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Release of *Baculovirus oryctes* into *Oryctes monoceros* Populations in the Seychelles

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On three islands of the Seychelles archipelago, the baculovirus of *Oryctes rhinoceros* was introduced into populations of *Oryctes monoceros* and became established. The percentage of infected beetles fluctuated between 20 and 50 and a modest population reduction (ca. 30%) was recorded. On a separate group of islands (Praslin group) the virus was found to have survived from a previous release made in 1973; on these islands the rate of infection was between 70 and 90%. © 1986 Academic Press, Inc.

KEY WORDS: *Oryctes monoceros; Oryctes rhinoceros; Baculovirus oryctes;* Biological control; Microbial control.

INTRODUCTION

Beetles of the genus *Oryctes* are pests of palms in most of the Old World tropics, particularly affecting seedling coconut and oil palms. The main economic species in Asia is *Oryctes rhinoceros;* this species has a high fecundity for the genus (Hurpin and Fresneau, 1973) and has been able to colonize many Pacific and some Indian Ocean islands (Bedford, 1980). In Africa, a complex of species is involved, of which *Oryctes monoceros* is the most important (Mariau, 1967); in the Seychelles islands, *O. monoceros* is the only species present.

Among many control agents tested against *Oryctes,* one which has proved effective is a baculovirus (Baculoviridae, subgroup "C," Matthews, 1979) isolated in Malaysia from *O. rhinoceros* by Hüger (1966). This virus was characterized by Payne (1974) and Payne et al. (1977) and Monsarrat et al. (1973). In the Ivory Coast it was found not to affect *O. monoceros* (see Julia and Mariau, 1976), but in Seychelles the same species was susceptible (Windsor, 1975).

Virus was originally introduced into Seychelles in 1973 because the *O. monoceros* population was very high, particularly on the islands of Praslin and La Digue, where attacks by the wood-boring lymexylonid, *Melittomma insulare,* had killed many trees. Releases were made on Mahé, Praslin, and La Digue, using artificial breeding sites following the methods used in Mauritius (Monty, 1978). In 1975 a few remnants of dead larvae found on Praslin were sent to the Institute of Virology, Oxford, England, where the presence of virus was confirmed serologically. No infection was found on Mahé or La Digue at that time, or during subsequent surveys on Praslin. The confirmed sample of virus was regarded as doubtful because of subsequent problems with the antiserum used reacting with healthy insect protein (I. A. D. Robertson, unpubl.).

MATERIALS AND METHODS

Insects. Live adult beetles were collected from coconut plantations by employing ethyl chrysanthemumate (Stauffer Chemicals, New Jersey) traps (Bedford, 1973), from their feeding burrows in the crowns of seedling coconut palms, and from dead coconut palm logs. The latter also provided all the larval stages. Adult beetles were immediately placed into individual clean glass tubes. Larvae were collected in nylon bags, all the larvae from one log being placed in one bag.

In the laboratory, adult beetles were kept

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in plastic beakers until they died, at which time they were processed or frozen intact at $-20°C$. Larvae were maintained in a mixture of manure and sawdust in individual containers.

The identification of the beetle was confirmed as *O. monoceros* by Dr. R. Madge of the Commonwealth Institute of Entomology, with the reservation that there were consistent differences between *O. monoceros* in the Seychelles and East Africa. The Seychelles beetle is larger and its pronotal cavity is less strongly punctate.

Virus. The midgut was dissected out of the dead (frozen or fresh) beetle and homogenized in 1 ml of cold distilled water. The homogenate was assayed for presence of virus by the enzyme-linked immunosorbent assay (ELISA) technique or by cross-over electrophoresis (or else was frozen at $-20°C$).

Serological assay. Antiserum was produced in rabbits by intramuscular injection of purified virus particles plus Freund's adjuvant and was supplied by the Institute of Virology, Oxford, England. Two injections had been given, at weekly intervals, and serum was collected at weekly intervals up to week 4 (Payne et al., 1977).

In the ELISA test, the double antibody method of Clark and Adams (1977) was used; polystyrene plates (Titertek Ltd.) were sensitized by coating with immunoglobulin G (IgG) diluted 1/100 in Na_2CO_3 buffer, *p*H 9.6. IgG was prepared from serum by precipitation with an equal volume of saturated ammonium sulfate solution. Midgut macerate diluted 1/10 and 1/100 in phosphate-buffered saline (PBS) was applied, followed by conjugate at a dilution of 1/400. The conjugate was prepared by coupling IgG with alkaline phosphatase (Sigma) in the presence of 0.06% glutaraldehyde. The substrate, *p*-nitrophenyl phosphate (Sigma), at a concentration of 0.67 mg/ml in diethanolamine buffer (9.7% v/v, *p*H 9.8), was incubated in the wells for 1 hr at 37°C. All other steps were incubated overnight at 4°C, and between each step the wells were washed with PBS-Tween. The absorption at 405 nm was measured manually in a Pye-Unicam SP6 spectrophotometer.

Cross-over electrophoresis was carried out on Cellogel strips at 120 V for 30 min with equipment supplied by Whatman-BDH Ltd. Ten microliters of the midgut macerate was applied to the cathodic side, and 1 μl of neat antiserum on the anodic side. Following two washes in 0.85% w/v saline, the Cellogel strips were stained in 0.5% w/v Napthalene Black (Acid Black 2, Whatman-BDH) in methanol:acetic acid:water (45:10:45) and destained in methanol:acetic acid:water (47.5:5:47.5). Extracts from virus-infected beetles gave a distinct blue line.

Larval extracts could not be assayed by either of the above methods nor by Ouchterlony double immunodiffusion tests, probably owing to oxidation of the extracts. In the absence of any more definite criteria, a flaccid cadaver was considered as virus infected. Fungal infections resulted in a stiff cadaver; this simple distinction was confirmed by bioassay of larval extracts in adult beetles.

Infection with virus. Virus was obtained from live infected male *O. rhinoceros* from the Philippines and from field-collected beetles from Praslin Island. Inoculum for the 1973 release had been sent as frozen *O. rhinoceros* larvae from Samoa.

The inoculum used for infection of adult beetles was a midgut macerate prepared and assayed as described above. Fifty microliters of each of two virus-positive extracts were diluted 1/10 in 10% sucrose solution. Ten microliters of this mixture was applied to the beetles' mouthparts.

Larvae were infected by mixing virus-infected adult midgut or larval extract with their food.

Estimation of population density. The density of the beetle population was assessed by palm damage surveys (Bedford, 1976; Hammes, 1974). Although this method is good for comparisons, there are many assumptions involved in calculating the actual beetle population. The method was therefore "calibrated" by making an absolute estimate of the beetle population of a small island by the mark–release–recapture method or Lincoln Index (Southwood, 1978). Ethyl chrysanthemumate traps were used to capture the beetles, which were marked before release by filing a groove across the elytron with a scalpel. Experiments had shown this to have no effect on the beetles' longevity. The proportion of marked to unmarked beetles recaptured is assumed to be the same as the proportion of captured beetles in the whole population.

The damage to palms was assessed from a sample of 40 palms in each of four ecological zones. The density of palms was also estimated, and the area of each zone measured from the Department of Overseas Survey (1:10,000) map (Table 2).

Virus release and monitoring of results. For field releases of virus, beetles reared from larvae in the laboratory were infected as described above. Some were retained in the laboratory to determine whether infection had been successful. The remainder

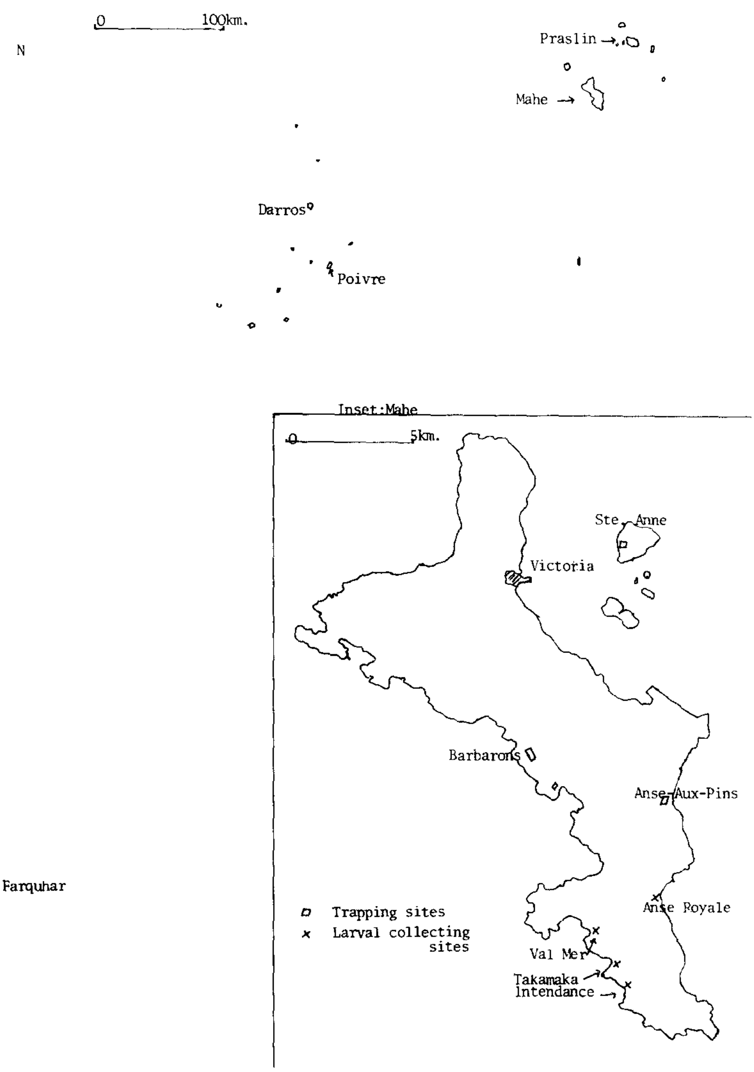

FIG. 1. Map of Seychelles.

TABLE 1
DISTRIBUTION OF *Baculovirus oryctes* IN SEYCHELLES ARCHIPELAGO

Island	Date	% Infected with virus (*N*)	Mean damage per palm	Population density/ha
Ste. Anne	June 81	0 (12)	1.35	8.6
	June 83	35 (273)	1.01	5.1
Mahé	Dec 81	0 (25)	2.41	10.9
	July 83	30 (800)	1.51	6.9
Praslin	Dec 82	76 (123)	1.81	8.2
La Digue	Oct 82	60 (15)	—	—
Cousin	Dec 82	Present (3)	0.5	2.3
Aride	May 83	Present (12)	0.9	4.1
Curieuse	Dec 80	Present (2)	Variable	—
Frégate	Jan 83	0 (2)*	0.2	0.9
Silhouette	Mar 83	0 (4)	0.4	1.8
Farquhar	Mar 82	0 (8)*	1.1	5.0
Desroches	Mar 82	0 (0)*	0.65	2.9

* Larvae collected from these islands were free of virus.

were placed in an open wooden box containing moist sawdust; this was suspended above the ground to avoid predation by rats. Most beetles dispersed from the boxes within 3 days.

Samples of the beetle population collected from coconut plantations as described above were assayed for presence of virus for several months before the initial virus release began and continuously thereafter. Trap sites and collecting areas are shown in Figure 1.

RESULTS

The susceptibility of *O. monoceros* to the baculovirus of *O. rhinoceros* was confirmed in the laboratory before release took place. However, the findings are not presented, as under local conditions the determination of LD_{50}s was not possible, virus could not be purified and, owing to interference from insect proteins, ELISA tests did not give quantitative results.

In a crude bioassay, the virus from Praslin compared favorably with the strains from Philippines and Samoa, so this local strain was selected for field release; it was considered preferable to utilize virus from *O. monoceros* than to introduce an exotic virus strain.

Beetles from various islands in the Seychelles were checked for virus infection (Table 1). On Mahé and the outlying coralline islands, the beetle populations were free of virus, but on the Praslin group the beetles were infected, often at very high infection rates.

The results of damage surveys on Ste. Anne island are shown in Table 2, with a population estimate by the Lincoln Index. This allows the damage survey to be calibrated so that population estimates can be inferred on other islands.

Table 3 shows both the total number and proportion of infected beetles released. For Mahé, a figure for the population in the Barbarons area is also given as this is where releases took place. It is unlikely that any of the released beetles would have dispersed beyond this area, as the flight activity of infected beetles is reduced (Zelazny, 1976).

The results of monitoring by ethyl chrysanthemumate traps are shown in Figure 2 (Ste. Anne) and Figure 3 (Mahé, Barbarons). On Ste. Anne, the virus was first detected about 10 weeks after release of virus-infected beetles. The overall infection rate was 34.8% ± 6 (N = 273); the fluctuations in the recorded infection rate were not statistically different from this. On Mahé, the virus took 16 weeks to become established and 10 months to reach a 30%

TABLE 2

ESTIMATE OF ADULT BEETLE POPULATION ON STE. ANNE ISLAND: COMPARISON OF PALM DAMAGE SURVEYS WITH LINCOLN INDEX

1. Lincoln index[a]	No. beetles caught = 80				
	No. marked and released = 89		$N = \frac{80 \times 89}{4} = 1780$		
	No. recaptured = 4				
Applying Bailey's correction (Begon, 1979)			$N = \frac{80 \times 90}{5} = 1440$[c]		
2. Damage survey	Height above sea level (m)	Area (ha)	No. of palms[b]	Mean damage per palm	No. of palms × damage
Ecological zone					
Coastal coconut plantation; Palms over 60 years old; Sandy soil, grass	<5	15	2290	1.52	3480
Coastal coconut plantation; Palms 20–30 years old; Sandy soil, grass	<5	12	1800	2.8	5040
Coastal coconut plantation; Red soil, understorey of *Litsea glutinosa* and *Cinnamonum zeylanicum*	<25	40	6000	1.3	7800
Hillside coconut, scrub; Red soil; palms giving way to *Tabebuia pallida*; understorey of *C. zeylanicum* and *Chrysobalanus icaco*	25–75	78	11700	2.70	31590
					47910[c]

[a] Many of the assumptions involved in the theory did not strictly apply here. (The releases were continuous, not discrete; more beetles were released than captured; may have been deaths during releases.) The numbers involved are rather low to give a good estimate.

[b] The spacing between palms was approximately 8 m, giving a density of 160/ha.

[c] No. of cuts per beetle = 47910/1440 = 33.

TABLE 3
NUMBER AND PROPORTIONS OF VIRUS-INFECTED *Oryctes monoceros* RELEASED

Place	Total released	Area (ha)	Beetle density	Estimated wild population	Proportion
Ste. Anne	131	145	8.6[a]	1440	1:11
Mahé Barbarons	278	400	10.9[b]	4360	1:16
Mahé	278	14,000	10.9[b]	152600	1:549

[a] Estimated by Lincoln Index.
[b] Estimated from damage survey.

infection rate. The overall rate was 30.6% ± 4 (N = 800) which was not significantly different from the rate on Ste. Anne.

The spread of the virus to the southeast was monitored using the traps at Anse-aux-Pins (Fig. 1) on the opposite coast of Mahé from the release site. The spread southwards was monitored by collections from dead logs and palm crowns (Table 4), and these data have been used to calculate a gradient of dispersal (Fig. 4). Following logarithmic transformation of both percent infection and distance in meters the slope of this line was $b = -0.35$ initially, and progressed to a two-component form. The factor b is similar to that computed for the spread of baculovirus in *O. rhinoceros* in Tongatapu (Entwistle et al., 1983). The rate of spread of virus disease in Seychelles was 1.5 km/month measured from the time of virus release, and 4 km/month from the establishment of the epizootic at the release site.

No trends in the number of beetles trapped are apparent in Figures 2 and 3. However, a reduction in damage of 35% was recorded by the method of Hammes (1974) (Table 5).

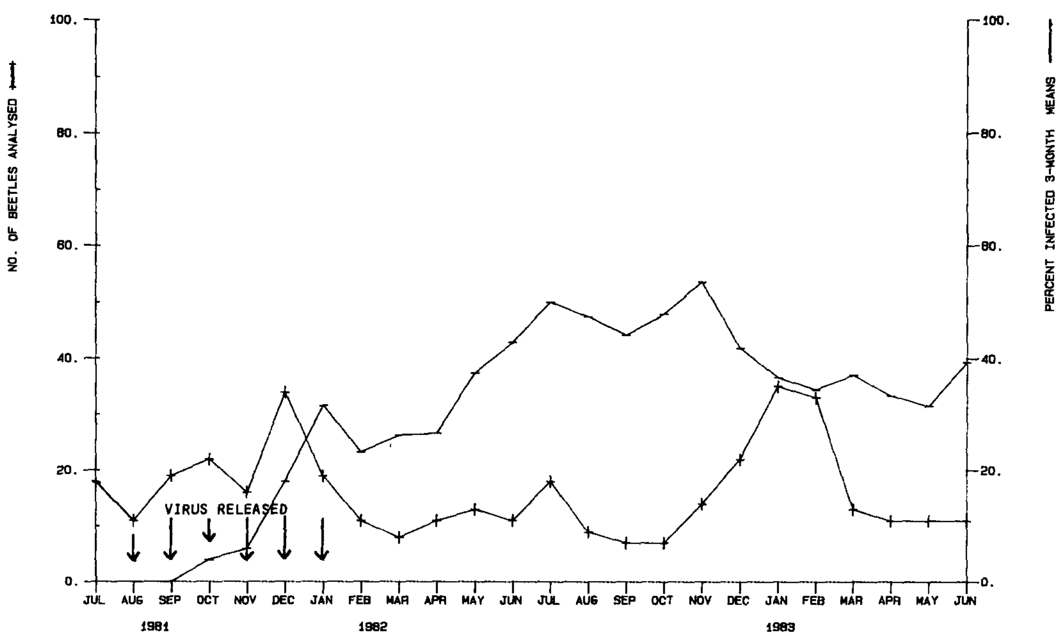

FIG. 2. Ste. Anne infection data. Beetles from traps, July 1981–June 1983. Virus released August 1981.

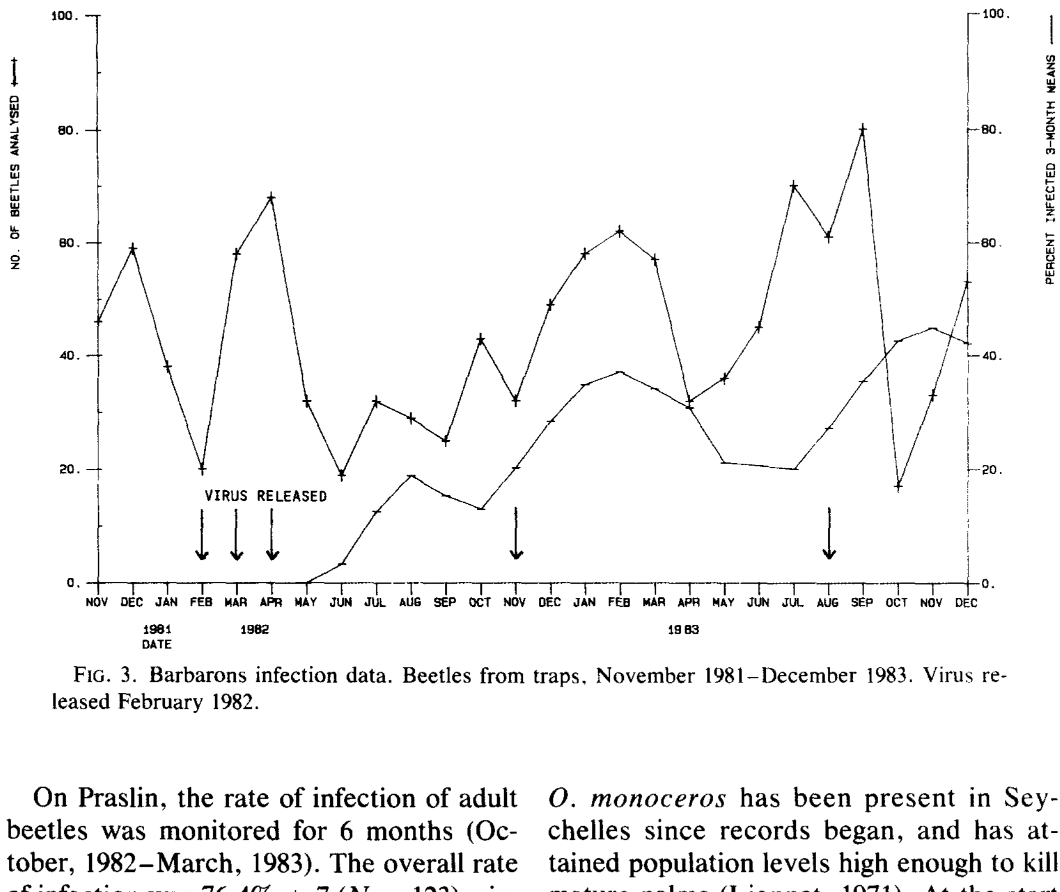

FIG. 3. Barbarons infection data. Beetles from traps, November 1981–December 1983. Virus released February 1982.

On Praslin, the rate of infection of adult beetles was monitored for 6 months (October, 1982–March, 1983). The overall rate of infection was 76.4% ± 7 (N = 123), significantly higher than the infection rates on Ste. Anne and Mahé.

DISCUSSION

All previous epizootiological studies of *B. oryctes* have been concerned with *O. rhinoceros* (Bedford, 1982). In those areas where the beetle had become established, introduction of the virus rapidly caused a reduction in population, e.g., Mauritius (Monty, 1978), Fiji (Bedford, 1976), and Wallis (Hames, 1971). In areas where the beetle is endemic, populations were lower where the virus was present than where it was absent (Zelazny, 1977). Thus, the virus is a significant mortality factor for *O. rhinoceros,* although the beetle can still cause problems when breeding sites are abundant. The possible advantages of rerelease of virus into *O. rhinoceros* populations are discussed by Marschall and Ioane (1982). *O. monoceros* has been present in Seychelles since records began, and has attained population levels high enough to kill mature palms (Lionnet, 1971). At the start of the present work there were only modest populations and damage levels were commensurately low. Presumably, had the initial beetle populations been higher, a greater percentage reduction would have been achieved.

When virus is introduced to a population, it eventually either disappears or becomes established. Once the percentage of infected hosts rises above a certain level, the population declines to a greater or lesser extent, until the virus incidence also declines. They may lead to fluctuations in the host insect population with concurrent variations in virus incidence, or to an equilibrium between insect and virus. The baculovirus became established on Mahé and Ste. Anne with little fluctuation; it took longer to establish in *O. monoceros* in Seychelles than in *O. rhinoceros* in Papua New Guinea (Gorrick, 1980). The equilibrium in-

TABLE 4
COCONUT LOGS: NUMBERS OF LARVAE COLLECTED AND PROPORTION INFECTED BY VIRUS

Region	Place[a]	Date	No. collected	% Dying of virus[b]
East Mahé	Anse-aux-Pins	July 82	42	0
East Mahé	Anse-aux-Pins	June 83	105	9.5
South Mahé	Takamaka	Nov 82	140	5.0
South Mahé	Val Mer	Dec 82	69	0
South Mahé	Intendance	Jan 83	144	2.1
South Mahé	Baie Lazare	Jan 83	63	1.6
South Mahé	Val Mer	Mar 83	33	0
SE Mahé	Anse Royale	July 83	154	10.4
Praslin	Grand'Anse	Dec 82	79	2.5
Praslin	Grand'Anse	June 83	84	22.6

[a] See Fig. 1.
[b] See Materials and Methods.

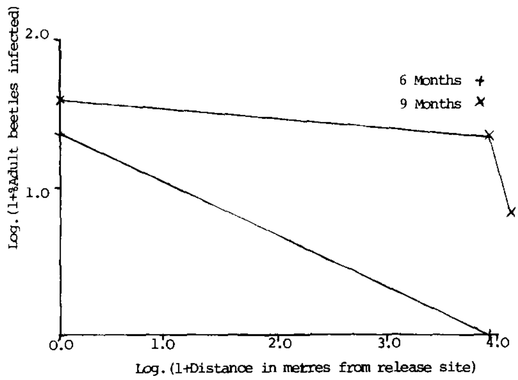

FIG. 4. Gradient of dispersal.

fection rate was similar for Seychelles *O. monoceros* to that observed for *O. rhinoceros* in Philippines and in Samoa (Zelazny, 1977; Marschall and Ioane, 1982). The rate of dispersal was also similar to that observed for *O. rhinoceros* in Tonga (Young, 1974).

The apparent discrepancy between populations of adult beetles as estimated from the catch in ethyl chrysanthemumate traps and as estimated from palm damage, has also been reported for *O. rhinoceros* in Western Samoa (Marschall and Ioane, 1982). Both approaches provide only indirect estimates; in theory the traps measure density compounded with flight activity (it is not known whether ethyl chrysanthemumate simulates a feeding or a mating attractant but see Sabatini, 1979), and palm damage measures density compounded with feeding activity. Trap catches show more variation than damage throughout the year. From observation on the abundance of the larval instars, it does not appear that the population varies greatly throughout the year. Therefore, feeding damage is considered to be the more reliable estimate of adult numbers. It is possible that flight activity increases to compensate for falling populations in order for breeding contacts to be maintained. In contrast to *O. rhinoceros*, adult activity in *O. monoceros* has a tendency to increase with higher rainfall. In common with *O. rhinoceros*, there was a tendency for the proportion of females

TABLE 5
DAMAGE SURVEYS—SEPTEMBER, 1983

Place	No. of palms surveyed	Mean damage in upper fronds (No. of cuts/ No. of fronds)	Mean damage in lower fronds (No. of cuts/ No. of fronds)	% Change
Ste. Anne	80	9.3/50	14.5/50	−36
Barbarons (nr Hotel)	80	15.6/50	24.0/50	−35
Barbarons (nr Hybrids)	40	15.3/50	24.1/50	−37

trapped to rise as the rate of infection in the population increased.

The high infection rate on Praslin Island is in contrast to that observed on the other islands, and those for *O. rhinoceros* [apart from a report of 84% infection in Tonga using ELISA (Young and Longworth, 1981)]. The possibility of resistance or tolerance of the beetle, and of attenuation of the virus, are at present under investigation. The answer may lie in ecological or climatic differences between Mahé and Praslin; alternatively, the island of Praslin may be small enough for local extinction of virus to occur, leading to fluctuations in the infection rate. The relationship between numbers of infected beetles and population reduction needs to be determined. This should enable accurate control recommendations to be made, and the value of integrated control measures to be estimated.

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