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Studies on controlling the teak defoliator outbreaks by seeding the baculovirus, HpNPV in epicenter populations

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ABSTRACT

The *Hyblaea puera* nucleopolyhedrovirus (HpNPV) is an ideal biocontrol agent for management of the teak defoliator, *H. puera* because of its host specificity, virulence and eco-friendly nature. However, application of HpNPV in extensive teak plantations is quite difficult owing to the rugged terrain of the plantation and height of the trees. The project was undertaken in the above context to develop a landscape level teak defoliator management strategy using the virus combining the knowledge on the population dynamics of the insect and the vertical transmission characteristics of the pathogen.

Vertical transmission of HpNPV (parent to offspring transmission) influencing different biological characters of the host was parameterized using a 76.81 kbp isolate of HpNPV under laboratory conditions. Infection of the fifth instar larva with a sub lethal dose of one hundred inclusion bodies of the virus revealed reduction in the survival of the larvae (20-40%), pupation (28%), adult emergence (27-66%), fecundity (50-78%), egg laying period (2 days), hatchability of the eggs (40%) but no change in the sex ratio. The reduction in reproductive potency due to vertically transmitted HpNPV from the parent to F1 generation inflicted collapse of the population in the F2 generation. This was further supported by sublethal dosing of HpNPV in a natural epicentre population in the Kariem-Muriem teak plantations, Nilambur, Kerala during March 2008 which also vertical transmission of HpNPV.

The trials on probable resistance of *H. puera* larvae to sublethal virus infection showed that the successive offspring generations were more susceptible to virus infection thereby ruling out the possibility of such a phenomenon in *H. puera*.

The results of this study indicated that one time low dose application of HpNPV during the epicentre phase of the teak defoliator population could contribute to the reduction in the insect population not only in the parent population but also in the F1 generation. This method of HpNPV application in the teak defoliator epicentres may be practiced for management of the teak defoliator at landscape level.

1. INTRODUCTION

1.1. HOST PLANT – *Tectona grandis*

The teak, *Tectona grandis* is one of most widely cultivated tropical timber tree has its distribution spread across the tropical and subtropical regions in the Asian, African and American continents as well as many islands in the Pacific and Atlantic oceans (Nair, 2007). It is the most widely planted tree species in India with major plantations spread out in the states of Kerala, TamilNadu, Karnataka, Madhyapradesh, Maharashtra, Gujarat, Rajasthan, Manipur and parts of Uttaranchal and Orissa. Kerala is credited for establishing the first teak plantation in the country during 1840 in Nilambur and has now about 76,202 ha of forest under teak. Apart from this teak is also grown under private sector though not extensive.

1.2. PEST INSECT – *HYBLAEA PUERA* (CRAMER)

The teak defoliator was first recognized as a pest of teak by Bourdillon (1898) who was then the British Forester of the Konni Forest Division (Kerala). The life history of this insect has been studied in some detail in India and Burma (Stebbing, 1908a) and neatly summarized by Beeson (1941). *H. puera* moths are comparatively small, with a wingspan of 3 to 4 cm, and have a characteristic resting posture that conceals the black-and-orange-yellow hindwings under the grayish-brown forewings. Normally the lifecycle of *H. puera* is completed in 3 to 4 weeks with five larval instars and the exact length being determined primarily by climatic conditions, particularly temperature. The neonate larva eats a shallow depression on the surface of the tender leaf and protects itself with strands of silk. The first and second instar feed mainly on the leaf surface. Starting with the third instar, the larva cuts out a leaf flap, folds it over, fastens it with silk, and feeds from within. The larvae consume the entire leaf leaving only the major veins of the tender leaf. Under normal conditions, leaf-feeding stage lasts 10 to 12 days and total developmental period contains 19 to 36 days (Nair, 1988; Sudheendrakumar, 1994, 2003).

Economic impact

Several attempts have been made in the past to quantify the economic impact of the teak defoliator. Defoliation by this insect pest does not kill teak trees, but assumed to cause a heavy loss in volume increment. During 1930's rough estimates based on several assumptions indicated

the loss at 6 to 65 percentage of the potential volume increment in teak plantations (Mackenzie, 1921; Beeson, 1928; Minchin, 1929). However, in 1941, based on fewer assumptions Beeson estimated a 13 per cent loss. Based on a 5-year experimental study in 4 to 8 year old teak plantations at Nilambur, Kerala, Nair *et al.* (1985) showed that natural defoliation by *H. puera* could result in a loss of about 44 percentage of the potential volume increment. This study unequivocally established the economic usefulness of control of the teak defoliator. Data from the permanent teak plots maintained at Nilambur to demonstrate the effect of protecting teak plantations against the teak defoliator indicated substantial gain in the height and girth in trees protected against teak defoliator (Varma *et al*., 1998). Referring to the current pest problems in native as well as exotic teak plantations, Nair (2003) reported that the teak defoliator is the widespread and the most serious pest of teak.

Control options

A range of control methods was suggested in the past by various workers to manage the teak defoliator. Extensive surveys have been made to gather information on the interrelationship between the teak pests, their natural enemies and other caterpillar hosts of the natural enemies (Stebbing, 1908ab; Beeson and Chatterjee, 1935abc). Beeson and Chatterjee (1939) published a brief account of the parasites of *H. puera* and *Eutectona machaeralis* recorded from Nilambur during 1937 and 1938. Among the parasites of *H. puera,* the tachinid fly *Palexorista solennis* Wlk. was the dominant species during the year 1937 with respect to parasitic efficiency (8 to 59 per cent). However, in 1938 ichneumonid *Eriborus gardeneri* Cush. was registered as the dominant species (30 per cent). Attempts were made by Forest Research Institute, Dehra Dun to use parasitoids as biological control agents against *H. puera* (Beeson and Chatterjee, 1939). *Trichogrammatoidea* sp. (Hymenoptera: Trichogrammatidae) and *Apanteles maleavolus* Wlkn. (Hymenoptera: Braconidae) were released against *H. puera* at Nilambur during 1938. In the same period, attempts were made to breed the braconid parasite, *A. machaeralis* Wlkn. and the tachinid, *Bessa remota* Alder. obtained from Burma on *H. puera* larvae. However, this was not successful as majority of the host larvae died due to some disease. None of the above biocontrol studies was later repeated or continued.

Based on information on the abundance and host range of the insect parasites associated with *H. puera* and *E. machaeralis,* a scheme for the biological control of the teak pests using silvicultural

measures was suggested to augment the efficacy of natural enemies by maintaining natural enemy reserves (Beeson, 1941). The suggestions included subdivision of the planting area into blocks of 8 to 16 hectare and maintaining strips of existing forests in between as natural enemy reserves, improvement of these reserves by promoting desirable plant species and eliminating undesirable ones and maintaining a varied flora of desirable species under teak canopy. Desirable plants are those, which support alternate hosts of the parasites of teak pests, and undesirable ones are those which serve as alternate hosts of the teak pests themselves. Another proposal made in the scheme was the introduction of selected species of natural enemies in localities, where there is a deficiency in the natural enemy complex. This scheme of biological control was not tested experimentally or practiced because of various practical difficulties. During 1942 to 43, Khan and Chatterjee (1944) studied the role of undergrowth in teak plantations as a factor encouraging parasites of *H. puera*, but no conclusive result was reported in this study.

Chemical insecticides were field-tested against the teak defoliator first in 1965 in Konni Forest Division wherein, 76 ha of Government teak plantations were aerially sprayed with Endrin (Basu-Chowdhury, 1971). Again, in 1978, an experimental spraying of chemicals was undertaken by Madhya Pradesh State Forest Development Corporation in Barnawapara Project Plantation (Singh *et al*., 1978). Malathion, Carbaryl and Fenitrothion were sprayed from air as ULV and LV sprays. Post spraying evaluation of pest populations showed very high kills. However, the practice of aerial spraying of chemical insecticides was discontinued due to obvious reasons.

The possibility of using “tree resistance” as a tool to manage teak defoliator had also been examined in the past. Mukhtar-Ahmad (1987), evaluated 20 clones of teak from Kerala, Tamil Nadu and Andhra Pradesh for their natural variation in susceptibility to teak defoliator and reported one clone (TNT 6) resistant to the teak defoliator and one clone showing superior growth (KLK 2) and suggested development of a hybrid using TNT 6 and KLK 2. Based on studies carried out in Kerala, Nair *et al*. (1997) reported that there is little scope for breeding for *H. puera* resistance in teak as the commonly observed escape of some trees from the teak defoliator attack was not due to genetic resistance, but due to phenological resistance.

The past two decades were exemplified by a renewed interest in the role of biological control agents in combating the teak defoliator which would be economically as well as ecologically acceptable. Based on the studies on the parasitoids of teak defoliator at Nilambur, Sudheendrakumar (1986, 1990) reported 4 species of larval parasitoids namely, *Palexorista solennis* Walk. (Diptera: Tachinidae), *Sympiesis hyblaeae* Surekha, *Eriborus gardeneri* Cush. (Hymenoptera: Ichneumonidae) and *Stictopisthus* sp. (Hymenoptera: Ichneumonidae) from Nilambur and found that, the percentage of parasitism caused by those indigenous parasitoids was very low. Nair *et al*. (1995) observed that the indigenous parasitoids of *H. puera* were unable to respond numerically to host density increase due to the high mobile nature of the host population. Massive aggregation of outbreak populations and shifting foci of infestations would facilitate the successive generations of the insect to escape from the parasitoid populations built up during the previous generation.

Based on a survey carried out in teak plantations in Nilambur on entomopathogens of forest insects Sudheendrakumar *et al*. (1988) reported a disease on *H. puera* caused by a Nucleopolyhedrovirus. Preliminary pathogenecity trials indicated that the virus was highly infective to its host.

1.3. PATHOGEN - THE *HYBLAEA PUERA* NPV (HPNPV)

Investigations on the spectacular nature of the epizootics in *H. puera* population in teak plantations at Nilambur, Kerala, revealed that a Nucleopolyhedrovirus (HpNPV) is the causative (Sudheendrakumar *et al*., 1988). Based on a weekly sampling carried out in teak plantations in Nilambur, Parambikulam, Ranni, Konni, Punalur, Malayattoor, Trichur, and Trivandrum Forest Divisions, Mohamed-Ali *et al*. (1991) reported that HPNPV infection was prevalent in all the teak plantations visited.

Bioassays carried out by Mohamed-Ali *et al*. (1991) against third and fourth instar *H. puera* larvae revealed that the virus is highly pathogenic. The estimated median lethal concentration and the median lethal dose values were 796 POBs per ml and 1,427 POBs per larva respectively. Nearly 35 per cent infectivity was lost due to exposure to UV light of *ca*. 250 nm for 15 min at 10 cm distance from the UV source. Heating the virus at 40 °C for seven days did not markedly reduce infectivity. Less than 10 per cent inactivation occurred when virus was held at pH 4 and

9.2 for 6 h. There was no cross infectivity to larvae of *Eutechtona machaeralis*, *Eligma narcissus* and *Atteva fabriciella*. A fourth instar larva yielded a mean of 3.8 x 10^8 POBs and the productivity ratio was 5,530.

In another study on HpNPV, Nair *et al*. (1998) addressed some of the aspects including disease transmission, epizootiology and field efficacy etc. These studies established the safety of the virus for field application. Disease transmission studies indicated intra-host persistence of the virus and transovarian transmission from parental generation up to the second filial generation under controlled laboratory conditions. Preliminary field studies were carried out in a plot with 100 trees at Nilambur in the year 1993, for HpNPV against the teak defoliator (Nair *et al*., 1996). During the year, there were four major peaks of defoliator infestation from March to June. One-time foliar application of a crude preparation of HpNPV at the rate of 10^5 POBs per ml of the spray fluid, at the earliest sign of each infestation, gave 70 to 76 per cent protection of foliage during the first two infestations. A reduced foliage protection of 33 to 43 per cent obtained during the third and fourth infestations was attributable to occurrence of rain soon after application of the spray. In protected trees a basal area increment was enhanced by 41 per cent, indicating the efficacy of HpNPV as a biocontrol agent against teak defoliator (Nair *et al*., 1996). Field trials revealed that HpNPV has little environmental persistence and does not persist even for a week when applied onto teak foliage. Although baculoviruses are credited to persist in forest soil for long periods and inoculative release has been advocated in the forest ecosystem, there was no evidence to indicate persistence of heavy doses of HpNPV polyhedra for one year to the next.

During 1992 - 1995, Nair *et al.* (1998) made attempts to standardize methods for mass production of HpNPV. Since large number of larvae could be collected from teak plantations during natural pest outbreaks, methods were developed to mass-produce the NPV using wild larvae. A large number of third instar larvae were collected along with the infested leaves and transported to the laboratory in large cloth bags. In the laboratory, the larvae were transferred into fresh leaves sprayed with HpNPV inoculum and maintained in large plastic buckets and cages (1 m x 1 m x 1 m). The dead larvae were harvested from the fourth day onwards and allowed to putrefy for one week in distilled water in conical flasks. The putrefied larvae were then macerated, filtered and sedimented. The sedimented OBs were separated out and stored as

stock suspension. But the high percentage of parasitism and bacterial contamination encountered became major drawbacks in the virus mass production programme. To overcome these draw backs, attempts were made to produce NPV from laboratory maintained *H. puera* larvae. Fourth instar larvae were used for virus multiplication and from inoculation onwards the procedure was same as described above. The laboratory-reared larvae were found to be high yielding than field collected ones (7.5×10^7 POBs per larva against 4×10^6 POBs per larva) and the risk of parasitism as well as bacterial contamination was very low but all those were at the cost of hiked expense. Ahmed (1995) observed an average POB yield per larva of 1.97×10^9 POBs at death. During the same period, attempts were made to characterize the HpNPV. Scanning electron micrographic studies revealed the size of the HpNPV polyhedra as 9 μm. Restriction Endonuclease analysis and SDS-PAGE analysis were carried out and confirmed the identity of the virus (Nair *et al*., 1998). Rabindra *et al*. (1997) carried out tests for cross-infectivity of HpNPV against three agricultural pests - *Helicoverpa armigera* (Hub.), *Spodoptera litura* (Fab.) and *Amsacta albistriga* (Wlk.) and the silkworm *Bombyx mori* (L.). The results showed that HpNPV was not cross-infective to any of these larvae tested.

Sudheendrakumar *et al.* (2001) reported the interaction of five primary variables- the host (*H. puera*), the virus (HpNPV), environmental factors, host tree (teak) and spray technology within a control window concept proposed by Evans (1994). The control window is a conceptual model that provides a means of bringing these primary variables together quantitatively to provide a framework for determining optimal dosages of microbial insecticides. Information on host biology including larval feeding habits and larval distribution patterns on host tree were generated. Following a series of ranging bioassays, dosage mortality relationships for larval stages were found out. Virus productivity relationships for larval stages were assessed for semi-purified virus. As part of the above study, experiments were carried out to understand the effect of sunlight on virus persistence and activity of HpNPV on foliage. The observations on virus decay indicated that sunlight has a striking effect on virus survival as mortality dropped from initial 97 per cent to 70 per cent in 6 h. The rate of decay continued up to about 24 h, after which it was stabilized at 13 per cent mortality up to 12 days. When subjected to artificial rainfall (200 mm per h) there was rapid loss of virus activity and the mortality rate dropped from 90 per cent to 15 per cent. No effect of either leaf surface or leaf volatiles on HpNPV was observed. Studies on virus application system indicated that ground based ULV sprayer Stihl SR 400 is effective

for trees up to 14 m height. A theoretical model for dosage estimation was predicted and the parameters predicted by the model were field-tested in 1997 and 1998 and the optimal dosage rates to achieve >95 per cent mortality of the target larvae were calculated.

Various methods of mass production of HpNPV were tried and finally a combination of individual larval feeding on virus contaminated leaf, followed by rearing on semisynthetic diet gave a better yield with minimal bacterial contamination. The mean yield achieved was approximately 2 x 10^8 POBs per larva at dosage 5 x 10^5 POBs per larva for semipurified virus. When compared to some other Baculoviruses produced in homologous hosts (*Helicoverpa zea* - 10^5 (Shieh, 1989); *Mamestra brassicae* - 2.2 x 10^3 POBs (Kelly and Entwistle, 1988); *Spodoptera exigua* -1.2 x 10^6 POBs (Smits and Vlak, 1988) the productivity ratio of 4 x 10^2 POBs for HpNPV was significantly low.

1.4. SUBLETHAL DOSE EFFECT

It is known that larvae getting sublethal NPV infection are able to complete their development and carry the disease to future generations either mostly by external contamination (Murray and Elkinton 1989, Murray and Elkinton 1990), as a low level, persistent infection (Kukan and Myers 1997) or in an inactive, latent form (Hughes et al. 1993). The sublethal effects may range from deformed pupae to lower development, lower weight, reduced reproduction, and shorter life span (Goulson and Cory, 1995; Rothman and Myers 1996; Peng, et al., 1997; Milks et al., 1998; Myers et al., 2000; Monobrullah and Shankar, 2008). A number of studies have shown that pupae that have survived exposure to NPV as larvae are smaller than untreated controls and the moths emerging from these pupae have reduced fecundity (Rothman and Myers 1996). Similarly, several studies have demonstrated that overt infection can be transmitted from parent to progeny (Kukan 1999, Kukan and Myers 1999), although the source of this infection is not clear. Shapiro and Robertson (1987) found persistence of NPV in adults and pupae of individuals surviving inoculation with virus as second instars. They also found that moths that survived exposure to virus at levels that killed 80% of the infected larvae had reduced fecundity, and vertically transmitted virus to offspring at a relatively high level (4.7–11.5%). However, Murray and Elkinton (1989) found that adults surviving virus challenge only transmitted virus to progeny at

very low levels (2.0%) and found no difference in pupal size or fecundity for individuals surviving exposure to virus as fourth instars (Murray et al. 1991).

Sublethal infection could influence the fecundity of moths by reducing the amount of fat and other nutrients available for egg or sperm production or through some associated cost of resistance mechanisms either when initially fighting off infection or in maintaining it at a nonlethal level (Myers and Kukan 1995). However, sublethal infection could also influence fecundity through an interaction with the hormonal balance of the individual during development (Myers et al, 2000). Infection of gypsy moth with NPV slows larval growth and inhibits larval molting and pupation (Burand and Park 1992). Most insect baculoviruses contain a gene (*egt*) that encodes the enzyme ecdysteroid UDP-glucosyl transferase which catalyzes the sugar conjugation of ecdysteroids (O'Reilly and Miller 1990). Fifth instar gypsy moth infected with NPV do not show the increase in ecdysteroid titer before pupation that occurs in control caterpillars (Park et al. 1993).

The sublethal effects also is suspected to arise the problem of development of insect resistance against viruses. Resistance to viruses has been observed in various insects like *S. frugiperda* (Fuxa *et al*., 1988), *T.ni.* (Milks and Myers, 2000; Milks et al., 2002), *M. californicum pluviale* (Dyar) (Rothman and Myers, 1996), *P. operculella* (Sporleder et al., 2007)

1.5. TRANSMISSION

One of the important characteristics of NPV is its ability to transmit itself horizontally and vertically. In horizontal transfer, pathogens are transmitted among individual hosts within a generation and between generations as environmental contamination, while vertical transmission occurs from parents to offspring (Andreadis, 1987). However, in field populations of insects, transmission of pathogens probably consists of a combination of horizontal transmission and vertical transfer (Fine, 1984). Such details of transmission, as whether the pathogen is transferred from host to host, survives in the environment, or is passed through the life stages of the insect, could influence the effectiveness of pathogens in control programs for forest or agricultural pests.

Vertical transmission

Transmission through adult is a fascinating adaption to long range environmental transport and possibly to transport in difficult local situations (e.g., trees) to initiate foci of infection (Fuxa, 2004). The levels of vertical transmission in lepidopteran species reported by earlier workers include 38-48% in *S. littoralis* (Abdul Nasr, et al., 1979), 5-12% in *L. dispar* (Shapiro and Robertson, 1987), 4-14% in *S. frugiperda* (Fuxa and Richter, 1991, 1993) and 10-15% in *T. ni*. (Fuxa, et al., 2000). Several studies have demonstrated that overt infection can be transmitted from parent to progeny (Kukan 1999, Kukan and Myers 1999), although the source of this infection is not clear. The vertical transmission may have evolved as a major means of long-distance transport in NPVs (Fuxa and Richter, 1991, 1993), as documented in the *S. frugiperda* and *A. gemmatalis* NPV systems and such a transport can also explain how NPVs initiate foci of infection in virgin areas. Studies on mechanism of transgenerational transmission of nucleopolyhedrovirus in *Lymantria dispar* L. in Western Siberia were carried out both in laboratory and field and found that occult virus can provide an important route of transgenerational NPV transmission, particularly in Western Siberia where gypsy moths migrate by female flight and can move away from trees contaminated by virus from previous larval infections (Ilyinykh et al., 2004).

Vertical transmission or passage from parent to offspring can take place by either transovum or transovarial routes. In the latter case, the virus is in a non-infective and non-replicate state in the host without causing overt disease but it can be transformed to a replicate and infective state when the host is stressed (Fuxa et al., 1992). Vertical transmission of *Neodiprion sertifer* nuclear polyhedrosis virus was studied in samples of sawfly populations and in transmission tests with and without stressors have shown that exposure of larvae to extreme temperatures, dry foliage, and chemical stressors caused no activation of latent infections (Olofsson, 1989). In the laboratory culture of *T. ni* larvae, prevalence rates of cytoplasmic polyhedrosis, nuclear polyhedrosis, and the late-instar disease were significantly greater at 95–100% relative humidity (RH) than at RH levels of 75% or below. These same three diseases killed significantly more insects in crowded rearing conditions (four or five larvae per cup) than in uncrowded conditions (one to three larvae per cup) (Fuxa et al, 1999).

While evaluating transovum transmission efficiencies of multiply embedded form of *A. californica* nucleopolyhedrovirus (AcMNPV) and singly embedded form of *H. zea* nucleopolyhedrovirus (HzSNPV) in tobacco budworm, *H. virescens* (F.), presence of abundant polyhedra on the chorion of eggs revealed by scanning electron microscopy was observed when females indirectly contaminated with virus. (Nordin et al., 1990). Persistent low levels of infection found in caterpillars reared from surface-decontaminated eggs contributed to the persistence of virus in low-density populations (Kukan, 1999). Transovarial and venereal transmission were proved in the case of BmNPV infected insects as the mating of BmNPV infected females with BmNPV uninfected males resulted in significant reduction in fecundity ($P < 0:01$) and hatching of eggs ($P < 0:001$) due to transovarial transmission of BmNPV and mating tests of uninfected females and infected males confirmed venereal transmission as there was a significant reduction in hatching of eggs ($P < 0:01$). Further, among the F1 hybrid offspring (infected female -uninfected male) that were infected transovarially, larval progeny died at first and second instar stages, whereas those infected venereally developed acute lethal infection late and died by the end of third and fourth instar stage (Khurad, et al., 2004). Venereal transmission of deformed wing virus (DWV) and subsequent vertical transmission of the virus to the progeny of DWV infected queens of honey bees was demonstrated (Yue et al., 2006; Chen et al., 2006; de Miranda and Fries, 2008). However no evidence was found for venereal or transovum (including transovarial) transmission of the parasite *Nosema* sp when larvae of gypsy moth, *Lymantria dispar* was infected (Goertz et al, 2007).

Horizontal transmission

In horizontal transfer, pathogens are transmitted among individual hosts within a generation and between generations as environmental contamination (Andreadis, 1987). Transmission depends on the interactions between infected and susceptible individuals, and the rate at which contacts result in new infections. This density dependence is more evident in pathogens that are transmitted horizontally rather than vertically (Andreadis, 1987) and it was supported by mathematical models (Brown, 1987). The effects of stage structure and host density on baculovirus horizontal transmission were examined in the laboratory using larvae of the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae). The insects were reared under different instar combinations which showed a greater risk of infection when late instar combinations were

used (Vasconcelos, et al., 2002). Studies on gypsy moth showed a non linear transmission of LdMNPV suggesting that spatial clumping and heterogeneity in behaviours such as feeding rate or the ability to avoid pathogen influenced the horizontal transmission (D'amico et al., 2005). The rate of horizontal transmission of HaSNPV variants in caged field plot experiments were found higher when higher instars were used as infectors and high density infectors were used (Zhou et al., 2005). In gypsy moth, *Lymantria dispar* L. (Lepidoptera, Lymantriidae), exclusion of feces from the rearing cages resulted in a 58% decrease in horizontal transmission (Goertz et al., 2007). Evaluation of the quantity and infectivity of MdSGHV released by individual infected house flies clearly showed that deposition of oral secretions and excreta onto a shared food substrate is the main route of natural MdSGHV transmission among adult house flies as revealed by the transmission electron micrographs of crops from infected flies (Lietze, 2009). Large concentration of virus (Hz-2V) particles at the terminal abdominal segment of infected of *Helicoverpa zea* female moths suggested that it may serve as a source of virus that can be transmitted horizontally between moths during mating. (Burand et al., 2004). Two alternative routes for nucleopolyhedrovirus transmission were investigated using *Mamestra brassicae* larvae: cannibalism of infected larvae and the release of virus prior to the death of the diseased host. The low frequency of cannibalism observed suggested that this is not a relevant transmission route while larvae were shown to transfer viable virus to the environment before death through either defecation or regurgitation (Vasconcelos, 1996).

The transmission characteristic of HpNPV has not been studied in detail. It is expected that such information will be useful in fine-tuning the application protocol of the virus in the biocontrol of the teak defoliator. The present study was undertaken with the following objectives:

1. Modeling of horizontal and vertical transmission dynamics of HpNPV.
2. Seeding of epicentre populations using formulated HpNPV and monitoring of NPV disease incidence in outbreak populations.

2. GENERAL MATERIALS AND METHODS

2.1. STUDY AREA

The laboratory experiments were carried out in the Entomology Laboratory of the Kerala Forest Research Institute (KFRI) Subcentre at Nilambur. The field studies were carried out in teak plantations coming under Nilambur North and South Forest Divisions [$11^0$10' N and $11^0$25' N & $76^0$10' E and $76^0$25' E (Figure 2.1)]. The teak plantations at Kariem-Muriem, Vazhikadavu Forest Range under North Nilambur division was selected for carrying out the sublethal application of HpNPV in the teak defoliator population.

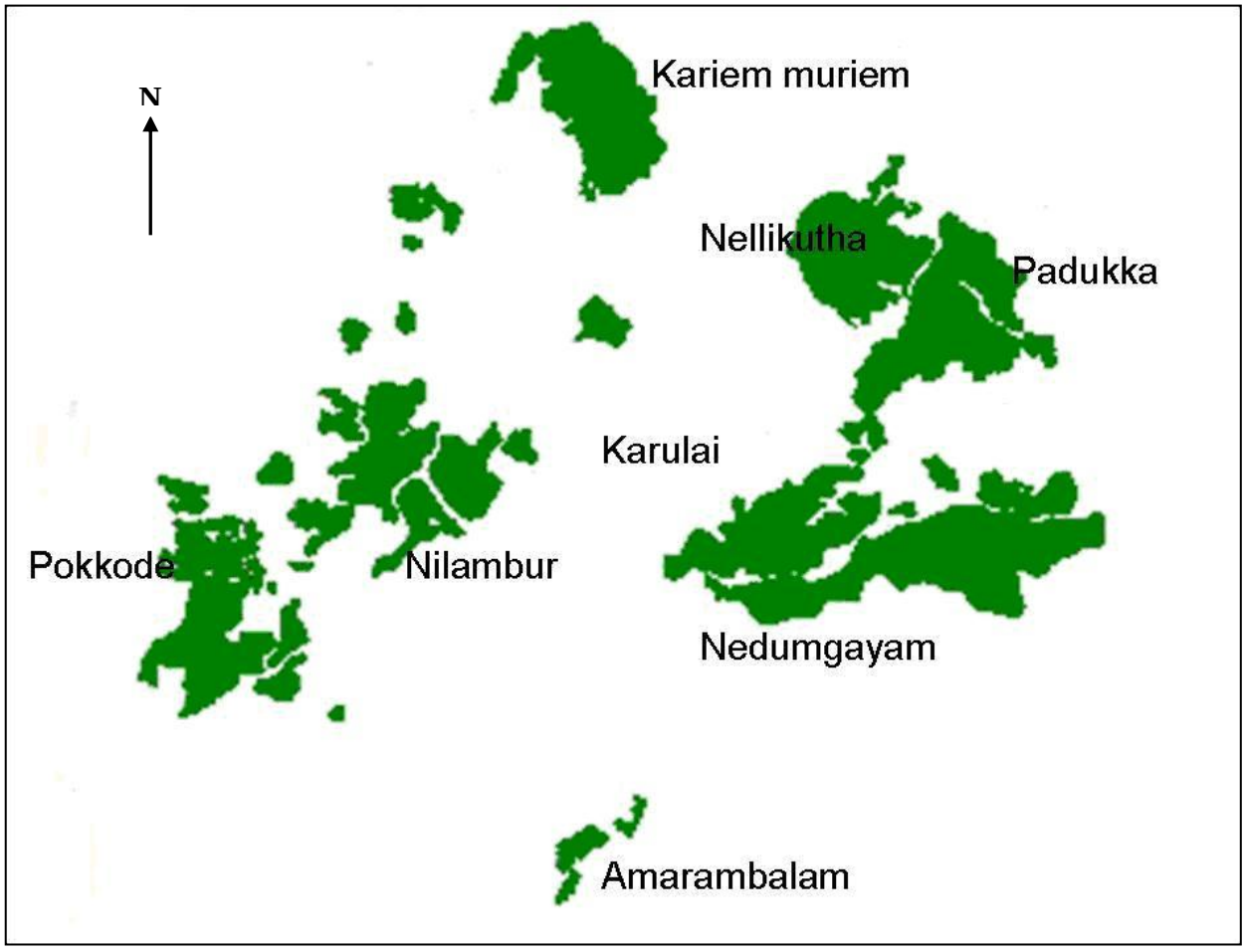

Figure 2.1: Map showing Nilambur teak plantations

2.2. INSECT CULTURE

For obtaining larvae required for various bioassays, a continuous culture of *Hyblaea puera* was maintained in the laboratory of the Kerala Forest Research Institute (KFRI), Subcenter, Nilambur throughout the study period. For establishment of the host culture, *H. puera* pupae were collected from the teak plantations at Nilambur and brought to the laboratory. Pupae were surface sterilized by soaking in 5 per cent sodium hypochlorite solution for 5 min and washed under tap water for another 2 min. The pupae were then air dried and allowed to emerge. The newly emerged moths were fed with 10 per cent (v/v) honey solution provided on sponge pieces. Sponge pieces soaked in diluted honey will be provided anew every day. It was found that fecundity increased with the time allotted for free movement before mating and hence, the moths were transferred in to a cage on the day of emergence. On the second day, moths were sexed, pairs were set and transferred to wide mouthed bottles (20 x 10 cm) covered with cotton cloth, which served as substratum for oviposition.

Egg laden cloth was removed daily and sterilized by soaking in 2 per cent sodium hypochlorite solution for five minutes and air-dried. When the eggs were about to hatch, the cloth was transferred to glass bottles provided with fresh tender teak leaf. *H. puera* has five larval instars. Until third instar, the larvae were reared on leaf in groups of 100 in glass bottles (20 x 10 cm). Every day morning, fresh tender teak leaves were provided. In every two days, glass bottles were changed and checked for dead larvae and contamination. From third instar the larvae were reared individually on semi synthetic diet (Mathew *et al*., 1990) in plastic rearing tubes (5.5 cm x 2.3 cm). The tubes were closed with perforated cap and kept inverted in a slanting position in aluminum trays. The larval rearing room was facilitated with a temperature of 28 ± 4 °C and a Relative Humidity (RH) of 60 ± 10 per cent.

2.3. HpNPV INOCULUM PREPARATION

The HpNPV used for the studies was amplified using fifth instar *H. puera* larvae. The OBs were isolated from larval cadavers by adopting slight modification of the method used by Biji (2006). The abdominal epithelium of the samples were incised and the fluid oozed out was vortexed for 10 s with twice the amount of water and filtered through 4 layers of muslin cloth. The filtrate was then subjected to centrifugation at 1500 rpm for 2 min to remove insect debris. The supernatant again centrifuged at 10,000 rpm for 7 min to suspend the NPV as pellet. The

supernatant was discarded and the pellet thus obtained was made upto a known volume using distilled water and concentration of occlusion bodies were determined by improved Neubauer's haemocytometer (0.1mm depth).

2.4. BIOASSAYS

Bioassays were carried out using leaf disc method (Biji, 2004). From the above aliquot, serial dilutions were made with distilled water to obtain desired concentrations. 10 µl of NPV solution was placed on a 0.5 cm^2 leaf disc of tender teak leaf and fed to fifth instar larvae of *H. puera* which were starved for 3 h. After 2-3 h, larvae that had eaten the whole leaf disc were transferred to artificial diet. Control insects were treated in the same way but 10 µl of distilled water replaced the virus dose.

3. FIELD MONITORING FOR *H. PUERA* OUTBREAK

3.1. INTRODUCTION

The infestation of the teak defoliator *H. puera* is a regular annual phenomenon in teak plantations throughout Kerala. Under normal conditions, leaf-feeding stage lasts to 10-12 days and total developmental period contains 19-36 days. Earlier studies by revealed that in large teak plantations like Nilambur, *H. puera* outbreaks began in comparatively small epicentres which may be 0.6 to 12 ha in area (Nair, 2007). The epicentres were not constant over the years and were selected by a group of migrating moths for their oviposition. The population build up that will occur in the epicentres results in the extensive outbreaks.

3.2. MATERIALS AND METHODS

Study area

The study was carried out in about 8500 ha of teak plantations (latitudes $11^0$10'N and $11^0$25'N and longitudes $76^0$10'E and $76^0$25'E) in Nilambur, Kerala during the pest incidence season in 2007 and 2008.

Epicentre detection and outbreak recording

Regular weekly field visits were made after the first summer showers beginning on the month of March. After the incidence of first epicentre infestation, regular fortnight observations were made which is correlated with the teak defoliator life cycle. Whenever an outbreak was located, the extent of infestation was estimated.

Temporal pattern of the outbreaks was examined to distinguish the origin of the outbreak. This was done based on the duration of the each larval instar, egg, adult and pupal period which together constitute about 21 days. Thus if a second outbreak was not happened between the 20^{th} and 26^{th} day (adding the oviposition period of 5 to the normal cycle, Sudheendrakumar, 1994) it was suggested that the outbreak was not caused by the previous moths but by the immigrating moths although the borderline cases may be suspect because of possible variation in the generation time under natural conditions.

3.3. RESULTS

Description of outbreak pattern

The chronology of teak defoliator outbreak during 2007 is shown in Table 3.1. The first epicentre of 2007 occurred in Kariem-Muriem plantations on 20th April 2007 in an area of 12.5 h. In the next 2 days 2 other epicentres were also observed – one at Kariem-Muriem itself (2 h) and another at Mundakkadavu of Vazhikkadavu range (27 h). The next phase of attack spread in the entire Kariem- Muriem area and other parts of Nilambur. Three peaks of outbreaks were observed which spread over 200 ha. Total 1260 ha of area were infested in the year. Six outbreak populations of the pests were observed during the year and the final population collapsed owing to a massive baculovirus epizootic during the month July 2007. There were four generations of the pest present in 2008 and the population collapse was occurred by a massive viral epizootic as in the last year occurred during the month of June 2008.

In 2008, the epicentre at Kariem-Muriem occurred one month earlier than the previous year and extended to an area of about 22 h. The second and third epicentres extending to about 10 ha and 6 ha were also observed in Kariem-Muriem and Vettilakkolli plantations respectively. Peaks of outbreaks extending over 100 h occurred only once and it extended to an area of about 700 ha in the Kariem-Muriem plantation. A total of 976 ha of area was infested in the year 2008 until the end of May. Four outbreak populations of the pest were observed in the field during the period March-May 2008. The chronology of teak defoliator infestation during 2008 was shown in Table 3.2. The comparison of infestation and rainfall of two years was shown in figure 3.1.

Table 3.1. Chronology of outbreaks of teak defoliator in 2007

Sl.No	Place	Probable oviposition date	Area (ha)
1	Kariem -Muriem	17-21 Apr	12.50
2	Kariem-Muriem	19-23 Apr	2.00
3	Mundakkadavu	18-23 Apr	27.90
4	Nellikkuthu	2-8 may	9.00
5	Kariem – Muriem	2-8 may	500.00
6	Chelakkadavu	5-11 May	3.00
7	Kalkkulam	6-10 Ma	1.00
8	Nedungayam – Kanjirakkadavu	5-13 May	60.00
9	Cherupuzha	11-17 May	2.00
10	Aruvakkode	15-17 May	2.77
11	Karimpuzha	13-16 May	4.00
12	Valluavassery	11-16 May	8.00
13	Amarambalam old	11-May	11.00
14	Amarambalam old	9-14 May	21.50
15	Emangad	9-14 may	8.90
16	Nellikkuthu	29 May- 4 June	22.00
17	Kariem-Muriem	29 May- 4 June	200.00
18	Thannippoyil	30 May- 05 June	18.00
19	Annunda	1-6 June	19.75
20	Athikkal	30 May- 05 June	14.00
21	Kariem-Muriem	18-24 June	300.00
22	Karimpuzha	12-18 June	2.00
23	Kariem-Muriem	30 June-04 July	11.00
	Total area infested		1260.32

Table 3.2. Chronology of outbreaks of teak defoliator in 2008

Sl. No	Place	Probable oviposition date	Area (ha)
1	Kariem -Muriem	19-23 mar	22.00
2	Kariem - Muriem	17-21 mar	10.00
3	Vettilakkolli	15-19 mar	6.00
4	Naripoyil	19-23 mar	10.00
5	Kanakutha	19-23 mar	3.00
6	Kurambilangodu	19-23 mar	7.00
7	Erampadam (1984 plntn)	19-23 mar	10.00
8	Erampadam (2003 plntn)	19-23 mar	15.00
9	Kariem Muriem	31 mar-4 apr	700.00
10	Vettilakkolli	31 mar-4 apr	40.00
11	Vettilakkolli	28 apr-2 may	55.00
12	Emangad	31 mar-4 apr	8.00
13	Amarambalam	31 mar-4 apr	33.50
14	Nedungayam	23-27 apr	20.00
15	Conoly (Elamchery)	23-27 apr	6.00
16	Mayiladi	23-27 apr	15.00
17	Kariem-muriem	19-23 may	12.00
18	Vettilakkolli	19-23 may	4.00
	Total area infested		976.5

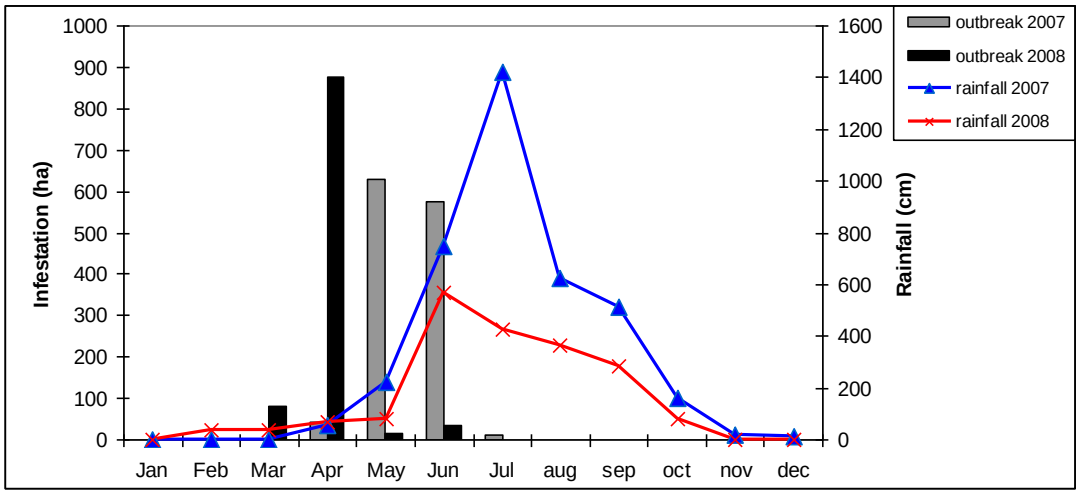

Figure 3.1: Comparison of rainfall and outbreak pattern of the years 2007 and 2008

3.4. CONCLUSION

The survival of teak defoliator particularly the early instar larvae depends on availability of tender foliage. The phenology of teak is influenced by rainfall. Early outbreak of teak defoliator is also known to have a strong correlation with premonsoon showers. In 2007, there was only 2mm of rainfall in the month of February and the monsoon showers started in April. But in the year 2008, there was heavy raining from the month of February itself. This resulted in early flushings and outbreak started in March itself. In 2007, the total area infested was about 1260 ha while in 2008 it was about 976 ha.

In 2007, the three epicenters first observed in the month of April were of independent origin. Similarly, the infested areas of Cherupuzha, Aruvakkode, Karimpuzha, Valluvassery and Amarambalam old plantations during the month of May had no known origin suggesting that the population might have developed from progenies of immigrant moths. In 2008, the three epicenters located on Kariem-Muriem and Vettilakkolli plantations during the month of February were of independent origin. All the other population could be related to the progenies of previous epicenters. In both years, the population decline had occurred by a massive viral epizootic. In July 2007, the epizootic completely wiped out the sixth generation of the teak defoliator population in the Kariem-Muriem plantations in about 31 ha causing 90 per centage mortality of the population. In 2008, the viral epizootic brought about the devastation of the fourth generation of teak defoliator present in 35 ha of Kariem-Muriem plantation during June.

4. LABORATORY EVALUATION OF VERTICAL TRANSMISSION

4.1. INTRODUCTION

Vertical transmission is commonly observed phenomenon with insect viruses especially with nucleopolyhedroviruses. The mechanisms may either be transovum or transovarial. Vertical transmission of insect pathogens in nature is not critical for maintaining their populations in nature nor does it correlate with disease prevalence but rather it functions to transport these pathogens in the ecosystem and provide foci of new infections (Fuxa et al., 1992). Vertical transmission of NPVs upto 6 generations were found in the case of *S. frugiperda* although the percentage of vertical transmission was found declined in late generations (Fuxa, et al 1992). The vertically transmitted NPV also has been associated with possible sublethal effects (Fuxa et al., 1999). The sublethal effects may affect both the biological and reproductive parameters of insects (Myers et al 2000; Khurad, et al., 2004)

4.2. MATERIALS AND METHODS

H. puera larvae were obtained from the continuous culture maintained at Kerala Forest Research Institute, Subcentre Nilambur. HpNPV used for the study was prepared and enumerated as described in Chapter 4.3. From this aliquot, serial dilutions were made with distilled water to obtain concentrations of $1x\ 10^2$, $1x\ 10^3$, $1x\ 10^4$, $1x\ 10^5$ and $1x\ 10^6$ OBs/ml. and bioassays were made using leaf disc method (Chapter 4.4). The experiments were carried out with 50 larvae per treatment dose and 100 larvae in control. Larvae within a small weight range were used for the experiments.

Mortality due to infection during the larval stages was recorded. Larvae that survived were weighed as pupae and reared to adults. The adults were paired as four sets and kept for mating: (1) infected male & infected female (C1), (2) uninfected female & infected male (C2), (3) uninfected male and infected female (C3) and (4) uninfected male and female (C4). Eggs laid on each day were counted and 20 eggs from each pair of moth were separated along with mull cloth and 10 of them were surface sterilized with 0.1% NaClO and dried and both were kept in tender teak leaf. The newly hatched larvae were reared on fresh leaves and their mortality due to the transmission of infection was recorded at succeeding larval stage. Any dead larvae were stained

and observed microscopically. Survived insects were reared through the adult stage of F1 generation. Pupae were collected and the adults were mated. Their oviposition rate is also noted.

Data analysis

Arcsine transformed values of the percentage mortality, percentage pupation, percentage emergence and sex ratio were subjected to one-way ANOVA to investigate the statistical difference between the doses and crosses. Comparison of larval weight and pupal weight were also done. The period of egg laying and oviposition rate of each cross on each dose were also analysed by using one-way ANOVA. The analysis was performed using the software SPSS 10.00.

4.3. RESULTS

Experiments conducted in the project aimed at quantifying the effect of sublethal dosages on the growth and reproductive parameters of the insect. The results are presented below under each of the twelve parameters quantified.

1. Larval survival

Mortality due to *H. puera* nuclear polyhedrosis virus started from 60hrs post infection. Parental mortality was observed to be higher in treatments and seen to be increasing according to the doses ranging from 60%-87%, with no NPV deaths in control.

2. Pupation and pupal weight

There was a reduction in overall pupation of 28% in the treated insects as compared to untreated control. The difference in body weight between pupae and larvae of HpNPV treated and untreated insects are given in Table 4.1. As can be seen, the all untreated insects lost weight as pupae while the treated insects had higher body weight as pupae than as larvae. This is an interesting observation which indicates the relatively low metabolism in sublethaly infected insects.

Table 4.1: Difference between pupal and larval weight of insects treated with HpNPV

Dose	N	Weight (g) (SD)
1 x 10^2	43	.08±.0056[a]
1 x 10^3	34	.08±.0068[a]
1 x 10^4	25	.10±.0089[a]
1 x 10^5	29	.09±.0069[a]
1 x 10^6	12	.08±.01[a]
control	137	-.07±.0081[b]

3. Adult emergence

The percentage of adults emerged from HpNPV treated and untreated insects are given in Table 4.2.

Table 4.2: Percentage adult emergence in insects sublethaly treated with HpNPV

Dose	N	% (SD)
1 x 10^2	64	72.86±4.34[b]
1 x 10^3	41	64.33±4.05[ab]
1 x 10^4	42	60.0±6.08[ab]
1 x 10^5	34	62.33±5.04[ab]
1 x 10^6	18	34.2±19.24[a]
control	168	98.33±0.88[c]

Emergence rate of adults from treated pupae were found to be varied significantly in the lowest and highest doses and in control. There was also a gradation in adult emergence as the dose increases though it was not a significant one. Control showed 98% emergence whereas emergence varied 34 – 72% in virus treated pupae.

4. Adult longevity

It was observed that the male moths which were sublethally infected lived for an average of 8.27 days, while infected females lived for 9.47 days. Untreated males lived for 10.2 days and females

lived for 11 days. The longevity of moths get reduced by 2 days in the case of both males and females.

5. Egg laying period

Table 4.3 presents the information on the period of egglaying . it can be seen that there is considerable reduction in the period of egg laying between treated and untreated insects. It can be inferred that the sublethal infection reduces the egg laying period by moths by 2-3 days.

Table 4.3: Period of egg laying of insects sub lethally dosed with HpNPV

Dose	**N**	**Days (SD)**
1 x 10^2	13	3.53±.38[a]
1 x 10^3	10	2.8±.53[a]
1 x 10^4	12	4.42±.26[a]
1 x 10^5	9	3.55±.053[a]
1 x 10^6	3	3.0±1.0[a]
control	18	6.1±0.43[b]

6. Fecundity

The fecundity of moths observed when crosses were made between treatments and between treatment and control is given in Table 4.4. It can be seen that all treatments had considerably lower fecundity than the untreated insects. Lowest fecundity was observed when both the sexes were infected (C1). When only the female was infected, the next lowest fecundity was observed and when infected male was crossed with untreated female, the fecundity was only next to that of the cross between untreated male and female.

Table 4.4: Fecundity of parent generation in different crosses

Dose	**cross**	**N**	**eggs**	**mean**	**No of eggs**
1 x 10^2	1	8	1875	234.4	159.6±43.2[a]
	2	9	1421	157.9	
	3	5	215	43	
1 x 10^3	1	8	1010	126.25	115.2±43.75[a]
	2	3	744	248	
	3	5	90	18	
1 x 10^4	1	7	1529	218.4	211.1±59.69[a]
	2	4	678	169.5	
	3	2	487	243.5	
1 x 10^5	1	6	789	131.5	139.0±53.61[a]
	2	2	433	216.5	
	3	3	307	102.3	
1 x 10^6	1	2	0	0.0	60.0±40.83[a]
	2	2	329	164.5	
	3	2	35	17.2	
control		3	1848	616	491.8±94.03[b]
		3	688	229.3	
		3	1718	572.7	

(Cross 1-IM x IF, cross 2- IM x CF, cross 3- CM x IF, N = no: of pairs)

7. Hatchability of F_1 eggs

The percentage of eggs hatched which was obtained from treated and intreated moths is presented in Table 4.5. There is substantial reduction in hatchability from 73% to 12% owing to the sublethal infection.

Table 4.5: Hatchability of eggs obtained from HpNPV treated and untreated moths

Sample	**Hatchability**	
	N	**%**
Treatment	790	12.3 [a]
Control	550	73.8 [b]

Table 4.6: Percentage mortality and pupation in the F1 generation owing to sublethal HpNPV infection in the parent generation.

Dose	**Mortality**		**Pupation**	
	N	**% Mortality**	**N**	**%Pupation**
1 x 10^2	134	84.42±3.78[a]	28	15.58±3.78[a]
1 x 10^3	96	89.92±4.39[a]	11	10.08±4.39[a]
1 x 10^4	224	81.33±5.97[a]	47	18.66±5.97[a]
1 x 10^5	158	83.22±4.89[a]	33	16.77±4.89[a]
1 x 10^6	39	91.00±9[a]	8	9.00±9.00[a]
Control	1100	7.79±5.46[b]	1076	92.20±5.47[b]

8. Survival of F_1 larvae and pupation

Table 4.6 shows the incidence of mortality and percentage pupation in F1 generation when insects were sublethally treated with HpNPV in the parent generation. It can be seen that while there was only 7% mortality in untreated insects, 80-90% mortality was observed insects which were treated. While 92% of the late instar larvae pupated in the untreated control, only 10-15% pupation was observed in the treatment lots. This clearly indicates the transmission of HpNPV into subsequent generations.

9. Sex ratio of F1 moths

The sex ratio of adult teak defoliator moths of the F1 generation which were the offsprings of the insects which received sublethal doses of HpNPV is given in Table 4.7. There was no significant

difference between either the different doses of treatment or with the untreated control. It can be inferred that sublethal infection of HpNPV does not have an impact of sex ratio of the F1 generation.

10. Fecundity of F_1 moths

Table 4.8 shows the substantial reduction in oviposition rate from over a 1000 eggs to just above 300 eggs in the treatements. The reduced egglaying could be an effect of the sublethal infection carried over to the F1 generation from the parental generation. This further proved and quantified the vertical transmission of HpNPV in teak defoliator population.

Table 4.7: sex ratio F1 moths

Dose	**N**	**M/F (SD)**
1 x 10^2	47	2.6 ± 1.22^a
1 x 10^3	27	0.76 ± 0.12^a
1 x 10^4	26	1.2 ± 0.2^a
1 x 10^5	22	1.41 ± 0.46^a
1 x 10^6	8	1.66 ± 1.33^a
Control	186	1.08 ± 0.09^a

Table 4.8: Comparison of oviposition rate of insects in F1 generation

	Oviposition	
Sample	**N**	**No. of eggs**
Treatment	3	313^a
Control	3	1315^b

11. Survival of F_2 insects

The impact of carried over sublethal infection on the second filial generation of insects is presented in Table 4.9. It can be seen that that when both the sexes were infected, the only one male moth was emerged during the F1 generation and hence the population could not proceed to

the f2 generation. When the male was the infected sex, mating and, egg lalying occurred in the F1 generation but there was cent percent mortality in the F2 larval stages and thus the F2 generation perished. When only the female was infected, the F1 moths laid eggs but none of the eggs emerged to continue the F2 generation. Thus all of the treated lots of insects failed to progress to the F2 generation while in the control set, more than 70% eggs hatched, and nearly 93% larvae survived into the pupal stage when the observations terminated.

The impact of vertical transmission of different biological and reproductive parameters of the pest is shown in figure 4.1.

Table 4.9: percentages of hatchability, mortality and pupation of insects in the F2 generation

Cross	**% hatchability**	**% mortality**	**% pupation**
Infected male | infected female	Nil	nil	Nil
Infected male | control female	8.6	100	Nil
control male | Infected female	0	nil	Nil
control male | control female	73.2	6.7	93.1

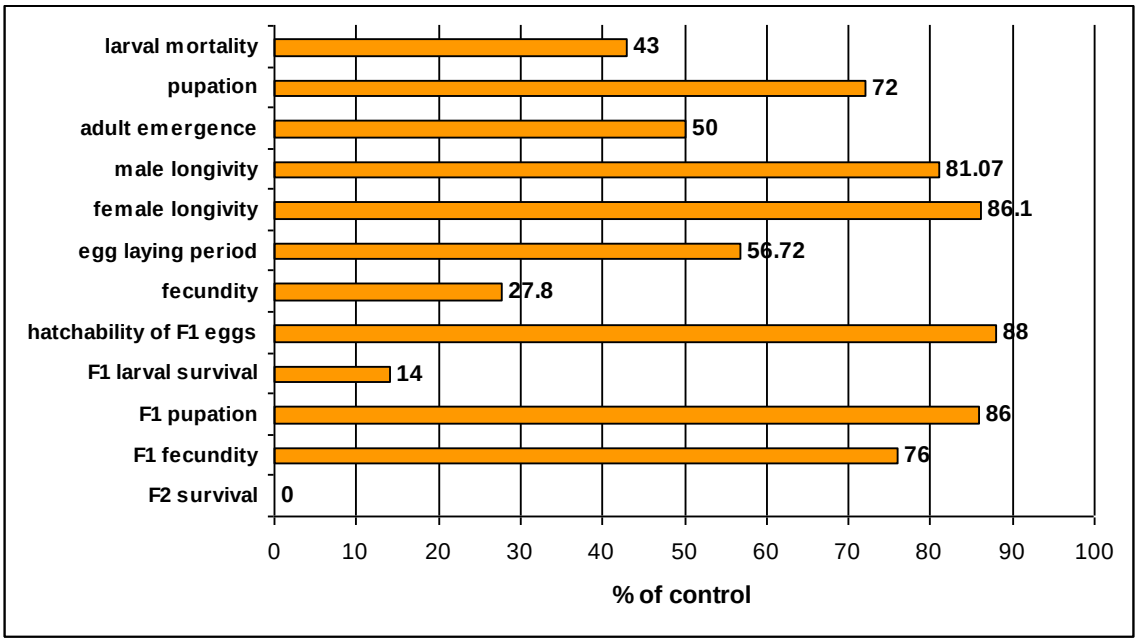

Figure 4.1. Impact of sublethal dosing of HpNPV on 12 biological parameters of *H. puera*

4.4. DISCUSSION

In the case of *H. puera*, the common mode of entry of NPV is per os as in the case of other lepidopteran insects. Usually a successful infection depends on the ingestion of sufficient virus to initiate replication in the host. In the experiment we used larvae of the same age i.e., early fifth instar so that the age of the larvae could not pose any problem to the possible transmission effects as found in the case of cabbage looper, *T. ni* larvae in which the time of treatment did have an influence, and sublethal effects were greatest when caterpillars were treated at third and fourth instar (Milks et al., 1998). Parental mortality was observed to be higher in treatments and seen to be increasing according to the doses ranging from 60%-87%, with no NPV deaths in control. Milks et al., 1998 have the opinion that the debilitating effects of TnSNPV did not appear be dose-dependent. In the experiment we found that the mortality due to vertical transmission did not showed any dose dependency in the F1 generation.

The difference between body weight between pupae and larvae of HpNPV treated and untreated insects (treated insects had higher body weight as pupae, Table 4) is an interesting observation which indicates the relatively low metabolism in sublethally infected insects. But the opposite effects have been explained in most of the studies and which is also correlated with reduced fecundity. The pupae that have survived exposure to NPV in their larval stage are smaller than untreated controls and the moths emerging from these pupae have reduced fecundity (Rothman and Myers 1996). However, Murray and Elkinton (1989) found that adults surviving virus challenge only transmitted virus to progeny at very low levels (2.0%) and found no difference in pupal size or fecundity for individuals surviving exposure to virus as fourth instars (Murray et al. 1991). Infection of gypsy moth with NPV slowed larval growth and inhibits larval molting and pupation (Burand and Park 1992). Most insect baculoviruses contain a gene (*egt*) that encodes the enzyme ecdysteroid UDP-glucosyl transferase which catalyzes the sugar conjugation of ecdysteroids (O'Reilly and Miller 1990). Fifth instar gypsy moth infected with NPV do not show the increase in ecdysteroid titer before pupation that occurs in control caterpillars (Park et al. 1993).

Sublethal infection could influence the fecundity of moths by reducing the amount of fat and other nutrients available for egg or sperm production or through some associated cost of

resistance mechanisms either when initially fighting off infection or in maintaining it at a nonlethal level (Myers and Kukan 1995). However, sublethal infection could also influence fecundity through an interaction with the hormonal balance of the individual during development (Myers et al, 2000). The reduction in fecundity was maximum when both the sexes are infected. This may be due to the improper vitellogenesis in the infected oocytes (Khurad et al., 2004) by reducing the amount of fat and other nutrients available for egg or sperm production or through some associated cost of resistance mechanisms either when initially fighting off infection or in maintaining it at a nonlethal level (Myers and Kukan 1995). Such maternal effect of transmission in the form of transovum and transovarial and venereal routes of infection was also recorded from *B.mori* (Khurad et al., 2004), *H. virescens* (Nordin et al., 1990), honey bee (Yue et al., 2006; Chen et al., 2006; de Miranda and Fries, 2008). However no evidence was found for venereal or transovum (including transovarial) transmission of the parasite *Nosema* sp when larvae of gypsy moth, *L. dispar* was infected (Goertz et al, 2007).

Sublethal infection also have profound effect on adult longevity though it is not a significant one but the adult emergence and egg laying period of the treated ones were found significantly low as compared to the untreated ones.

5. VERTICAL TRANSMISSION OF HpNPV IN FIELD POPULATION OF *H.PUERA*

5.1. INTRODUCTION

Baculovirus epizootics resulting in high larval mortalities and population depression is known in several tropical lepidopteran species including the gypsy moth, *L. dispar*; the Douglas-fir tussock moth, *Orgyia pseudotsugata*; the spruce budworm, *Choristoneura fumiferana*; the western spruce budworm, *Choristoneura occidentalis*; the jackpine budworm, *Choristoneura pinus*; the pine beauty moth, *P. flammea* and the fall webworm, *Hyphantrea cunea* (Moscardi, 1999). In India, the occurrence of nuclear polyhdrosis virus in poplar defoliator, *Clostera cupreata* (syn.*Pygaera fulgurita*) (Ahmed and Sen-Sarma, 1984), babul defoliator, *Taragama siva* (*Streblote siva*) (Ahmed, 1992) and teak defoliator, *H. puera* (Sudheendrakumar et al., 1988) were reported.

Long term studies on landscape level population dynamics of *Hyblaea puera* carried out by KFRI have shown that the origin of large scale outbreaks is preceded by the occurrence of localized, high density epicentre populations. These epicentres are an ideal setting for adopting control operations since they occur in small patches and the subsequent generations from these epicentres cause large-scale outbreaks. The latter aspect has been tested by using molecular techniques (RAPD analysis of genome using nuclear and mitochondria specific markers) wherein it is found that the outbreak populations share the same gene pool as that of epicentre populations. One of the unique and economical ways of preventing large-scale outbreaks of the teak defoliator is to suppress the epicentre populations using HpNPV. An attempt was made to evaluate the impact of sublethal dose of HpNPV on an epicenter population of *H. puera*. It was hypothesized that the sublethal infection would allow a portion of the insect population to carry over the virus through vertical transmission to the next generations and there by help to reduce the pest population.

5.2. MATERIALS AND METHODS

The field application of HpNPV was done on 26th March 2008 in an epicentre area located at Kariem-Muriem teak plantation when most of the population was of 4th and 5th instars. Larval samplings were done before and after 72 hrs post application of the virus to observe the intensity

of NPV incidence. 2700 µl of the virus sample of the strain LST containing 6.5 x 10^7 POBs/ml was diluted with 280 litres of water. Plantowet @ 2 ml per litre was added in the mixture as adjuvant. Within the epicentre, spraying was done using High Volume Sprayer and occasionally workers were engaged to climb up the tree. The spraying was done in such a manner that point application and wind assisted broadcast spraying was often done to assure the whole area get covered under spray with minimum effort and also to take advantage of the possible horizontal transmission.

Six days after field spraying, 100 pupae observed healthy on external appearance were collected separately from the treated and nearby untreated Vettilakkolli plantation. Emergence from the pupae and the sex ratio of emerged moths were noted. Three crosses were set during the mating phase- infected male x infected female (T1), uninfected female x infected male(T2), and uninfected male x infected female (T3). The cross T4 between uninfected male x female served as the control. Eggs laid on each day were counted and one set of eggs from each pair of moth were separated along with mull cloth and another set was surface sterilized with 0.1% NaClO and dried. Both sets were kept in tender teak leaf. The newly hatched larvae were reared on fresh leaves and their mortality due to the transmission of infection was recorded. The viral deaths obtained from the progeny of each cross were kept at -20^o C. The larval progeny that died due to infection were examined for NPV. Survived insects were reared through the adult stage to next generations and their biological and reproductive characters were also noted in the coming generations.

5.3. RESULTS

Before the virus application, the density of larvae varied from 0-56, with an average of 9.96±11.46/twig (fourth instar dominating - 58%) and 3 days after NPV field application it was 10-17, with an average of 4.59±4.23/twig (fifth instar dominating – 96%). The average larval mortality in the sample collected before NPV application and in the sample collected after NPV application was 0.13±0.51 (N=36) and 0.24±0.5/twig (N=45), respectively. The larval sample collected from the field 3 days after NPV application and subsequently reared in the laboratory recorded 54.23±36.73% mortality.

The emergence of pupae from treated and control plots were 57% and 58% respectively. Though the fecundity of moths under different mating groups were not significant ($F_{3,23}$ = 0.92, P > 0.05), maternal influence on fecundity was thereby evident. The oviposition rate of different groups were in the order T1<T3<T2<T4. There was also a significant difference in the percentage hatchability ($F_{3,10}$ = 30.55, P < 0.0001), NPV incidence ($F_{3,10}$ = 3.89, P < 0.05) and pupation ($F_{3,10}$ = 6.06, P < 0.05) between different mating groups (Table 5.1). Because of the very low number of pupae obtained in some crosses and vertical transmission of NPV was proved, the experiment was not continued beyond F1 level.

Table 5.1: Effect of NPV on fecundity, %hatchability, % NPV incidence and % pupation (mean±SD) of F1 generation of field collected samples

Cross	**Fecundity**			**Hatchability**	**Mortality**	**Pupation**
	N	**n**	**fecundity**			
T1	7	2	139±179.61	3.05±4.31	17.63±24.94	27.3± 38.7
T2	6	3	303.67±138.69	10.68±10.87	29.36±25.45	15±25.98
T3	7	5	224.8±169.33	29.53±6.71	42.07±6.78	16.04±10.97
T4	7	4	338.25±234.06	52.33±4.41	10.05±2.46	63.63±4.58

T1 - Infected male X Infected female; T2 - Infected male X Uninfected female;

T3 - Uninfected male X Infected female; T4 - Uninfected male X Uninfected female

N = No. of pairs set

n = No. of pairs which laid eggs

5.4. DISCUSSION

During the field application of HpNPV, the 4th instar larvae were predominant and after 3 days of spraying most of them had reached the 5th instar stage. The stage structure of the host can influence the pathogenicity of the virus (Evans, 1998; Biji, 2006) the older larvae being less susceptible to disease. This could be the reason for not getting a very high mortality rate occurred after 3 days of field inspection (0.13 to 0.24 % NPV incidence/twig). But the fifth instar larvae brought to the laboratory recorded 54% mortality leaving 46 % alive. In vertical

transmission experiment larval survival is important as the surviving population can transmit the virus to the next generation. In this context fourth instar stage proved an appropriate time for the sublethal HpNPV application in the field. Long-term effect of NPV spraying in natural condition of the host resulted reduction in pupal weight, fecundity, egg fertility and alteration in sex ratio in F1 generation of Gypsy moth (Il'inykh *et al*., 2009). The induced NPV epizootic in satin moth (*Leucoma salicis*) and its long-term monitoring over 20 years have showed that it affects the healthiness of pupae, adults and offspring and reproductive potential of females (Ziemnicka, 2008). The change of biological and reproductive characters (pupation, adult emergence, mating systems and fecundity) of the offspring generation of the *H. puera* (Table 5.1) at the cost of viral resistance would be sufficient enough for collapsing the population, without further HpNPV augmentation.

6. HOST INSECT RESISTANCE TO SUBLETHAL HpNPV DOSAGE

6.1. INTRODUCTION

One of the characteristic of use baculovirus in insect management programme is their host specificity, although which can be related to natural resistance to their pathogens. (Narayanan, 2004). Resistance to baculovirus can be defined as the development of an ability in a strain of insects to tolerate doses of virus that would cause disease or prove lethal in the majority of individuals in a normal population of the same species (Fuxa, 1993). Resistance to baculoviruses has been observed in field populations of *S. frugiperda* (Fuxa *et al*., 1988). At the beginning of the season, the larvae are susceptible to the NPV, but later in the season, there is a trend towards reduced susceptibility and increased heterogenicity after exposure to the virus. The following work was done to look for indications on resistance build up if any when sublethal infection occurs in teak defoliator larvae.

6.2. MATERIALS AND METHODS

The *H. puera* larvae used for the experiment were obtained from the continuous culture maintained at KFRI Subcentre, Nilambur. The early fifth instar larvae used for the study was inoculated with a dose 1 x 10^2 POBs/ml of HpNPV (LST isolate) by leaf disc method. Ten microlitres of NPV solution was placed on a 0.5 cm^2 leaf disc of tender teak leaf and fed to fifth instar larvae of *H. puera* which were starved for 3 h. After 2 h, larvae that had consumed the whole leaf disc were transferred to an artificial diet. Insects set as control were treated in the same way but ten micro liters of distilled water replaced the virus dose. The larvae were kept at 26+4ºC and 60+10% RH in rearing tubes (5.5cmx 2.3cm) with a perforated lid inside a BOD incubator. The first filial generation of the insects obtained from the above said generation was divided into two batches randomly. One set was challenged with HpNPV using the same dose and procedure as above. The second set was untreated. Insects were reared through the generations according to standard procedures.

6.3. RESULTS

The effect of repeated administration of HpNPV in successive generations of teak defoliator is presented in Table 6.1. It can be seen that insects which were untreated in both the generations

had a mortality of 2.9% and pupation rate of 62.4 %. When only the F1 generation was treated with HpNPV, there was a mortality incidence of 47% and pupation rate of 38%. However, when HpNPV was administered in both the generations, there was 70% mortality and 13% pupation rate in the first filial generation. Insects untreated during the fist filial generation but treated during the parent generation gave similar values as that of insects treated only in the F1 generation.

Table 6.1. Mortality and pupation rates of H. puera larvae tested fro resistance against HpNPV

Parameter	**Parent**	**Treated parent**		**Untreated parent**	
		Treated F1	**Untreated F1**	**Treated F1**	**Untreated F1**
% Mortality	22	70.83	31.68	47.06	2.97
% Pupation	95	13.1	32.92	38.24	62.38

6.4. DISCUSSION

Observing the F1 generation, it was seen that the insects had become more susceptible to HpNPV rather than becoming resistant to infection in any way. There was very high mortality when HpNPV was applied consecutively in two generations. In *S. frugiperda* the LD_{50} increased from 4.1 to 18.7 PIB/insect in a laboratory colony exposed to the NPV at LD_{80} for 7 generations, where as in a control colony not exposed to NPV the LD_{50} was 5.9 PIB/insect after 7 generations (Fuxa et al., 1998). Laboratory experiments to determine the possible coevolution of *T. ni* and its nucleopolyderovirus (TnSNPV) showed 4.4 and 22 times resistance to TnSNPV which was not accompanied by increased virulence of TnSNPV or change in the restriction profile of viral DNA when digested with *BamHI, EcoRI, HindIII, PstI, SalI, SstI* or *XhoI* (Milks and Myers, 2000). The current study has the limitation that it could be continued upto only one filial generation However, experiments spanning more generations are required to answer the question of resistance buildup in an unequivocal manner.

7. CONCLUSIONS

1. HpNPV dosages around a hundred POBs (sublethal) when ingested by teak defoliator larvae, resulted in about a mortality of f 60% of the population leaving 40% of the population to the next generatiuon.

2. The insects that survived the above said dose, retained the baculovirus in their body and vertically transmitted it to the next generation.

3. In the process of vertical transmission, the biological parameters like rate of pupation, adult emergence, male and female longevity, oviposition period and fecundity got reduced while the sex ratio remained unchanged.

4. One -third of the offsprings which received the vertically transmitted baculovirus died and the survivors showed reduced pupation rate, adult emergence rate, fecundity and longevity.

5. HpNPV when vertically transmitted reduced the reproductive potential of the insect during the parent and first filial generation so as to inflict cent percent mortality in the second filial generation.

6. Observation on resistance to HpNPV in the teak defoliator larvae ruled out such a possibility upto F1 generation under laboratory condition.

7. It is concluded that application of sublethal dose of HpNPV during the epicentre phase of the eruptive outbreaks of the teak defoliator can cause viral epizootics which in turn can suppress population buildup and prevent large scale outbreaks. This method of HpNPV application may be practiced for management of the teak defoliator in the landscape level.

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