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Management of coconut rhinoceros beetle (*Oryctes rhinoceros*) by augmentation of *Oryctes* baculovirus (Kerala isolate) in Little Andaman Islands

G SHYAM PRASAD [1*], V JAYAKUMAR[2] and T V R S SHARMA[3]

Central Agricultural Research Institute, Port Blair, Andaman & Nicobar Islands 744 101

ABSTRACT

A study was conducted during 2002–05 for optimization of virus dosage for efficient dispersal of virus to ensure maximized disease transmission, suppression of beetle (*Oryctes rhinoceros* L). To suppress the pest, baculovirus was released for the first time in 1987 at Hut bay, Little Andaman, which eventually brought down palm damage to insignificant levels within 4 years of release. However during 2000–01 fresh outbreak of coconut rhinoceros beetle was noticed warranting release of virus through infected beetles at a dose of 130 µg of Crude Virus Preparation/beetle for efficient dispersal of virus. Release of 135 virus inoculated beetles at 3 locations in Little Andaman's resulted in over 99% reduction in palm damage by 36th month of augmentation. Examination of trapped adults confirmed 3.6-fold increase in prevalence of virus with the reduction in breeding sites.

Key words: Baculovirus, Little Andaman, *Oryctes rhinoceros*

The Little Andaman's located between 6–14 N and 92–94 E longitude has a total geographical area of 732 km^2. Major part of the arable area is under coconut (*Cocos nucifera* L.) and red oil palm (*Elaeis* Jacq.). However coconut rhinoceros beetle (*Oryctes rhinoceros*) greatly limits coconut production by damaging crown region of the palms (10 – 40%). *Oryctes* baculovirus, the pathogen of *O. rhinoceros* is naturally present in coconut-growing tracts of Asia including India (Zelazny 1977b, Mohan *et al.* 1983). The biological suppression of coconut rhinoceros beetle by *Oryctes* baculovirus has been successful in many parts of the world (Mohan and Pillai 1993, Prasad *et al.* 2008).

The Kerala isolate of *Oryctes* baculovirus (OBV-KI) was introduced for the first time into the Little Andaman Islands during 1988 in 1 location for suppression of *O. rhinoceros*. This led to dramatic reduction in palm damage (Jacob 1996). However, replacing old palms with new plantings led to increase in the beetle population, thereby leading to increase in the palm damage due to availability of abundant breeding sites. Thus warranting re-release of *Oryctes* baculovirus (OBV-KI) to suppress the beetle population.

The present paper reports optimization of virus dosage for efficient dispersal of virus to ensure maximum disease transmission, suppression of the beetle and augmentation of baculovirus in Little Andaman's during June 2002–August 2005.

[1]Senior Scientist (Entomology), (E mail: shyamprasad @nrcsorghum.res.in), NRC for Sorghum, Hyderabad 500 030; [2]Scientist Sr. Scale (Pathology), (E mail:jkpath@rediffmail.com); [3]Head, Field crops, (E mail: tvrs@indiatimes.com)

MATERIALS AND METHODS

The *Oryctes* baculovirus Kerala isolate (OBV-KI) was propagated on second and third instar rhinoceros beetle grubs. For bioassay OBV-KI, infected midguts of grubs were extracted, homogenized in chilled phosphate buffer (50 mM, *p*H 7.6) and clarified by centrifugation at 5 000 rpm for 10 min. (Mohan and Pillai 1993). The supernatant was passed through 0.45 m membrane filter. The virus was expressed as weight of virus infected tissue/ml. The crude virus preparation was stored at – 20°C till further use.

The healthy adults required for the study were reared in insectary of Department of Entomology, Central Agricultural Research Institute, Port Blair (India). Insects were reared from egg to late third instar grubs in 1 m diameter concrete ring filled with sterilized cowdung and sawdust (1: 1). The final instar grubs were collected and allowed for pupation in 5.0 kg volume perforated plastic containers containing sterilized sawdust. On emergence, the beetles were transferred to plastic container containing bits of coconut frond as feed. The optimum dosage of virus ensuring maximum longevity of the infected beetle along with reduced fecundity was determined for effective dissemination of virus among the natural population over a long time.

Bioassays were done using a week-old laboratory reared *O. rhinoceros* beetles. Droplets of crude virus preparation in

Table 1 Effect of OBV (KI) infection on the longevity and fecundity of *Oryctes rhinoceros* adults

Dose of CVP (mg/ 0.1 ml)	Longevity		Longevity reduction %		Fecundity Eggs/female	Fecundity reduction (%)
	Male	Female	Male	Female		
5	38.0[b]	43.4[b]	34.0	29.4	16.5[c]	71.7
50	21.5[c]	23.3[c]	62.6	62.1	9.8[b]	83.2
500	17.9[d]	20.5[d]	57.3	66.6	1.4[a]	97.6
5000	13.2[e]	16.3[e]	77.1	73.5	0[a]	100
Healthy	57.6[a]	61.5[a]			58.5[d]	
CD (P=0.05)	1.6	1.7			1.6	

Means followed by the same letter is not significantly different by LSD $P = 0.05$

10% sucrose solution ranging from 5×10^{-3} to 5.0 mg infected tissue/0.1 ml was applied on the mouth parts of upturned beetles in 2 split doses on successive days. The inoculated beetles were confined individually in plastic containers with moist autoclaved saw dust and bits of coconut frond. The beetles were observed daily for OBV infection and confirmed by Giemsa staining. The bioassay was replicated twice and the median effective dose computed by probit analysis (Finney 1975).

Two healthy males were paired with an infected female. The hatching of laid eggs and disease symptoms in the developing grubs were observed till the grubs pupated. Longevity and fecundity of infected female beetles and longevity of male was recorded. The adult longevity and fecundity data was subjected to ANOVA and means were separated by LSD (Gomez and Gomez 1978).

Droplets of dilutions of 130.0 mg CVP/0.1 ml, the ED_{50} mixed with 10% sucrose was fed to beetles on 2 successive days. The inoculated beetles were confined to container with autoclaved saw dust and fed with coconut frond bits. The virus inoculated beetles were released at dusk in coconut grooves. Totally 135 virus infected beetles were released at 3 experimental locations during June 2002 – July 2003.

The monitoring of virus prevalence in natural population at Little Andaman was carried out at pre- and post-augmentation of the virus by deployment of Sime RB pheromone traps. The presence of baculovirus in adults was determined mainly by visual observation of the midgut and its contents. Disease diagnosis in grubs collected from the breeding sites was done periodically based on the external symptoms and also by visual inspection of the guts. The decline in the breeding sites (sawdust, oil palm bunch refuse heaps, dead palms) was monitored for recording occupancy by the grubs.

The reduction in frond and crown damage was assessed at 8 – 10 months interval by random selection of 300 marked palms (100 palms in each replication) at all the 3 locations during 2002–05.The extent of palm damage (%) was computed from number of damaged leaves, spindles to the total number of leaves, spindles that had emerged since the previous observation. The data were subjected to ANOVA (Gomez and Gomez 1978).

RESULTS AND DISCUSSION

Adult longevity

An effective dose (ED_{50}) of 130.20 mg of CVP; log ED 50 = 2.12; Slope (b) = 1.52; Intercept (a) = 1.79; df = 2; c^2 = 0.62 indicating homogeneity was optimum to ensure maximum longevity of the infected beetle coupled with reduced fecundity thus, facilitating effective spread of virus among the natural population.

Exposure of beetles to graded doses of crude virus preparation led to reduction in longevity of the beetles to varying degrees depending on the concentration of crude virus preparation (Table 1). The average longevity of healthy male and female beetles was 57.6 and 61.5 days respectively. The reduction in longevity of male and female relative to healthy beetles was 69 and 67% at 500 mg of Crude Virus Preparation (CVP). At 5 000 mg of CVP the longevity reduction in male, female was 77 and 70% respectively. The male and female beetles exposed to 50 mg of crude virus preparation showed relatively longer life span of 21 and 23 days with average reduction of 37 and 34% respectively. Longevity of male and female were statistically at par at all the doses of crude virus preparation.

The average fecundity of healthy beetle was 58.5 eggs with 80% hatching. During short-life span of 16 and 20 days there was 100 and 99.4% reduction in fecundity at 5 000 and 500 mg of CVP, whereas at 50 and 5 mg recorded 83 and 72% reduction in fecundity during life span of 23 – 43 days. Thirty two per cent of eggs failed to hatch. Zelazny (1977a) reported that the drastically reduced fecundity greatly contributed to population decline in the field.

Pre- and post-virus release palm damage assessment

Prior to release of OBV-KI infected beetles, ie in June 2002 frond and spindle damage was 55.1 and 44.4% respectively (Table 2). Observations after 24 months of virus release, i e in May 2004 recorded a remarkable decline in palm damage well below ETL of 10% (crown damage 4.4% and spindle damage 0.7%). There was a progressive decline in palm damage from June 2002 to September 2005 by declining from 55.1 to 0.4% and 44.1 to 0.1% in frond and spindle damage, respectively at all the 3 locations. There was a significant reduction in palm damage by 24 months of release and the frond and spindle damage was below 8%. The reduction in spindle damage was rapid compared to leaf damage, whereas the leaf damage was the cumulative damage which includes previous spindle damage also resulting in higher damage figures. The most significant results in the experiment was the reduction in frond and spindle damage

Table 2 Impact of OBV (KI) release on *Oryctes rhinoceros* in Little Andaman's

Observation	Frond damage (%)			Mean	Spindle damage (%)			Mean
	L_1	L_2	L_3		L_1	L_2	L_3	
Pre-release								
June 2002	62.5^c	56.3^c	46.5^c	55.1^c	51.5^c	43.8^c	37.8^c	44.4^c
Post-release								
July 2003	34.3^b	28.8^b	22.8^b	28.6^b (48.1)	18.5^b	10.9^b	10.3^b	13.3^b (70.0)
May 2004	7.2^a	3.5^a	2.6^a	4.4^a (92.0)	1.1^a	0.7^a	0.4^a	0.7^a (98.4)
Sept. 2005	0.7^a	0.2^a	0.3^a	0.4^a (99.3)	0^a	0.3^a	0a	0.1^a (99.7)
CD (*P*= 0.05)	9.9	11.3	12.3	7.1	9.5	6.6	6.3	6.8

Means followed by the same letter(s) are not significantly different by LSD *P*=0.05
Figures in parentheses represent per cent reduction in frond/spindle damage over pre-release damage
L_1, Hutbay; L_2, Butler bay; L_3, R K. Pur

by 99.0% within 3 years after the release of OBV-KI infected beetles. This trend agreed with the previous report (Prasad *et al.* 2008).

Observations during September 2005 showed establishment of baculovirus in the natural population. The infected beetles released in 2002 and 2003 have spread the disease to the succeeding generations as indicated by the first observation made 12 months after the release and subsequent observations. Transmission of virus occurs by the visits of the infected female for oviposition, and males for mating. Thus, the beetles are regarded as productive flying virus reservoirs disseminating virus in an efficient and ideal manner (Huger 2005).

The drastic decrease in the population of beetles and grubs suggested occurrence of epizootic in a span of 12 months following the release of virus. Similar epizootic outbreaks of OBV disease have been reported in India (Mohan and Pillai 1993, Jacob 1996).

Prevalence of virus disease in grubs and beetle population

Larval population in all the breeding sites showed decline by the first year of release of baculovirus. Observation of beetles in traps, 12 months after the release of virus recorded 40.5% infection. In September 2005, 67.6% of trapped beetles were infected by virus, coinciding with the lowest levels of beetle and larval population. There was a progressive decline in the site occupancy ratio from 0.85 to 0.05 (Table 3). It was observed that many of the breeding sites had been abandoned by the beetles. The increase of infection among the grubs can be attributed to release of 135 infected beetles.

Table 3 Post-release baculovirus prevalence in natural population of *Oryctes rhinoceros*

Observation	Site occupancy ratio	Adult baculovirus prevalence (%)
Pre- release		
Jun 2002	0.85 (n = 55)	18.4 (n =103)
Post -release		
July 2003	0.61 (n = 39)	40.5 (n =121)
May 2004	0.24 (n = 42)	55.1 (n =69)
Sept. 2005	0.05 (n = 60)	67.6 (n =71)

*Data on annual basis

The mean prevalence of baculovirus infection in beetles was 18.4% in 2002, which stabilized to 67.6% by September 2005 after the 2 releases made in 2002, 2003 (Table 3). Overall there were 3.6-fold increase in the virus prevalence among beetle population. Such a trend was reported by earlier workers with varying degrees of incidence in different parts of the world, viz 50% in trapped beetles in Minicoy two- and a half year after introduction of OBV-K I, 43 to 63%, 8 years after the introduction of OBV into Western Samoa (Zelazny 1977b) and between 30–50% after 15 years (Marschall and Ioane, 1982). Zelazny (1977b) reported 4–29% virus incidence in the Philippines and Indonesia and the highest incidence of 84% were recorded in trapped beetles in Tonga (Young and Longworth 1981), 7 years after OBV was introduced into this Island. Damaging outbreaks have been reported to occur, especially after natural disasters and

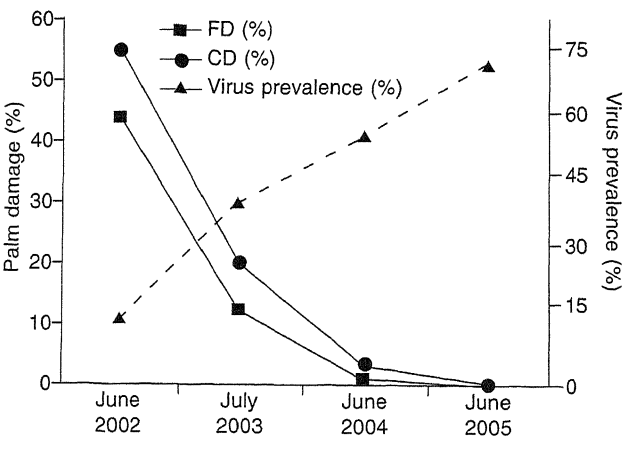

Fig 1 Palm damage reduction in Little Andaman's due to release of baculovirus

other forms of land clearances that result in large amount of rotting compost (Jackson *et al.* 2005).

Following the epizootic the larval and beetle populations, breeding site occupancy depicted decline and subsequently stabilized at low levels and virus incidence in natural population increased (Fig 1). Similar observations had been made in many South Pacific Islands (Young and Longworth 1981).

Management of *O. rhinoceros* needs an ongoing sustained effort and the current system can be improved by increased speed of action of virus and greater reliability. Experiences from past releases of OBV-KI in these islands suggest that inundative releases have to be made as and when coconut palm damage exceeds threshold levels of 10%.

Consequent to December 2004, tsunami about 5 000 ha of coconut palms have suffered permanent damage in A & N Islands generating huge quantity of organic matter (coconut stumps), which are potential breeding sites for beetles to multiply. Recent survey shows outbreak of *O. rhinoceros* in southern group of islands (Car Nicobar, Nancowry group and Katchal), which has witnessed severe devastation. In such situation inundative release of OBV-KI infected *O. rhinoceros* is suggested for effective management of *O. rhinoceros.*

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