Possibility of resistance to a baculovirus in populations of the coconut rhinoceros beetle (*Oryctes rhinoceros*)

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Summary. In the Indonesian provinces of North and South Sulawesi and East Java, in Southern Luzon (the Philippines) and in the Maldives, similar conditions exist for the development of populations of the coconut pest, *Oryctes rhinoceros*. Yet population levels can vary greatly, and there has been a negative correlation between the presence and prevalence of the baculovirus disease of *Oryctes* and the damage caused by this pest. In the Maldives the virus was completely absent and its introduction in 1984/85 reduced the *O. rhinoceros* population significantly. In East Java and South Sulawesi the disease was present but occurred rarely, raising the possibility of host resistance to the virus in these areas. Evidence for resistance to the virus disease was obtained from laboratory trials in the Philippines. Compared to earlier tests in Western Samoa, the response of *O. rhinoceros* adults to inoculations with the baculovirus was markedly lower. Furthermore, virus-infected adults transmitted the disease less often to healthy partners than previously observed with Samoan beetles. Trials with larvae showed no evidence for resistance but confirmed that baculovirus isolates can differ significantly in their virulence.

POSSIBILITÉ DE RÉSISTANCE À UN BACULOVIRUS CHEZ LES POPULATIONS D'ORYCTES DU COCOTIER *(ORYCTES RHINOCEROS)*

Résumé

Les provinces indonésiennes du nord et du sud de Sulawesi et de l'est de Java, le sud de Luzon (Philippines) et les Maldives présentent des conditions analogues pour le développement des populations d'un ravageur du cocotier, *Oryctes rhinoceros*. Pourtant, l'importance de ces populations peut varier de beaucoup, et on a observé une corrélation négative entre la présence et la prévalence de la maladie à baculovirus d'*Oryctes* et les dommages causés par ce ravageur. Aux Maldives, le virus était totalement absent et son introduction en 1984/85 a entraîné une nette réduction de la population d'*O. rhinoceros*. Dans l'est de Java et le sud de Sulawesi, la maladie était présente mais les cas étaient rares, ce qui donne à penser qu'il pourrait y avoir une résistance de l'hôte au virus dans ces zones. Des preuves de la résistance à la maladie virale ont été obtenues au cours d'essais en laboratoire effectués aux Philippines. Par rapport aux tests effectués précédemment au Samoa-Occidental, la réponse d'*O. rhinoceros* adulte à l'inoculation du baculovirus a été nettement plus faible. En outre, des adultes infectés par le virus ont transmis la maladie à des partenaires sains moins souvent que dans les observations faites précédemment sur des oryctes au Samoa. Les essais effectués sur des larves n'ont donné aucune preuve de leur résistance mais ont confirmé que la virulence des isolats de baculovirus peut varier considérablement.

POSIBILIDAD DE RESISTENCIA A UN BACULOVIRUS EN POBLACIONES DEL ESCARABAJO RINOCERONTE DEL COCOTERO *(ORYCTES RHINOCEROS)*

Resumen

En las provincias indonesias de Sulawesi septentrional y meridional y Java oriental, en el sur de Luzón (Filipinas) y en las Maldivas existen condiciones análogas para el desarrollo de poblaciones de la plaga de los cocoteros formada por *Oryctes rhinoceros*. Sin embargo, los niveles de población pueden variar mucho, y se ha observado una correlación negativa entre la presencia y la prevalencia de la enfermedad del baculovirus del *Oryctes* y los daños ocasionados por esta plaga. En las Maldivas no existía en absoluto el virus, y su introducción en 1984/85 redujo considerablemente la población de *O. rhinoceros*. En Java oriental y Sulawesi meridional la enfermedad estaba presente, pero se observaba raramente, aumentando la posibilidad de resistencia del hospedante al virus en esas zonas. En Filipinas se demostró en pruebas de laboratorio la resistencia a la enfermedad vírica. En comparación con pruebas anteriores realizadas en Samoa Occidental, la respuesta de los adultos de *O. rhinoceros* a inoculaciones con el baculovirus fue considerablemente inferior. Por otra parte, los adultos infectados por el virus transmitían la enfermedad a otros sanos con una frecuencia inferior a la observada anteriormente en los escarabajos de Samoa. En los ensayos realizados con larvas no se obtuvieron pruebas de resistencia, pero se confirmó que los baculovirus aislados pueden tener una virulencia considerablemente distinta.

The rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), is a serious pest of coconut palms in many tropical parts of Asia and the Pacific. Damage can be so severe that raising young palms is extremely difficult and yields of mature palms are greatly reduced. Most cultural and chemical control methods are either impractical or insufficient. One of the more promising techniques has been the

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use of a baculovirus disease, which was discovered in 1963 in Malaysia (Huger, 1966) and infects specifically *O. rhinoceros*. The introduction of this disease into the South Pacific became an example of highly successful classical biological control (see Bedford, 1981 or Waterhouse and Norris, 1987 for reviews). Populations have dropped to insignificant levels in many areas and are expected to remain low unless beetles develop resistance to the disease.

The authors' present research raises the possibility that various levels of resistance to the baculovirus already exist in the *O. rhinoceros* populations of the Philippines and Indonesia. The problem appears to be especially severe in Java and South Sulawesi, where continuous widespread outbreaks of the pest occur. The question of resistance has been addressed by comparing the field populations of *O. rhinoceros* in different parts of Asia and conducting laboratory studies in the Philippines.

As it would have involved high quarantine risks to introduce *O. rhinoceros* from other countries into the Philippines for direct comparison of its susceptibility, three trials conducted earlier in Western Samoa were meticulously repeated (Zelazny, 1976, 1979). Ten virus isolates from the Philippines and Western Samoa were compared at that time and there was evidence that *O. rhinoceros* larvae showed differences in their susceptibility toward them. In this study different virus isolates were also compared, but only one of the isolates previously used is still available. This isolate, labelled PA by Zelazny (1979) but PV505 by Crawford *et al.* (1985) was used, and two additional Philippine and two Samoan isolates were studied.

Methods

The damage to coconut palms, expressed in *O. rhinoceros* cuts per frond; the density of *O. rhinoceros* populations, expressed in cuts per hectare on the upper palm fronds; the average number of breeding sites per hectare; and the percentage of virus-infected *O. rhinoceros* adults were determined as part of recently completed or ongoing ecological studies in the Maldives and in Indonesia (Zelazny *et al.* unpublished). The methods used have been described earlier (Zelazny and Alfiler, 1986). Baculovirus infections had been determined by bioassay tests in Western Samoa and the Philippines but were done by gut examinations in Indonesia and the Maldives. The results were compared with earlier field observations from the Philippines and Samoa. Data from Western Samoa on the percentage of upper fronds damaged (Zelazny, 1977) were converted into cuts per frond, using highly significant correlations from data collected in the Maldives.

For the susceptibility experiments, *O. rhinoceros* larvae were collected in the field in Southern Luzon (provinces of Albay and Camarines Sur) and reared on a mixture of decaying sawdust and dry cow dung. Only insects reared from eggs were used in the trials. The procedures for inoculating larvae and adults with the baculovirus were identical to those used earlier (Zelazny, 1979) except for two modifications: larvae were kept in sawdust and cow dung instead of kapok wood and cow dung, and one of the lower dosages was replaced by a higher one.

Briefly, the baculovirus isolates PA (PV505), V2 and X from the Philippines and SAM and S2A from Western Samoa, were multiplied in third instar *O. rhinoceros* larvae. Isolates PV505, S2A (a subisolate of SAM), and subisolates of V2 and X (labelled V2/3B and X2B) have been characterized by Crawford *et al.* (1985), and Crawford, Zelazny and Alfiler (1986). Isolate SAM originated from Western Samoa but had been passed through *Oryctes monoceros* in the laboratory.

Virus-killed larvae (frozen shortly after their death and stored for several weeks) were blended and mixed with dry larval food to give final concentrations of 0.2, 2, 20 and 200 ppm. Ten young third instar larvae were kept individually for five weeks in each mixture and in untreated food. The trial was repeated four times on different dates and with freshly prepared mixtures.

Adult beetles were injected with 0.1 ml of sterile water containing either 0, 0.05, 1, 10 or 200 picolitres (litre 10^{-12}) of haemolymph which had been collected from larvae with advanced symptoms of virus infections (isolates V2, X or SAM) and had been stored frozen for several weeks. Adults were bioassayed (Zelazny, 1978) two weeks after inoculation and the experiment repeated five times. LD_{50} values were estimated for larvae and adults using the maximum likelihood method.

Transmission from virus-infected to healthy beetles was studied by inoculating *O. rhinoceros* adults as above but using a higher dosage, 200 µl of haemolymph (Zelazny, 1976). At one or two weeks after inoculation each adult was kept in 500 ml of larval food together with an uninfected partner, and the beetles were separated again after one day or three days. Table 1 shows the seven combinations of sexes, times after inoculation, and days kept together that were used. Only virgin beetles were used when adults of the opposite sex were paired. After two weeks, females were dissected to see if they had mated and all adults were bioassayed for virus infections. Pairs of the opposite sex that had failed to mate and pairs in which the inoculated partner did not become infected were discarded.

Results

The average *O. rhinoceros* damage to coconut palms and the presence and prevalence of the baculovirus disease are negatively correlated in the four countries where sufficient data were obtained (see Table 2). This confirms that in these countries the disease is one of the most important factors determining the size of the pest population. Other environmental factors, such as weather conditions, or the presence of natural enemies, did not appear to contribute much to the observed variations in *O. rhinoceros* populations, with the exception of the density of *O. rhinoceros* breeding sites.

The results in Table 3 represent an attempt to quantify the correlation between *O. rhinoceros* population density (expressed in *Oryctes* cuts per ha on upper palm fronds), *O. rhinoceros* breeding density (expressed in breeding sites per ha) and prevalence of the baculovirus disease (expressed in percentage of virus-infected adult beetles). Data on breeding site density are not available from Western Samoa and East Java. In the remaining areas the data were divided into different groups to maximize variation in the breeding density, but geographically adjacent areas were always combined in the same group. In the Maldives the islands were grouped according to whether the baculovirus had been released or not.

The resulting multiple regression model is shown in Table 3. The model is significant and reflects the augmentative influence of the breeding sites on the population and the regulating influence of the baculovirus disease.

Although it will be useful for understanding and managing pest populations, such a simple model cannot be expected to represent accu-

TABLE 1. **Transmission of the baculovirus from infected to healthy *Oryctes rhinoceros* adults**

Weeks after inoculation	Days kept together as pairs	Percentage cases of virus transmission (no. of pairs)			
		Philippine beetles			Samoan beetles [1]
		Isolate V2	Isolate SAM	Isolate X	
1	1	24 (33)	9 (33)	9 (34)	12 (25)
2	1	6 (36)	0 (33)	6 (31)	60 (15)
1	3	3 (34)	9 (32)	12 (33)	44 (25)
2	3	14 (36)	18 (33)	18 (40)	68 (18)
2	3	47 (32)	57 (30)	36 (33)	80 (25)
2	3	9 (33)	19 (32)	6 (33)	40 (25)
2	3	9 (32)	28 (32)	3 (33)	52 (25)

[1] *Source:* Zelazny (1976).

TABLE 2. **Damage caused by *O. rhinoceros* to coconut palms and prevalence of the baculovirus disease in four countries**

Country	Location	Year	*O. rhinoceros* cuts per frond	Virus-infected adult beetles (%)
Western Samoa	Upolu	1970/75	0.21	43.0 (2 383)[1]
Philippines	Southern Luzon	1982/84	0.23	28.1 (462)
Indonesia	North Sulawesi	1987/88	0.15	20.0 (130)
Indonesia	South Sulawesi	1987/88	0.32	4.4 (298)
Indonesia	East Java	1988	0.36	0.3 (392)
Maldives	Meemu, Laviyani and North Ari Atolls	1984-88	0.81	0 (504)
Maldives	Meemu, Laviyani and North Ari Atolls	1988	0.23	42.7 (1 042)

[1] Figuras in parentheses = numbers examined.
Source: Zelazny, 1973, 1977; Zelazny and Alfiler, 1986; Zelazny *et al.* (Unpublished)

rately the complex ecology of this pest, nor even the interactions between the above three variables. For example, the incidence of the baculovirus itself depends on the population size—the disease will spread better at higher pest densities than at lower.

It is important to understand the reason for the wide variation in the natural incidence of the virus disease. The data from South Sulawesi and East Java in particular stand out, where a high beetle population should have actually promoted the spread of the disease. It has proved impossible to attribute the low natural prevalence of the virus in South Sulawesi and East Java to any environmental factors. The results from the laboratory trials in the Philippines raise the possibility that resistance to the virus disease might account for such low natural disease incidence. Compared to the earlier trials in Western Samoa, virus concentrations about 20 times higher were needed to infect 50 percent of the

TABLE 3. **Correlation between *O. rhinoceros* cuts per ha (Y), number of *O. rhinoceros* breeding sites per ha (X_1) and percentage of virus-infected *O. rhinoceros* adults (X_2) in three countries**

N. area	Grouping for breeding density	Cuts/ha Y	Breeding sites/ha X_1	Infected adults X_2 (%)
1. Philippines	low	288.1	2.3	20.9
2. Philippines	medium	291.5	3.1	34.4
3. Philippines	medium	262.8	3.4	12.1
4. Philippines	high	505.3	6.9	46.5
5. North Sulawesi	low	194.9	0.8	35.7
6. North Sulawesi	medium	208.6	3.2	18.1
7. South Sulawesi	low	270,6	2.3	0
8. South Sulawesi	high	476.4	9.6	1.4
9. Maldives, before virus introduction	low	958.9	3.2	0
10. Maldives, before virus introduction	high	1 679.8	12.5	0
11. Maldives, after virus introduction	low	349.8	3.1	41.9
12. Maldives, after virus introduction	high	490.9	9.2	43.1

Multiple regression model:
$Y = 280.2 + 75.2\,X_1 - 7.4\,X_2$

Probability of: $X_1 = 0.016$
$X_2 = 0.177$

Total regression $= 0.019$
$r^2 = 0.584$

adult beetles in the Philippines (see top of Table 4). (One of the 150 control adults was found to be virus-infected and the responses were adjusted accordingly with Abbott's formula.)

The results of the transmission trials also indicate lower susceptibility in the Philippine population, where virus-infected beetles passed the disease on to healthy beetles less frequently in 20 out of 21 groups compared to corresponding earlier trials with Samoan beetles (see Table 1). In this experiment none of the 246 control adults gave a positive bioassay result. Infected females transmitted the baculovirus consistently more often to healthy males than vice versa. In both trials the three baculovirus isolates tested did not give significantly different results.

The results of the susceptibility tests with larvae (bottom of Table 4) did not provide clear evidence of resistance in larvae and the LD_{50} values overlapped with those determined earlier in Western Samoa. The responses did, however, differ from the four virus isolates tested. Regression lines between percent mortality in probit scale and dosage in log scale did not deviate significantly from parallel lines and covariance analysis showed significant differences between isolate S2A and the isolates V2 ($F = 104.6$, d.f. 1, 5, $P < 0.005$) and PA ($F = 11.2$, d.f. 1, 5, $P = 0.02$) as well as between isolates X and V2 ($F = 7.7$, d.f. 1, 5, $P = 0.04$). Two out of 200 control larvae died during the trial and the mortality rates were adjusted accordingly.

TABLE 4. **Estimated LD_{50} concentrations[1] of baculovirus for *Oryctes rhinoceros* larvae and adults originating from Western Samoa and the Philippines**

Isolate	Origin of isolate	Philippine population	Samoan population[2]
Tests with adult beetles			
SAM	Western Samoa	8.2	—
V2	Southern Luzon, Philippines	9.3	—
X	Bugksuk Island, Philippines	13.3	—
PB	Leyte Island, Philippines	—	0.2
PC	Mindanao Island, Philippines	—	0.4
SA	Western Samoa	—	0.9
Tests with larvae			
S2a	Western Samoa	5	—
X	Bugsuk Island, Palawan, Philippines	18	—
PA	Southern Luzon, Philippines	29	11
V2	Southern Luzon, Philippines	54	—
PB	Leyte Island, Philippines	—	3
PF	Leyte Island, Philippines	—	6
SA	Western Samoa	—	11
SB	Western Samoa	—	13
SC	Western Samoa	—	14
SD	Western Samoa	—	15
PE	Central Luzon, Philippines	—	16
PC	Mindanalo Island, Philippines	—	21
PD	Mindanao Island, Philippines	—	22

[1] For adults, LD_{50} concentrations are expressed in picolitres (litre 10^{-12}); for larvae in ppm.
[2] *Source:* Zelazny (1979).

Discussion

Frequent outbreaks of *O. rhinoceros* in Java and South Sulawesi are apparently the result of a low natural prevalence of the baculovirus disease. This situation causes concern as the baculovirus is considered the most important natural control agent of the pest. The authors' research points to two possible reasons for the low disease incidence-resistance in the host population to the disease and the presence of virus strains with low virulence. Evidence for resistance has been found among *O. rhinoceros* adults in the Philippines. The adult beetles are spreading the disease in nature and the reduction of their lifespan and fecundity represents the main impact of the virus on the population. If the problem is caused by virus strains of low virulence then the release of the more virulent strains will be no easy solution. However, there appears to be no reason why such less virulent strains should have developed and established themselves. On the other hand, natural selection for disease resistance is commonly found among animals and plants.

At this stage it is important to confirm the resistance hypothesis by comparing side by side the susceptibility of *O. rhinoceros* adults originating from various parts of Southeast Asia. Unfortunately, this is not possible in a coconut-growing country since it would cause unacceptably high quarantine risks. The assistance of another country is therefore needed to study where, and at what levels, resistance to the baculovirus occurs.

REFERENCES

BEDFORD, G.O. 1981. Control of the rhinoceros beetle by baculovirus. *In* Burges, H.D., ed. *Microbial control of pests and plant diseases,* p. 409-426. London, Academic Press. 949 pp.

CRAWFORD, A.M., ASHBRIDGE, K., SHEEHAN, C. & FAULKNER, P. 1985. A physical map of the *Oryctes* baculovirus genome. *J. Gen. Virol.*, 66: 2649-2658.

CRAWFORD, A.M., ZELAZNY, B. & ALFILER, A.R. 1986. Genotypic variation in geographical isolates of *Oryctes* baculovirus. *J. Gen. Virol.*, 67: 949-952.

HUGER, A.M. 1966. A virus disease of the Indian Rhinoceros Beetle, *Oryctes rhinoceros* (Linnaeus), caused by a new type of insect virus, *Rhabdionvirus oryctes* gen. n., sp. n. *J. Invert. Pathol.*, 8: 38-51.

WATERHOUSE, D.F. & NORRIS, K.R. 1987. *Biological control—Pacific prospects.* Melbourne, Intaka Press. 454 pp.

ZELAZNY, B. 1973. Studies on *Rhabdionvirus Oryctes*. III. Incidence in the *Oryctes rhinoceros* population of Western Samoa. *J. Invert. Pathol.*, 22: 122-126.

ZELAZNY, B. 1976. Transmission of a baculovirus in populations in *Oryctes rhinoceros. J. Invert. Pathol.*, 27: 221-227.

ZELAZNY, B. 1977. *Oryctes rhinoceros* populations and behaviour influenced by a baculovirus. *J. Invert. Pathol.*, 29: 210-215.

ZELAZNY, B. 1978. Method of inoculating and diagnosing the baculovirus disease of *Oryctes rhinoceros. FAO Plant Prot. Bull.*, 26: 163-168.

ZELAZNY, B. 1979. Virulence of the baculovirus of *Oryctes rhinoceros* from ten locations in the Philippines and Western Samoa. *J. Invert. Pathol.*, 33: 106-107.

ZELAZNY, B. & ALFILER, A.R. 1986. *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) larva abundance and mortality factors in the Philippines. *Environ. Entomol.*, 15: 84-87.