Introduction and Field Comparison of Baculovirus Strains Against *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) in the Maldives

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ABSTRACT Five baculovirus strains were introduced in 1984–1985 into virus-free island populations of *Oryctes rhinoceros* (L.) in the Maldives, either singly or in combination. Pest populations were monitored by palm damage surveys for up to 4 yr on 22 islands, some of them deliberately untreated. The method used allowed estimates from a single survey of the number of beetle attacks during each of 18 mo (3rd–20th month before the survey date). Such surveys gave continuous records for *O. rhinoceros* attacks during 1983–1987. Most islands where the virus was released showed a highly significant reduction in palm damage. The prerelease pest density on a given island influenced the efficacy of the virus introduction. Adjustments were made for this effect when the performances of the different virus strains were compared. Strain X2B gave consistently better reduction in pest populations and more virus-infected beetles compared with strain V2/3B; the remaining strains gave intermediate control. These results agreed with earlier laboratory tests, in which the virulence of both strains was compared by infecting *O. rhinoceros* larvae. Two islands where three strains were released simultaneously also experienced a high damage reduction; here, X2B predominated eventually over strains V2/3B and S2A.

KEY WORDS Insecta, *Oryctes rhinoceros*, baculoviruses, microbial control

Oryctes rhinoceros (L.) is one of the most serious and common pests of coconut palms in tropical Asia and the Pacific. Adult beetles cause damage by boring into the heart of the palms and feeding on the young, developing fronds. When a palm dies, the decaying trunk serves as a breeding place for the insect. The highest populations are found in areas where the baculovirus of *Oryctes* does not occur naturally. The deliberate introduction of this virus has caused significant damage reduction (Bedford 1981).

DNA restriction fragment patterns have shown that genetic variation is common in isolates of this baculovirus (Crawford et al. 1986). Moreover, laboratory studies demonstrated significant differences in the virulence of baculovirus isolates and strains against *O. rhinoceros* larvae (Zelazny 1979, Zelazny et al. 1989). In adults of *O. monoceros* (Olivier), the infection rate appears to be greatly influenced by the correct mixture of baculovirus strains (Purrini 1989).

The success of a control technique using the baculovirus is, therefore, likely to be influenced by the strain of virus released. However, it is difficult to judge the potential of a strain from laboratory tests because many factors, other than infectivity to larvae, could contribute to its performance under field conditions. Field trials with different virus strains ideally should be done in virus-free areas because determining the strain in each infected beetle sampled will be impractical. Another important factor is the size of the trial areas. *O. rhinoceros* adults disperse quickly over large areas (Zelazny & Alfiler 1987), which can result in masking of population reduction and mixing of beetles from different treatments.

In the islands of the Maldives, *O. rhinoceros* populations reached extremely high levels and were free of the baculovirus disease (Zelazny 1983). In addition, the presence of many small isolated islands provided ideal conditions for research on the microbial control potential of different baculovirus strains.

We here describe the results of trials in the Maldives, during which five different baculovirus strains were released on three atolls and pest populations were studied on 22 islands. The establishment of the strains and the occurrence of genomic changes in one of the atolls was described earlier (Crawford & Zelazny 1990).

It was not possible to monitor the *O. rhinoceros*

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Table 1. Experimental islands and released baculovirus strains with pre- and postrelease damage by *O. rhinoceros*

Island[a]	Atoll	Baculovirus strain released	Avg no. attacks/ha		Post-/pre-release	Deviation from regression in Fig. 5[c]
			Prerelease	Postrelease[b]		
(A) Thuvaru	Meemu	X2B[d]	41.5	9.1**	0.219	−0.079
(B) Ukulas	North Ari	X2B	36.5	10.1**	0.278	−0.065
(C) Himandhoo	North Ari	X2B	38.6	8.9**	0.231	−0.093
(D) Feridhoo	North Ari	X2B	33.2	9.8**	0.296	−0.075
Average	—	—	—	—	—	−0.078
(E) Kuda-us-fushi	Meemu	V2/3B[d]	44.8	12.3**	0.274	+0.004
(F) Kohlufushi	Meemu	V2/3B	74.2	18.3**	0.247	+0.236
(G) Guraidhoo	Laviyani	V2/3B[d]	28.8	25.2	0.877	+0.467
(H) Kanifushi	Laviyani	V2/3B[d]	14.4	12.2	0.849	+0.313
Average	—	—	—	—	—	+0.255
(I) Maalhos	North Ari	Bu27	35.3	13.1**	0.371	+0.018
(J) Mathiveri	North Ari	Bu27	37.9	9.7**	0.256	−0.074
(K) Rasdhoo	North Ari	Bu27	31.7	7.7**	0.244	−0.141
Average	—	—	—	—	—	−0.066
(L) Raiymandhoo	Meemu	S2A[d]	52.5	7.7**	0.147	−0.055
(M) Ohluvelifushi	Laviyani	S2A[d]	40.3	12.9**	0.320	+0.011
Average	—	—	—	—	—	−0.022
(N) Thoddoo	North Ari	MSA	40.9	9.9**	0.243	−0.061
(O) Mulaku	Meemu	Mixed[d]	45.3	8.9**	0.197	−0.068
(P) Muli	Meemu	Mixed[d,e]	45.3	11.6**	0.257	−0.008
(Q) Mafilaafushi	Laviyani	X2B[d,f]	26.8	2.7**	0.100	−0.327
Kureli	Meemu	None	12.5	14.0	1.122	—
Madivaru	Laviyani	None[g]	3.9	3.8	0.969	—
Maduvvari	Laviyani	V2/3B[h]	47.9	38.9	0.813	—
Difushi	Laviyani	S2A[h]	20.8	33.6**	1.614	—
Hukurudhoo	S. Ari	None	51.6	46.8*	0.907	—
Average	—	—	—	—	1.085	—

[a] Letters identify the islands shown in Fig. 5.
[b] 1–2.5 yr after the releases. Significant changes to prerelease figures are indicated by * (=1% significance level) or by ** (=0.1% significance level), Mann-Whitney *U* test (Siegel 1956).
[c] The figures quantify the deviation of the damage reduction from the predicted levels. For examples on how these deviations are obtained, see Fig. 5.
[d] Strain(s) identified from samples after release.
[e] Virus spread naturally from Mulaku within 2 mo.
[f] X2B released, but unknown (possibly mutated) strain recovered.
[g] V2/3B recovered; apparently natural spread.
[h] Virus did not become established.

populations continuously after the release. However, by using a survey method that takes advantage of the regularity of the growth of coconut palms and the consistency of the place of attack of *O. rhinoceros* (Zelazny & Alfiler 1987), it was possible to obtain estimates of the number of beetle attacks for each month over a period of >4 yr.

Materials and Methods

The origin and characteristics of baculovirus strains V2/3B, X2B, S2A, and MSA have been described (Crawford et al. 1986). These strains can be distinguished by their restriction endonuclease profiles when the enzymes Bam HI, Hind III, and Eco RI are used. The identification of the strains before and after the release and some minor genetic changes which were observed during the course of the observation have been described by Crawford & Zelazny (1990). An additional strain, Bu27, isolated from Bugsuk Island, Palawan, Philippines, in 1984 was used. The Eco RI and Bam HI restriction profiles of this strain were identical to that of strain X2B except for the following differences: for Eco RI, band I was closer to band H, and band K appeared as a doublet; for Bam HI, the position of bands G–I matched those of strains V2/3B, S2A, and MSA but not that of X2B. Virus inoculum was prepared either from the guts of infected beetles from the Philippines (Zelazny 1978) or from infected tissue culture fluid (Crawford & Sheehan 1984). *O. rhinoceros* adults were field-collected from virus-free islands, fed with two or three drops of inoculum, and released by hand or by letting them fly from open containers at dusk.

The experimental islands, ranging in size from 10 to 60 ha, and the strains released, are included in Table 1. Maps of the islands are shown in Fig. 1–3. In Meemu Atoll and North Ari Atoll, 40–51 inoculated beetles were released per island; in Laviyani Atoll, this number varied between 12 and 43. On the island of Mulaku, strains V2/3B, X2B, and S2A were released simultaneously in 17 inoculated beetles per strain. The island of Muli is separated from Mulaku by about 1.5 km of sea. No infected beetles were released on Muli, but the

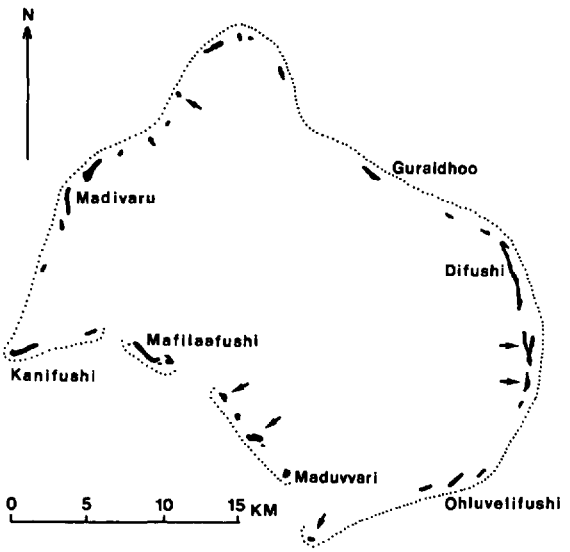

Fig. 1. Map of Laviyani Atoll, Republic of the Maldives. Experimental islands are named; arrows indicate additional (not experimental) virus releases.

virus spread to the island shortly after its release on Mulaku. Each atoll contained one isolated, untreated island.

On each island listed in Table 1, 40 palms were marked and surveyed periodically by climbing and

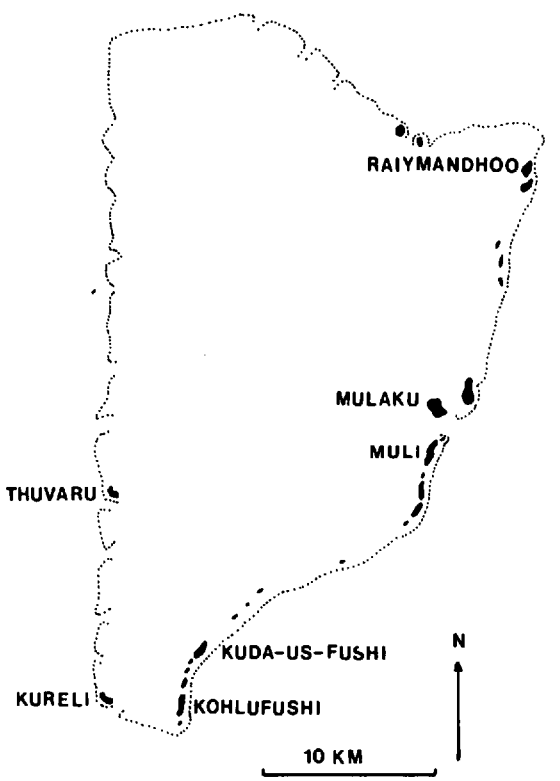

Fig. 2. Map of Meemu Atoll. Experimental islands are named.

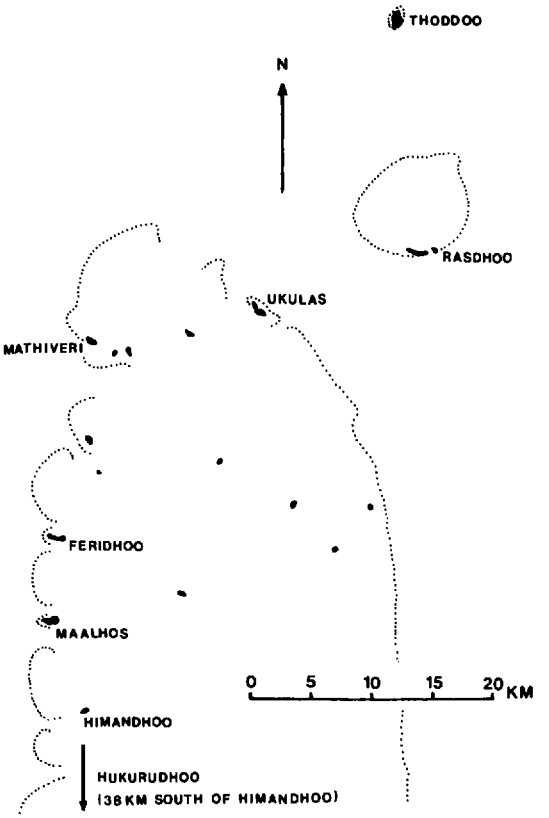

Fig. 3. Map of North Ari Atoll. Experimental islands are named.

other methods described earlier (Zelazny & Alfiler 1987). Briefly, the position of each cut in the fronds caused by *O. rhinoceros* (there are no other pests or conditions in the Maldives that can cause such cuts) was recorded, as well as the sequence of the fronds from the top (youngest) to the bottom (oldest). The youngest frond was marked with paint during the first survey, and these marks served as references during subsequent surveys as well as indicators of the rate of frond production. These data, together with the fact that the point of attack varies little, make it possible to estimate the month during which a given cut was caused by the beetle. An estimate of the monthly number of attacks represents the density of feeding adults in the population. It is not necessarily closely correlated with the density of breeding adults, that of the total adult population, or the number of larvae, but it is obviously the most important parameter for evaluating what beneficial effect the introduction of a given baculovirus strain has on reducing the palm damage. A single survey gave monthly estimates of the number of beetle attacks 3–20 mo before the survey was done. The first survey was done just before the virus release (Meemu Atoll, February 1984; Laviyani Atoll, January 1985; Ari Atoll, June 1985), and the final survey was done in February–March 1988.

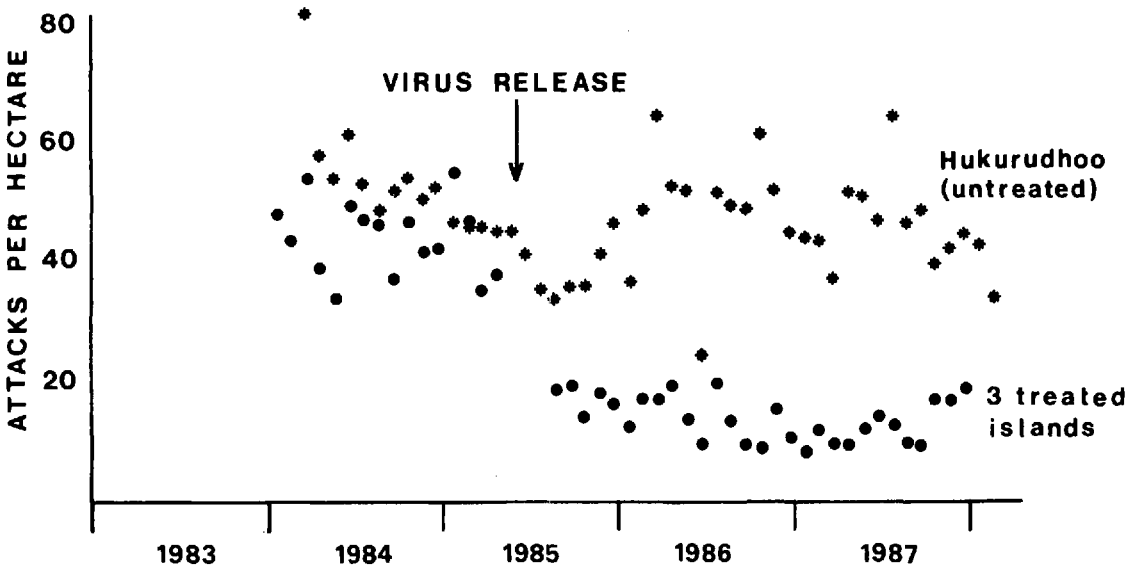

Fig. 4. Estimated monthly attacks per hectare by *O. rhinoceros* on Hukurudhoo (untreated, asterisks) and on three other islands of Ari Atoll (solid circles) where baculovirus was released in June 1985.

Adult beetles, periodically collected from palms, were dissected, and their midguts were examined (Zelazny 1978) to determine the prevalence of baculovirus infections. To confirm that the baculovirus was indeed absent from the islands of the Maldives at the time of its introduction, *O. rhinoceros* adults and larvae collected from palms and breeding sites were examined before the releases on 15 of the experimental islands and on 12 other neighboring islands. The virus strains present after the introduction were determined in selected samples by restriction endonuclease analysis. Collection of other data from the listed islands included estimates of the density of palms and of breeding sites (pieces of decaying trunks of coconut palms and other trees containing *O. rhinoceros* stages). Nonparametric statistics were used routinely for comparing means because the data sometimes were found to be not normally distributed.

Results

During prerelease surveys in 1984 and 1985 and up to 1 mo after the virus was introduced, no sign of baculovirus infections was found among 504 adult beetles and among larvae present in 494 breeding sites. The *Oryctes* baculovirus became established in all but 2 (Maduvvari and Difushi) of the 19 treated islands. In 1988, virus strains were analyzed from nine treated islands (Table 1). One isolate (the only one obtainable from Mafilaafushi, Laviyani Atoll) was a new, previously unidentified strain, here designated as XMS. It differs from the released strain X2B by lacking the Hind III restriction endonuclease site at map unit 52.5 between Hind III fragments M and S, and by a deletion of approximately 100 base pairs from the Bam HI fragment H (see Crawford et al. 1986). Because no additional isolates were available from Mafilaafushi, it is unclear whether this strain represents a mutation from X2B or whether it (instead of X2B) was accidentally introduced from the Philippines. The strain is of great interest, however, because Mafilaafushi showed an outstanding damage reduction. During the 1988 survey, the baculovirus also was recovered from one of the three untreated islands (Madivaru), where it had spread from an unknown source.

Fig. 4 gives examples of monthly beetle attacks on the untreated island of Hukurudhoo, Ari Atoll, and on three neighboring islands where the baculovirus was released. The monthly records were grouped into prerelease (averages, see Table 1) and postrelease records; the latter were further divided into 6-mo periods following the introductions. The periods 1–2.5 yr after the release showed, on average, the greatest reduction in the number of attacks. However, any subsequent increases were, on average, small and were only statistically significant on four islands. Because of the late release dates in Ari Atoll, no records were obtained from those islands later than 2.5 yr after the introductions. The average number of monthly attacks 1–2.5 yr after the releases are given in Table 1 as "postrelease" records. For untreated islands, the reference "time of release" was that on neighboring, treated islands. Among the three untreated islands and the two islands where the disease did not become established, one (Difushi) showed a highly significant increase in the number of monthly attacks and another one (Hukurudhoo) a modest but significant decrease. All islands where the baculovirus became established showed a highly significant reduction in the number of attacks in the

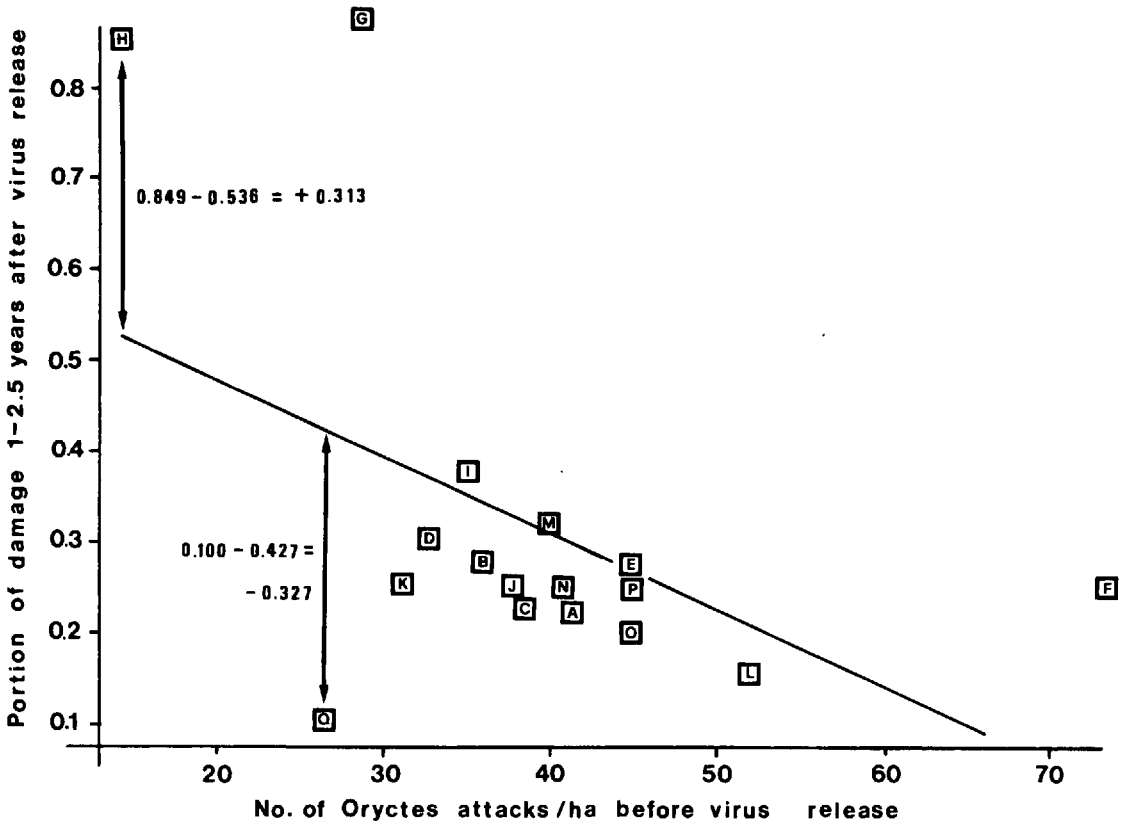

Fig. 5. Linear regression and correlation on 17 islands between the average monthly number of *O. rhinoceros* attacks before the virus introduction and the portion of damage 1–2.5 yr after the release. The value 1 is equal to the pre-introduction damage level. Letters refer to the list of islands in Table 1, which gives also the deviations from the regression line. Two examples for calculating the deviations are illustrated.

postrelease period, except two islands where strain V2/3B was introduced. Dividing the postrelease by the prerelease records gives portions indicating the relative reduction in the number of attacks (next to last column in Table 1). A value of 1 indicated no change in the damage level; this was close to the average portion on untreated islands and on those where the disease did not become established.

These portions of attacks did not show consistent differences between the released virus strains (Table 1). However, to compare the performance of the strains, we must consider that various environmental factors are likely to influence the degree of population reduction. For example, the spread of diseases is commonly dependent on the host density. This appeared also to be the case during the baculovirus introduction. The degree of damage reduction (1–2.5 yr after the releases) was significantly negatively correlated ($r = -0.514$; $P = 0.035$; $n = 17$) with the average number of attacks per hectare before the releases (Fig. 5). On the other hand, correlations between damage reduction and palm density ($r = 0.169$; $n = 17$), size of the island ($r = -0.277$; $n = 17$), or number of breeding sites per hectare before the virus releases ($r = 0.214$; $n = 11$) were not significant.

To compare the relative performance of the different virus strains, therefore, we must take the prerelease population density into account. We consider the deviation of the postrelease portion of attacks from the regression line shown in Fig. 5 as the most suitable measure for the performance of a given virus strain. These deviations from the "predicted" levels of reduction are given in the last column of Table 1; the mechanism for calculating them is illustrated in Fig. 5. A negative figure means that the population was reduced more than predicted by the regression in Fig. 5, and a positive figure means the reduction was less than predicted.

The differences from the predicted reductions were surprisingly consistent for the different virus strains released. All islands where strain X2B was released had negative values, whereas all islands where strain V2/3B was released had positive values. This indicated that which strain was released was indeed a major factor in causing deviations from the regression line in Fig. 5. Less information was obtained from the other strains which were released on fewer islands. On the islands of Mulaku

Table 2. Prevalence of baculovirus infections among *O. rhinoceros* adults collected from palms on islands where different virus strains had been released

Virus strain	% Infected adults[a]								
	1–6 mo	n_s	n_b	6–12 mo	n_s	n_b	>12 mo	n_s	n_b
X2B	26.7	4	116	70.7	2	41	40.7	4	140
V2/3B	15.0	2	20	34.5	4	84	36.5	2	63
S2A	44.4	2	36	41.7	1	12	48.4	1	31

[a] Percentages are at given time periods after beetles were released. n_s, number of samples examined; n_b, number of beetles examined.

and Muli, which were exposed to three strains, strain X2B predominated over strains V2/3B and S2A after the release (Crawford & Zelazny 1990). Again, the deviation from the predicted reduction was negative in both cases.

Nonparametric statistics with the deviations from the regression in Fig. 5 would show a significant difference at the 5% level if strains X2B, V2/3B, and Bu27 are compared using the Kurskall-Wallis *H* test (the equivalent to the parametric analysis of variance) as well as if strains X2B and V2/3B are compared with the Mann-Whitney *U* test (Siegel 1956). Higher significance would result if the deviations of Mulaku and Muli are included under strain X2B (because this strain predominated on these two islands).

Table 2 gives the prevalence of the disease after the introduction of three strains (few beetles were dissected from islands with the remaining two strains). The number of samples was too small for detailed statistical analyses. As the virus had already reduced the number of beetles considerably, more frequent sampling might have had a significant effect on the populations. However, the data indicate a lower disease prevalence on islands where strain V2/3B was released compared with islands with strains X2B or S2A. If the samples after 6 mo are compared for strains X2B and V2/3B (six each), the differences are indeed significant at the 5% level (Mann-Whitney *U* test). The density of feeding adults and the prevalence of the baculovirus are not necessarily directly correlated. The former is a complex product of the effect of the disease on the various sectors of the pest population, including the larvae, over a number of generations.

Discussion

We have demonstrated that strains of the baculovirus can differ in their effect on *O. rhinoceros* populations under field conditions. The degree of difference observed was large enough to suggest that during a control program that uses the virus, the strain selected can have an important influence on the results. Progress already has been made in developing a virus inoculum of a specific strain suitable for use by coconut farmers and extension staff (Crawford & Sheehan 1984, Zelazny et al. 1987).

The clearest difference was observed between strains V2/3B and X2B, with X2B showing more virulence under field conditions as indicated by a consistently better damage reduction when adjusted for prerelease pest densities; a higher prevalence of virus-infected beetles; and in islands where a mixture of strains were released, X2B predominated eventually over strains V2/3B and S2A (Crawford & Zelazny 1990).

In earlier laboratory studies (Zelazny et al. 1989), the virulence of isolates X, V2, and S2A was compared against *O. rhinoceros* larvae and adults. The isolates X2B and V2/3B released in the Maldives represent a further degree of purification by two additional end-point dilutions, although there was no evidence of heterogeneity in the original isolates X and V2. These tests showed that strain V2 was significantly less virulent to larvae than strains X and S2A. However, the dosage–mortality and transmission tests with adult beetles showed no significant difference between strains V2 and X.

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