JOURNAL OF INVERTEBRATE PATHOLOGY 29, 210–215 (1977)

Oryctes rhinoceros Populations and Behavior Influenced by a Baculovirus

BERNHARD ZELAZNY

UNDP/FAO Cadang-Cadang Research Project, Guinobatan Experiment Station, Guinobatan, Albay, 4908, Philippines

Received January 14, 1976

Oryctes rhinoceros adults stopped feeding and flew less frequently, and the males mated less often after they became infected with the baculovirus of *Oryctes*. Monitoring the larval and adult population in Western Samoa over several years gave evidence that the incidence of the virus is dependent on the density of the *O. rhinoceros* population. Male beetles were more frequently infected than female bettles. Adding more virus-infected beetles to the *O. rhinoceros* population of Nukunonu Atoll, where the virus had become established 5 years earlier, reduced the population significantly. The data indicated that the reduction was caused mainly by a decrease in the number of eggs deposited.

INTRODUCTION

The Indian rhinoceros beetle *Oryctes rhinoceros* damages coconut palms seriously in many parts of the Indo-Pacific region. Deliberate introductions of the baculovirus of *Oryctes rhinoceros* into Tonga and Fiji have caused significant depressions of the beetle populations (Young, 1974; Bedford, 1976). Virus infections occurred predominantly among adults, causing shortening of the life span and sterility in females (Zelazny, 1973 a, b). The disease is transmitted readily between the beetles during mating (Zelazny, 1976). This paper describes the effect of the virus on the behavior of adults, and on the beetle populations of Upolu Island (Western Samoa) and Nukunonu Atoll (Tokelau Islands), where it was introduced in 1967 (Marschall, 1968, 1970). The baculovirus of *Oryctes rhinoceros* was originally described by Huger (1966) as *Rhabdionvirus oryctes*.

MATERIAL AND METHODS

Effect of the virus on the behavior of adults. The experiments were conducted in Western Samoa with laboratory-reared beetles which were inoculated with the virus either by injecting 0.1 ml of water containing 2×10^{-8} ml (low dosage) or 2×10^{-4} ml (high dosage) of hemolymph from larvae with advanced virus infections or by letting the beetles swim for 10 min in a 10% suspension of ground up, freshly virus-killed larvae. The feeding, flight, and mating behaviors of the inoculated beetles were compared with those of control beetles which had been either injected with water or left untreated. Virus infections were detected by bioassay tests (Zelazny, 1972) at the end of the experiments. On the average, 56% of the beetles injected with the low dosage of the virus and 11% of the beetles inoculated by letting them swim in a virus suspension failed to become infected, and the data obtained from these beetles were discarded.

Monitoring the incidence of the virus in the O. rhinoceros *population of Upolu.* The incidence of virus infections in the larval and adult population was monitored over 4 and 2 years, respectively. Sampling was done as described earlier (Zelazny, 1973b). On the average, 178 (157–214) larval sites were examined twice a year from three areas covering about 10 km² each. In four

ISSN 0022-2011

locations, adults were caught with 50 traps (Bedford, 1973) four times a year for 5 weeks, and the beetles trapped singly (on the average, 114 per trapping period) were bioassayed. The *O. rhinoceros* damage on 643 marked palms in 33 locations, randomly selected along the main roads of Upolu, was taken as an indication of the size of the *O. rhinoceros* population 8 months before each reading, the median time at which the damage had been caused.

Addition of the virus to the O. rhinoceros *population on Nukunonu Atoll.* The coconut-growing islets of Nukunonu Atoll (Tokelau Islands), which are arranged in an ellipse 8 × 13 km in size, were divided into two treatment groups and one control group. The northern (90 hectares) and southern islets (40 hectares) were treated by catching rhinoceros beetles with 366 and 278 attractant traps, respectively, and by collecting larvae every 2 to 3 months. All female beetles and larvae obtained were killed. The southern islets were further treated by releasing each week about 20 male beetles which had been caught in the traps and which had been injected with hemolymph from virus-infected larvae. This program was continued for 20 months. The western and eastern islets were left untreated, separating the two treatment groups by 4.5 and 10.5 km on both sides. One-thousand palms were marked in the three groups, and their *O. rhinoceros*-caused damage was recorded at the beginning of the experiment and 23 months later.

RESULTS

Effect of the Virus on the Behavior of Adults

Of the 332 control beetles used in the experiments reported, two proved to be infected with the virus.

The effect of virus infections on feeding behavior was examined by offering pieces of sugarcane to freshly emerged, individually kept beetles twice a week. At the age of 3 weeks, 106 beetles were inoculated with the virus while 139 were kept as controls. One week after inoculation, beetles injected with the high dosage of the virus were feeding (24 of 47) significantly ($P < 0.007$) less frequently than the control beetles (128 of 136) and also less frequently than beetles injected with the low dosage of the virus or inoculated by letting them swim in a virus suspension (46 of 56). Two weeks after inoculation nearly all virus-infected beetles stopped feeding (Fig. 1).

In a second experiment, the feeding behavior of virus-infected beetles was studied under field conditions by tethering beetles to the crowns of coconut palms. After 1 week, 16 of 32 control beetles, 2 of 25 beetles inoculated with the virus 7 days earlier, and none of 27 beetles inoculated 14 days earlier had bored into the palms for feeding.

The flight activities of 121 virus-infected and 113 control beetles were studied by

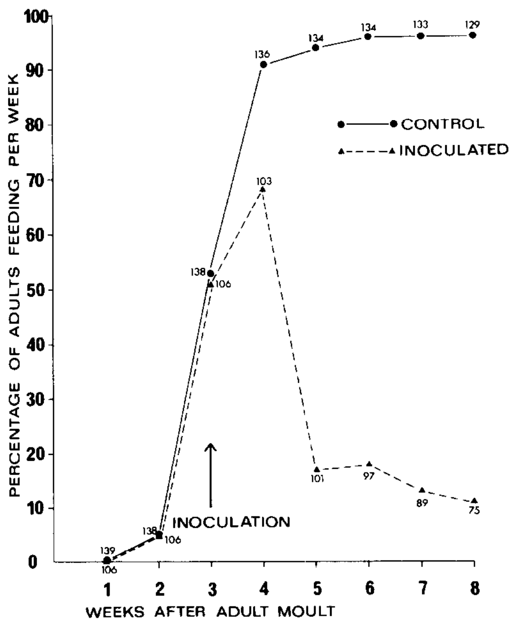

FIG. 1. Feeding frequencies of virus-infected (inoculated) and uninfected (control) *Oryctes rhinoceros* adults. The beetles were observed from the time of their adult moult. At the age of 3 weeks, 106 were inoculated with the virus (arrow). The numbers on the curves are the numbers of beetles alive during the different times of the experiment.

recording the number of beetles flying out of open tins during one night. The results are given in Table 1. The flight activity decreased considerably in both groups, possibly because the beetles were not fed. As compared to the control beetles, 13.8 ± 1.9% fewer virus-infected beetles were flying on the average. This difference was significantly higher than zero (P = 0.0008, 5 df).

Mating was studied by injecting beetles with the high dosage of the virus only, then, after either 1 or 2 weeks, pairing them for 2 days with uninfected beetles of the opposite sex. The females were then dissected for the presence of sperm. In pairs containing uninfected females, 40 of 52 males inoculated with the virus 2 weeks earlier but 37 of 38 uninfected males mated. This difference was significant at the 5% level. Mating was not significantly reduced in 53 males (87%) and in 26 females (88%) inoculated 1 week earlier, as well as in 29 females (93%) inocualted 2 weeks earlier.

Monitoring the Incidence of the Virus in the O. rhinoceros *Population of Upolu*

Between 1971 and 1974 the percentage of larval sites containing virus-infected larvae fluctuated between 5.6 and 11.2 (average, 8.2). The *O. rhinoceros*-caused damage on coconut palms seemed to follow (P = 0.06) these fluctuations over most of the period (Fig. 2, upper half).

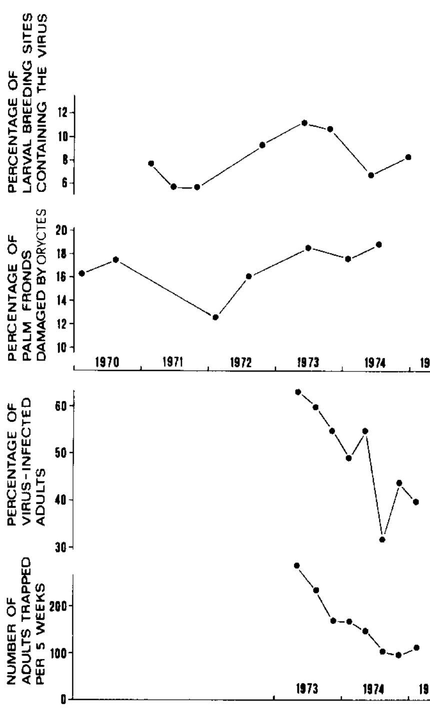

FIG. 2. Studies on the larval and adult population of *Oryctes rhinoceros* on Upolu, Western Samoa. In the upper half, the virus incidences in 151 to 214 larval sites from three areas are compared with the *O. rhinoceros* damage on the fronds above horizontal of 643 marked palms in 33 separate locations. The first two palm damage values are quoted with permission from Young (1971). The lower half gives the incidences of virus in adults (average 114) trapped in four locations during 5-week periods and the total numbers of trapped beetles.

Between 1973 and 1975 the percentage of adults infected with the virus decreased from 63 to 35 (average, 51) corresponding significantly (P = 0.05) to a decline in the number of beetles trapped (Fig. 2, lower half).

Significantly (P = 0.001) more males (60.7%, N = 239) than females (52.1%, N = 999) were found infected with the virus. There was a positive but insignificant (P = 0.09) correlation between the virus incidence and the percentage of female beetles trapped (average, 78%).

TABLE 1

FLIGHT ACTIVITY OF *Oryctes rhinoceros* INOCULATED WITH THE BACULOVIRUS OR UNTREATED

Time after inoculation (days)	Virus-infected beetles flying during one night (%)	Control beetles flying during one night (%)	Difference (%)
3	29 (N = 121)	44 (N = 113)	15
6	13 (N = 113)	33 (N = 112)	20
9	16 (N = 99)	32 (N = 106)	16
12	12 (N = 95)	27 (N = 102)	15
15	13 (N = 84)	23 (N = 100)	10
18	6 (N = 77)	13 (N = 99)	7
Average	16	35	13.8 ± 1.9

TABLE 2

CHANGES IN THE *Oryctes rhinoceros* POPULATIONS OF NUKUNONU ATOLL DURING LARVAL COLLECTIONS AND BEETLE TRAPPING (ISLETS OF GROUPS 1 AND 2) AND AFTER ADDING VIRUS-INFECTED MALE BEETLES (ISLETS OF GROUP 2 ONLY)

Group	January 1973 to May 1973	June 1973 to October 1973	November 1973 to April 1974[a]	May 1974 to September 1974
		Average number of beetles trapped per month[b]		
1	240 ± 32	201 ± 31	176 ± 4	149 ± 10[c]
2	82 ± 11	63 ± 3	25 ± 2[c]	14 ± 3[c]
		Number of larvae found		
1	3248	1301	2790	1111
2	3197	193	322	571
		Percentage of female beetles trapped		
1	65[e]	57	64	61
2[d]	55[f,g]	45[g]	48[g]	51
	Percentage of fronds above horizontal damaged by *O. rhinoceros* on marked palms			
	January 1973			December 1974
1	3.7			2.4
2	6.5			1.9
Control group	1.5			1.9

[a] No records were obtained in January 1974.
[b] Deviations from the means are given as standard errors.
[c] Significantly ($P < 5\%$) smaller than the mean of the previous 5-month period.
[d] Released and recaptured males are excluded from these data.
[e] During the first month, 66% females were trapped.
[f] During the first month, 71% females were trapped.
[g] Significantly ($P < 5\%$) lower than the percentage of females trapped in Group 1.

Adding the Virus to the O. rhinoceros *Population of Nukunonu Atoll*

Beetles naturally infected with the virus were found in all parts of Nukunonu Atoll in January 1973 (39%, $N = 189$). However, it was impossible to monitor the virus incidence during the following 20 months of adding virus-infected male beetles to part of the population, and the trial was evaluated by the number of beetles trapped (Table 2).

On the islets where only larvae were collected and beetles were trapped, the number of trapped beetles decreased during the trial from 240 to 149 beetles per month (or from 2.67 to 1.66 beetles/hectare/month). On the islets where, in addition to larval collections and beetle trapping, virus-infected male beetles were released, the number of beetles trapped decreased significantly ($P = 10^{-5}$) more, from 82 to 14 beetles per month (or from 2.05 to 0.35 beetles/hectare/month). The significant decline started about 10 months after the beginning of the trial. The number of larvae collected also decreased more on the islets where virus was added (Table 2).

At the beginning of the trial, the percentages of female beetles trapped were similar in both treatment groups, 66 and 71%, but changed during the trial to significantly ($P < 0.05$) fewer females being trapped on the islets where virus-infected males had been added (Table 2).

The rhinoceros beetle damage to coconut palms is given in Table 2, representing the beetles attacks made between 3 and 13 months before the reading. On the islets where virus was added, the damage was highest at the beginning and declined significantly during the trial. On the islets were only beetles and larvae were removed, the palm damage was medium high at the beginning of the trial, and at the end had declined slightly, whereas the damage was lowest on the control islets where it increased slightly during the trial.

The data on palm damage and beetle and larval collections suggest that the two treatment groups had different periods of peak *O. rhinoceros* breeding. So on the islets where virus release had occurred, the beetles population was higher than in the other treatment group before the trial (higher palm damage) but lower at the beginning of the trial (lower beetle catches per hectare), whereas the larval population was higher on the islets where virus release had occurred at the beginning of the trial. However, since the trial lasted twice as long as one breeding cycle of *Oryctes rhinoceros*, about 10 months, the end results would have been little influenced by different breeding patterns.

DISCUSSION

After becoming infected with the baculovirus of *Oryctes*, rhinoceros beetle adults stop feeding and fly less frequently, males mate less frequently, and, as reported earlier (Zelazny, 1973a, b), females stop egg laying. Since the virus is often transmitted during mating (Zelazny, 1976), less mating of the virus-infected males could have caused the slightly lower incidence of virus infections observed among the females. No significant difference was observed earlier (Zelazny, 1973b). A lower incidence of virus infections among females corresponds with the data reported by Cumber (1957) and Bedford and Maddison (1972, 1973) which indicate that a new introduction of the virus increases the percentage of female beetles in the population. My observations further suggest a positive correlation between the incidence of the virus and the percentage of female beetles.

Only small fluctuations in the *O. rhinoceros* population and the incidence of the virus were observed during 1971 to 1975, possibly due to constant climatic conditions in Western Samoa. Still, evidence was obtained that the incidence of the virus disease is dependent on the density of the *O. rhinoceros* population.

Maddison (1974) studied the effect of adding the virus to an *O. rhinoceros* population in which the virus had already become established some time ago. No significant changes were detected; possibly the trial period was too short. My Nukunonu data suggest that the *O. rhinoceros* population is little affected by careful larval collections or trapping of beetles but that the additional releases of virus-infected beetles can reduce the population significantly. After the releases of virus-infected males, the percentage of trapped females dropped, indicating that the released males infected a significant number of females. The data suggest that the virus releases reduced the larval population immediately and reduced the adult population after a delay of 10 months (about one beetle generation), implying that the releases caused a significant decrease in the number of eggs deposited. In the laboratory, the females stop depositing eggs when they become infected with the virus (Zelazny, 1973a).

Releasing more virus-infected beetles into areas where the disease has already been established should be further investigated as means of controlling the beetle. Males as well as females could be used for the release since the virus is transmitted readily between both sexes (Zelazny, 1976). The releases would cause little additional damage since the infected beetles stop feeding. However, it could be difficult to obtain large numbers of beetles for the releases.

ACKNOWLEDGMENTS

Messrs. M. Kaisa and S. Peni assisted in the experiments. Mr. C. Schipper provided large numbers of healthy beetles and larvae. The experiments on Nukunonu Atoll were financed by the New Zealand

Administration of the Tokelau Islands. Comments on the manuscript by Dr. D. Meadows, UNDP/FAO Coconut Research and Development Project, Philippines, are gratefully acknowledged. The studies described in this paper were carried out while the author was employed as Insect Ecologist in the UNDP/FAO Project on Research on the Control of the Coconut Palm Rhinoceros Beetle in Apia, Western Samoa.

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