Seeding baculovirus HpNPV in the epicentre populations of teak defoliator, *Hyblaea puera* to prevent large-scale outbreaks

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The defoliation caused by *Hyblaea puera* can result in approximately 44% reduction in the annual volume increment of teak, a major timber tree. Several management options used in the past to control the pest were not effective due to high cost and environmental problems. In the present study we have used the vertical transmission characteristics of *Hyblaea puera* nucleopolyhedrovirus (HpNPV) for cost-effective and environment-friendly control of the most devastating pest. First, a laboratory experiment was conducted to verify the vertical transmission of HpNPV and later field spraying was done to study the effect under natural conditions. Vertical transmission of HpNPV in the laboratory ranged between 13% and 59%. The sublethal transmission caused reduction in pupation, adult emergence, male and female longevity, egg-laying period, fecundity, hatching of F1 eggs, F1 mortality, F1 pupation and F1 fecundity, but had no influence on the F2 survival compared to the control. Spraying of HpNPV in the epicentre population of the pest resulted a viral epizootic at the F2 generation and led to collapse of the host population. Seeding of HpNPV in the epicentre populations of the teak defoliator has been proved to be an economical and environment-friendly method for management of the pest.

Keywords: Baculovirus, epicentre populations, field spraying, *Hyblaea puera*, teak, vertical transmission.

TEAK, *Tectona grandis*, is a widely cultivated tropical timber tree in the Asian, African and American continents and many Pacific and Atlantic islands[1]. Natural teak forests cover about 29 million hectare (m ha) and planted teak covers 4.35–6.89 m ha (ref. 2). However, natural teak forests have declined and deteriorated, while planted teak forests face several threats, including pest attack[2]. This includes defoliators and skeletonizers, and several diseases that damage young teak plants resulting in huge economic loss[1]. The teak defoliator, *Hyblaea puera* is recognized as the most serious pest of teak causing a reduction of approximately 44% in annual volume increment per hectare per year due to defoliation[1]. Biology and population dynamics of *H. puera* are well known[1,3]. Three levels of population density have been recognized within the annual cycle of occurrence of *H. puera* at the landscape level[4]. These include very low-density populations known as endemic (during natural defoliation of teak in December–February), first high-density populations in small patches known as epicentres (during fleshing of teak after premonsoon in February–March), and waves of high-density, large-scale infestations that become epidemic, also known as outbreaks (late April–July). During the non-outbreak season, *H. puera* is not prevalent in plantations, though theoretically it can complete several generations per year[1]. Spatio-temporal distribution of infestation and several behavioural characteristics of *H. puera* suggest density-dependent short-range and long-range migrations[1,4]. The role of such migrations in *H. puera* outbreaks has also been confirmed by recent molecular studies[5].

Several silvicultural, chemical and biological control methods against the pest have failed due to their non-target impacts, cost-intensive and/or hazardous nature[1]. Later, a baculovirus, the *Hyblaea puera* nucleopolyhedrovirus (HpNPV) was discovered[6]. This is considered as a major breakthrough in teak defoliator management due to its specificity, speed to kill and environment-friendly nature. Following this, 'Hybcheck' – a freeze–dried powder formulation containing HpNPV was developed[7]. Application of this product could help amplify the natural inoculum load in the ecosystem and thereby provide a tool for the effective management of teak defoliator. However, HpNPV application in extensive teak plantations requires manpower and specific equipment, resulting in increased cost of application. This encouraged the need to develop a landscape-level management strategy to control *H. puera* using the virus combining knowledge on the population dynamics of the insect and vertical transmission characteristics of the pathogen.

Nucleopolyhedroviruses (NPVs; family Baculoviridae) have shown considerable potential as practical insect control agents in agriculture and forestry[8,9] due to their vertical and horizontal transmission leading to population

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collapse in hosts[10–12]. The horizontal transmission of baculovirus via susceptible larvae ingesting occlusion bodies (OBs) persisting in the environment can be effective at high host densities[12], while the vertical transmission of baculovirus could contribute to the persistence of virus in low-density populations[11]. Both maternal and paternal routes of vertical transmission of baculovirus were confirmed through mating experiments in several lepidopterans[11,13,14]. Despite numerous challenges in the field, including high ultraviolet levels, high levels of vertically transmitted covert infections of nucleopolyhedrovirus were reported in tropical migratory pests[12]. Vertical transmission of the pathogens to the next generations results in sublethal effects which may range from deformed pupae[15] to poor development, lower weight, reduced reproduction and shorter life span[16]. The functional relationship of sublethal effects with insect disease indicates that non-mortality effects may significantly influence the population dynamics of the host[17]. Thus knowledge on the efficiency of vertical transmission is also important for understanding its potential in the long-term control of the pest as has been reported in many species, including satin moth and gypsy moth[18,19].

Population outbreaks of *H. puera* begin from small epicentres (1–1.5 ha), and spread by long-range and short-range migrations[1,4]. Therefore, developing methods for managing the initial epicentre populations by seeding sublethal HpNPV instead of lethal doses of baculovirus could be the most practical way to limit pest outbreaks and thereby minimize the economic loss due to defoliation. The present study aims to: (a) quantify the vertical transmission of HpNPV, (b) explore the remote effects of baculovirus transmission in disease spread while spraying HpNPV in the epicentre populations of the host and (c) explore the implications of the results in the management of the teak defoliator.

Materials and methods

Insects and HpNPV inoculum

The laboratory experiment on the vertical transmission of HpNPV was carried out using the insect population maintained in the Entomology Laboratory of Kerala Forest Research Institute, Sub Centre at Nilambur, Kerala, India. Methods used for establishment and maintenance of *H. puera* culture are available elsewhere[4,6,20]. The HpNPV used for various experimental purposes was originally isolated from diseased larvae collected from the teak plantations of Nilambur[6] and maintained in the above Laboratory following standard methods[7,20].

Experimental sites

HpNPV spraying experiments were carried out in the epicentre populations of *H. puera* at the Kariem–Muriem teak plantations (11°22.7′–11°25.7′N lat. and 76°16.44′–76°18.47′E long.) in Nilambur North Forest Division, Kerala. This plantation (1000 ha) comprises trees that are more than 25 years of age with an average of 8 m distance between them. The average annual rainfall of the region is above 4000 mm. The average day time temperature is above 32°C and relative humidity is above 70%. Vettilakolli teak plantation in the Nilambur North Forest Division located about 8 km from the experimental site was used as the control site. The age and spacing of trees and environmental conditions were similar in both the sites.

Vertical transmission of HpNPV in the laboratory

Five concentrations of HpNPV (1×10^2, 1×10^3, 1×10^4, 1×10^5 and 1×10^6 OBs/ml) were prepared from the stock suspension by serial dilution with distilled water. Volume of 10 µl of OB suspensions was placed on a 0.5 cm^2 leaf disc of tender teak leaf and fed to fifth instar larva of *H. puera* 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, except that the OB inoculum was replaced with distilled water. Five replicates each with 20 larvae for all five concentrations as treatment and one set of 30 larvae for each concentration as control were used in the experiment.

Mortality due to infection during the larval stages was recorded. The survivors from the concentrations that resulted in less than 50% larval mortality were included in the subsequent experiments. The larvae that survived were weighed as they entered into the pupa stage at their first day of pupation and reared to adults. Three crosses per concentration with ten replicates were set during the mating phase, i.e. infected male × infected female (IM × IF), infected male × healthy female (IM × HF) and healthy male × infected female (HM × IF). Another set of the healthy male × healthy female (HM × HF) with ten replicates served as the control. Each pair of moths was transferred to separate glass bottles (20 × 10 cm) covered with a white muslin cloth (which also served as the substratum for oviposition) and fed with 10% diluted honey. Eggs laid on each day were counted and the rearing bottles were changed daily. In order to understand the transovum (outside egg) and transovarial (within egg) transmission of HpNPV, sets of sterilized (with 0.1% sodium hypochlorite solution) and unsterilized eggs, each with 20 eggs and 30 replicates were monitored. The surface-sterilized and unsterilized eggs were later air-dried and kept in tender teak leaf for hatching.

The newly hatched larvae (referred to as the F1 generation) were reared on young leaves until the third instar and later transferred individually to artificial diet in plastic rearing bottles (5.5 cm × 2.5 cm) kept at 28° ± 4°C and relative humidity of 60 ± 10%. The mortality due to transmission of infection was recorded at succeeding

larval stages. Dead larvae obtained during the progression of the experiment were Giemsa-stained and observed microscopically for viral infection. Surviving insects were reared till the collapse of the population during the F2 generation. Various parameters were studied to estimate the cost associated with the surviving HpNPV challenge, specifically percentage pupation, adult emergence, adult longevity, egg-laying period and the influence on fecundity, hatching of eggs and virus mortality.

Seeding of HpNPV in the epicentre population

The field application of HpNPV was done in the epicentre population developed after the pre-monsoon showers (last week of March) using the virus stock diluted to a concentration of 6.5×10^2 OBs/ml. Plantowet (2 ml/l) was added in the mixture as an adjuvant. Within the epicentre, spraying was done using a high-volume sprayer from the ground and occasionally by climbing the trees. Point application and wind-assisted broadcast spraying was done to ensure that the whole area was covered under spray with minimum effort (Figure 1 *a*). The seeding was done in about 2 ha covering more than 150 trees. Since the epicentre is located only in one site in the entire landscape during this period, seeding was restricted to this single site.

Larval sampling was done prior to and three days after the application of the virus. Trees were randomly selected in the epicentre area. Twigs (consisting of at least four leaves) were defined as the basic sampling unit. Care was taken to take samples from three different levels of tree height, i.e. top, middle and bottom, and a total of nine samples was collected from each tree (three each from different tiers). The number of larvae per twig was scored individually (Figure 1 *b*). The larvae with characteristics of virus infection were scored separately (Figure 1 *c*). The larvae sampled after three days of field spraying were brought to the laboratory and kept individually in rearing tubes containing artificial diet to examine the probable NPV mortality.

Pupae were collected after six days of field application of HpNPV separately from the virus-treated area at Kariem–Muriem and an untreated plantation at Vettilakkolli (Figure 1 *d*). Hundred pupae found healthy on external appearance were collected from the treated and control plots, and brought to the laboratory to observe and compare the possible HpNPV transmission that can occur under natural conditions. In the laboratory, adult emergence and sex ratio of the emerged moths were noted. Four crosses were set during the mating phase with seven replicates each, with the same procedure adopted for the laboratory experiment. All the plantations in the landscape were regularly monitored to detect viral epizootic, if any, following HpNPV field application for about three months.

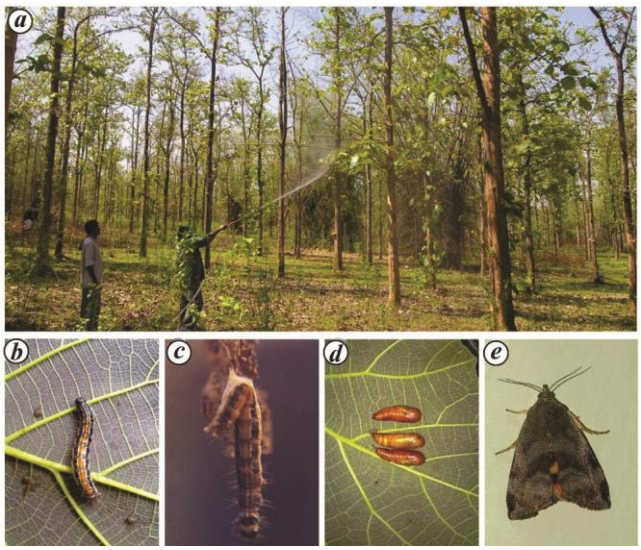

Figure 1. ***a***, Field application of HpNPV in the Kariem–Muriem teak plantation, Nilambur, Kerala, India. ***b***, Healthy *Hyblaea puera* larva. ***c***, HpNPV infected larvae with characteristic posture. ***d***, *H. puera* pupae. ***e***, *H. puera* adult.

Data analysis

The per cent mortality, pupation and adult emergence and sex ratio were subjected to one-way ANOVA with Tukey's multiple comparison tests. Larval and pupal weights were compared using one-way ANOVA. The period of egg-laying and oviposition rate of each cross at each concentration were also analysed with one-way ANOVA using SPSS 16.00 (ref. 21).

Results

Vertical transmission of HpNPV in the laboratory

Mortality due to HpNPV started 60 h post-inoculation. The percentage mortality due to HpNPV infection increased with increasing dosage (1×10^2 OBs/ml = 41%, 1×10^3 OBs/ml = 48%, 1×10^4 OBs/ml = 63%, 1×10^5 OBs/ml = 78% and 1×106 OBs/ml = 87%). No NPV deaths were found in the control. The survivors from two treatments (1×10^2 and 1×10^3 OBs/ml) were used for further experiments. Table 1 shows a comparison of the biological traits of the sublethally infected larvae with the untreated control. The sublethal HpNPV infection significantly affected pupation ($F_{2,12} = 82.25$, $P < 0.001$), pupal weight ($F_{2,211} = 201.84$, $P < 0.001$) and pupa-to-larva weight ($F_{2,211} = 4010.63$, $P < 0.001$) of the parent generation. Total adult emergence from the pupa ($F_{2,12} = 7.79$, $P < 0.007$) and female moth emergence ($F_{2,12} = 7.01$, $P < 0.01$) were significantly low, while there was no significant difference in male moth emergence ($F_{2,12} = 0.89$, $P = 0.435$) among the treatments. The female and male longevity ($F_{2,78} = 20.29$, $P < 0.001$ and $F_{2,78} = 10.70$,

Table 1. Effect of sublethal HpNPV infection on various biological parameters of the parent generation

Parameter	Treatment		
	10^2 OBs/ml	10^3 OBs/ml	Control
Pupation (%)	43.00 ± 4.64^a	34.00 ± 4.00^a	91.33 ± 1.33^b
Pupal weight (g)	0.30 ± 0.01^a	0.28 ± 0.01^a	0.13 ± 0.01^b
Pupal weight – larval weight (g)	0.08 ± 0.004^a	0.08 ± 0.001^a	-0.07 ± 0.001^b
Adult emergence (%)	74.75 ± 7.38^a	67.12 ± 7.84^a	97.84 ± 0.89^b
Male emergence (%)	38.91 ± 4.38^a	40.24 ± 5.82^a	47.65 ± 4.69^a
Female emergence (%)	35.84 ± 4.19^a	26.88 ± 4.85^a	50.18 ± 3.97^b
Sex ratio (male : female)	1.07 : 1.00	1.44 : 1.00	1 : 1.1.00
Adult longevity (male; days)	8.23 ± 0.22^a	8.33 ± 0.30^a	10.20 ± 0.25^b
Adult longevity (female; days)	9.37 ± 0.27^a	9.62 ± 0.34^a	11.00 ± 0.24^b

Within a row, means (± SE) followed by the same letters are not significant (Tukey's test, $P \leq 0.05$). OB, Occlusion bodies.

$P < 0.001$ respectively) were also significantly low in sublethally infected larvae 174 (1×10^2 and 1×10^3 OBs/larvae respectively) compared to the control.

Table 2 shows comparison of the different growth and reproductive parameters of the F1 generation obtained from different mating pairs descended from the sublethally infected larvae (1×10^2 and 1×10^3 POBs/larvae). The fecundity of different crosses set during the mating phase of the adult did not show any clear maternal influence. However, there was significant reduction in fecundity compared to the control ($F_{6,74} = 29.83$, $P < 0.001$). The egg-laying period of the females of different crosses was also significantly low compared to the control ($F_{2,78} = 10.70$, $P < 0.001$). The transovum and transovarial modes of transmission tested using sterilization of the egg surface revealed that there was a significant reduction in hatching of sterilized and/or unsterilized eggs compared to the control ($F_{6,74} = 338.43$, $P < 0.001$ and $F_{2,74} = 14.08$, $P < 0.001$ respectively). However, there were no significant difference in the hatching of sterilized or unsterilized eggs ($F_{11,90} = 0.83$, $P = 0.616$). Also, there was no HpNPV mortality in the control. The larvae that originated from the sterilized or unsterilized eggs did not show any significant difference in mortality due to sublethal HpNPV infection ($F_{11,24} = 1.06$, $P = 0.432$). As there was no difference among the studied traits due to transovum and transovarial transmission, the data were pooled for further analysis. The pupation rate of the larvae originating from different mating pairs was also different compared to the control ($F_{6,20} = 58.56$, $P < 0.001$). The experiment was stopped at this stage because of the low number of pupae obtained in the treatments.

Vertical transmission of HpNPV in the epicentre population

Before HpNPV application, the density of *H. puera* larvae averaged 17.44 ± 0.61 larvae/twig (range: 0–41, $n = 180$ twigs) with the fourth instar larvae dominating (58%). After three days of NPV field application, the density was 5.52 ± 0.31 larvae/twig (range: 0–16, $n = 180$ twigs) with 96% fifth instar larvae. The percentage viral infection due to HpNPV before and three days after field spraying was 0.4 ± 0.03 twig (range: 0–3.70, $n = 180$ twigs) and 2.50 ± 0.57 twig (range: 0–50.00, $n = 180$ twigs) respectively. However, mortality due to HpNPV infection increased to 56.65% in the 203 subsamples of larvae collected randomly from 20 trees after HpNPV spraying, when reared individually in the laboratory.

The emergence of adults from treated and control plots was 67% and 58% respectively. Though fecundity of the moths of different crosses was not significantly different between the groups ($F_{3,23} = 0.68$, $P > 0.05$), there was a maternal influence. Thus, oviposition rate of different crosses was in the order IM × IF < HM × IF < IM × HF < HM × HF. Though there was an effect of sublethal virus on different parameters, adult longevity ($F_{3,23} = 0.80$ and $F_{3,23} = 2.43$ for male and female respectively; $P > 0.05$) and the percentage of hatchability ($F_{3,23} = 2.62$, $P > 0.05$), HpNPV incidence ($F_{3,23} = 2.62$, $P > 0.05$) and pupation ($F_{3,10} = 2.05$, $P > 0.05$) were not significantly different among different mating pairs (Table 3). Due to the very low number of pupae obtained in some crosses and vertical transmission of NPV being proved, the experiment was stopped at the F1 level.

HpNPV epizootic

An epizootic of HpNPV was observed after two months of field spraying in the Kariem–Muriem plantation at the same place where the HpNPV spraying was carried out. The viral epizootic devastated insect population had spread out in 42 ha area. The epizootic populations were characterized by dead or morbid larvae with flaccid bodies hanging from the tree top. The larval density of the area was 2.08 ± 1.38 larvae per twig (range: 0–6 larvae). The percentage HpNPV incidence per twig was 58.27 ± 6.84 (range: 0–100). It was also noted that most of the dead larvae were of late instars, i.e. third to fifth instars. Differential Giemsa staining of insect tissues in the laboratory revealed the presence NPV polyhedra.

Table 2. Comparison of the effect of sublethal HpNPV infection on the growth and reproductive parameters of the F1 generation of different mating pairs

	Concentration			Mating pair			
	102 OBs/ml			103 OBs/ml			
Parameter	IM × IF	IM × HF	HM × IF	IM × IF	IM × HF	HM × IF	Control HM × HF
Fecundity	234.30 ± 13.72[b]	157.90 ± 10.33[b]	43.40 ± 4.48[a]	126.25 ± 7.38[e]	248.43 ± 14.86[e]	18.33 ± 1.20[d]	472.77 ± 33.29[c,f]
Egg-laying period (days)	3.60 ± 0.16[b]	3.40 ± 0.16[b]	3.60 ± 0.16[b]	2.63 ± 0.18[d]	3.00 ± 0.22[d]	2.83 ± 0.17[d]	6.13 ± 0.06[a,e]
Hatching–sterilized (%)	11.00 ± 1.00[a]	3.00 ± 0.82[a]	14.00 ± 1.63[a]	10.63 ± 1.48[d]	12.86 ± 1.01[d]	12.50 ± 1.12[d]	73.83 ± 0.81[b,e]
Hatching–unsterilized (%)	11.00 ± 1.25[a]	13.50 ± 1.50[a]	11.57 ± 1.50[a]	10.00 ± 1.34[d]	13.57 ± 2.10[d]	10.38 ± 1.54[d]	73.83 ± 1.21[b,e]
Male longevity (days)	8.10 ± 0.43[a]	8.80 ± 0.33[a]	7.80 ± 0.36[a]	8.50 ± 0.57[d]	8.86 ± 0.34[d]	7.50 ± 0.50[d]	10.20 ± 0.25[a,e]
Female longevity (days)	9.20 ± 0.51[a]	9.50 ± 0.48[a]	9.40 ± 0.48[a]	9.88 ± 0.61[d]	9.71 ± 0.52[d]	9.17 ± 0.70[d]	11.00 ± 0.24[a,d]
Mortality–sterilized (%)	86.31 ± 0.60[a]	84.72 ± 3.50[a]	78.52 ± 0.74[a]	87.78 ± 6.19[d]	88.89 ± 5.56[d]	93.33 ± 6.67[d]	0.00[b,e]
Mortality–unsterilized (%)	86.31 ± 0.60[a]	81.48 ± 3.70[a]	95.83 ± 4.17[a]	81.11 ± 1.11[d]	84.92 ± 8.29[d]	85.00 ± 7.64[d]	0.00[b,e]
Pupation (%)	13.69 ± 0.60[a]	16.99 ± 3.22[a]	13.75 ± 1.99[a]	15.25 ± 3.23[d]	13.25 ± 6.88[d]	10.37 ± 5.79[d]	92.33 ± 0.65[b,e]

Within a row, means (± SE) followed by same letters are not significant (Tukey's test, $P \leq 0.05$); IM, Infected male; IF, Infected female; HM, Healthy male; HF, Healthy female.

Table 3. Effect of HpNPV on fecundity, hatching, NPV incidence and pupation (mean ± SE) in the F1 generation of field-collected samples

	Mating pair			
Parameter	IM × IF	IM × HF	HM × IF	HM × HF
Fecundity (No. of eggs)	139.00 ± 1.27	303.60 ± 80.07	224.80 ± 75.72	371.17 ± 1.57
Male longevity (days)	8.57 ± 0.97	8.33 ± 0.76	7.86 ± 0.88	7.00 ± 0.38
Female longevity (days)	7.57 ± 0.69	9.17 ± 0.70	10.29 ± 0.61	8.71 ± 0.89
Hatching (%)	3.05 ± 4.31	10.68 ± 1.87	29.53 ± 6.71	52.33 ± 4.41
HpNPV (%)	17.63 ± 24.94	29.36 ± 25.45	42.07 ± 6.78	10.05 ± 2.46
Pupation (%)	27.30 ± 38.70	15.00 ± 25.98	16.04 ± 10.97	63.63 ± 4.58

The biological parameters of different mating pairs did not differ significantly (Tukey's test, $P < 0.05$).

Discussion

Vertical transmission of HpNPV in the laboratory

The vertical transmission rate in *H. puera* ranged between 13% and 59% based on NPV concentration, similar to other insect–baculovirus systems (Supplementary Table 1). In *H. puera*, viral transmission starts with the contaminated food as in other lepidopteran insects and the infection passes through both sexes to their progeny. Parent mortality was found to increase according to the viral concentration (41–87%) and decrease with the age of the larvae, as found in previous studies[20]. Such dose dependency of larval death was also reported in several species, including *Bombyx mori*[13], *Helicoverpa armigera*[22] and *Trichoplusia ni*[23], and larval age dependency in *T. ni* and *Lymantria dispar*[23,24]. Studies on the sublethal effects of HpNPV in successive generations have shown its apparent influence on pupation, adult emergence, mating success, fecundity, egg-laying period, hatchability and survival of larval offspring (Table 1). The reduction in pupation rate (approximately 28%) of sublethally treated *H. puera* larvae compared to the control is similar to that of *L. dispar*, *Mamestra brassicae*, *T. ni* and *Spodoptera litura*, in which infection with NPV resulted in slow larval growth, and inhibition of larval molting and pupation[23–25]. Pupae with more weight from sublethally HpNPV-infected larvae than uninfected ones may be due to the relatively low metabolism in sublethally infected insects. This is supported by the fact that most insect baculoviruses contain a gene (*egt*) that encodes the enzyme *ecdysteroid UDP-glucosyl transferase* which catalyses the sugar conjugation of ecdysteroids and thereby influences the molting of infected larvae to pupae[24,25].

Sublethal infection also affected the adult emergence and egg-laying period of the treated insects, which may be due to the persistence of NPV in adult and pupae of individuals surviving inoculation with virus, as found in other lepidopteran–NPV systems[26]. Sublethal infection could influence fecundity through an interaction with the

hormonal balance of the individual during development. This may be due to the improper vitellogenesis in the infected oocytes[13] by reducing the amount of fat and other nutrients available for egg or sperm production, or through associated cost of resistance mechanisms either when initially fighting off infection or in maintaining it at a non-lethal level[27]. The sex ratio of adults that emerged from the survivors of virus-challenged *H. puera* larvae was not significantly different, making it clear that both sexes are equally susceptible. At the same time the longevity of treated female adults was slightly higher than male adults, a general pattern in Lepidoptera[28].

According to Kukan[11], overt infections in subsequent generations can result from both transovum and transovarial modes of transmission. Therefore, vertical transmission through adult host is an adaptation for long-range environmental transport[29]. The present study has demonstrated biparental transmission of HpNPV to filial generations. It has also demonstrated that both transovum and transovarial routes of transmission occur in *H. puera*. The lack of significant differences in various biological parameters indicates the absence of clear evidence for maternal effect on vertical transmission in *H. puera*. Though maternal effect of transmission in the form of transovum and transovarial, and venereal routes of parasite infection was reported for a number of insects, including *B. mori*[13], *H. virescens*[30] and *S. exigua*[31], no evidence was found for venereal or transovum (including transovarial) transmission in others such as *L. dispar*[32]. The biparental mode of transmission of virus is more advantageous than a female-oriented one, due to equal chances of viral infection spread if either male or female is infected.

The F1 generation had 78–93% mortality without further inoculation of HpNPV and with a reduction in hatching, pupation and fecundity, which demonstrates the existence of vertical transmission of HpNPV in *H. puera*. The high progeny mortality and sublethal effects indicate a higher vertical transmission of HpNPV. Therefore, possible chances of viral resistance due to sublethal infection reported in other systems can be ruled out[33].

Vertical transmission of HpNPV in the epicentre population

Viral infection rate in natural *H. puera* populations was 0.25% and seeding increased NPV infection in the field to 5.83% after 72 h of virus application. At the time of field application of HpNPV, the fourth instar larvae were predominant (58%) and after three days of spraying most of them developed to fifth instar (96%). The age of the host can influence the pathogenicity of the virus[34], and this could be the reason for the lack of high mortality rate after three days of field application. However, mortality of the larvae brought to the laboratory increased to 56.65% by the end of the generation. Such high mortality rates after NPV application under natural conditions were also found in the case of other species such as *S. litura* and *L. dispar*[19,35]. In field conditions, high mortality rates are expected due to the augmented effect of HpNPV on susceptible larvae, horizontal transmission power of HpNPV, or both.

The changes in the biological and reproductive characters (pupation, adult emergence, mating systems and fecundity) of the offspring generation of *H. puera* due to seeding of the sublethal virus in the host lead to collapse of the successive generation without further HpNPV augmentation (Table 3), as found in the gypsy moths after NPV spraying[19]. Two months after HpNPV seeding, an epizootic was observed at the experimental site. It was assumed that the HpNPV epizootic had occurred among the F2 progenies of survivors of the previous population subjected to sublethal HpNPV infection through spraying considering the insect life span of 24–29 days. More than 50% of the sampled insects were found infected with HpNPV. The observed epizootic occurred at the end of the third population level (i.e. epidemic) and the heavy monsoon that followed resulted in low-density endemic populations hindering further monitoring of the effect of HpNPV seeding in the field. The epizootic without continuous augmentation of HpNPV could probably be due to a combination of horizontal and vertical transmission of the baculovirus in the field, as hypothesized by Fine[36].

Conclusion

The timing, frequency and patterns of spraying of virus as a bioinsecticide will be influenced by its spread and persistence in the host population. Information on the epizootiology of HpNPV obtained in the present study needs to be incorporated in the management of teak defoliator. The existing protocol insists on timely detection of infestation of teak defoliator for successful use of HpNPV for its management and spraying of HpNPV in the entire area of infection for killing all individual pests making it costly and time-consuming[37]. The present study on the vertical transmission in both laboratory and the field indicates that the survivors of sublethal HpNPV dose infection could act as carriers of HpNPV to next generations through both maternal and paternal routes. Based on the existing knowledge on the biology, population dynamics and migration characteristics of *H. puera*, seeding of the virus HpNPV in sublethal doses in epicentre populations would be the most practical and economical method to control large-scale population outbreaks of the teak defoliator. The same method can be considered for similar pests as well.

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