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Bio-suppression of coconut rhinoceros beetle, *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) by *Oryctes* baculovirus (Kerala isolate) in South Andaman, India

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Abstract

Coconut rhinoceros beetle (*Oryctes rhinoceros* L.) is the major constraint in the production of coconut across the Andaman & Nicobar Islands. To suppress the pest population, baculovirus was released for the first time in 1987 at four locations in South Andaman, which brought down palm damage to insignificant levels within 48 months of release. However, during 1999–2000 large-scale felling of old coconut palms to be replaced with fresh ones by the Andaman Plantation Development Corporation (APDC) resulted in a fresh outbreak of coconut rhinoceros beetle. This warranted release of virus through infected beetles to suppress the pest population. A dose of 130 μg crude virus preparation (CVP)/beetle ensured optimum longevity coupled with low fecundity for efficient dispersal of virus. Release of such pre-infected beetles (795) at five locations in South Andaman resulted in over 90% reduction in palm damage within 23 months of release. Examination of experimental and adjoining palm groves confirmed the prevalence of virus among the sampled beetle population, which registered three-fold increases since the release of virus. There was a

drastic reduction to the extent of 91% in the occupancy. However, the recent tsunami in December 2004 in addition to destroying 5000 ha of coconut area has generated huge amounts of organic matter, which has resulted in the rhinoceros beetle outbreak. Augmentation of virus is suggested for effective management of the pest.

Keywords: *Oryctes rhinoceros*; Baculovirus

Article Outline

1. Introduction

Globally, India is the major producer of coconut, *Cocos nucifera* L., with an annual production of nearly 15,000 million nuts (Nampoothiri and Thomas, 2000).

The Andaman & Nicobar group of islands, located between 6–14°N and 92°–94°E longitude has a total geographical area of 8,29,300 ha. The major part of the arable area (about 24,746 ha**)** is under coconut palms. However, the coconut rhinoceros beetle (*Oryctes rhinoceros*) greatly limits coconut production on these islands by damaging the crown region of the palms (10–40%) (Anonymous, 2005).

Oryctes baculovirus (*Baculovirus oryctes*), the entomopathogen of *O. rhinoceros*, was discovered from the wild population in Malaysia (Huger, 1966), is naturally present in other parts of Asia including India ([Zelazny, 1977a] and [Zelazny, 1977b]; Mohan et al., 1983). The biological suppression of coconut rhinoceros beetle by *Oryctes* baculovirus has been demonstrated in many parts of the world (Purrini, 1989; Mohan and Pillai, 1993; Jacob, 1996). The virus-infected adult beetles act as reservoirs of virus, spreading the disease in their natural habitats (Huger, 1973).

Oryctes baculovirus introduction is reported from Western Samoa in 1967 (Marschall, 1970). Further introductions of the virus were made in Fiji (Bedford, 1976) during 1970–1974, on Wallis Island (Hammes, 1971) in 1970–1971, on Tonga Island (Young, 1974) in 1970–1971, on Tokelau Island ([Zelazny, 1977a] and [Zelazny, 1977b]) in 1967–1973 and in Mauritius (Monty, 1978) in 1970–1972, resulting in reduction in palm damage. In 1978–1979, the virus was released in Papua New Guinea (Gorick, 1980).

The Kerala isolate of *Oryctes* baculovirus (OBV-KI) was introduced for the first time into the Andaman Islands during 1987 in four locations for suppression of *O. rhinoceros*. This led to about 90% reduction in palm damage within 43 months of virus release (Jacob, 1996). However, during 1999–2000 large-scale felling of coconut palms by M/s Andaman Plantations Development Corporation for new planting over an area of 1500 ha led to an increase in the beetle population, thereby causing an increase in the palm damage due to availability of potential breeding grounds. This warranted the release of *Oryctes* baculovirus (OBV-KI) to suppress the beetle.

The present paper presents the study carried out at CARI, Port Blair, from June 2001 to August 2005. The work included standardization of virus dosage for inoculation of adult rhinoceros beetle with the objective of efficient dispersion of virus over prolonged periods to ensure maximum disease transmission and population suppression of beetles leading to reduced palm damage.

2. Materials and methods

2.1. Virus

OBV-KI was propagated on second and third instar rhinoceros beetle grubs (Zelazny, 1972). For bioassays OBV-KI-infected midguts of grubs were removed and homogenized in chilled phosphate buffer (50 mM, pH 7.6) and clarified by centrifugation at 5000 rpm for 10 min (Mohan and Pillai, 1993). The supernatant was passed through 0.45 μm membrane filters. The virus was expressed as weight of virus-infected tissue/ml. The crude virus preparation (CVP) was stored at −20 °C.

2.2. Rearing of *O. rhinoceros* adults

The disease-free beetles for the study were reared in the insectary of the Department of Entomology, Central Agricultural Research Institute, Port Blair, India. Insects were reared from egg to late third instar grubs in 1 m diam. concrete rings filled with sterilized cow dung 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 containers containing bits of coconut frond as feed.

2.3. Standardization of virus dose

The dosage of virus which when administered would give maximum longevity of the infected beetle coupled with reduced fecundity was determined to ascertain effective spread of virus among the natural population over a long period.

Bioassays were performed using 1-week-old laboratory-reared *O. rhinoceros* beetles. Droplets of CVP in 10% sucrose solution ranging from 5×10^{-3} to 5.0 mg infected tissue/0.1 ml was dispensed on mouth parts of upturned beetles. Totally, 0.2 ml was fed in two split doses on successive days. Twenty beetles were exposed to each concentration. The inoculated beetles were confined individually in plastic containers with moist autoclaved sawdust. Bits of coconut frond were offered as feed. The beetles were observed daily; OBV infection was confirmed by Giemsa staining (Zelazny, 1978). Longevity and fecundity of infected female beetles and longevity of male beetles were recorded. The bioassay was replicated twice and the median effective dose computed by Probit analysis (Finney, 1975) using MSTAT-C version 2.10. Two healthy males were allowed to mate with an infected female. The percent hatching of eggs laid by adult females and disease symptoms in the developing grubs were observed till pupation. The life span of beetles post-virus treatment was taken for calculating longevity. The difference in longevity of males and females was analyzed by single-factor analysis of variance

(ANOVA). The male and female longevity and fecundity data were subjected to ANOVA in randomized block design. The means were compared by LSD (p=0.05%).

2.4. Release of virus-infected beetles

Droplets of dilutions, 130.0 μg CVP/0.1 ml, the ED_{50} mixed with 10% sucrose, were fed to beetles in split doses on two successive days. The inoculated beetles were fed with coconut frond bits and confined to a container with autoclaved sawdust. The virus-inoculated beetles were field released at dusk. Totally, 795 virus-infected beetles were released at five experimental locations (viz. Minnibay, Mithakhadi, Bambooflat, Garacharma, Sippighat) over an area of 53 ha at the rate of 15 beetles/ha during June 2001–July 2002.

2.5. Monitoring of baculovirus prevalence in natural populations of *O. rhinoceros*

The monitoring of virus prevalence in natural population at five locations in South Andaman was carried out by deployment of Sime RB pheromone traps before and after the augmentation of virus. The presence of baculovirus in adults was determined mainly by visual observation of the midgut and its contents (Zelazny, 1978).

Disease diagnosis in grubs collected from the breeding grounds such as sawdust dumps at three saw mills, dead standing palms and decomposing cow-dung heaps was based on external symptoms and also by visual inspection of the guts (Huger, 1966). The grubs were dissected and the midgut epithelial smear stained with Giemsa was observed for confirmation of disease (Mohan et al., 1983).

2.6. Pre- and post-baculovirus release damage assessment

The reduction in frond and crown damage was assessed at 8–10-month intervals by random selection of 300 marked palms (100 palms in each replication) at all the five locations during 2001–2005.

The extent of palm damage (%) was computed from the number of damaged leaves, and spindles to the total number of leaves and spindles that had emerged since the previous observation. The data on percent damage were transformed into arcsine values and subjected to ANOVA in randomized block design and means compared by LSD at the 5% level (Gomez and Gomez, 1978).

All the data were analyzed using the MSTAT-C (ver. 2.10) statistical software.

3. Results

3.1. Adult longevity

Exposure of beetles to graded doses of CVP led to reductions in the longevity of beetles to varying degrees depending on the concentration of CVP (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 μg of CVP. At 5000 μg of CVP the longevity reduction in male and female beetles was 77% and 70%, respectively. The male and female beetles exposed to 50 μg of CVP showed relatively longer life spans of 21 and 23 days with average reduction of 37% and 34%, respectively. Longevity of male and female beetles were statistically non-significant at all doses of CVP (F=0.082).

Table 1.

Effect of OBV-KI infection on the longevity and fecundity of *O. rhinoceros* adults

Dose of CVP (µg/0.1 ml)	Male longevity		Female longevity		Fecundity	
	Days	Percent reduction	Days	Percent reduction	Eggs/female	Percent reduction
5	38.0±0.6[b]	34.0	43.4±0.6[b]	29.4	16.5±0.5[c]	71.8
50	21.5±0.5[c]	62.6	23.3±0.4[c]	62.1	9.8±0.4[b]	83.2
500	17.9±0.5[d]	68.9	20.5±0.4[d]	66.6	1.4±0.3[a]	99.4
5000	13.2±0.4[e]	77.1	16.3±0.4[e]	73.5	0[a]	100
Healthy	57.6±0.7[a]	–	61.5±0.9[a]	–	58.5±1.1[d]	–
CD (0.05)	1.6	–	1.7	–	1.6	–

Means followed by the same letters are not significantly different by LSD (0.05%).

The average fecundity of healthy beetles was 58.5 eggs with 80% hatching. During the short life span of 16 and 20 days there was 100% and 99.4% reduction in fecundity at 5000 and 500 µg of CVP, whereas at 50 and 5 µg 83% and 72% reduction in fecundity during the life span of 23–43 days was recorded. Only 68% of eggs laid by infected beetles hatched and developed into grubs.

3.2. Pre- and post-virus release palm damage assessment

Before release of OBV-KI-infected beetles, frond and spindle damage was 63.6% and 51.5%, respectively (Table 2). Observations after 23 months of virus release recorded a remarkable decline in palm damage well below an ETL of 10% (crown damage 7.2% and spindle damage 6.8%). At all the five locations there was a progressive decline in palm damage and by August 2005 the mean palm damage was merely 1.7% and 0.5% in terms of frond and spindle damage, respectively. Damage was more on palms, which were in the periphery of the grooves.

Table 2.

Effect of OBV-KI release on *O. rhinoceros* population and palm damage in South Andaman

	Frond damage (%)						Spindle damage (%)			
	L1	L2	L3	L4	L5	Mean	L1	L2	L3	L4
Pre-release										
July 2001	64.4	54.5	60.3	75.2	63.5	63.5	42.7	39.6	54.6	68.0
	(53.3)[d]	(47.5)[d]	(50.9)[e]	(60.1)[d]	(52.8)[d]	(52.9)[d]	(40.8)	(39.0)	(47.6)	(55.5)
Post-release										

July 2002	22.5	15.3	24.9	31.9	28.7	24.7	13.3	14.3	17.4	26.6
	(28.3)[c]	(22.9)[c]	(29.8)[d]	(34.4)[c]	(32.4)[c]	(29.5)[c]	(21.4)	(22.2)	(24.6)	(31.0)
June 2003	6.7	8.5	5.3	6.8	8.5	7.2	4.1	2.5	8.4	9.5
	(15.0)[b]	(16.8)[b]	(13.2)[c]	(15.1)[b]	(16.9)[b]	(15.4)[b]	(11.6)	(9.1)	(16.8)	(17.9)
June 2004	5.5	1.7	2.6	4.6	5.2	3.9	0.7	0.2	0.6	3.7
	(13.5)[b]	(7.5)[a]	(9.3)[a]	(12.4)[ab]	(13.0)[a]	(11.1)[ab]	(5.1)	(2.4)	(4.6)	(11.1)
August 2005	1.4	1.1	1.5	1.8	2.6	1.6	0	0	0.5	1.2
	(6.5)[a]	(5.7)[a]	(7.0)[a]	(7.7)[a]	(9.3)[a]	(7.2)[a]	(0)	(0)	(4.1)	(6.3)
Mean	20.1	16.2	18.9	24.1	21.7	20.2	12.2	11.4	16.34	21.85
	(23.3)	(20.1)	(22.1)	(25.9)	(24.8)	(23.2)	(15.7)	(14.5)	(19.5)	(24.4)
CD (p=0.05)	3.8	3.3	3.7	5.5	3.8	4.4	2.7	2.7	3.4	4.5

Means followed by the same letter(s) are not significantly different by LSD at 0.05%.

Figures in parentheses are arcsin transformed values.

L1—Mithakhadi, L2—Minnie Bay, L3—Bambooflat, L4—Garacharma, L5—Sippighat.

3.3. Prevalence of virus disease in grubs and beetle population

Larval population in all the breeding sites showed a decline by the 12th month of release of baculovirus. Observation of grubs in breeding sites 12 months after the release of virus recorded 24.3% infection. In 2005, 37.6% of grubs were infected by virus, coinciding with the lowest levels of beetle and larval populations. There was a progressive decline in the site occupancy ratio from 0.95 to 0.08 (Table 3). It was observed that many of the breeding grounds had been abandoned by the beetles.

Table 3.

Effect of baculovirus virus release on site occupancy of *O. rhinoceros*

Observations	**Site occupancy ratio**	**Prevalence of baculovirus (%)**	
		Grubs	**Beetles**
Pre-release			
July 2001	0.95	11.30 (n=292)	21.17 (n=85)
Post-release			
July 2002	0.81	17.11 (n=187)	33.02 (n=109)
July 2003	0.52	24.35 (n=78)	50.00 (n=96)
July 2004	0.21	32.94 (n=85)	57.14 (n=77)

August 2005	0.08	37.68 (*n*=69)	66.07 (*n*=56)

Data on an annual basis.

The mean prevalence of baculovirus infection in beetles was 21.2% in 2001, which steadily increased to 66.0% by 2005 following releases made in 2001 and 2002 (Table 3). Overall there were three-fold increases in the virus infection among the beetle population.

4. Discussion

OBV-KI infection effectively reduced the longevity and fecundity in *O. rhinoceros* beetles. Based on the dose–response curve, a concentration of 130 μg CVP of OBV-KI was arrived at for infecting *O. rhinoceros* beetles, meant for field release. This dosage of virus was chosen to ensure maximum longevity of beetles 37–44 days post-infection, coupled with drastically reduced fecundity enabling the infected beetles to spread the virus into a large number of breeding sites through fecal contamination, and to others through mating. (Zelazny, 1977a) and (Zelazny, 1977b) noted that the drastically reduced fecundity greatly contributed to population decline in the field.

Observations during July 2005 showed clear evidence of the establishment of baculovirus in the pest population. All infected beetles, released in June 2001 and 2002 with a life span of 37–44 days, ought to have contributed to the disease to the succeeding generations, as indicated by the first observation made 12 months after the release and subsequent ones. Transmission of virus to grubs in breeding sites 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 diseased beetles contaminate the organic matter with excreta containing virus and healthy grubs contract the disease by feeding on the virus-contaminated organic matter (Bedford, 1980).

The sharp decrease in the population of beetles and grubs suggested the occurrence of an epizootic in a span of 12 months, following the release of virus. Similar epizootic outbreaks of OBV disease have been reported in South Pacific Islands (Marschall, 1970; Bedford, 1976; Hammes, 1971; Young, 1974; Marschall and Ioane, 1982), Papua New Guinea (Gorick, 1980), Mauritius (Monty, 1978; Marschall and Ioane, 1982) and India (Mohan and Pillai, 1993; Jacob, 1996).

The reduction in spindle damage was more rapid than that of leaf damage because damage to leaves was heavier and, besides, previous spindle damage would have resulted in damage marks on the 10–11 leaves unfolding from it in the next 11 months. The decrease in damage to fronds and spindle was drastic by the second year of the release of virus. The most significant results in the trial were the reduction in frond and spindle damage by 95.8% and 99.0%, respectively, 4 years post-release of OBV-KI-infected beetles. The mean decrease in palm damage and increase in virus prevalence of baculovirus following inundative release were indirectly related. There was a significant reduction in palm damage by 23 months of release and the frond and spindle damage was below 8% (Fig. 1). This trend agreed with the previous reports (Purrini, 1989; Bedford, 1976; Hammes, 1971; Young, 1974).

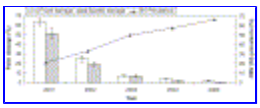

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Fig. 1. Impact of innundative release of OBV-KI on palm damage in South Andaman, A & N Islands, India.

The incidence of OBV-KI in 38.5% of sampled grubs by the fifth year of release of virus was high as compared with reports in Minicoy where 7.5% of larval breeding sites were infected 3 years post-introduction of virus (Mohan and Pillai, 1993), 7.3% in Western Samoa 4 years post-release of OBV (Zelazny, 1973), 5.6–11.2% in Western Samoa 4–8 years post-release ([Zelazny, 1977a] and [Zelazny, 1977b]) and 14.6% in Tonga 7 years post-release (Young and Longworth, 1981). The increase in infection among grubs is attributed to release of a higher (795) number of beetles. Similarly, a virus incidence of 66.0% in beetles was observed by the fifth year of virus release; 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 2.5 years post-introduction of OBV-KI, 43–63%, 8 years after the introduction of OBV into Western Samoa ([Zelazny, 1977a], [Zelazny, 1977b] and [Zelazny, 1973]) and between 30% and 50% after 15 years (Marschall and Ioane, 1982). Bedford (1976) recorded 57–68% virus incidence in beetles in Fiji. (Zelazny, 1977a) and (Zelazny, 1977b) reported 4–29% virus incidence in the Philippines and Indonesia, and the highest incidence of 84% was 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 other forms of land clearances that result in large amounts of rotting compost (Jackson et al., 2005).

A density-dependent relationship between *O. rhinoceros* and OBV-KI was evident from the fluctuations of their levels during the course of the study. Following the epizootic, the larval and beetle populations, breeding site occupancy and virus incidence in breeding grounds showed a co-ordinated decline and subsequently stabilized at low levels thereafter. Similar observations had been made in many South Pacific islands (Young, 1974; [Zelazny, 1973], [Zelazny, 1977a] and [Zelazny, 1977b]; 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 the earthquake followed by tsunami during December 2004, about 5000 ha of coconut palms have suffered permanent damage generating huge quantities of organic matter (coconut stumps), which are potential breeding grounds for beetles to multiply. A recent survey shows an outbreak of *O. rhinoceros* in the Southern group of islands (Car Nicobar, Nancowry group and Katchal), which has witnessed severe devastation. In this situation, inundative release of OBV-KI-infected *O. rhinoceros* alone could provide an effective solution for managing *O. rhinoceros*.

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