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Ecology of baculovirus-infected and healthy adults of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) on coconut palms in the Philippines

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Abstract. 1. Males and females of *Oryctes rhinoceros* (L.), a serious pest of coconut palms, congregate in decaying coconut trunks to mate, oviposit and prepare the wood for the young larvae. A baculovirus disease is often transmitted from beetle to beetle in this environment and transmission to and from larvae probably also occurs.

2. Young beetles are mainly found feeding on palms, where they also frequently contract the virus disease, presumably through mating with diseased partners.

3. Monitoring beetle populations in the Philippines with attractant traps and by collections from palms showed seasonal fluctuations which were often inversely correlated with the incidence of the virus disease. Disease prevalence matched closely the proportion of females in the population. Possible explanations for this correlation and for the seasonality of the disease prevalence are outlined.

Key words. Insecta, *Oryctes rhinoceros*, baculovirus, microbial control.

Introduction

The rhinoceros beetle, *Oryctes rhinoceros* (L.), is a major threat to coconut farmers in South-East Asia. Although the baculovirus of *Oryctes* has been recognized to be an important natural control agent (review by Bedford, 1981), serious pest outbreaks can still develop even if the disease is present in the beetle population. This happens typically during replanting of coconut groves when beetles breed in the decaying trunks of felled palms.

Recently, studies have investigated the ecology of *O.rhinoceros* larva populations and the role of the baculovirus in suppressing them (Zelazny & Alfiler, 1986). The number and type of breeding places were found to be the most important variables influencing the disease incidence. For example, a limited number of dead standing palms can effectively promote contact between adults and larvae and increase the transmission rate of the virus. The virus disease has a large impact on *O.rhinoceros* adults, which actively spread the disease to breeding places and among themselves (Huger, 1973; Zelazny, 1973a, b, 1976, 1977). Virus-infected beetles stop feeding and egg-laying after 1 week, and die prematurely after 4–5 weeks. Therefore, the disease prevalence among adult beetles influences the present and future pest populations and determines the rate of transmission to the breeding sites. The baculovirus is not transmitted through the pupal stage and newly emerged beetles are not carriers until they are themselves infected.

The present paper describes studies in the

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Philippines on the prevalence of the baculovirus disease in *O.rhinoceros* adult populations and on factors influencing the disease incidence. They were conducted in locations where initial ecological studies have been done already (Zelazny & Alfiler, 1987). During these studies, catches of adult beetles with attractant traps were shown to be of beetles searching for breeding places. The results also suggested that after leaving their pupation site, *O.rhinoceros* adults spent about 5 weeks feeding on coconut palms. This was followed by dispersion over longer distances (more than 500 m) and a period of about 7 weeks spent in breeding sites and on occasional additional visits to palms.

Materials and Methods

O.rhinoceros adults were caught with attractant traps in five locations in the Philippine provinces of Albay and Camarines Sur in South-Eastern Luzon between 1980 and 1983 (Zelazny & Alfiler, 1987). The locations (Table 3) each contained fifty coconut cap traps (Bedford, 1973), baited with ethylchrysanthemumate. Workers checking the attractant traps weekly emptied the trap content into glass jars without touching the beetles, to minimize cross-contamination. In or near each trapping location three 9 ha plots were surveyed for *O.rhinoceros* breeding sites over the same period. In addition beetles were collected regularly from coconut palms in one of the five locations. Care was taken to avoid cross-infections between larvae and beetles during the collections from breeding sites (Zelazny & Alfiler, 1986).

The presence of baculovirus infections in beetles trapped individually and in those collected from breeding sites was investigated by bioassay (Zelazny, 1978). Using sterilized instruments a beetle was cut up and fed to two mass-reared larvae which were observed for 4–5 weeks. For each beetle, two other larvae from the same breeding container were kept as controls. Females found in breeding sites were checked to see if they had mated by examining the content of their spermatheca.

To study the effect of adding virus-infected and healthy beetles to the population, releases of marked beetles were conducted at Guinobatan on two occasions. In December 1983, 145 laboratory-reared beetles were infected by feeding them small amounts of gut homogenate from virus-diseased beetles and released. In October 1984, 578 laboratory-reared healthy beetles, 5 weeks old, were released in the same location. The beetles were marked by scratching a cross on the pronotum with a small file.

The data collected during a large-scale breeding site survey in Southern Luzon (Zelazny & Alfiler, 1986) were further analysed to obtain information on the ecology of *O.rhinoceros* adults and the baculovirus.

Results

Breeding behaviour and virus transmission in breeding sites

Males and females often occur together in breeding sites. Males help females to prepare the wood for the eggs and the small larvae. The decaying wood is chewed into small particles and packed tightly around the eggs. In total, seventy-six pairs of mature beetles were found in breeding sites. Pairs of the opposite sex were significantly more common than expected by chance ($\chi^2=16.5$, 2 d.f.) and the data in Table 1 indicate that typically the female arrives first in the breeding site and the male follows after some eggs have been deposited. The males seem to stay longer and chew up more wood for the young larvae. Mating also can take place in the breeding sites and the prevalence of the baculovirus among males and females found together in the breeding sites was higher than in beetles found singly (Table 1). For groups of adults larger than two the infection rate was still higher (on average 43.7%, $n=87$).

The data from the large-scale breeding site survey indicated that in twenty-one locations (where a total of at least fifteen beetles was collected from breeding sites) the percentage of virus-infected adults correlated with the number of larval sites containing virus ($r_s=0.655$, $P=0.001$), suggesting that adults can infect larvae and vice versa.

Releases of diseased and healthy beetles

Earlier surveys indicated that virus transmission between *O.rhinoceros* adults is also common in live palms, especially in areas where dead standing palms are rare (Zelazny *et al.*,

Table 1. Condition of *O.rhinoceros* adults in breeding sites and association with eggs and newly hatched larvae.*

	Breeding sites containing:				
	♀	♂♀	♂	♂♂/♀♀	>2
Total no. of breeding sites found	114	54	114	22	27
Percentage of sites without eggs	40.4[a]	50.0[bc]	64.9[c]	36.4[ab]	37.0[abc]
Percentage of sites with eggs but no newly hatched larvae	57.9[a]	42.6[b]	23.7[c]	50.0[ab]	51.9[ab]
Percentage of sites with eggs plus young first instar larvae	1.8[a]	7.4[ab]	11.4[b]	13.6[ab]	11.1[ab]
Percentage of females mated	95.0[a]	97.4[a]	–	100[a]	96.9[a]
Percentage of females infected with baculovirus	18.5[a]	26.9[a]	–	50.0[a]	40.0[a]
Percentage of males infected with baculovirus	–	34.0[a]	19.3[b]	39.1[ab]	46.3[ab]

* Figures in the same line followed by the same letter are not significantly different at the 5% level (Wilcoxon signed-rank test).

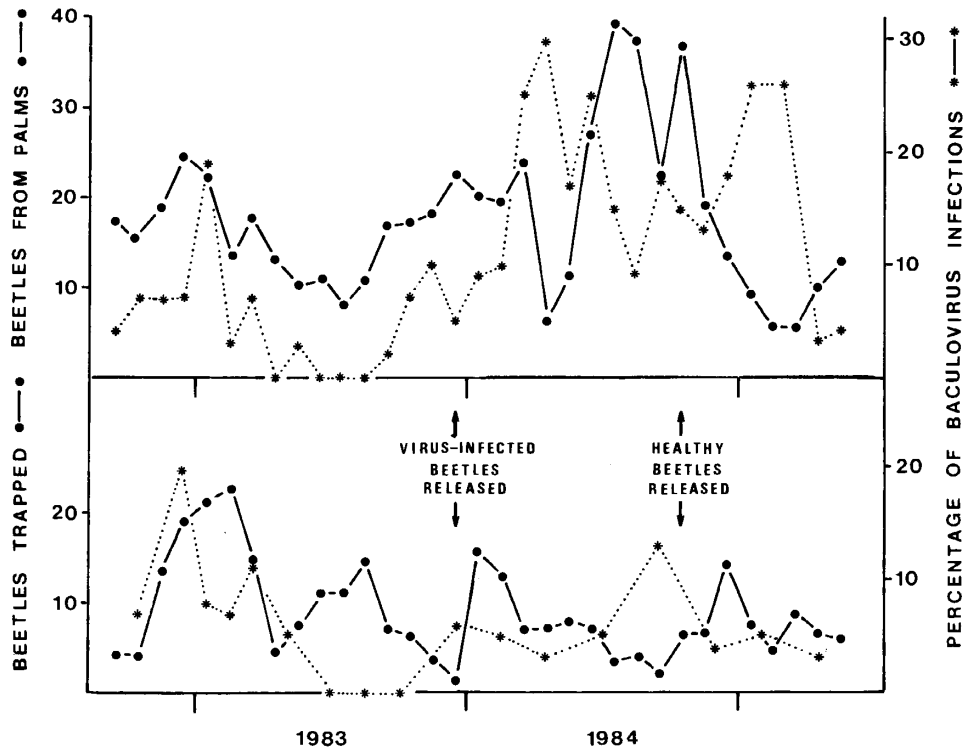

Fig. 1. *O.rhinoceros* adult population monitored in Guinobatan by traps and palm collections, and prevalence of the baculovirus disease among the beetles. Times of releases of virus-infected and healthy beetles are indicated by arrows. Recaptures are excluded from the presented data. During periods of low populations, data from several months were grouped to determine the baculovirus incidence. Palm collection data are from a central 6 ha blocks, but traps were operated also in surrounding areas (total 50 ha). Beetles found on palms in the surrounding areas are included in the disease incidence figures.

Table 2. Baculovirus infections among recaptured *O.rhinoceros* adults which had been marked and released uninfected at the age of 5 weeks.

Weeks after release	Recaptured from:	Total	Virus infected
1	Palms	17	6 (35%)
2	Palms	16	2 (12%)
> 2	Palms	11	2 (18%)
> 2	Breeding sites	15	0 (0%)

unpublished). The release of 145 virus-infected beetles in Guinobatan, which had a very low density of dead standing palms, was followed by an increase in the incidence of disease among the wild beetles found in palms, but not among beetles searching for breeding sites (trap catches, see Fig. 1). A peak in virus infections was observed 4 months after the release. However, the increase appeared small considering that the average palm damage in the 50 ha release area (reported by Zelazny & Alfiler, 1987) was 189.3 *O.rhinoceros* cuts per hectare before the release which corresponded to only about one feeding adult per hectare. This supports earlier observations that fast and long distance dispersal is frequent in this species (Zelazny & Alfiler, 1987).

The releases of marked, healthy, unmated beetles in the same location gave further evidence of transmission in palms (Table 2). Curiously, this release was also followed by a marked increase in the infection rate among the wild, unmarked beetles feeding on palms. Again a peak in virus infections was observed 4 months after the release (Fig. 1). It is possible that the higher population density resulting from the release could have accelerated the disease transmission.

Natural disease prevalence among O.rhinoceros adults

Prevalence of the baculovirus disease among beetles from traps and breeding sites was closely correlated (Table 3). Beetles from palms were examined only in Guinobatan and there had a similar infection rate. The disease incidence among larvae had approximately similar trends. Breeding activities of *O.rhinoceros* females seem to go through two minima each year (Fig. 2), the first one coinciding with the annual dry season (March–May) in Southern Luzon. However, the number of males trapped only showed one clear annual cycle. Also, the prevalence of baculovirus infections among trapped beetles showed only one clear annual minimum and maximum. It matched closely the ratio of females among the trap catches. Virus infections among males and females showed similar trends with females typically having a higher infection rate. However, during the months of March–May (dry season) males had either a similar or a higher infection rate (data not shown).

Discussion

Adult beetles, the main carriers and main hosts, appear to become infected with the virus via

Table 3. Prevalence of the baculovirus disease in five locations among larvae (breeding sites) and adult beetles.

Location	% of larval sites with baculovirus (1982/83)	% virus-infected adults from: Traps* (1981–83)	Breeding sites (1982–84)	Palms (1982–83)
Guinobatan	4.9 (n=81)	6.8[a] (n=703)	6.2 (n=16)	5.7 (n=681)
Mauraro	0.9 (n=106)	7.6[ab] (n=669)	8.7 (n=23)	–
Paulog	25.4 (n=59)	12.5[b] (n=678)	32.0 (n=25)	–
Baao	4.9 (n=346)	18.8[c] (n=610)	13.2 (n=38)	–
Calabanga	16.3 (n=473)	38.4[d] (n=318)	44.8 (n=165)	–

* Monthly records were compared with the Wilcoxon signed-rank test, averages followed by the same letter in this column are not significant at the 5% level.

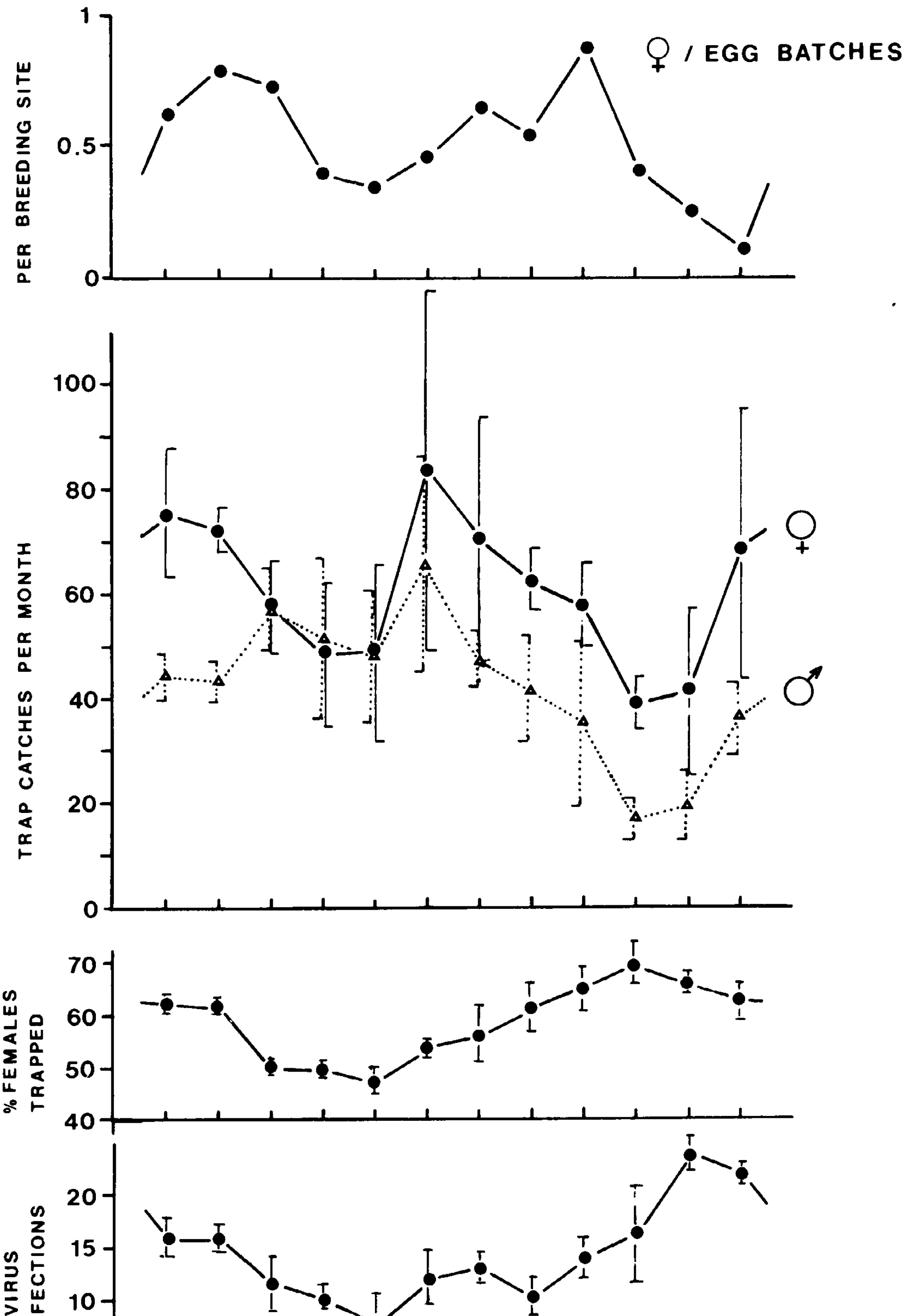

Fig. 2. Average seasonal variation (between September 1980 and August 1983) in *O.rhinoceros* breeding activity, trap catches of females and males, and baculovirus prevalence and sex ratio among trapped beetles in five locations in Southern Luzon. Breeding activity (top) is expressed in the number of females, egg-batches or groups of first instar larvae found per breeding site in various areas of Southern Luzon (Zelazny & Alfiler, 1986). Trapping data were averaged over 3 years, for percentages through arcsin transformations. Vertical lines indicate the standard errors of these averages.

two routes, during mating and when in coconut trunks for breeding. The spread of the disease among young adults which are mainly found feeding on palms, is likely to be attributable to contact during mating. For example, many of the young, healthy and unmated beetles which were released soon became infected. As the palm damage was low in the experimental area, accidental contact with the virus during the normal feeding process seems unlikely. Evidence that mating can take also place in palms had been obtained earlier (Zelazny, 1975).

The rate at which adults spread the disease to, or become infected in, breeding sites depends on the ecological conditions, especially on the type and density of breeding sites. For example, dead standing palms promote the spread of the disease, but a high number of lying trunks reduces contact between infected and healthy beetles (Zelazny & Alfiler, 1986). Among the five locations listed in Table 3, three (Guinobatan, Mauraro and Baao) had a high density of lying trunks and a low density of dead standing palms with a resulting low prevalence of the virus disease among adults and larvae. Paulog had a medium density of dead standing palms and a low density of lying trunks, whereas Calabanga had a high density of both lying trunks as well as dead standing palms. Virus infections were high among adults and larvae in the latter two areas.

The incidence of baculovirus infections among adult beetles appeared to be inversely correlated to the density of the adult population, as indicated by both trap catches as well as palm collections (Fig. 1). This negative correlation is also evident if the seasonal fluctuations of trap catches (especially those of males) are compared with those of the virus prevalence (Fig. 2). The data did not permit conclusions on the reasons for this negative correlation or the seasonal fluctuations of the disease prevalence and we are limited to discussing the most likely explanations.

Disease transmission is commonly dependent on the host density, with a higher disease incidence (after a certain time lag) when the host density increases. However, an increase in the density of *O.rhinoceros* adults through the emergence of larger numbers of young beetles from the breeding sites will, no doubt, lower the disease prevalence initially, because newly emerged beetles are uninfected. Since females live on average longer than males, older beetles have a surplus of females and the emergence of young beetles would probably also lower the proportion of females. A seasonal peak in emergence of young beetles (between March and May) could then explain the seasonality of virus prevalence and the proportion of females, as well as the close correlation of both. Unfortunately it was not possible to draw conclusions from the data on the seasonality of emerging young beetles, but two observations give some support to this hypothesis.

(1) Data collected by Soebandrijo *et al.* (1982) in East Java seem to suggest that the highest number of *O.rhinoceros* pupae occurred at the end of the dry season with maximum adults trapped (by light) 2 months later at the beginning of the rainy season. If a similar pattern occurs in Southern Luzon, one would expect maximum number of pupae in March–May, maximum adult emergence in April–June and a peak in beetles caught with attractant traps (those searching for breeding sites) in May–July. Trap catches in March–May were probably not good indicators of the adult density because the dryness of the traps affected their attractivity (Zelazny & Alfiler, 1987).

(2) Schipper (1976) found that a temperature difference of 3°C causes a substantial increase in *O.rhinoceros* pupation 1–2 months later. In Southern Luzon the minimum daily temperature drops near the end of the year and reaches its lowest level in January–March. Such a mechanism could cause a peak in adult emergence between March and May and might override any seasonality in number of eggs deposited. Indeed, without the temperature influence, the two peaks in egg-laying might only cause minor peaks in pupation because of the large variability in the development time of the insect.

Therefore, a seasonal peak in the number of young beetles emerging from pupation sites in April–June could be the reason for the low disease prevalence during that period and for the high virus-incidence in October to December among trapped beetles. Both, the release of infected as well as healthy beetles caused a marked peak in virus infections after a similar time lag, 4 months (Fig. 1). The negative correlation between trap catches and disease prevalence could therefore be an artefact.

An additional factor which might play a role in the correlation between sex ratio and disease prevalence is the laboratory observation that females transmit the baculovirus more frequently to males than vice versa (Zelazny *et al.*, 1989). Clearly, more data are needed to support these suggested mechanisms for the seasonality of *O.rhinoceros* populations and the prevalence of the baculovirus disease.

Introductions of the baculovirus into disease-free islands has lowered the pest population density to 10–20% of the pre-release levels, while 40% and more of the adult beetles became infected (Bedford, 1981; Zelazny *et al.*, 1990). The overall incidence of virus infections in the Philippines was lower than in Western Samoa (Zelazny, 1973b) but higher than in many areas of Indonesia. The possibility of the Philippine and Indonesian *O.rhinoceros* population possessing some degree of resistance to the disease has been discussed earlier (Zelazny *et al.*, 1989).

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