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Microbial pathogens of the coconut pest *Oryctes rhinoceros*: influence of weather factors on their infectivity and study of their coincidental ecology in Kerala, India

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Summary

The rhinoceros beetle, *Oryctes rhinoceros* L., is an economically important pest of the coconut palm. Management of this pest has been accomplished using microbial agents *viz*., *Oryctes* virus (OrV) and an entomofungal pathogen *Metarhizium anisopliae*. Recently an opportunistic bacterial pathogen *Pseudomonas alcaligenes* has also been noticed to cause septicaemia in the grubs when under stress. To unravel the influence of abiotic weather factors and the interactions amongst these microbial pathogens, a 3 year study was conducted from September 1996 to August 1999 in three of the southern districts of Kerala, India. Of the 6627 grubs and 307 adults collected from various breeding sites of the pest, 5% of the grubs and 22% of the adults had natural virus infection, 3% larvae died of *M. anisopliae* mycosis and 20% larvae succumbed to bacterial septicaemia. *Oryctes* virus infection in grubs and adults was negatively correlated to minimum temperature (correlation co-efficient, $r = -0.4$, and -0.6 respectively, sample size, $n = 0$). Increase in relative humidity increased the fungal activity ($r = 0.8$) whereas, maximum temperature had a negative impact ($r = -0.7$). Occurrence of virus infection in grubs and adults was positively correlated ($r = 0.6$), supporting the contention of active transmission of the virus pathogen between these two stages. The bacterial septicaemia in the grubs was marginally correlated with virus infection and *P. alcaligenes* undermined the efficiency of the virus pathogen.

Introduction

The rhinoceros beetle, *Oryctes rhinoceros* L. is distributed in most of the coconut-growing countries in the world. It is one of the major economically important pests of this plantation crop (Nair 1986), which is grown in 0.982 million hectares in Kerala out of the total of 1.8 million hectares in India, accounting for 54.7% of the area, and contributing 42.3% of the production (Singh 1998). Suppression of this insect and its damage to the coconut palm can be brought about, to a great extent, by the application of a non-occluded virus, specific to *Oryctes* (Bedford 1980; Alfiler 1984; Mohan & Pillai 1993) and an entomofungal pathogen *Metarhizium anisopliae* (Nirula *et al*. 1955, 1956; Young 1974; Abad *et al*. 1992; Moslim *et al*.1999).

Besides the manipulation of the habitat of the pest to improve *Oryctes* virus infection (Zelazny *et al*. 1992) for effective utilization of these bioagents for the regulation of the pest population, it is very important that the effect of biotic and abiotic factors on the insect pathogens and the 'coincidental' ecology of these pathogens must be understood. In addition to this, their interactive patterns should also be determined so that accurate decisions regarding manipulation of the pathogens and the host will provide a firm base for the integrated management of the pest. Harris & Dent (2000) observed that in the priorities of the biopesticide field, studies on the pathogen ecology were very low and stressed that they were as important as searching for the pathogens, because ecology could eventually become one of the causes of failure of the biopesticide system under development. With this aim, a survey was conducted in three southern districts of Kerala, India, *viz*. Alleppey, Quilon and Kottayam from September 1996 to August 1999, to monitor the prevalence of natural *Oryctes* virus infection and *Metarhizium* disease in the *O. rhinoceros* population and the factors that govern them. Besides the viral and fungal diseases, which are proven biocontrol agents of this coleopteran pest, an opportunistic bacterial infection was also observed to be prevalent in this insect, especially in larval stages. The present work gives some information on the influence of the weather parameters and coincidental ecology of these microbial pathogens of *O. rhinoceros*.

Materials and methods

Collection of pest from field

Fortnightly collections of *Oryctes* grubs and adults, starting from September 1996 to August 1999, were made from coconut gardens in Krishnapuram, Kappil, Purakkad and Thottappally areas of the Alleppey District; Ayiramthengu, Oachira, Choonad, Thodiyur, Thazhava and Karunagappally in the Quilon District and Vazhappally in the Kottayam District of Kerala, India. The common breeding sites were farmyard manure, cattle waste pits, coir waste dumps, dislodged coconut logs, decaying trunks and stumps of coconut trees. In addition to these, vermicompost pits also harboured the immature stage of this pest (unpublished). The collected insect samples were transferred to the laboratory and screened immediately for abnormal grubs. The healthy ones were maintained in moist sterilized coir waste (Gopal & Sathiamma 2000) for further studies. Weather data *viz*. average maximum and minimum monthly temperature, relative humidity and rainfall were taken from the Agrometeorological unit based in the Research Institute in which these studies were conducted.

Diagnosis of the microbial diseases

Daily observations were made for the exopathological symptoms of *Oryctes* virus infection (Zelazny 1972, 1973; Mohan *et al*. 1983) in the grubs and adults, and it was confirmed microscopically by checking the presence of hypertrophied nuclei in midgut epithelial tissue/haemolymph cells by 3% Giemsa staining (Zelazny 1972). Mycosis of the grubs by *Metarhizium* was scored according to the criteria elucidated by Nirula *et al*. (1955) and Young (1974). Symptoms of occurrence of bacterial septicaemia by opportunistic *Pseudomonas alcaligenes* were recorded following the description made by Gopal & Gupta (2002).

Statistical analysis

Correlation co-efficient (r) were used to investigate the influence of abiotic weather factors on the incidence of microbial infections as well as the coincidental occurrence amongst the pathogens, with the sample size, $n = 10$.

Results

Weather

The trend of the weather parameters is represented in Figure 1. From the 3 years' data, it is seen that the average monthly maximum temperature was highest from January to May (annual range, 30.3–34.3 °C; mean for the period, 33.6 °C) and that the average monthly minimum temperature during December–February (annual range, 20.9–24.5 °C; mean for the period, 21.7 °C) and in July (22.3 °C) fell below the annual mean (23 °C). Relative humidity was observed to be high between July and November (annual range, 93–96%; mean for the period, 96%). Average monthly rainfall was high from May to October (annual range, 5–447 mm; mean for the period, 316 mm) and then declined.

Field collection data

A total of 6627 grubs and 307 adults were collected over the period of 3 years from the various breeding sites from the coconut gardens. Grubs which showed lethargy and developed translucent midgut line (15–20 days after collection from field) were dissected to check the

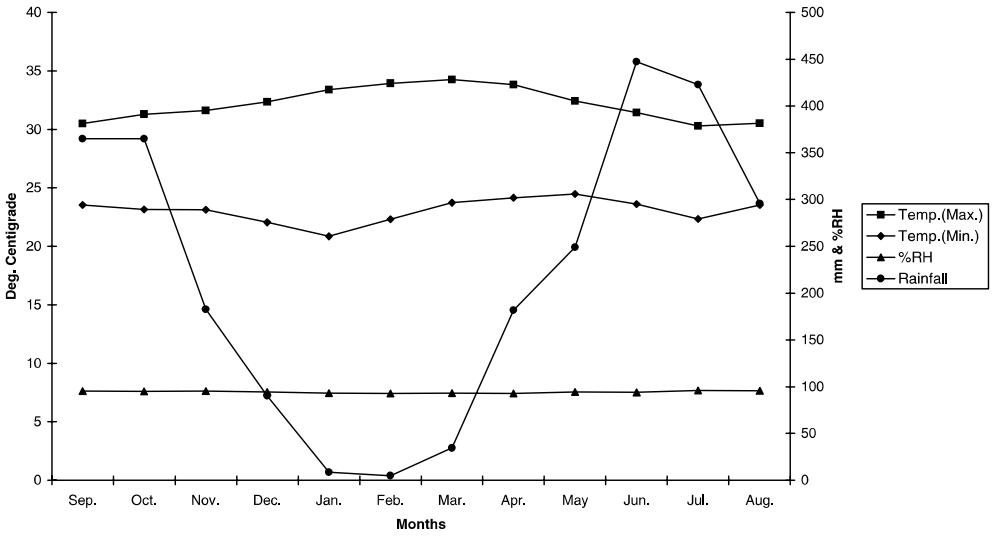

Figure 1. Monthly average weather data for 3 years (September 1996–August 1999).

Table 1. Correlation of occurrence of the microbial infections in *O. rhinoceros* grubs and adults to abiotic factors.

Microbial pathogens	Weather			
	Temperature (maximum)	Temperature (minimum)	Relative humidity	Rainfall
Correlation of *Oryctes* virus in grubs to	0.199	−0.420	−0.194	−0.259
Correlation of *M. anisopliae* in grubs to	−0.735*	0.069	0.816*	0.519
Correlation of *Pseudomonas alcaligenes* in grubs to	0.345	−0.065	−0.120	−0.494
Correlation of *Oryctes* virus in adults to	0.166	−0.625**	−0.249	−0.225

* Significant at $P = 0.05$; ** significant at $P = 0.01$.

presence of high haemolymph content and swollen midgut filled with white mucoid fluid. Smears were made from midgut epithelial tissue and haemolymph cells of such samples and stained with 3% Giemsa stain. Presence of pink hypertrophied nuclei confirmed natural virus infection in 5% of the grubs and in 22% of the adults. Approximately 3% of grubs had mummified and later produced green-coloured spores externally. These were stained with cotton blue in lactophenol and confirmed as that of *M. anisopliae* var *major* based on their size and shape. No adults died due to the fungal infection, although they are reported to be susceptible. Infection by *P. alcaligenes* was scored when grubs suddenly became transparent, flaccid, yet retained their body shape and structure, developed chalky white spots in abdomen segments having unvoided faecal pellet in the half open rectum, and followed by browning at the moribund stage. When such grubs were dissected they showed dried lumpy food in the midgut and putrefied fat bodies. *Pseudomonas alcaligenes* colonies in large numbers were detected in haemolymph, fat bodies and midgut contents, giving off a characteristic odour while growing on nutrient agar plates. More than 19% of the field-collected and laboratory-maintained grubs died due to this infection.

Correlation of microbial pathogens with abiotic factors

Incidence of *Oryctes* virus in the immature stages had a marginal negative correlation (correlation co-efficient, $r = -0.4$, sample size, $n = 10$) with average minimum temperature, otherwise there was no significant influence of any other weather factors (Table 1), whereas, this factor proved to be significantly negatively correlated with the occurrence of viral infection in adults ($r = -0.6$, $n = 10$). The *Metarhizium* infection is positively correlated at significant level to the relative humidity ($r = 0.8$, $n = 10$) and negatively to the average maximum temperature ($r = -0.7$, $n = 10$). Average maximum temperature had a positive influence on bacterial infection, whereas rainfall had negative impact (Table 1). Figure 2 shows that virus incidence peaked during October and February in the larval stage, and during February in adults. November and July are favourable for a *Metarhizium* epizootic. Appearance of opportunistic *P. alcaligenes* coincides with that of viral

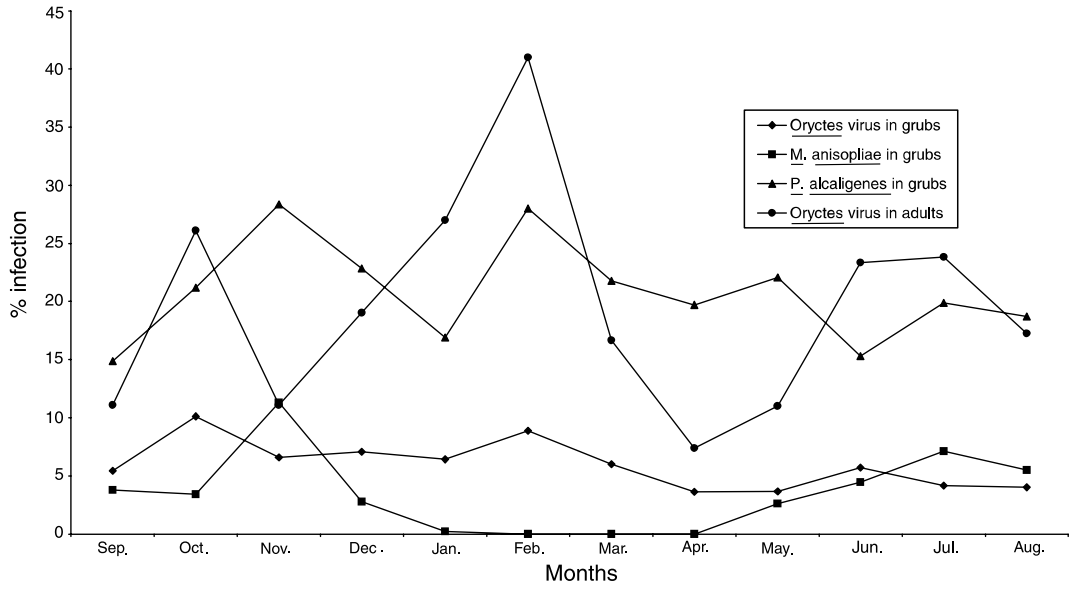

Figure 2. Monthly average trends for a period of 3 years (September 1996–August 1999) of different microbial pathogens of *O. rhinoceros*.

Table 2. Correlation amongst the occurrence of microbial pathogens in the *O. rhinoceros* grubs and adults.

Microbial pathogens of *O. rhinoceros*	*Oryctes* virus in grubs	*M. anisopliae* in grubs	*P. alcaligenes* in grubs	*Oryctes* virus in adults
Correlation of *Oryctes* virus in grubs to	–	−0.13	0.39	0.62*
Correlation of *M. anisopliae* in grubs to	–	–	0.18	−0.26
Correlation of *P. alcaligenes* in grubs to	–	–	–	0.21
Correlation of *Oryctes* virus in adults to	–	–	–	–

* Significant at $P = 0.01$.

infection in grubs, mostly during September–November and February (Figure 2).

Correlation amongst microbial pathogens

The statistical analysis of co-occurrence of three microbial pathogens depicted a significant positive correlation with incidence of *Oryctes* virus in grubs and adults ($r = 0.6$, n = 10) (Table 2). A marginal positive correlation was observed ($r = 0.4$, n = 10) between the virus and *P. alcaligenes* infection in the larval stages of *Oryctes* (Table 2).

Discussion

The non-occluded virus *Baculovirus oryctes* was till recently considered as part of the Baculovirideae, however, currently this pathogen has been removed from this group and put in the *Oryctes* virus group (Evans & Shapiro 1997). Viral infection in immature stages and adult *Oryctes* peaks almost during the same period, September–March in the case of grubs and December–March in adults. This supports the contention that when the viral infection is present in any of these two stages, the usual route of its perpetuation from diseased beetles to healthy beetles and grubs, and from diseased grubs to healthy immature stages and adults, takes place frequently, thereby increasing the natural occurrence of the disease. It has been reported that the life cycle of the *Oryctes,* from egg to adult, takes place in 6 months (Lever 1969). In Kerala, India, the peak adult emergence coincides with rainfall i.e. June and July, which indicates that mating and egg laying would have occurred approximately 6 months earlier or even more (December–February). This lends support to our observation that the virus occurrence was higher in adults during the December–February period when the mating and egg-laying activities would have brought a greater number of beetles' interactions increasing the possibility of virus transfer from diseased individuals to the healthy ones. This period (December–February) also experiences lower minimum temperature. Hence, a strong negative correlation is observed between the virus incidence in the adult beetles and the temperature. Zelazny & Alfiler (1991) mention that the virus was most prevalent among male and female beetles found together in the breeding sites. In addition to this, the nocturnal habit of the adults makes them highly active during early evening and the dawn period (Ohler 1984), which facilitates beetles meeting and transmitting the virus. Villacarlos & Betonio (1990) also reported a negative correlation with virus incidence and temperature, but a significant positive influence of rainfall, which was not observed in our studies.

Growth of any fungus is enhanced when temperature is near the optimum for the species and relative humidity and rainfall are high. *Metarhizium* also responds to these criteria, causing mycosis during such weather periods in *Oryctes* grubs. Death of grubs due to an entomofungal agent reduces the virus inoculum (Hochberg & Waage 1995) hence we observe that virus incidence in adults decreases along with that of the grubs.

Abundance of bacterial infection due to gut residence of the opportunistic *P. alcaligenes* is usually seen during the weather period when the temperature is on the rise. In our earlier work (Gopal & Gupta 2002) we have noted that for this bacterial infection to occur in these grubs, one of the predisposing biotic stress factors is the presence of *Oryctes* virus disease in the host. Figure 2 shows the approximate coincidence of *P. alcaligenes* and virus mortality in grubs.

For the effective control of rhinoceros beetle, use of the virus and *M. anisopliae* is recommended as components of integrated pest management. Application of *M. anisopliae* in the breeding sites of the pest during high humidity and rainfall period results in good control of the grubs. Effectiveness of the virus is somewhat reduced because of the fungal infection, but the recovery by the virus is quick once the fungal infection declines. Moreover, the average *Metarhizium* infection was only 3%, too little to vitiate the viral inoculum load. It is actually the occurrence of the opportunistic infection by *P. alcaligenes* that seriously undermines the virus transmission in the field, as 20% of grubs died of it. This death results in diminished availability of the virus for its natural perpetuation. A salient feature of *P. alcaligenes* infection was that it reduced during May–July when the maximum temperature decreased and rainfall increased. During the same period a small peak in viral infection in *Oryctes* was also seen (Figure 2).

This study reveals that biotic and abiotic factors play a very important role in the ecology of microbial infections of *O. rhinoceros*. Although all three microorganisms ultimately killed the host, only the viral and fungal agents are established biocontrol pathogens. The bacterial disease has a negative influence in the effective

control of the rhinoceros beetle by the *Oryctes* virus as it leads to the reduction in viral inoculum. It thus acts as an antagonist of the viral pathogen. More detailed field level studies elaborating augmentation of bioagents during the lean pathogen activity periods with minimal interference from each other, combined with habitat management of the pest, need to be undertaken in different ecozones to test the strength of this basic information on microbial pathogens of *O. rhinoceros*.

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