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Field efficacy of nuclear polyhedrosis virus for protection of teak against the defoliator, *Hyblaea puera* Cramer (Lepidoptera : Hyblaeidae)

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ABSTRACT : In the year 1993, a 100 tree plot in a 17 year old teak plantation at Nilambur in Kerala was experimentally protected from *Hyblaea puera* Cramer, a serious defoliator of teak plantations, using a naturally occurring baculovirus (*Hp*NPV) reported earlier from this species. During the year, there were four major peaks of defoliator infestation from March to June. One-time foliar application of a crude preparation of *Hp*NPV at the rate of 1 x 10^5 POB/ml of the spray fluid, at the earliest sign of each infestation, gave 70-76 per cent protection of foliage during the first two infestations. A reduced foliage protection of 33-43 per cent obtained during the third and fourth infestations was attributable to occurrence of rain soon after application of the spray. In protected trees, the basal area increment was enhanced by 41 per cent, indicating the efficacy of *Hp*NPV as a biocontrol agent against the teak defoliator.

KEY WORDS : Baculovirus, *Hp*NPV, *Hyblaea puera*, teak defoliator

The teak defoliator, *Hyblaea puera* Cramer (Lepidoptera : Hyblaeidae) is recognized as the most serious pest of the teak tree, *Tectona grandis* Linn.f. (Beeson, 1941). In Kerala, *H. puera* outbreaks occur almost every year, causing one to three severe defoliations during the early part of the growth season (Nair, 1988). Nair *et al.* (1966) estimated that a net annual gain of 3 m^3 of wood volume per hectare can be obtained in young teak plantations, by protecting them against the defoliator.

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Chemical as well as biological methods have been tried in the past to control the teak defoliator (Mathur, 1960; Basu-Chowdhury, 1971; Singh, 1980; Nair *et al.*, 1989, 1995), but none have been found satisfactory. In forests, the scope of chemical control is limited because of environmental concerns, but biological control using naturally occurring insect pathogens is promising (Burges, 1981). In a study on natural mortality agents of the teak defoliator, Sudheendrakumar *et al.* (1988) isolated a viral pathogen which was identified to be a nuclear polyhedrosis virus (*Hp*NPV) causing death of larvae within 72 h in laboratory tests. In the present study, the field efficacy of *Hp*NPV and the feasibility of using it to protect a teak stand continuously over a period of one year was examined.

MATERIALS AND METHODS

The study was carried out in the year 1993 in a 17 year old teak plantation at Kariem-Muriem, Nilambur (latitude 11°22'-11°25' N, longitude 76°16'-76°18'E). Two plots of about 100 trees, each of comparable growth and stand composition, were selected. A buffer strip of the plantation, 100 m wide, was left between the two plots. Both the plots were kept under surveillance by daily visual observation during the critical periods. Trees in the experimental plot were sprayed with a preparation of *Hp*NPV whenever infestation occurred. The control plot was left untreated. A total of five virus applications were made to control five distinct infestations when the larvae were in the first or second instar.

A stock suspension of the polyhedral occlusion bodies was prepared as described below. Field collected third or fourth instar host larvae were fed in the laboratory with teak leaves treated with POB suspension. Dead larvae were collected after 72 h and suspended in distilled water and allowed to putrefy for 7-8 days. The macerated suspension was filtered through muslin cloth and centrifuged at 1000 rpm for 2 min and the sediment discarded. The filtrate was then centrifuged at 5000 rpm for 20 min. The pellet was suspended in water and centrifuged at 5000 rpm for 20 min and this process was repeated thrice. The pellet obtained finally was suspended in distilled water and stored. The spray fluid was prepared by diluting the stock with non-chlorinated water to a concentration of 1 x 10^5 POB/ml. Before spraying, a wetting agent (0.2% Tween 80) was added.

Each tree within the treatment plot was individually sprayed using a rocker-sprayer, in the morning hours. The quantity of spray fluid applied per tree ranged from 0.75-1.75 litres, depending on the total foliage present. Rain occurred during the third, fourth and fifth trials. In the third trial, fairly heavy rain occurred on the day of spraying (17.6 mm) as well as on the second (26.6 mm) and third day (2.4 mm) after spraying. In the fourth trial, rainfall ranging from 10.5 mm to 25 mm occurred on the day of treatment as well as during the next four days. The period of fifth trial was characterised by rainfall on all days (mean 21 mm). The treatment effect was assessed by scoring the leaf damage,

counting the surviving larvae and measuring the girth increment of the trees.

To measure leaf damage, 14 well-infested trees were marked in each plot on the first day of each spray. As the level of infestation varied depending on flushing intensity, only well infested comparable trees were chosen. From each tree, eight shoots were sampled randomly, two days and 4-6 days after treatment. Percentage leaf loss was scored visually into one of five class intervals (0-5, 6-25, 26-50, 51-75, 76-100) and using the mid-point of the score interval, the mean percentage leaf loss per shoot was calculated. The average of 8 shoots gave the percentage leaf loss for the tree. These values were transformed using Taylor's power law to stabilize the variance (Southwood, 1978) (for this set of data, the value of Z was $x^{0.3}$). The significance of the difference in the mean leaf damage between treated and untreated trees was analyzed using multiple analysis of variance. In trial No.3 (Tables 1 and 2), an additional estimate of foliage loss was made after the infestation was over, in order to assess the total impact. For this, the tree was taken as the unit instead of the sampled shoots, and the damage was scored into the same score classes.

To assess mortality of larvae due to treatment, counts of larvae were made from the shoots sampled for estimating leaf damage. Counts were made prior to each treatment (including eggs) and twice after treatment. The actual counts were transformed using Taylor's power law to stabilise the variance (for this set of data the value of Z was $x^{0.2}$). The significance of the differences was tested by multiple analysis of variance, using pretreatment counts as covariates.

To measure growth of trees, the girth at breast height (GBH) of all the trees in the two plots was measured prior to the first treatment and at the end of the year. From the initial and final GBH of each tree, the corresponding basal area was calculated. The basal area increments were compared by analysis of variance using the initial basal area as covariate.

RESULTS AND DISCUSSION

There were four major and one minor, discrete infestations during the year, as indicated by the insect counts in the untreated plots (Fig.1).

In the first trial (Table 1), four days after treatment, foliage loss was only 14 per cent in the treated trees compared to 46 per cent in the untreated trees. This difference was statistically significant ($P < 0.01$). Seventy per cent of the potential leaf loss was prevented by the treatment. The protection afforded to the foliage was not reflected in the post-treatment count of larvae (Table 2); larval numbers had declined in both treated and untreated trees with no statistically significant difference between the two.

In the second trial, six days after treatment the foliage loss was only 10 per cent compared to 42 per cent in the untreated trees (Table 1). The difference

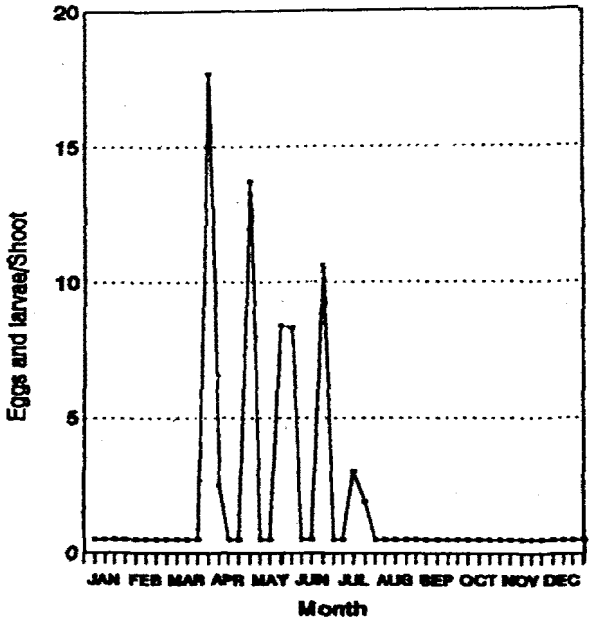

Fig 1. Outbreaks of *Hyblae puera* in the experimental plantations during 1993 as indicated by counts of eggs and larvae in the untreated plots

was statistically significant ($P < 0.01$). The degree of protection worked out to 76 per cent. The larval counts showed significant difference after 2 days, but not after 6 days.

In the third trial, only 43 per cent of the foliage loss was prevented by the treatment. Although the difference between treated and untreated plot was significant, the level of protection was not satisfactory. The larval number differed significantly between treated and untreated on the sixth day but not on the second day after treatment (Table 2). Foliage loss assessment made for the whole trees on completion of larval feeding (9 days after treatment) gave 95 per cent mean leaf loss in untreated trees compared to 74 per cent

Table 1. Efficacy of *Hp*NPV for control of *Hyblaea puera* measured by protection from defoliation in five consecutive field trials in 1993

Trial No. & Date of application	Mean percentage leaf loss				Per cent protection
	2 days after treatment		4-6 days after treatment		
	Untreated	Treated	Untreated	Treated	
1 (23 March)	-	-	46[a*]	14[b*]	70
2 (20 April)	1[a]	15[b]	42[a]	10[b]	76
3 (17 May)	29[a]	41[a]	67[a]	38[b]	43
4 (12 June)	23[a]	31[a]	60[a]	40[b]	33
5 (9 July)	3[a]	2[a]	3a	2[a]	33

All the values were statistically adjusted for initial variability and rounded off to the nearest integer

* Within each set of untreated and the corresponding treatment, the difference between the values followed by the same letter are not statistically significant

in the treated; the level of protection worked out to 22 per cent.

In the fourth trial also, the foliage loss significantly differed between treated and control, but the level of protection was only 33 per cent (Table 1). The larval count showed no significant difference (Table 2). In the fifth trial, the infestation level was too low (Fig.1) to arrive at any meaningful conclusion on the effectiveness of treatment.

tree is an index of growth in wood volume. With a mean initial basal area of 208 cm^2 per tree per year, the mean increment of basal area over the year was 24 cm^2 in the treated plot compared to 17 cm^2 in the untreated plot. The difference was statistically significant ($P < 0.01$). The gain in basal area increment per tree due to treatment was 41 per cent. Among the three methods used for assessing the treatment effect, defoliation scoring is the most practicable.Although in this study we

Table 2. Population estimates of *H. puera* in the untreated and treated trees

Trial No.	Pre-treatment (eggs and larvae/shoot)		Post-treatment (larvae/shoot)			
	2 days		4-6 days			
	Untreated	Treated	Untreated	Treated	Untreated	Treated
1	17.7	16.2	6.3[a]	5.2[a]	2.8a	2.2[a*]
2	13.7	19.8	11.0[a]	3.7[b]	3.0[a]	1.0[a]
3	8.4	20.9	8.2[a]	4.8[a]	8.0[a]	1.2[b]
4	0.8	9.7	14.1[a]	15.8[a]	3.9[a]	4.2[a]
5	3.4	10.7	2.0[a]	4.5[a]	0.5[a]	0.7[a]

* Within each set of the untreated and treated trees, the difference between values followed by the same letter are not statistically significant

The lower level of foliage protection obtained in the third and fourth trials in comparison to the first and second was attributable to the occurrence of rain during the experimental period which would have caused partial washing off of the POBs from the foliage. This suggests the need of adding suitable sticking materials in the POB suspension before field application.

The increment in the basal area of a

scored the leaf damage in sampled shoots, visual scoring of the whole tree for defoliation as we did in the third trial, is simpler. This is best done after the feeding is complete and the larvae have descended to the ground for pupation. Sampling of the surviving larval population to estimate the fall-off of number due to treatment was less efficient than defoliation scoring, apparently due to high inter tree variability in larval numbers, and dispersal of larvae

during the experimental period. Sampling of dead larvae is impracticable because they are easily dislodged from the trees. Although the effectiveness of NPV was also demonstrated by tree growth measurement, this method can be applied only for long-term studies.

The results show that 70-76 per cent of the leaf damage caused by *H. puera* can be prevented by timely, one-time foliar application during each outbreak of a crude preparation of *Hp*NPV polyhedral suspension containing 1 x 10^5 POB/ml applied at the rate of 0.75 to 1.75 litres/ tree (depending on the foliage level) using a high volume sprayer. Compared to many other baculoviruses, the *Hp*NPV is quick acting and causes significant mortality of larvae in about 3 days (Mohammed Ali *et al.*, 1990). While the present study establishes the feasibility of using *Hp*NPV for controlling *H. puera* infestation, further refinements in the spray formulation, dosage and application technology, etc. are necessary for its economic and effective use.

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