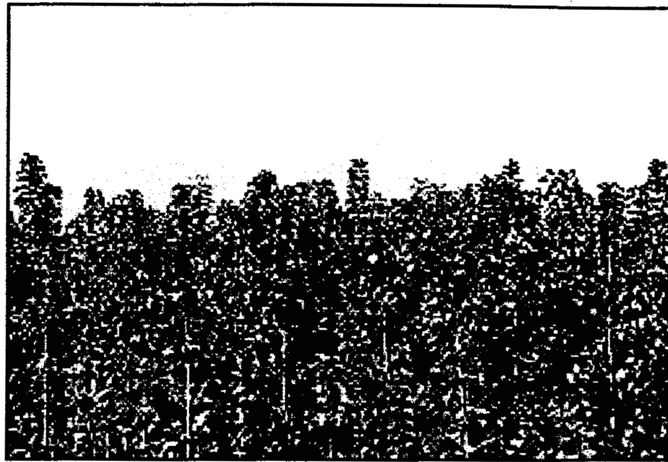


Fig. 2.3. View of a young teak plantation

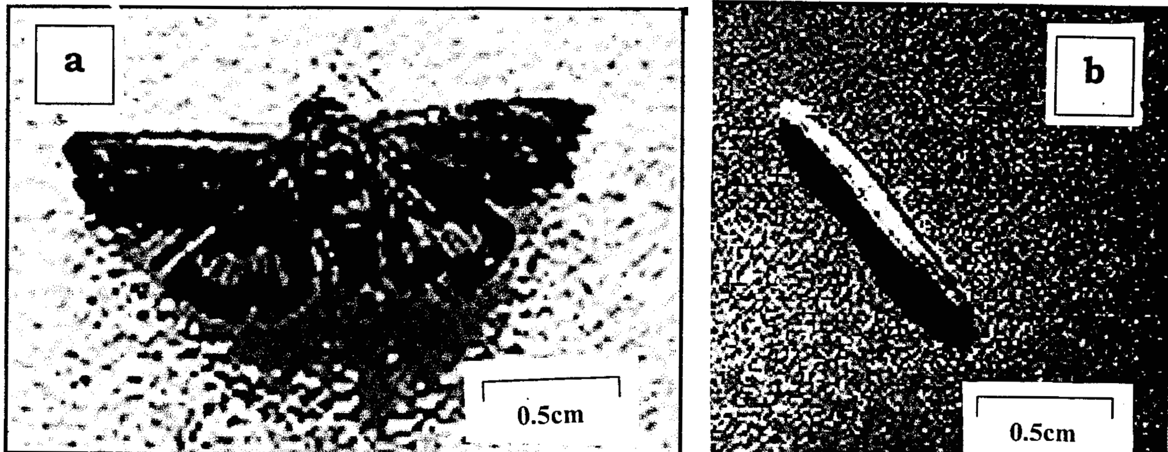


Fig. 2.4 (a) Hyblaea moth : (b)Fifth instar larva

In addition to the above general observations, ten linear plots of 20 trees each were established in the study area to gather more detailed information. Each plot was established at the meeting point of two adjacent grids. Two shoots each from the top, middle and bottom levels of each tree were observed at fortnightly intervals and the following information recorded.

a) Presence or absence of larvae
b) Number of old leaves (damaged and undamaged)
c) Number of tender leaves (damaged and undamaged)
d) Percentage of damage (in the first four leaf pairs of the shoot)

2.3. MONITORING OF WEATHER PARAMETERS

Measurement of weather parameters was made to study the influence of weather on population trend of the pest and on NPV disease incidence. An automated weather station (Campbell Scientific Inc. , USA) was installed at a height of 5 mt above the ground on top of a tower (Fig. 2.5) at Ambalakkunnu in Kariem-Muriem. It became operational in June 1993. Rainfall, temperature, relative humidity, wind speed, wind direction, and light intensity were logged at 30 seconds intervals. Except for rainfall which was added up on a daily basis, all other weather parametres were averaged hourly. A computer software (Graphterm, support software) supplied by Campbell Scientific Inc. was used to summarize the data. Technical problems caused some discontinuity in data collection.

Fig. 2.5 Automatic weather station set up in the study site at Kariem-Muriem,

2.4. LABORATORY CULTURE OF THE HOST INSECT *HYBLAEA PUERA*

A continous culture of *H. puera* was maintained in the laboratory throughout the project period in order to facilitate various experimentation. The following methods were used.

The culture was started from field collected pupae. The pupae were brought to the laboratory and maintained in glass bottles (8cm x 18cm) covered with cheese cloth. The emerging adults were kept in pairs in separate glass bottles and provided with 10% honey solution for feeding. The adults usually mated on the first day of emergence and started laying eggs from the second night onwards. The eggs were laid on the cloth covering the bottles. Theys were collected with a hair brush and washed in 0.5% sodium hypochlorite solution for 10 minutes. After air drying, the eggs were kept for emergence in glass bottles along with freshly collected tender teak leaves. The teak leaves were also sprayed with 0.5% sodium hypochlorite solution and air dried. The larvae fed on tender teak leaves until they reached the third instar. The third instar larvae were then transferred individually into sterilized glass tubes (2.5cm x 8cmj containing an artificial diet (Mathew, *et al.,* 1990) (Fig. 2.6). The tubes were covered with sterilized cotton balls. They were then kept inverted in a slanting position in aluminium trays.

Fig. 2.6. *Hyblaea* larva reared in the artificial diet

The larva usually makes a web over the diet and start feeding on the diet. By keeping the tubes in an inverted position, the diet can be kept free of faecal pellats. The faecal pellets were removed daily from the tubes. On pupation, the pupae were removed and transferred to sterilized glass bottles (8cm x 18cm). Adults start emerging in about 4 to 6 days. One generation

is completed in about 26 days. Eggs hatch in about two days. Among the larval instars, 1st instar, 2nd instar and 3rd instar take 2 days each and 4th and 5th instar take 3 days each. The pupal period is 5 days and the adult life span is 7 days. Moths emerging from field collected pupae lay over 600 eggs, but the fecundity of lab reared insects is lower.

Over 45 successive generations of *H. puera* were successfully reared out in the laboratory. Although all possible measures were taken to prevent contamination, death due to diseases were often noiticed in the culture. Presence of NPV and/or bacteria were often noticed on microscopic examination. However, routine microscopic examination could not be made due to practical difficulties. Sometimes, the occurrence of disease resulted in loss of the laboratory stock. The culture was then restarted with field collected pupae. During the later stage of the project period, the incidence of disease was drastically reduced by following more stringent hygeinic measures. These included reducing the number of larvae handled per person by providing additional labour. In spite of all these precautions, NPV deaths were often encountered in the culture, possibly indicating intra- host persistence of the virus (see Section 5.3).

3. PEST POPULATION TRENDS AND WEATHER DURING THE STUDY PERIOD

3.1. PEST POPULATION TRENDS

Detailed monitoring of the field populations of *H. puera* was necessary to facilitate epizootiological studies of NPV to test the efficacy of NPV for control of *H. puera* population under field conditions and to develop a suitable protocol for timely detection of pest population outbreaks. The methods used for population monitoring are described in Section 2.1.

Detailed monitoring of the field populations of *H. puera* was necessary to facilitate epizootiological studies of NPV, to test the efficacy of NPV for control of *H. puera* populations under field conditions and to develop a suitable protocol for timely detection of pest population outbreaks

Earlier studies (Nair and Sudheendrakumar, 1986; Nair and Mohandas, 1996) had shown that although a low density, endemic population of the teak defoliator exists in teak plantations throughout the year, outbreaks occur each year starting with the appearence of the pre-monsoon rains, first in small discrete patches, then in larger patches, and subsequently flaring up to sudden widespread outbreaks. Within a large plantation area, much variation can be expected in the incidence of infestation in space and time. The present study was designed to bring out this variation which has practical implications in the application of control measures.

The infestation pattern observed during the 4 - year study period is described below (see Fig.3.1).

Year 1992

The first outbreak occurred in the first fortnight of July when a small patch of 1.6 ha was completely defoliated. This patch was infested when most of the remaining plantation also had a comparable foliage status. A much larger infestation of about 130 ha occurred in the second fortnight of July but a smaller infestation occurred in the first half of August (14 ha). It was followed by a larger infestation covering 114 ha in the second half of August. There was a further decline (to 85 ha) and subsequent increase (to 170 ha) in the first and second fortnights of September. A major infestation extending to 239 ha. occurred in the first fortnight of October. The last infestation of the year occurred in the second fortnight of October (59.56 ha). Thus there were eight distinct infestations during the year, although of them did not occur in a given place.

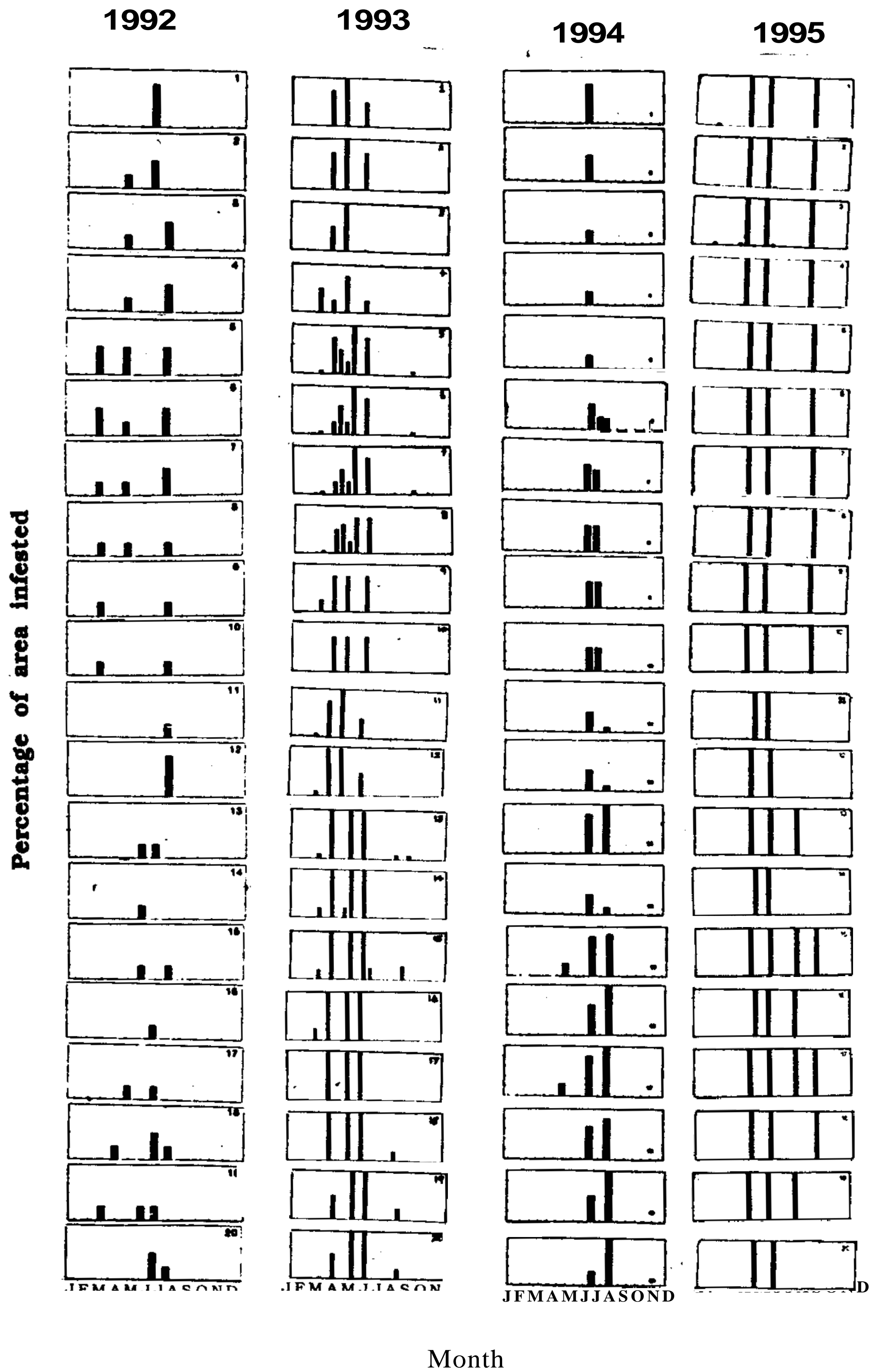


Fig.3.1. Pattern of defoliator infestations at during the years 1992-95 (The bars indicate percentage of area infested in each of the twenty grids in a given fortnight)

Year 1993

The first outbreak of the year. which occurred in early March was confined to an area of 0.89 ha. There was a gradual build up from then onwards until the second fortnight of May when almost the entire area was infested. Presence of outbreak populations continued upto July beyond which they were absent except for the small patchy infestations in September and October.

Year 1994

The early infestations occurred in the first fortnight of April. Unlike in the previous two years, the initial patch was quite large covering an area of 23.9 ha The peak infestations occurred in the second fortnights of June and July. There were no infestation peaks in September or October.

Year 1995

In 1995. the early infestations occurred in the second fortnight of February in small patches covering a total area of 3.5 ha. Massive infestations occurred in May, covering 872.5 ha in the first fortnight and 653.75 ha in the second fortnight. In the first fortnight of June the entire Kariem-Muriem plantations were under infestation. There were extensive but low density infestations in August and October.

Frequency of outbreaks

The frequency of outbreaks was not uniform among the grids: the number of infestations in each of the 20 grids during the study period is given in Table 3.1. In general, 9 to 15 infestations occurred over the 4-year period, with grid no.15 registering the highest of 19 infestations. Grids 13 and 17 had next highest infestation.

Analysis of location of the first outbreak during each year showed that some sites were more prone to the first attack (Fig. 3.2). Obviously some site characteristic. other than foliage status is responsible for succeptibility to infestation. Identification of these characteristics will be helpful for monitoring pest incidence for timely application of control measure as well as to understand the causation of outbreaks.

Pattern of outbreaks between years

There was considerable variation in the pattern of outbreaks between years. In 1993 and 1995 the intensity of outbreak was high, leading to total defoliation in

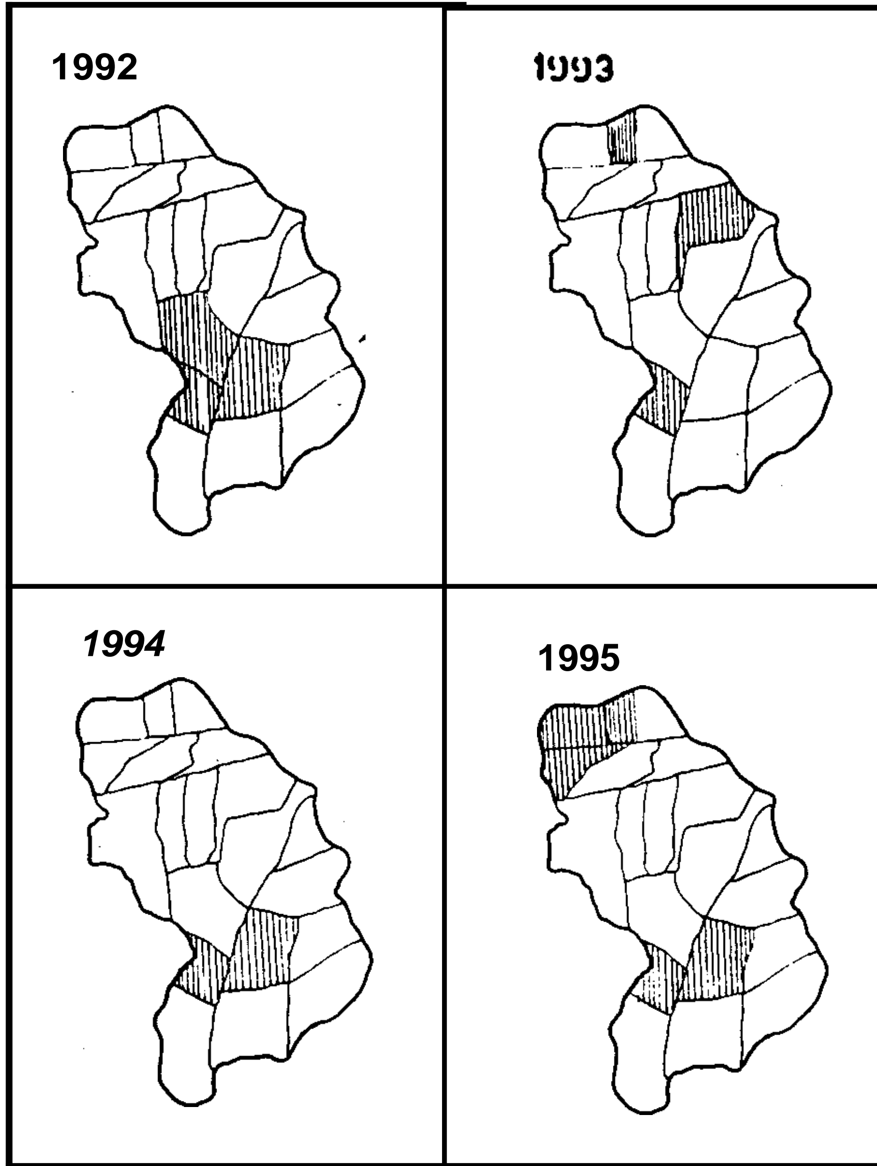


Fig.3.2. Grids infested during the first outbreak at Kariem-Muriem

Table 3.1 Frequw=ency of outbreaks at Kariem-Muriem during the study period

Grid Number	1992	1993	1994	1995	Total
1	1	4	1	4	10
2	2	4	1	5	12
3	2	4	1	7	14
4	2	4	1	5	12
5	3	4	1	3	11
6	3	5	2	3	13
7	3	5	2	3	13
8	3	5	2	3	13
9	2	5	2	3	12
10	2	4	2	3	11
11	1	4	2	2	9
12	1	4	2	2	9
13	4	6	2	3	15
14	1	5	2	2	10
15	4	7	3	5	19
16	1	5	2	3	11
17	3	3	3	6	15
18	4	4	2	3	13
19	4	55	2	3	14
20	2	5	2	2	11

most of the grids but in 1992 and 1994 total defoliation did not occur in any grid. The number of outbreaks was also higher in 1993 and 1995. compared to the other two years. Fig. 3.3. shows the total area defoliated at Kariem-Muriem during each of the outbreaks. It can be seen that the major infestations occurred during May and June in all the years except 1992. In most years small-scale outbreaks occurred during the later part of the year.

Discussion

This study shows that a large area of teak plantations gets infested every year repeatedly. It is evident that there is considerable variation in the outbreak pattern of teak defoliator between places and between years. From the management point of view, the findings that sites of the first occurrence of outbreak remain constant between years, is helpful. Identifying the physical factors accounting for this phenomenon will drastically reduce the area to be monitored during the initial part of the year.

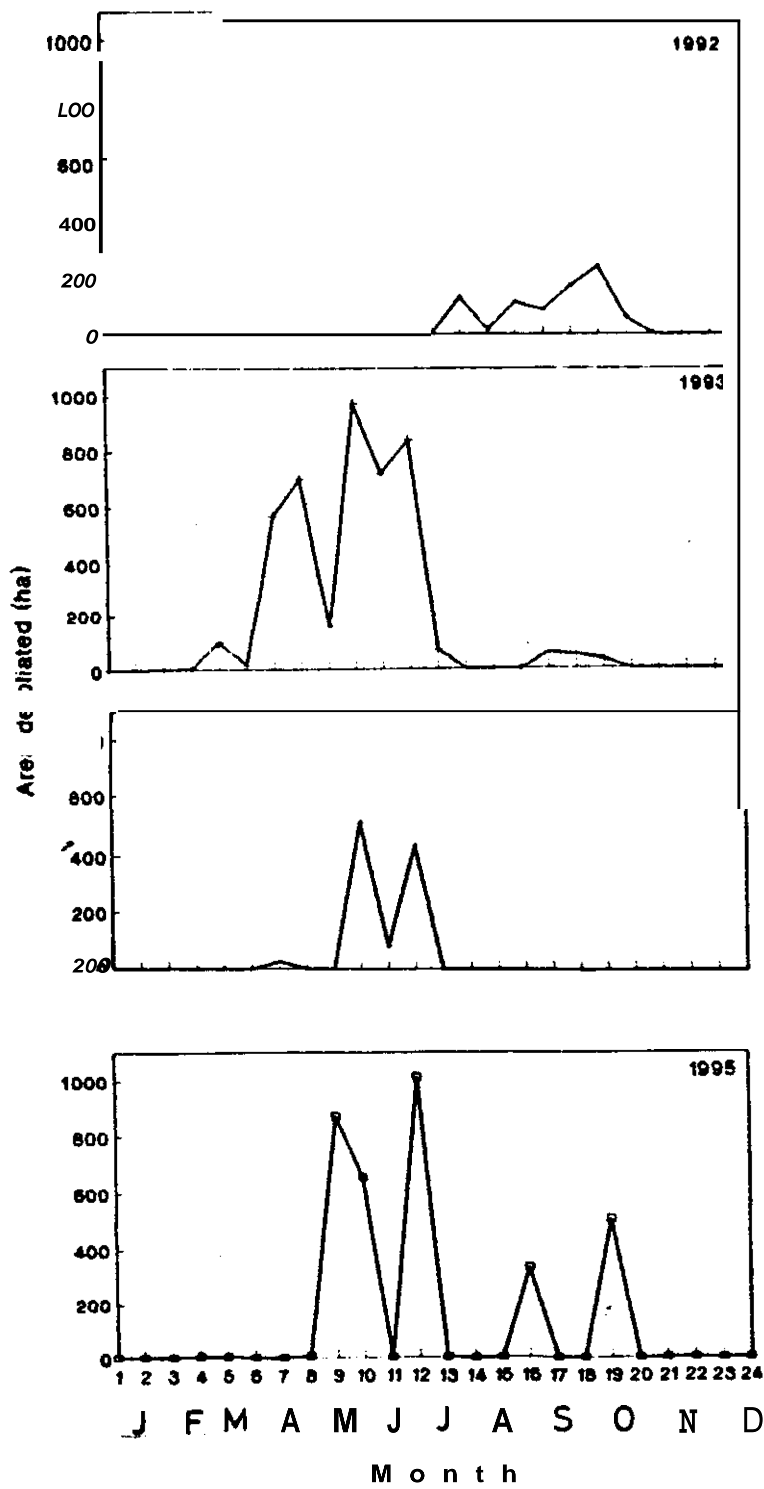


Fig.3.3. Area under defoliation at Kariem-Muriem during the study period, 1992-1995

Table 3.2. Weather data at Kariem-Muriem Teak Plantation collected at 0800 h IST.

Date April 1994	Time (h)	Temperature (°C)		RH %		WD avg. deg.	WS ms^{-1}	SR (MJ) m-2 d-1	Rainfall mm d-1
		Maximum	Minimum	Minimum	Maximum				
1	800	35.75	26.72	91.9	50.4	152	8.1	22.7	0.0
2	800	34.33	25.97	91.7	51.6	150	6.2	17.5	0.0
3	800	35.66	23.79	93.4	47.7	192	7.7	20.2	29.5
4	800	36.04	25.29	91.2	40.8	168	6.3	22.3	0.0
5	800	36.75	24.60	95.0	41.4	164	7.3	21.0	20.1
6	800	33.95	23.96	93.3	54.4	163	5.8	19.6	0.0
7	800	35.71	24.79	92.5	44.2	181	7.1	22.6	3.8
8	800	32.69	24.73	92.8	60.6	161	6.5	17.6	0.0
9	800	30.86	23.17	95.5	66.4	178	4.3	8.2	12.9
10	800	35.96	24.34	95.1	46.0	178	6.7	23.6	18.0
11	800	33.88	23.24	95.4	56.7	184	5.7	18.4	25.1
12	800	33.32	23.82	95.2	55.1	180	5.3	17.9	21.3
13	800	31.84	24.86	93.3	60.3	183	3.7	13.3	0.3
14	800	34.94	24.56	92.8	52.8	190	5.9	19.6	0.0
15	800	36.29	24.39	93.8	43.6	191	5.2	23.4	0.0
16	800	35.94	24.94	92.5	44.0	175	6.0	22.3	0.0
17	800	34.02	23.79	94.5	57.6	173	6.1	16.6	10.4
18	800	33.93	25.22	95.3	57.4	158	7.1	19.8	20.1
19	800	30.13	24.92	93.0	75.3	195	5.5	10.7	1.0
20	800	33.92	26.09	91.3	60.2	159	5.4	16.8	0.0
21	800	34.73	25.31	93.1	55.6	163	6.6	21.3	0.0
22	800	34.66	26.79	91.2	50.8	209	6.1	22.1	0.0
23	800	35.03	26.86	90.1	52.3	168	6.9	21.6	0.0
24	800	35.22	26.94	90.2	53.8	159	7.4	20.6	0.0
25	800	35.02	26.43	92.0	52.5	168	6.6	19.3	0.0
26	800	34.71	27.20	90.8	54.1	167	6.8	19.9	0.0
27	800	34.57	26.88	92.8	59.0	148	5.9	17.0	0.0
28	800	35.79	26.75	93.6	53.4	168	6.8	19.1	0.0
29	800	35.13	22.38	95.1	54.0	195	6.5	13.7	40.4
30	800	34.84	24.05	93.1	51.8	216	7.0	22.2	23.4

RH - relative humidity; WD - wind direction; WS - wind speed; SR - solar radiation

3.2. WEATHER

The weather parameters measured included temperature, rainfall, relative humidity, wind direction, wind speed and sunshine. A typical weather data set for April 1994 is given in Table 3.2. Analysis of the relationship between weather parameters and the pest and N W incidence is not yet complete, but specific correlation drawn are indicated. There have been some discontinuities in the weather data collection. Since the weather station was located in the middle of the plantation, safety of the equipment was a problem all through the study period. The sensors were tampered in some instances.

4. CHARACTERIZATION OF *H. PUERA* NPV AND STUDIES ON CROSS-INFECTIVITY

4.1. CHARACTERIZATION

The nuclear polyhedrosis virus of *H. puera* (HpNPV)was first recorded by Sudheendrakumar *et al.* (1988). Apart from the scanning electron micrographic studies revealing the size of the polyhedra, no further attempts have been made to characterise the NPV. Identification and characterization of baculoviruses is essential for their development as pest control agents. The most important methods used for characterization of baculoviruses are electron microscopic studies to reveal the ultrastructure and restriction enzyme (REN) analysis of DNA to determine the molecular weight of the genome. Although the project envisaged electron microscopic study of the HpNPV, this could not be carried out due to lack of facilities and suitable collaboration. REN analysis of viral DNA and electorphoretic analysis of polyhedral proteins were carried out in collaboration with Dr. S. Mathavan, in his laboratory at the Madurai Kamaraj University.

Materials and Methods

Polyhedral inclusion bodies (PIBs)of the virus obtained from late fourth instar larvae were used for the study. The PIBs were isolated from the insect cadavers by allowing them to putrefy in distilled water for 7-8 days. The suspension was filtered through muslin cloth. The filtrate was treated with 10% SDS (1/100 v/v) and centrifuged at 1000 rpm for 3 minutes. This was followed by centrifugation at 5000 rpm for 15 minutes, thrice.

Restriction Endonuclease analysis

Virions were isolated from purified PlBs by incubating them at 32° C in an alkaline lysis buffer (0.13M Na_2 CO_3 at pH 8) in the presence of 10% SDS (70ml/1µ l) for 1 hour. Undissolved PIBs were removed after sedimenting at 6000 rpm for 10 minutes. The virions were digested with proteinase-K (150µ g/ml) at 55° C, overnight. This was suspended in tris-EDTA buffer. Phenol, Chloroform, and iso amyl alcohol extraction was done. The aqueous phase was centrifuged at 10,000 rpm for 10 min at 4°C to remove impurities and then treated with 0.1 V of 3M sodium acetate and 2 V of 70% ethanol.

The DNA was subjected to single digestion with NdeI. Bam HI, BgI 11. and Hind III restriction enzymes for 4 hours at 37°C. The cleaved fragments were separated on 0.7% agarose gel using tris-acetate buffer system. The genome size of the DNA fragments was estimated by comparison with the marker DNA (Hind 111)and using DNA SIS computer package.

SDS-PAGE analysis of NPV proteins

PIBs were pretreated with 1% SDS. 0.05 M tris-EDTA and 0.05% mercaptoethanol for 30 minutes at pH 7.2 and dissociated with Laemmli's buffer. The Protein concentration was estimated by the method of Lowry *et al.* (1951) and analysed by SDS-PAGE (Laemmli, 1970). Gels were stained by silver nitrate.

Results and Discussion

The restriction enzymes Nde I. Bam HI, Bgl Il and Hind Ill cleaved the HpNPV genome into 6,9, 7 and 8 fragments respectively (Table4.1).The A and B fragments of Nde 1 were found to co-migrate. Two sets of co-migratingfragments. AB and CD. were observed in Bam HI restriction pattern. The size of the genome estimated were 100.63 kbp for NdeI. 99.36 kbp for Barn HI, 101.33 kbp for Bgl Il and 99.55 kbp for Hind 111 in single digestions.

Table 4.1. Estimated sizes (kbp) of REN cleaved HpNPV DNA fragments

Fragments	Enzymes				
	Nde I	Bam HI	BgI I1	Hind Ill	Marker DNA
A	32.59	23.13	27.46	25.51	23.13
B	32.59	23.13	24.29	23.13	9.12
C	16.02	13.83	16.82	19.97	6.56
D	10.31	13.83	10.83	8.70	4.37
E	6.21	8.40	0.06	7.21	2.32
F	2.91	7.73	6.48	6.88	2.00
G		3.75	5.39	5.26	0.36
H		3.45		2.89	
I		2.11			
No. of fragments	6	9	7	8	7
Estimated size	100.63	99.36	101.36	99.55	

The genome *size* of baculoviruses fall within the range of 88 to 160 kbp (Tanada and Kaya. 1993). The genome size of *H. puera* NPV appears to be very close to that of *Spilosoma obliqua* (103kbp) (Manickavasagam *et al.*. 1992).

The SDS-PAGE analysis of polypeptide profiles showed 22 polypeptide bands. The polyhedrin protein was estimated to be 30 kDa. In general, the molecular weight of the polyhedrin proteins range from 25 to 31 kDa (Tanada and Kaya, 1993).The molecular weight of HpNW polyhedrin protein (30 KDa) is the same as that of *Mythimna (Pseudalatia) separata* NPV (Mathad *et al.,* 1991).

This study has revealed the genome size and the characteristic of polyhedrin protein of HpNPV. Further electron microscopic studies are required to understand the morphotype of the virus - whether it is single embedded or multiple embedded.

4.2. CROSS-INFECTIVITY

Mohammed Ali et *al.* (1991) reported that *H. puera* NPV appeared to be specific and did not infect a few other forest pests tested, viz.. *Eutectona machaeralis. Eligma narcissus* and *Atteva fabriciella* We carried out further tests to check on the infectivity of HpNW to some other common lepidopteran species. viz.. *Helicocerpa (=Heliothisj armigera, Spodoptera litura, Amsacta albistriga* and *Bombyx mori.* In addition, the NPVs of *A. albistriga, A. litura* and *H. armigera* were tested for cross infectivity to *H. puera*

Fourth instar *H. puera* larvae maintained in the Iaboratory on artificial diet as described earlier (Section 2.2) were used for the studies. Cultures of other insects as well as their NPVs were obtained from the Entomology Laboratory, Tamil Nadu Agricultural University. Coimbatore through Dr. R.J. Rabindra who collaborated with us in this study. A suspension of the respective NPV containing 108 PIB/mI was prepared and sufficient quantity was applied on to the respective host leaves and the larvae were allowed to feed for 24hr. Three replicates of 10 larvae each were used for each experiment. An untreated control set and a set treated with the NPV of the same host were also included for each experiment. Daily observations were made for symptoms of disease development, if any. and mortality until death or pupation.

The results showed that the NPVs of none of the other species tested viz, *A. albistriga, B. mori, H. armigera* and *S. litura* were cross infective to *H. purea.* All the larvae fed on leaves treated with other NPVs pupated normally while 100% mortality of larvae occurred within 72hr when fed on leaves treated with HpNW.

The mean weight of *H. puera* larvae after 72 hr of feeding on untreated leaves and leaves treated with the NPVs of other species are shown in table 4.2. The results indicate that NPVs of other species had no effect on *H. puera* larvae.

Table 4.2. Weight of H. puera after 72 h of feeding with NPV of other insects

N W used for feeding	Mean weight of larvae (mg)
A. albistriga NPV	184.7
H. armigera NPV	201.4
S. litura NPV	195.3
Nil (control)	180.2

In general, the specificity of baculoviruses is a desirable attribute for its use as an ecofriendly pest control agent. Most baculoviruses are known to be specific to one or a narrow range of closely related species. The NW of the gypsy moth *(Lymantria dispar)* is reported to be very specific to gypsy moth larvae (Lewis, 1981). The NPV of *Helicoverpa* sp. can infect 7 species of *Helicoverpa* viz., *H. armigera. H. paradoxa H. peltigera, H. phloxiphaga, H. punctigera, H. virescens* and *H. zea.* but at the same time it did not infect 37 other insects (Ignoffo and Couch, 1981). The NPV of the alfalfa looper. *Autographa californica* has the widest host range. infecting several species of Lepidoptera (Hunter *et al*.. 1984) but this seems to be an exception. In the case of HpNPV, the results obtained so far indicate that it is host specific.

5. DISEASE EPIZOOTIOLOGY

5.1 SEASONAL INCIDENCE OF NPV DISEASE

Large-scale mortality of *H. puera* larvae due to virus disease has been observed many times during our several years of observations in teak plantations. Although systematic data have not been collected. such disease incidence often appeared to result in collapse of the local population. In a weekly sampling of larvae from 1987 to 1989 in teak plantations at Kariem-Muriem. Mohanadas (1995) showed that NPV disease occurred almost throughout the year whenever the host population was present, but the percentage incidence was higher following the initial host population outbreak. He did not encounter any epizootic incidence of NPV disease. An understanding of the seasonal incidence of the disease and of the conditions under which epizootics are initiated will be useful for developing strategies for managing the pest. A systematic study was therefore carried out to record the seasonal incidence of disease and examine its correlation with host density and weather parameters.

Materials and Methods

Study carried out in 1992 and 1993

The study area consisted of two teak plantation zones - Karulai and Kariem-Muriem of Nilambur Forest Division where pest incidence was regularly monitored. Whenever a discrete infestation occurred, a sample of larvae and pupae were examined to record disease incidence. Five trees were randomly selected from an infested area for carrying out larval sampling. The tree canopy was divided into top and bottom levels and four shoots (representing four directions) were sampled from each level. Diseased and healthy larvae present on each shoot were counted and recorded. Diseased larvae were taken to the laboratory and NPV infection was confirmed by examining the larval smear stained with Ciemsa stain. A pupal sampling was also made from the same site later. The pupae were collected from the soil and litter within 2 m radius of each of the five trees. Diseased pupae were examined for NPV infection.

Study carried out in 1994

In 1994 the study was limited to teak plantations at Kariem-Muriem and to the main outbreak period. Pest incidence was regularly monitored during the months

of May and June and 50 to 300 live larvae were collected from each distinct population. These larvae were observed in the laboratory for expression of NPV infection.

Study carriedout in 1995

NPV infection in the larval population during the non outbreak period of the pest was studied during the months of December 1994 and January to March 1995. Larval samples were collected from different plantations in Nilambur. As the larval numbers were very small, a thorough search had to be made to locate them. The collection target was 50 larvae and on certain occasions this required upto 3 1/2 hours of collection time. The larvae were observed in the laboratory for expression of NPV disease.

Results

Year 1992

Table 5.1 shows the incidence of NPV infection in larvae and pupae of 8 discrete populations of *H. puera* from June to September 1992. The disease was present in all the populations and in larvae. the percentage of infection ranged from 0.0001 to 4.09. In pupae the percentage of infection ranged from 7.1 to 39.7.

Table 5.1. Incidence of NPV disease in field populations of *Hyblaea puera* in Nilambur teak plantations in 1992

Sampling area	Sampling date	No. of larva sampled	% of larvae dead due to NPV	No. of pupae sampled	% of pupae dead due to NPV
Kariem-Muriem	18-06-1992	2348	4.09	82	13.41
Kariem-Muriem	12-07-1992	834	1.43	-	-
Karulai	14-07-1992	746	0.0001	545	17.20
Kariem-Muriem	17-07-1992	3354	0.92	544	22.79
Kariem-Muriem	20-07-1992	3139	0.28	697	39.72
Kariem-Muriem	11-08-1992	2163	0.36	197	14.73
Karulai	27-09-1992	1694	2.83	-	-
Kariem-Muriem	27-09-1992	2124	2.54	177	7.10

In 23 larval samples collected from March to December from various teak plantations across Nilambur. NPV infection was noted in 13 samples (Table 5.21. Within the sampling period of March to December incidence was noticed in all months except March and November to December. When present, the incidence ranged from 0.1 to 14.7%. with peak incidence in April and August.

Table 5.2. Incidence of NPV disease in field populations of *Hyblaea puera* in Nilambur teak plantations in 1993

Sampling area	Sampling date	No. of larvae sampled	% of larvae dead due to NPV	No. of pupae sampled	% of pupae dead due to NPV
Kariem-Muriem	04-03-1993	2193	0	171	0
Kariem-Muriem	23-03-1993	1093	0	205	0
Kariem-Muriem	13-04-1993	790	0.254		-
Kariem-Muriem	22-04-1993	675	9.412	210	2.381
Poolakkappara	12-05-1993	100	0		-
Kariem-Muriem	18-05-1993	854	0.117	-	-
Kariem-Muriem	19-05-1993	644	0.465	-	
Chaliyar	28-05-1993	856	0.467	105	5.71
Aravallikkavu	28-05-1993	100	1.000	622	8.36
Poochakkuthu	30-05-1993	787	0.127	-	-
Kariem-Muriem	14-06-1993	757	0.793		
Nedungayam	19-06-1993	801	0		
Kariem-Muriem	09-07-1993	422	2.84	-	-
Nedungayam	15-07-1993	94	5.32		
Nedungayam	15-08-1993	34	14.71	-	-
Kariem-Muriem	15-09-1993	153	3.97	149	16.10
Chaliyar	16-09-1993	402	0	-	-
Valluvasseri	18-10-1993	48	2.0	-	-
Panayangode	21-10-1993	20	0	-	-
Valluvasseri	24-10-1993	25	0	-	-
Nedungayam	28-11-1993	9	0	-	-
Kariem-Muriem	30-11-1993	26	0		
Mailady	01-12-1993	25	0		

NPV infection was also noted in the pupal samples corresponding to the larval samples in which infection was observed. When present, the percentage of infection ranged from 2.3 to 16.1. the highest being in May and September.

Year 1994

In 1994 there was no evidence of NPV infection in the 6 samples collected in May (Table 5.3). In June the infection rate ranged from 1 to 26%.

Table 5.3. Incidence of NPV deaths in field populations of *H. puera* in teak plantations at Kariem-Muriem

Date	No. of insects observed	% of larvae dead due to N W
13-05-1994	50	0
14-05-1994	100	0
15-05-1994	200	0
16-05-1994	100	0
17-05-1994	100	0
18-05-1994	100	0
04-06-1994	200	1.00
06-06-1994	250	11.60
08-06-1994	300	12.80
12-06-1994	171	4.76
28-06-1994	50	26.00

Year 1995

The 1995 study was carried out examining the status of infection in sparse population during the non-outbreak season (Table 5.4). Observations in December 1994 were also included here. The results gave evidence of NPV infection in the rarely sighted larvae during the off-season of the pest. In various samples the rate of infection ranged from 0 to 16.6%.

Table 5.4. NPV incidence in early 1995. including December 1994

Date of	Locality of collection	No. of larvae collected	**% death due to NPV**
13-12-1994	Panayangode	33	6.06
20-12-1994	Valluvasseri	41	0
28-12-1994	Nedungayam	41	0
03-01-1995	Nedungayam	37	2.70
11-01-1995	Nedungayam	36	5.56
18-01-1995	Nedungayam	40	16.6
01-02-1995	Nedungayam	43	10.0
07-02-1995	Nedungayam	38	0
14-02-1995	Nedungayam	32	0
21-02-1995	Nedungayam	30	0

Conclusion

The overall seasonal pattern of NPV infection in the *H.* puera population is presented in Table 5.5. Although NPV infection was not recorded in March and November, the trend indicates that infection is prevalent in the population throughout the year.

Table 5.5. Seasonal incidence of NPV infection in field populations of *H. puera* in teak plantations at Nilambur

March	Year				Overall infection
	1992	1993	1994	1995	
January	-	-		+	+
February				+	+
March	-	0	-	0	0
April		+		0	+
May	-	+	0	0	+
June	+	+	+	-	+
July	+	+			+
August	+	+			+
September	+	+			+
October		+			+
November	-	0	-		0
December		0	+		+

+ indicates presence of infection: 0 indicates absence of infection; - indicates absence of sample.

Compared to larval stages, viral infection was greater in the pupal stage. The overall average infection rate was 1.8% for larvae and 5.4% pupae during the year 1992-93. This is probably because in a sample of larvae, only those found dead at the moment of collection were accounted. Any incipient infection which would have been expressed only subsequently was therefore left out. In the case of pupae, however, they were observed in the laboratory for expression of infection until the moths emerged.

Irrespective of whether the population was dense or sparse, the intensity of infection was found to be highly fluctuating. Examination of the data presented in Section 3 on pest population trends and weather parameters will show that disease incidence was not correlated with the host density or temperature and rainfall.

During the study period 1992-95, no typical epizootic was observed in any of the study sites. However, it is difficult to conclude that an epizootic did not occur in any of the teak plantations in Nilambur. An epizootic may go unnoticed, if it occurs in a population of very early larval instars. Another reason for not encountering an epizootic could probably be the longer interval between sampling. What was observed in the study areas could be an enzootic situation which may lead to an epizootic under favourable conditions.

For estimating NPV infection in the host population, the methodology used in 1994 and 1995 appears to be more appropriate as incipient infection. If any, was expressed during the laboratory observation period could be recorded. An observation period of four days appears to be sufficient.

5.2. FIELD STUDIES ON PERSISTENCE OF HpNPV

The persistence of a microbial pathogen under field conditions is an important criterion which will determine its efficacy as an applied biological control agent. In this study, observations were made on the persistence of HpNPV when applied in the field.

Persistence between years

As described in Section 8, in the year 1993, a 100 - tree plot within a 17-year old teak plantation was continually protected from the defoliator using HpNPV. The plot was sprayed five times and the total inoculum sprayed during the year was about 1.02×10^{10} PIB per plot. which works out to 2.8×10^6 PIB/m^2 of ground. A similar 100 - tree plot in the same plantation which was left unsprayed served as the control.

In May 1994, when the first infestation of the next year occurred, larvae were sampled from both the treated and control plots and examined in the laboratory for disease incidence. Larvae were maintained in the laboratory under sterile conditions and dead larvae were microscopically examined to identify the cause of mortality. The sampling and examination was repeated during the second infestation in June 1994.

The results are presented in Table 5.6. There was no incidence of NPV infection either in the treated or control plot during the first infestation. In the second infestation. two out of 50 larvae showed N W death in the untreated plot, but none in the treated plot. These results indicate that NPV applied to the foliage repeatedly in the previous year did not lead to incidence of disease in the following year.

Table 5.6. Incidence of NPV in a plantation subjected to NPV spray in the previous year

Date of collection	Control						Treated					
	No. of larvae col.	No. of dead larvae	Casue of mortality				No. of larvae col.	No. of dead larvae	Cause of mortality			
			Hp	Ba	Pa	Oth			Hp	Ba	Pa	Oth
13-5-1994	**100**	11	**0**	**9**	**3**	**0**	**100**	**10**	**0**	**10**	**0**	**0**
15-5-1994	**100**	1	**0**	**0**	**0**	1	**100**	**3**	**0**	**2**	1	**0**
17-5-1994	**100**	1	**0**	1	**0**	**0**	**100**	1	**0**	1	**0**	**0**
04-6-1994	**50**	**0**	**0**	**0**	**0**	**0**	**50**	**6**	**0**	**6**	**0**	**0**
06-6-1994	**50**	**13**	**2**	**0**	**6**	**5**	**50**	**3**	**0**	**3**	**0**	**0**
08-6-1994	**50**	**8**	**0**	**8**	**0**	**0**	**50**	**3**	**0**	**3**	**0**	**0**

Col. - collected: oth - others; Hp - HpNPV; Ba - Bacteria; Pa Parasites

Although NPVs are believed to remain in the environment through persistence in the soil ('Thompson *et al.*. 1981).in practical terms, even a dose of 2.8 x 10^6 PIB/m^2 of area did not cause incidence of disease in the following year.

Short- term persistence

In order to investigate short-term persistence, starling from April 1994 onwards, small plots of 10 trees each in the teak plantations at Kariem-Muriem were sprayed with HpNPV at weekly intervals. The intention was to study the incidence of disease when infestations occurred in the plots. A concentration of 1 x 10^5 PIB/mI was used and each tree received a spray volume of about a litre.

The first infestation of the year occurred on 10 May in a plot treated with NPV on 5 May. From this plot. samples of larvae were collected on 13. 15 and 17 May and maintained in the laboratory on teak leaves under sterile conditions. Larvae which were found dead within four days of collection were microscopically examined for presence of NW. Control larvae were also collected from untreated plots and subjected to similar examination.

The results presented in Table 5.7 shows that there was no incidence of NPV in the treated or control plots. Since the infestation occurred on 10 May, the newly hatched larvae must have been present since 11 to 12 May. Larvae collected on 13. 15 and 17 May must have fed on leaves treated with N W on 5 May. If the PlBs had persisted on the foliage for a period of 6 to 12 days, these larvae could have acquired them. The absence of disease incidence in the sampled larvae shows that the PIBs did not persist in the foliage even for 6-12 days.

The results of the above two experiments indicate that PIBs have very little viability underfield conditions and that environmental persistence of the virus is not a major factor contributing to incidence of the disease.

5.3. LABORATORY STUDIES ON DISEASE TRANSMISSION

Perpetuation of the N W disease in the insect population requires transmission of the polyhedral inclusion bodies (PlB) between generations either through the environment or the host itself. As shown above, environmental persistence does not appear to be a major factor. Two types of host-mediated transmission are generally recognized - (1) Virus carried through external contamination of eggs (transovum transmission) and (2) Viruscarried internally through the eggs (trans-ovarial transmission). Studies were carried out to elucidate the type of viral transmission taking place in *H. puera.*

Table 5.7. Incidence of NPV in a plantation subjected to NPV spray in the previous week

Date of collection	Control						Treated					
	No. of larvae col.	No. of dead larvae	Cause of mortality				No. of larvae col.	No. of dead larvae	Cause of mortality			
			Hp	Ba	Pa	Oth.			Hp	Ba	Pa	Oth
13-5-1994	100	12	0	9	3	0	66	9	0	9	0	0
15-5-1994	100	1	0	0	0	1	86	3	0	1	2	0
17-5-1994	100	1	0	1	0	0	100	2	0	1	1	0

Col. - collected; oth - others; Hp - HpNPV; Ba - Bacteria; Pa - Parasites

Methods

Fourth instar larvae from the laboratory culture were used for the study. A set of 120 larvae were individually kept in 6 x 2.5 cm glass tubes and provided with 2 ml of artificial diet mixed with 200 polyhedral inclusion bodies. A control set of 120 larvae were similarly fed with pure diet. Out of those, larvae which consumed the diet completely were used for further study. Such larvae from both the sets were transferred to new tubes and provided with fresh teak leaves. Mortality of the control and NPV-fed insects in the larval and pupal stages were recorded. On emergence the moths under each set were grouped in batches for oviposition. The Eggs obtained were used for further studies.

The eggs laid by the F1 generation moths from both control and NPV-fed sets were divided into two batches . One batch was sterilized by dipping in 2% sodium hypochlorite solution for 10 minutes and the other batch was kept unsterilised. Eggs from the control set were also divided into two batches and one batch was sterilised and the other batch was left unsterilised. On hatching, thirty larvae from each set were transferred to individual tubes and reared on teak leaves. Percentage mortality of larvae and percentage pupation were recorded. The pupae were divided into two batches and one batch was sterilized by dipping in 2% sodium hypochlorite solution and the other batch left unsterilized. Percentage emergence of moths was recorded. The whole experiment was later repeated using F2 generation.

Smears from all the dead larvae were stained with Giemsa stain to ascertain NPV infection.

Results and Discussion

In the parental set, 12.6% of the virus-fed larvae died due to NPV infection as against 7.8% in the control (Table 5.8). Similarly 6.9% of the pupae of the virus-fed set did not emerge as against 3.9% in the control.

In the F1 generation, viral death was higher than in the parental set. In the F1 progeny of virus-fed larvae, 36.7% died due to NPV infection irrespective of whether the eggs were surface-sterilized or not. In the control set, 13% of the larvae died due to NPV infection in the washed [surface sterilization of eggs) group, but none in the unwashed group. In the pupal stage of the F1 progeny of virus-fed larvae, 5.8% of insects in the washed group and 21% in the unwashed group died due to NPV infection. In the corresponding control set, 3.9% of pupae in the washed group and 31.8% of pupae in the unwashed group died due to NPV infection. In the F2 generation, in the virus-fed set, death due to NPV was still higher than in the F1 generation. However, as in the parental and the F1 generation, some death due to NPV infection also occurred in the untreated set. The details are given in Table 5.8.

Table 5.8. Transmission of NPV through successive generation from IV instar larvae fed with 200 PIBs/larvae

Generation	Stage of insect	% Mortality due to NPV			
		Treated		Control	
P	Larva	12.6 (103)		7.8 (103)	
	Pupa	6.9 (48)		3.9 (74)	
		washed	unwashed	washed	unwashed
F1	Larva	36.7 (30)	36.7 (30)	13.3 (30)	0 (30)
	Pupa	5.8 (17)	21.0 (19)	3.8 (26)	31.8 (22)
F2	Larva	52.5 (40)	47.5 (40)	0 (20)	20.0 (20)
	Pupa	6.3 16)	5.3 (19)	5.6 (18)	0 (14)

The figure in the parentheses indicates the total number of larvae/pupae under observation.

it may be seen from the results that NPV-fed larvae transmitted the NPV to F1 and F2 generations. The disease was manifested in insects originating from surface sterilized eggs and-pupae, both in the F1 and F2 generations, indicating transovarian trasmission of the NPV. Incidence of disease in the control set (not fed with NPV), although at a lower level, is also indicative of the presence of NPV in the parental larvae, possibly in an occult state, and its transovarian transmission to the F1 and F2 generation.

The occurrence of disease in successive generations of the field populations of *H. puera* in the absence of environmental persistence also suggests that the disease is perpetuated through host-mediated transmission.

5.4 EFFECT OF NPV ON REPRODUCTION OF *H. PUERA* MOTHS

Since there is an indication that NPV remains in *H. puera* in an occult state and is transovarially transmitted (Section 5.3), the effect of sublethal doses of NPV on the longevity and reproduction of the moth were studied.

Effect of feeding late instar H.puera larvae with NPV on fecundity of moths

Early fifth instar larvae from the laboratory culture were used in the study. Fifty larvae were kept individually in glass tubes 10 x 2.5cm and fed with fresh teak leaves treated with NPV suspension at 1×10^6 Pib/ml (Fig. 5.1). A control set fed on teak leaves treated with distilled water was also maintained. The fecundity of the moths which emerged from the control set and the treated set was recorded. he data were analysed using student's **t** test.

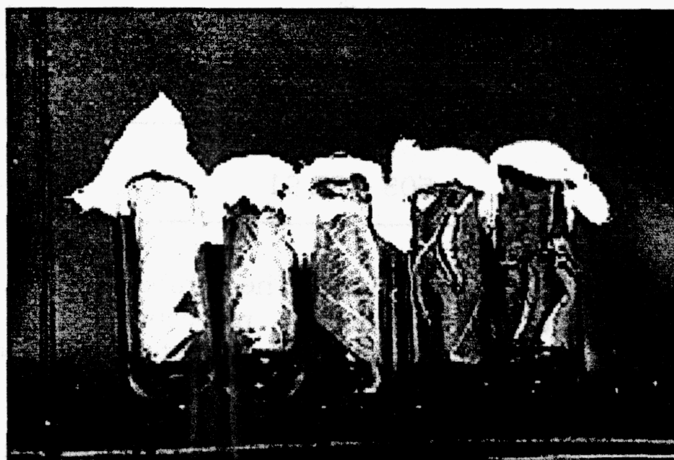


Fig. 5.1 *Hyblaea* larvae individually reared on leaf in tubes

The fecundity of nine moths of the treated and control sets are given in Table 5.9. There was no statistically significant difference, although the trend indicates lowered fecundity of NPV-fed adults. Further studies are needed to examine this. If the trend is confirmed it will mean that through intra-host persistence, NPV can reduce the fecundity of the moths and substantially affect the population *size* in the next generation.

Effect offeeding H. puera moths with NPV on reproduction

Freshly emerged moths from the laboratory culture were used for the study. Twenty three females were fed with 1:1 suspension of 10% honey and a crude suspension of the virus. The NPV-fed females were allowed to mate with normal males. A control set was also kept in which the moths were given 10% honey. The moths were kept under observation for mating. The number of moths mated and oviposited in each group was recorded.

Table 5.9. Effect of NPV on fecundity of moths which emerged from larvae fed with sublethal dose of NPV

No. of moths	Number of eggs laid	
	Treated	Control
1	200	140
2	586	1152
3	1104	1740
4	644	568
5	452	1078
6	234	1326
7	190	568
8	506	1074
9	654	1326
Mean	508 + SE	997 + SE 153

The results are presented in Table 5.10. The number of moths mated in the control and treated sets were 18 and 14 respectively. Out of the mated moths, only 6 laid eggs in the treated set as against 14 in the control.

Parameters observed	Treated	Control
No. of female moths observed	23	23
No. of moths mated	18	14
No. of moths oviposited	14	6

The results indicated that NPV feeding has an adverse effect on mating and oviposition behaviour of *H. puera* moths which may lead to collapse of the pest population. More critical studies are needed, using more purified virus in order to confirm these effects.

6. MASS PRODUCTION OF Hp NPV

Availability of sufficient stock of NPV was a pre-requisite for many of the proposed investigations.Therefore attempts were made to build up a large stock of the material from the early stage of the project and to standardize the rnass production methods. Since large numbers of larvae can be collected from teak plantations during the natural outbreaks, with little effort, methods were developed to mass produce the NPV using field- collected larvae.

The first attempt to mass produce HpNPV was made in June 1992 when the earliest outbreak of the teak defoliator occurred in the Kulathupuzha region in southern Kerala, about 250km away from the KFRI head quarters at Peechi A large number of third instar larvae were obtained by collecting the infested leaves. Leaves containing larvae were stored inlargecloth bags and transported to Peechi. At Peechi, the larvae were transferred into freshtender teak leaves sprayed with HpNPV inoculum and maintained in large plastic buckets as well as in cages (lm x lm x lm). The dead fourth and fifth instar larvae were collected fromthe third day onwards and tranferred to a amid flask containing distilled water and allowed to putrefy. After one week, the larvae were taken out, macerated and filtered through double layered cheese cloth. The filtrate was left for sedimentation of PIBs and after one week, the sedimented PIBs were separated by decanting resuspended in distilled water and stored as the stock suspension.

Such attempts to produce NPV were made several times subsecquently at Nilambur whenever outbreaks occurred. Dunng each collection, 15,000 to 20,000 larvae were collected and processed. Initially large plastic buckets (100 1.) were used to store larvae collected in the field. Infested leaves were plucked and stored in them. Dunng heavy outbreaks, 4 buckets full of infested leaves could be collected in about an hour by employing 4 to 5 labourers. Generally, the infested leaves were collected by climbing on the trees. The larvae were brought to the laboratory within one and a half hours after the collection started. The main problem encountered in this method of larval collection was death of a large number of larvae due to insufficient don in the bucket filled with infested leaves. In subsequent efforts, the plastic buckets were replaced with gunny bags. This resultedin improvement of don but caused some escape of larvae. Further improvements were made by using cloth of appropriate mesh size and filling the bags only halfway. Transportation was also effected as quickly as possible.

Larvae were collected fromfield with the help of local labour. People residing near the plantations were employed for this purpose. They were given training for collection of larvae from the field, Others were employed in the laboratory for retrieval of dead larvae. Trained local people were also employed for monitoring the pest population in teak plantations in order to make field collections at the appropriate time.

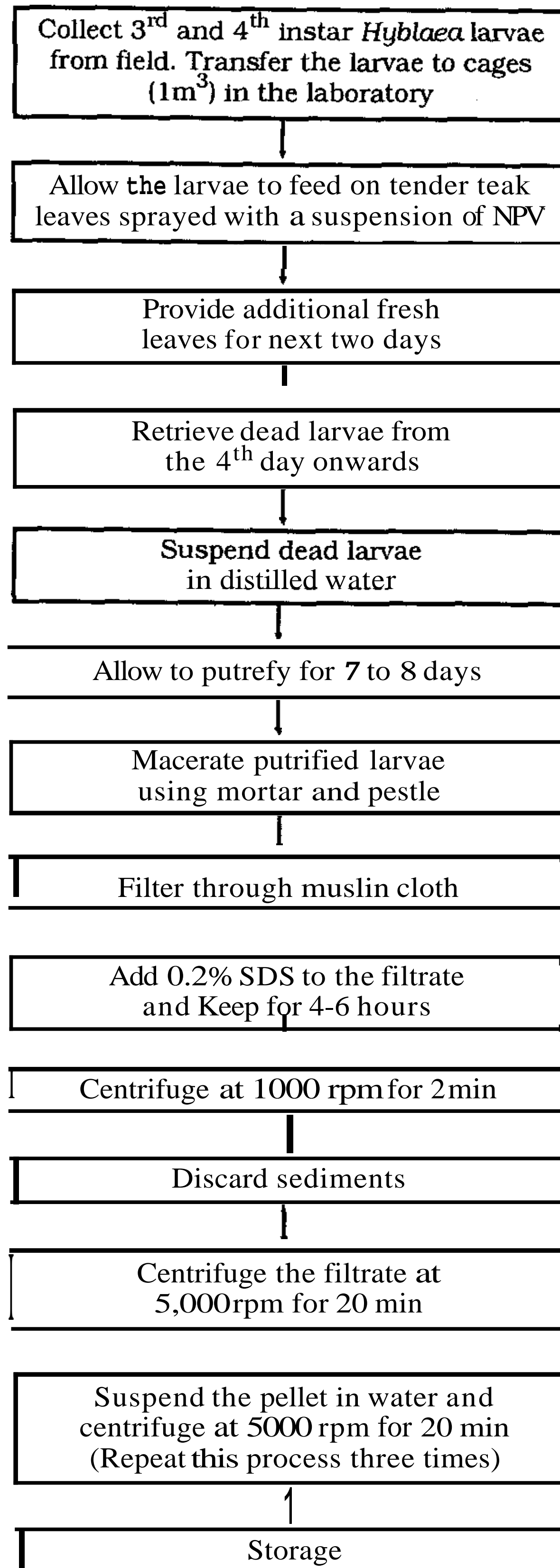
The larvae brought to the laboratory fromthe field in cloth bags were transferred to wooden cages(lm^3)with glass sides and wire-mesh top. Larvae were allowed to feed on tender leaves sprayed with a suspension of NPV polyhedra. Third or fourth instar larvae were mainly used for this purpose. Once in 4-6 hours, the larvae inside the cage were agitated to promote free movement and feeding. Proper aeration was necessary to prevent suffocation of larvae between the layers of leaves. Observations continued for three days within which mortality of the larvae occurred due to NPV infection. Dead larvae(Flg.6.1)were then separated and allowed to putrefy in distilled water for 7 to 8 days and then purified. The sequence of operations are shown in Chart 6.1.

Fig. 6.1 A *Hyblaea* larva dead due to NPV infection

The above method of mass production of NPV had some demerits. In the field collected larvae, parasites were often found in plenty. In addition, bacterial contamination was encountered often. Also, due to overcrowding in cages, premature death became a major mortality factor. Though most deaths occurred due to bacteria such larvae also showed presence of PIBs.

To overcome the contamination, attempt was made to produce NPV from cultures of *Hyblaea* larvae maintained in the laboratory. For this purpose, fourth instar larvae were used. Initially the larvae were reared in groups by allowing them to feed on NPV-sprayed tender teak leaves. The rest of the procedure was the same as described earlier. One major

Chart 6.1. The sequence of operations followed in the mass production of HpNPV



advantage of this method of NPV production from laboratory culture was lower level of bacterial contamination. Also the chances of parasitised larvae entering the culture was avoided.

Yield of PIBs

We obtained an average yield of 4 X 10^6 PIB/larva when field collected larvae were used and an average of 7.5 X 10^7 PIB/larva when laboratory reared larvae were used (Table 6.1). Both are rather low yields, although laboratory rearing was better. In the case of field collected larvae, the lower productivity was attributed to premature death of larvae due to bacterial infection and parasitism. In case field collected larvae are used for mass production of NPV it is advisable to collect the larvae in the first or second instar from the field and then rear them in the laboratory until the fourth instar, because parasitism is lower in early instars.

When large numbers of field collected larvae were handled at a time for mass production of NPV, considerable time was spent to retrieve the dead infested larvae. Delay in retrieving the dead larvae resulted in oozing out of body fluid and loss of PIB's.

This can be avoided by handling small number of larvae at a time or increasing the man power for retrieving dead larvae. The merits and demerits of the two methods of mass production are compared in Table 6.1.

Table 6.1 Comparison of two methods of mass production of HpNPV

Criteria	Field collected larvae	Laboratory reared larvae
Yield per larva	low ($4*10^6$ PIB/larva)	high ($7.5*10^7$ PIB/larva)
Contamination	high	low
No. of larvae	high	low
Expenditure per larva	low	high
Period of activity	seasonal	continuous
Need for trained-man power	low	high
Risk of parasitism	Yes	no
Monitoring of populations	needed	not needed

In another approach, attempt was made to produce the NPV from individually reared laboratory culture of larvae. In this method early fifth instar larvae were exposed to the NPV and the dead larvae retrieved after 3 days and homogenized. This method gave an yield of 1 X 10^8 PIB per larva which was much better than the other two methods. However, this method is time consuming, but can be adopted when a pure sample of the virus is required. Considering the high cost incurred for laboratory rearing of *H. puera* larvae and the comparative ease with which larvae can be collected from teak plantations during the natural outbreaks, use of field collected larvae remains the most practicable method for mass production of HpNPV at present. Collection of early instars from the field and rearing them in the laboratory upto the 4th instar before exposing them to NPV, and handling of the larvae in comparatively small batches by several teams of workers for quick harvest of the dead larvae can improve the yield of PIBs substantially. Further work is necessary to streamline the procedures and optimise the productivity.

7. DEVELOPMENT OF A MONITORING PROTOCOL FOR TIMELY APPLICATION OF HpNPV

Though defoliator outbreak is a regular annual feature in the teak plantations, it is often difficult to predict the exact time of outbreak each year. Therefore monitoring of field populations of the teak defoliator is a prerequisite for timely application of any control measure. This is true for application of HpNPV also because of the following reasons:

1. As shown in section 5.2 HpNPV has little environmental persistence. Therefore although inoculative spray has often been recommended in the case of NPVs of forest insects (Entwistle 1978), will not be effective against *H. puera.*

2. Generally early larval instars are more susceptible to NPV than later instars. Through monitoring early larval instars can be targetted.

3. In the case of *H. puera,* the onset of an outbreak is sudden and there is wide difference between the time of occurrance of infestation at different places. Monitoring of pest populations is therefore required for timely implementation of control operations in a given locality.

Since arrival of defoliator moths to the teak plantation is the first step in the initiation of an outbreak, the best indicator of a forthcoming infestation is the detection of moths. For this purpose we developed a unique light trap using a black- light tube, powered by a battery. The battery is charged using a solar photovoltaic system. A brief description of the light trap designed for the purpose is given below.

Trap Design

The light- trap system (fig. 7.1) consists of the trap, the collection cage and the solar photovoltaic (SPV) system. The basic trap unit is similar to the Pennusylvanian type. A twenty watt black- light florescent tube (24" long) acts as the light source. Four bottles are fvred on the periphery of the tube. A funnel is attached to the bottom part of the battle which leads into a walk in cage where insects will remain undisturbed and alive. The solar photovoltaic system consists of four 30W (nominal) SPV panels, a 12V 60 AH storage battery and an electronic control unit. Two models namely, the fixed and portable ones were designed to suite specific needs. The portable model can work 6 hrs daily while the fixed model can work for 12 hours daily without the battery being deep discharged.

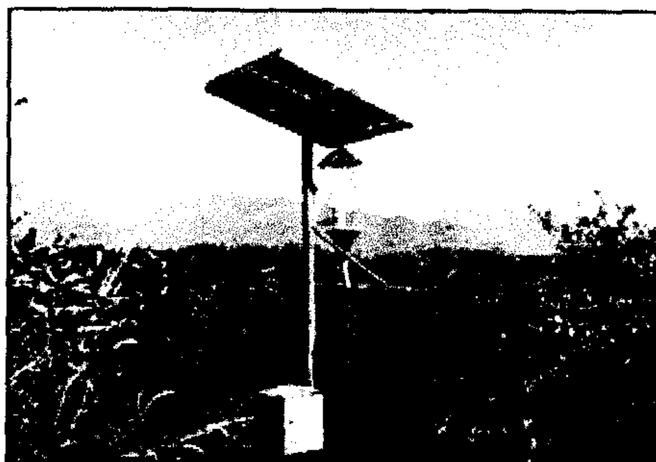


Fig. 7.1. A solar powered light trap system set up in the study site (Nilambur)

Efficiency of the trap

The light trap was established on the top of a hillock at Ambalakkunnu in Kariem- Muriem in June 1993. This site is in the centre of the 1000 ha teak plantation at Kariem-Muriem. Collection of moths was done almost continuously upto the end of 1995.

The number of moths collected in the trap during representative time periods of the years 1993, 1994 and 1995 is shown in Table 7.1. As part of the epizootiological studies, sampling of field populations of teak defoliator was done at Kariem-Muriem. Information was thus available on the life stage of the insect present in the field. From this information, dates of two phenomena in the life history of the insect, namely, egg laying and adult emergence were arrived at using the earlier known information on time required to complete each developmental stage. Our aim was to test the reliability of light trap catch as an indicator of the presence of adult moths in the field.

In June 1993, a fairly wide spread infestation occurred at Kariem-Muriem. Larvae collected from the infested area and reared in the laboratory, pupated and moths started emerging by 28 June. In the field, moths were collected in the trap from 24 June onwards after a period of zero catch. Table 7.1. shows that the increase in the number of moths in the field got reflected in the trap catch. It also indicates that the emergence had started four days before in the field than that in the laboratoy. The emergence of moths

Table 7.1. Dates of moth emergence and egg laying at Kariem-Muriem compared with the light-trap catch

Year 1993

Julian date	Egg laying	Moth emergence	Light-trap catch
174 (23June)		-	0
175		-	28
176		-	30
177	-	+	32
178	-	+	42
179		+	13
180	-	+	18
191	-	+	64
182			0
183	-	-	57
184	-	-	6
185			12
186		-	48
187	+	-	32
188	+		2
189	+	-	18
190	+	-	3
191	+	-	18
192		-	0
Year **1994**			
124 (4May)	-		0
125	-	-	8
126	+		8
127	+	-	0
128	+	-	0
129	+	-	9
130			0
131	-	-	0
132			0
133	-	-	4

Tab 7.1 contd..

Julian date	Egg laying	Moth emergence	Llght-trap catch
134	-		5
135		-	0
136		-	0
137	-	-	0
138	-	-	0
139		-	0
140		-	0
141	-	-	1
142			0
143			0
144			0
145	-		0
146		-	2
147			0
148			0
149	-	+	0
150		+	116
151		+	327
152	-	+	37
153	-	+	53
154			63
155			37
156	-	-	0
Year 1995			
56 (25February)	-		0
57			0
58	+	-	0
59	+	-	0
60	+	-	0
61	+	-	1
62	-	-	0
63	-	-	0
64	-	-	0

continued upto 30 June. No moths were collected in the trap on 1 July. A new area got infested at Kariem-Muriem during the period 7-9 July. Prior to this infestation, there was an increase in the number of moths trapped.

On 6 May, 1994 egg laying occurred in a small patch at Kariem-Muriem. Moths were trapped one day before the start of egg laying. This catch occurred after 15 days of zero catch. Here also the trap catch functioned as a good predictor. During the period of adult emergence from the infested area, an increase in the trap catch was observed.

In 1995, the first outbreak population started on 27 February. A single moth was collected on 2 March which was the first catch of the year. In this case, egg laying strated in the field, before any moths were collected in the light trap. Since only a very small area was infested (< 1 ha), the number of moths would have been low, leading to very small catch.

In all the cases described above, the light trap catch was representative of the density of adult moths in the field. When the density of moths was high, the trap catch was a good predictor of forthcoming outbreaks. Collection of defoliator moths on a day following days of zero catch is the indication for an incipient outbreak. Visual observations are needed to confirm the existance and extent of the outbreak. Following guidelines were developed for scouting for the infested area.

1. After moths have been caught in the light trap, daily observations should be made in the teak plantations to detect folds on tender foliage caused by the second instar larvae. These observations can be limited to areas with tender foliage.

2. If folds are detected, pluck out tender leaves and look for eggs or first instar larvae. The first instar larvae will be feeding by scraping the leaf near the veins. If these stages are found, make similar observations in transects radiating in four directions from the site where folds were detected. This is needed because egg laying may occur on more than one day at a given location and hence younger stages may escape undetected. The present study showed that *H. puera* infestations can be effectivelymonitored by (1) detection of moths using solar- powered light traps using a black light tube and (2) ground surveilance for early instar larvae, when moths have been detected in the light trap.

8. FIELD TRIALS OF HpNPV FOR CONTROL OF *HYBLAEA PEURA*

In an earlier study of Mohammed Ali *et al.* (1991)reported efficacy of HpNW under laboratory conditions. In the present study the efficacy of HpNW was tested under field situation. In a preliminary trial carried out in 1992. ten Hybleae infested trees were sprayed with a crude suspension of NW. This treatment appeared to be quite effective in reducing the defoliation. Based on the above encouraging results a planned field trial was carried out in 1993.the results of which are presented below.

Materials and Methods

Study plots and infestation monitoring

Two plots of about 100 trees each with comparable height and stand composition were selected at Kariem-Muriem. A buffer strip of the plantation, 100 m wide, was left between the two plots. Both the plots were kept under surveillance by daily visual observation during the critical periods. Daily moth catches in a solar powered light- trap installed within the plantation at a distance of 1 km indicated the critical period of infestation. N W was applied in the experimental plot whenever infestation was detected with in the plot. The control plot was left untreated. A total of five NPV applications became necessary during the year to control five distinct populations.

Preparation of NPV and spray application

A stock suspension of NPV was prepared during the previous year using field collected larvae and stored. It consisted of a filtered water suspension in which diseased insects were allowed to putrefy. Calibration of the PIB was done using a haemocytometer. The spray suspension was prepared by diluting the stock with non-chlorinated water to a concentration of 1×10^5 PIB/ml. Before spraying, a spreader-sticker (Tween 80)was added at a concentration of 0.2%. The NPV dosage was the same in all the five applications.

Each tree within the treatment plot was individually sprayed using a rocker-sprayer. The quantity of spray solution applied per tree ranged from 0.75 to 1.75 litre, depending on the total foliage present.

Treatment effect

The effect of NPV application was assessed by the following three criteria.

1. Leaf damage

Since the intensity of infestation varied between trees depending on the density of tender leaves, well-infested comparable trees were selected for damage assessment. Fourteen such trees were marked in each plot on the first day of each spraying. From each tree, eight shoots were sampled randomly to assess leaf damage. The damage assessment was done before spraying and twice thereafter, at an interval of 2 to 3 days. Damage was expressed as percentage leaf loss, based on visual scoring. For each leaf. percentage leaf loss was scored into one of five class intervals (0-5,6-25.26-50.5 1-75.76-100) and using the mid-point of the score interval, the mean percentage leaf loss per shoot was calculated. The percentage leaf loss for each sampled tree was calculated by averaging the values for the 8 shoots sampled. These values were transformed using Taylor's power law ($2 = x^{0.3}$) to stabilize the variance (Southwood.1978). The significance of the difference in leaf damage between treated and check trees was analysed using multiple analysis of variance. The pre-treatment damage scores were used as covariates.

2. Larval mortality

To assess the reduction in larval numbers due to treatment, larval counts were made from the shoots sampled as above. For each tree, the numbers recorded in the eight shoots were averaged to get the mean number of insects per shoot. Since the variance in the number of insects per shoot among the 14 trees sampled per date. over time, was found to be dependent on the mean, the actual counts were transformed using Taylor's power law ($2 = x^{0.2}$) for statistical analysis. The significance of the differences was tested by multiple analysis of variance in which the pretreatment larval counts were used as covariates.

3. Growth increment

Growth increment was another criterion used to assess the effect of treatment. The girth at breast height (GBH) of all the trees in the two plots were measured prior to the first NPV application and measurements were repeated at the end of the year. From the initial and final GBH of each tree, the corresponding basal area was calculated. The basal area increments were compared by analysis of variance taking the initial basal area as covariate.

Results

The trend of infestation and the effect of NPV treatment, in terms of protection from foliar damage, reduction in larval population and growth increment of the trees are given below.

Pest incidence

Fig. 8.1. shows the pest incidence in the study area during the year 1993. Five major infestations occurred during the year, all between March and July. Each infestation was caused by a discrete population of larvae which developed more or less synchronously and pupated in less than 10 days. NPV spray applications were made as soon as each of these infestations was detected, when most larvae were in the first or second instar. During the rest of the year, the pest population was negligible and consisted of mixed age groups.

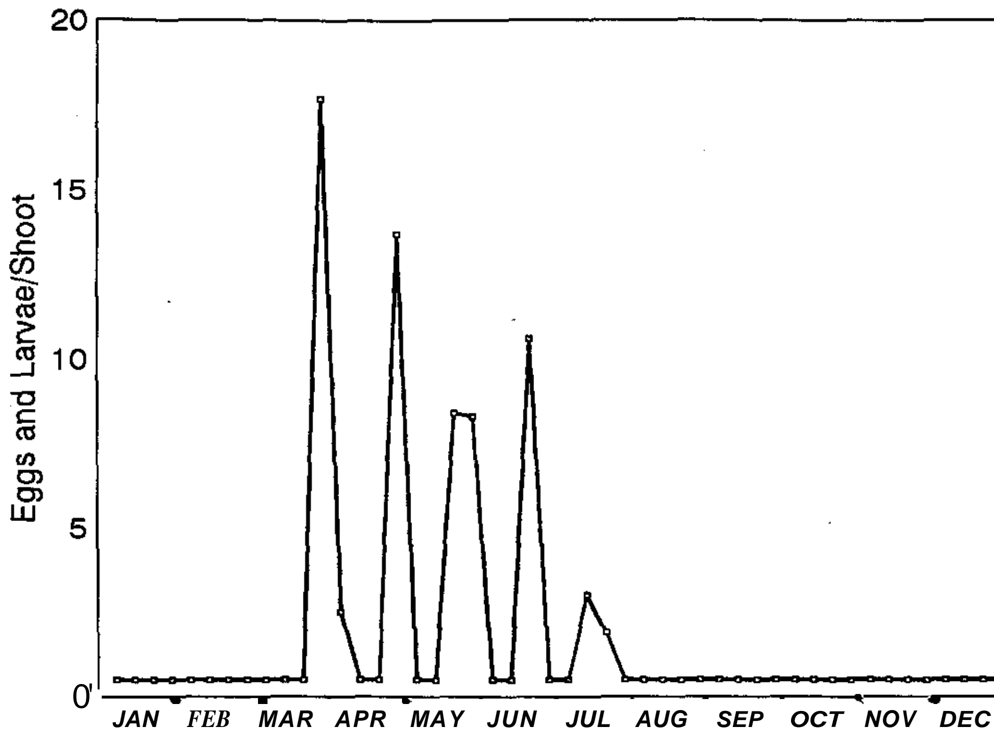


Fig. 8.1. *H. puera* population trend at Kariem-Muriem in 1993

Effect of treatment on foliage loss and population level

In the first trial (23 March 1993), 4 days after the treatment, the foliage loss was only 14% in the treated trees, compared to 46% in the untreated trees (Table 8.1). This difference was statistically significant ($P < 0.01$). It was known that no measurable damage had occurred prior to the treatment, although no damage scoring was done. The degree of protection afforded by the NPV treatment worked out to 70%. In other words, 70 per cent of the potential leaf loss mean number of insects per shoot was 17.7 in the

check trees and 16.2 in the treated trees (Table 8.2). Table 8.2 also shows the subsequent decline in the number of larvae 2 days and 4 days after the treatment. These numbers do not reflect the effect of treatment adequately.

Table 8.1. Effect of NPV spray on *H. puera* as measured by protection from defoliation in five field trials, in the year 1993.

Trial No. and date of NPV application	Mean percentage leaf loss				Percentage protection
	2 days after Treatment		4-6 days after treatment		
	Check	Control	Check	Control	
1 (23 March)	-		46[a]	14[b]	70
2 (20 April)	1[a]	15[b]	42[a]	10[b]	76
3 (17 May)	29[a]	41[a]	67[a]	38[b]	43
4 (12 June)	23[a]	31[a]	60[a]	40[b]	33
5 (9 July)	3[a]	2[a]	3[a]	2[a]	33

All the values were statistically adjusted for intital variability and rounded off to the nearest integer. The figures suuperscribed by some litter are not statistically significant at 5% level. Within each set of check and the corresponding treatment, the difference between the values followed by the same letter are not statistically significant.

In the second trial (20 April 1993), six days after the treatment, 42% foliage loss was recorded in the check trees compared to 10% in the treated trees. This difference was statistically significant ($P < 0.01$). The degree of protection obtained worked out to 76%. Most insects were in the first instar when the treatment was applied. The number of insects per shoot was significantly reduced in the treated trees when compared to the check trees two days after the treatment when most insects were in the second instar (Table 8.2). Six days after the treatment when most insects were in the fourth to fifth instar this difference was not statistically significant although the trend was evident.

the fourth to fifth instar this difference was not statistically significant although the trend was evident.

Table 8.2 Population estimates of *H. puera* (eggs and larvae) in the NPV field trials.

Trial No.	Mean number of insects per shoot per tree[1]					
	Prior to treatment		2 days after treatment		4-6 days after treatment	
	Check	Treated	Check	Treated	Check	Treated
1	17.7	16.2	6.3[a]	5.2[a]	2.8[a]	2.2[a]
2	13.7	19.8	11.0[a]	3.7[b]	3.0[a]	1.0[a]
3	8.4	20.9	8.2[a]	4.8[a]	8.0[a]	1.2[b]
4	10.8	9.7	14.1[a]	15.8[a]	3.9[a]	4.2[a]
5	3.4	10.7	2.0[a]	4.5[a]	0.5[a]	0.7[a]

[1] The figures represent the mean of 14 trees (8 shoots each) sampled on each date. The figures superscribed by some letter are not statistically significant at 5% level. Within each set of the check and the corresponding treatment, difference between the values followed by the same letter are not statistically significant.

An explanation for the lower effectiveness of the NPV spray in this trial may be found in the occurence of rainfall during this period (Table 8.3). Unlike in the previous trials, fairly heavy rainfall occurred on the first and third days of treatment. This would have caused partial washing off of the PIBs from the foliage resulting in lower effectiveness.

In this trial, an additional estimate of foliage loss was made by scoring all the individual trees in the check and treated plots on the 9th day of treatment after the feeding was completed.(It may be recalled that generally the final scoring was made on 8 shoots each from 14 sample trees, 4-6 days after the treatment. At this time most insects were

still in the 5th instar.) The mean foliage loss per tree was found to be 95% in the check plot and 74% in the treated plot, the difference between the two being similar to that obtained by scoring shoot samples.

Table 8.3. Rainfall during the experimental period.

Trial No. and Date of spraying	Rainfall (mm.) Days from the date of spraying					
	0	1	2	3	4	5
1 (23 March)	0.0	0.0	0.0	0.0	0.0	0.0
2 (20 April)	0.2	0.0	0.0	0.0	0.0	0.0
3 (17 May)	17.6	0.0	28.6	2.4	0.0	0.0
4 (12 June)	15.8	17.8	14.2	25.0	10.8	0.0
5 (9 July)	1.2	20.0	24.4	57.6	12.0	9.6

In the fourth trial (12 June 1993), when scored 4 days after the treatment, the foliage loss amounted to 60% in the check and 40% in the treated trees. Although the difference was statistically significant, here again the level of protection was low (Table 8.1). There was no significant difference in the number of insects between the check and treated trees either 2 days or 4 days after the treatment (Table 8.2).

In the fifth trial (9 July 1993) the infestation level was too low (Fig.8.1) to arrive at any meaningful conclusion on the effectiveness of treatment (Tables 8.1 and 8.2).The period was also characterised by continuous rainfall (Table 8.3).

Effect of treatment on tree growth

The basal area increment of trees in the check plot and the treated plot is given in Table 8.4. Over the year, the mean increment of basal area in the treated plot was 23.66 cm^2 per tree compared to 16.98 cm^2 per tree in the check plot. The difference was statistically significant ($P < 0.01$). The gain in basal area increment per tree due to treatment was 6.68 cm^2 which works out to 39.3%.

Table 8.4. Effect of protection afforded by NPV on growth increment of teak.

Plot	Basal area (cm^2)		
	Initial	Final	Increments
Check	231.33	250.06	16.98
Treated	185.28	207.89	23.66

[a]Adjusted for initial variability

Discussion

The results of this study show that 70-76% of the leaf damage caused by teak defoliator can be prevented by timely, one-time foliar application of NPV during each outbreak. This was achieved by using a spray concentration of $1x10^5$ PIB/ml at the rate of 0.75 to 1.75 litre per tree, depending on the foliage level, using a high volume sprayer. Compared to many other baculoviruses, the HpNPV is quick acting and causes significant mortality of larvae in about 3 days (Ali *et al,* 1991).While the present study establishes the feasibility of using HpNW for controlling the teak defoliator infestation, further refinements are necessary in the production of PIBs, its formulation and application. When heavy rain occurred during the infestation period, the NPV spray was less effective, apparently due to wash off of the N W from the foliage. Except for the use of a sticker spreader in the spray fluid, the NPV used in this study was a crude preparation. It is obvious that the effectiveness of HpNW can be increased by appropriate formulation incorporating desirable adjuvants like uv protectants, standardising the PIB concentration in the spray fluid, optimising the size of the spray droplets etc. (Young and Yearian.1986). Further research is needed to accomplish these objectives. However, since the PIBs must be ingested from the foliage where it is applied, it cannot be very effective during rains. Contact insecticides may be more effective during the rainy period. Therefore, for year round protection against the teak defoliator, an integrated management strategy need to be developed with NPV as the major component.

Among the three methods used for assessing the treatment effect, defoliation scoring is the most practicable. Although we scored the leaf damage in sampled shoots in this study, visual scoring of defoliation can be carried out for the whole tree. This is best done after the feeding is complete and the larvae have descended to the ground for pupation. Scoring of all the trees in

a plot is more dependable than scoring sample trees. Since teak defoliator infestation depends on the presence of tender foliage, and since there is considerable between-tree variation in the flushing intensity, particularly during the early season, sampling is less effecient.

Sampling of the larval population to estimate the fall-off of numbers due to treatment was less efficient than defoliation scoring because of high inter-tree variability in larval numbers, and the dispersal of larvae during the experimental period. Sampling of dead larvae is impracticable because they are easily dislodged from the trees.

Although in this study the effectiveness of NPV was also demonstrated by measurement of the growth increment of treated and untreated trees, this method can be applied only for long- term studies, In this study, despite the fact that two later applications were only partially effective due to rain, the basal area of the treated trees increased by 39% over that of untreated trees. Earlier studies (Nair *et al,* 1985) had shown that when given complete protection from teak defoliator the basal area increment was enhanced by 65% and volume by 80%.

Timely detection of infestation is a pre-requisite for successful use of NPV for control of teak defoliator outbreaks. In the present study,infestations were detected promptly by daily ground surveillance for signs of infestation during the critical periods when moths were collected in light traps. These methods need to be standardised for routine practice.

9. SCREENING COMMERCIAL PREPARATIONS OF *BACILLUS THURINGIENSIS* AGAINST *H. PUERA*

Bacillus thuringiensis has received increasing attention in recent years as a natural biocontrol agent. As early as the 1980,s. commercial products based on *B. thuringiensis* began to replace chemical pesticides for control of forest defoliators in North America (Frankenhuyzen. 1993).It is now extensively used against several forest pests including spruce budworm in Canada, gypsy moth in the USA and many other pests in Europe. Commercial products based on *B. thuringiensis* are now registered for use in India and some are produced indigenously.

Singh and Misra (1978) tested the effectiveness of a *B. thuringiensis* preparation, Thuricide against 26 species of forest insects under laboratory conditions. They reported 90% mortality of third instar larvae of the teak defoliator, when fed on teak leaves sprayed with 0.5% (30×10^9 spores/gm) of Thuricide. However, no further progress was made in the use of *B. thuringiensis* products against forest pests in India. In a recent study. Mohamed Ali. *et al.* (1991) reported presence of *B. thuringiensis* in a natural population of *H. puera* in Kerala. although the incidence rate was negligible. The use of *B. thuringiensis* against this pest, particularly In nurseries and young plantations of teak appears promising. We tested five commercial preparations of *B. thuringiensis* against *H. puera* for possible use as part of an IPM programme. All the products were tested in the laboratory and one was tested in the field.

9.1. LABORATORY TESTS

Materials and methods

Fourth instar H. puera larvae were used for the bioassay. The larvae were obtained from the laboratory culture maintained on artificial diet (Section 2.4). The test larvae were starved for two hours before the experiment.

All the five preparations tested were based on B. thuringiensis kurstaki. These included 3 WP formulations, viz.. Delfln (Sandoz (India) Ltd.). Bioasp and Biolep (Biotech International. New Delhi) and 2 liquid formulations viz., Dipel BL (Lupin Agrochemicals. Bombay) and an unnamed product (referred as TACF hereafter) of the Tuticorin Alkali Chemicals and Fertilizers Ltd.. Tuticorin. Of the above, Biolep is an asporogenous preparation.

A stock solution of 0.2% concentration of all the formulations were first prepared in distilled water from which the required concentrations were prepared by serial dilution. The concentrations tested were 0.2%, 0.05%, 0.0125% and 0.003%. The preparations were sprayed on fresh teak leaves using a hand sprayer, the leaves were air-dried and placed in sterilised bottles (5cmx8cm) into which the test larvae were released. Each test involved all the *B. thuringiensis* preparations at all concentrations with 10 larvae for each treatment, thus requiring a total of 200 larvae. For ease of handling and to ensure greater homogeneity of test larvae, only one replicate was run on each date. Three such replicates were run on three different dates and the data were pooled and analysed statistically. With each replicate a control set of 10 larvae was also maintained in which larvae were fed leaves sprayed with distilled water instead ofbacterial preparation. Larval mortality was recorded at 6, 12,18,24 and 48 hours after exposure to treated leaves. Mortality of larvae in the treated sets was adjusted for mortality in the control using Abbot's formula (Abbot, 1925). The data were subjected to probit analysis for estimating LT_{50} and LC_{50} values (Finney. 1977).

Results and Discussion

The percentage mortality of larvae at 6 hours after exposure to treated leaf is presented in Table 9.1. At the lowest concentration of 0.003% of *B. thuringiensis,* mortality ranged from 13-43% and at the highest concentration of 0.2%. it ranged from 43 to 67% depending on the product. The highest mortality of 73.3% was obtained at 0.05% concentration of bioasp.

Table 9.1. Mortality of *H. puera* larvae at 6 hours on exposure to different B.t products

Product	Percentage mortality at 6 hours				
	Concentration percentage				
	Nil	0.0003	0.0125	0.05	0.02
Control	4.0	-		-	-
Biolep		43.3	53.3	60.0	50.0
Dipel		23.3	56.6	50.0	60.0
Delfin		16.6	53.3	53.3	56.6
Bioasp		26.6	56.6	73.3	66.6
TACF	-	13.3	30.0	30.0	43.3

In general, with increase in the concentration. the percentage of larval mortality also increased. However. the dosage-mortality relationship was not consistent. For example. biolep gave 60% mortality at a concentration of 0.05%. but only 50% at 0.2% concentration. Similarly, bioasp gave a higher mortality at 0.05% in comparson to the lower mortality at 0.2%. This may have occurred due to inconsistency of feeding and/or feeding inhibition at higher concentrations of the biocide. However, such inconsistency was not noticed at or after 24 hours.

The LC_{50} values obtained for the different formulations are given in Table 9.2. Biolep showed the lowest LC_{50} value followed by Dipel. Bioasp, Delfin and TACF. The LT_{50} was minimum for Biolep and maximum for TACF (Table 9.3).

Table 9.2. LC_{50} of different B. t products evaluated against *H. puera* (6 hours after treatment)

Product	LC50	Fiducial limits	
	(gm/1000 ml)	Lower	Upper
Biolep	0.009	0.000015	5.144
Dipel	0.014	0.005	0.038
Bioasp	0.035	0.009	0.129
Delfin	0.047	0.015	0.152
TACF	0.492	0.030	8.031

Table 9.3. LT50 of different B.*t* products evaluated against ***H.*** puera (Data for 0.003% concentration)

Product	LT50	Fiducial limits	
	(hr.)	Lower	Upper
Biolep	6.60	3.60	12.05
Dipel	12.60	9.86	16.14
Delfin	15.03	10.78	20.89
Bioasp	15.04	7.93	28.51
TACF	24.38	19.41	30.62

Since immediate action of a biocide offer maximum leaf protection. the fastest acting product would be the most useful under practical conditions. However, LC_{50} is another important factor for selecting a product, considering the cost. Taking both LT_{50} and LC_{50} into consideration, Biolep is the most effective as it has the lowest values for both, ie., it provides fastest kill at the lowest concentration. Next to Biolep, Dipel was the most effective. TACF was the least effective, with others falling in between.

Bioasp which is an asporogenous product was third in effectiveness with respect to LC_{50} and fourth with respect to LT_{50} and Delfin was vice versa. An asporogenous preparation will not be perpetuated in the environment because it does not produce spores. Bioasp therefore has advantages in terms of safety to beneficial insects like the silkworm.

B. thuringiensis is one of the microbial pesticides which has shown much promise against agricultural pests. Once ingested, the larvae cease to feed within a few minutes and subsequent damage to the foliage is minimised. Thus *B. thuringiensis* can be regarded as a useful control agent for use in an IPM programme against the teak defoliator.

9.2. FIELD TRIAL

The asporogenous preparation of *B. thuringiensis kurstaki.,* namely Bioasp was selected for the field trial due to its safety. As the product does not contain viable spores, there will be no secondary build up of the pathogen in the sprayed area. This is important since *B. thuringiensis* is known to have a wide host spectrum including beneficial insects like the silkworm.

Methods

The trial was carried out in a 17-year old teak plantation at Poochakkuthu. in Nilambur. Two plots of 20 trees each with a buffer zone in between were selected. One of the plots was sprayed with 0.1% *B. thuringiensis kurstaki* in water using a rocker sprayer. A total of 60 liters of spray fluid was needed to spray 20 trees. The control plot was left untreated.

The efficacy of treatment was evaluated in three ways.

1. Larval sampling

The number of larvae present in the experimental plots were estimated prior to the spraying of *B. thuringiensis kurstaki* and twice at an interval of 24 hours after the

spraying. For each sampling, eight shoots were removed from each tree, 4 from top and 4 from bottom levels. The larvae present on the shoots were counted and recorded. Similar larval counting was also carried out in the control plot.

2. *Estimation of leaf loss*

Leaf loss in the experimental plots was estimated at specific time intervals. The percentage of leaf loss in the sampled shoots was visually scored and the mean percentage leaf loss per plot was estimated.

3. *Estimation of leaf loss at the end of the experiment*

Foliage loss of all the trees in the experimental plots were visually scored when all the live larvae present completed their feeding and pupated.

Results and Discussion

The number of healthy larvae in the treated and control plots during the sampling period are given in Table 9.4. In both the treated and control plots. larvae were predominantly in the fourth instar. Fifth and third instar larvae were also present in small numbers. During the first 24 hours after spraying, the larval number was reduced by 82.12% of the initial number in the treated plot while there was a reduction of 2.09% in the control plot. After 48 hours the reduction was 95.97% and 59.19% in the treated and control plots respectively. On both the days the reduction in larval number in the treated plot was statistically significant (P<.01).

Table 9.4. Number of healthy larvae during sampling

Plot	Time after spraying		
	00 hrs.	24 hrs.	48 hrs.
Control	1196	1171	488
Treated	2187	391	88

The percentage leaf loss estimated from the sampled shoots are given in Table 9.5. leaf loss of 60.79% in the treated plot and 72.35% in the control plot had occurred prior to the spray application. It can be seen that there was no increase in foliar damage in the treated plot after spraying. In the control plot the damage per

Table 9.5. Percentage leaf loss per shoot (mean of 96 shoots)

Plot	Time after spraying		
	00 hrs.	24 hrs.	48 hrs.
Control	72.35	83.82	89.39
Treated	60.79	56.37	57.61

increased to about 84% and 89% after 24 hr and 48 hr respectively. Between the plots there was a statistically significant (P<.01) difference in leaf loss.

The leaf loss estimated 4 days after treatment by visual scoring after all the insects had completed feeding showed that an average of 52.5% foliage was remaining in the treated plot while only 3.75% was left in the control plot. The results indicate that Bioasp is effective in controlling field populations of the teak defoliator. There was significant reduction in larval number and more significantly, there was very little foliage loss subsequent to spraying. This indicates fast kill of the pest.

10. GENERAL DISCUSSION AND CONCLUSIONS ON THE USE OF HpNPV FOR MANAGEMENT OF THE TEAK DEFOLIATOR

The main purpose of this study was to evaluate the usefulness of the naturally occurring Nuclear Polyhedrosis Virus of *H. puera* (HpNPV) for applied control of the pest. Various aspects pertinent to this central issue were investigated in this study.

The HpNPV reported first by the KFRl team (Sudheendrakumar, *et al.*. 1988) is a DNA virus belonging to the family Baculoviridae. Baculoviruses, reported from insects and a few other arthropods are not known to occur in other animal orders and are therefore considered to be very safe biocontrol agents (Evans. 1986).In addition, they have a very restricted host range and therefore pose no threat to non-target organisms, unlike other microbial control agents such as *Bacillus thuringiensis.* Earlier, scanning electron microscopic photograph of the organism and the characteristics of the disease had indicated the identity of the organism as NPV (Sudheendrakumar *et al.*. 1988).In this study, electrophoretic analysis of the viral DNA using restriction endonucleases and SDS-PAGE analysis of the polyhedrin protein showed its similarity to the NPV of other insects and confirmed the identity of the organism as baculovirus. Transmission electron microscopic studies need to be carried out to determine the structure of HpNPV - to determine whether it is single embedded or multiple embedded. Cross infectivity studies also established its high specificity. The HpNPV does not affect the teak skeletonizer *Eutectona machaeralis,* the *Ailanthus* pests *Eligma narcissus* and *Atteva fabriciella.* the silk worm *Bombyx mori* nor the agricultural pests *Helicoverpa armigera, Spodoptera* litura and *Amsacta albistriga* These studies confirm the safety of HpNPV for applied biocontrol of the teak defoliator.

Epizootiological studies (section 5) yielded very valuable information of practical value. It was shown that HpNPV has little environmental persistence in teak plantations. When applied on to the foliage the infectivity did not persist even for a week. Although, baculoviruses are credited to persist in soil for long period without loosing viability and inoculative release has been advocated for applied biocontrol. particularly in the forest ecosystem (Entwistle. 19861 there was no evidence to indicate persistence of heavy doses of NPV polyhedra from one year to

the next to enhance the incidence of disease. Thus the use of inoculative release of HpNPV for control of the pest or even prophylactic application of the virus can be ruled out. Disease transmission studies clearly established intra-host persistence of the virus and transovarian transmission from parental generation upto the second fllial generation under controlled laboratory conditions. This is consistent with the poor environmental persistence and the host population density-related triggering of epizootics under field conditions. These results indicate that it will not be possible to induce disease epizootic under field conditions by enhancing the inoculum load in the environment artificially. Inundative spray of polyhedra during the course of an infestation appears to be the only option. The study also showed (section 8) that 70 to 76% of the foliage loss caused by the pest can be prevented by timely one- time foliar applications of HpNPV during each pest outbreak. This was achieved by using a high volume spray of 0.75 to 1.75 litre of the spray fluid per tree, containing 1×10^5 PIB/ml. While the present study establishes the feasibility of using HpNPV for protection of teak plantaticns from the attack of ***H. puera.*** further refinements are necessary for its effective use in the field. These relate to appropriate formulation of the NPV to enhance protection from UV-inactivation and rainfastedness. determining instar-specific dosage-mortality relationships, and improved spray delivery technology. particularly the use of low-volume or ultra low volume spraying methods.

In this study, we also developed a unique method for mass production of HpNPV which makes use of the cheap availability of large number of larvae during the natural pest outbreaks in teak plantations. The larvae can also be reared in the laboratory in an artificialdiet we developed earlier. The merits and demerits of the two mass production systems are discussed in Section 6. Further refinements are necessary in both, but the present studies establish the feasibility of mass production systems. A combination of the two systems of production appears to be the most suited to take advantage of the merits of both.

An important factor which affects the success of field operations for control is our ability to apply the NPV at the right time during the course of pest outbreaks. While this is important for all insects, it assumes special significance in the case of the teak defoliator because of its unique population dynamics. During the early stages of outbreak, the infestations are discrete, but discontinuous in space, within a large plantation area. The present study has established a monitoring system which makes use of (1) a light trap for adult moths to signal the initiation of outbreaks and (2) a ground surveillance system for detection of early instar larvae on the

foliage. A new solar-powered light trap was designed and developed for use in remote forest areas where electricity is not available. The studies also showed that early outbreaks tend to occur in certain areas repeatedly over the years, indicating a relationship with geographic features in the mountainous teak plantation areas. Such insights have sharpened our ability to predict infestation-prone areas where control efforts need to be concentrated.

Based on the results of this study the stage is now ripe for establishing a pilot production facility for HpNPV, which will also give an opportunity to refine the methods and technologies indicated above for its successful field application. Towards this end, attention needs to be focused on the following.

Successful use of NPV under field conditions by the forest departments/private enterprises will require the availability of a formulated product. Research is needed to identify suitable additives, adjuvants, UV protectants etc, for developing a commercial formulation. The formulated product then needs to be tested for safety to non-target organisms.

While the commercially available product can be used straight away for protecting young plantations of teak by using conventional spray technology, it will be advantageous to standardise appropriate spray application technology to reduce the spray volume and to increase target specific delivery. Efforts are also needed to identify or develop suitable spraying mechanisms to reach taller trees.

When the NPV is produced on a mass scale we must ensure that the virus remains unchanged and free of any contamination. DNA fingerprinting can be used as a quality control mechanism during the production process.

It is clear from as indicated above that further developmental work is needed before the management of the teak defoliator using baculovirus becomes a reality.

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