ORYCTES VIRUS FOR BIOCONTROL OF RHINOCEROS BEETLES-*Oryctes rhinoceros*

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The rhinoceros beetle, *Oryctes rhinoceros* is a major pest of oil palm in Malaysia. As the replanting activities increase over recent years, infestation of oil palm with *Oryctes* correspondingly increases. The entomopathogenic *Oryctes rhinoceros* virus was discovered 40 years ago in Malaysia (Huger, 1966). It was then successfully introduced into many South Pacific countries to control *Oryctes* on coconut (Zelazny *et al.*, 1992). In Malaysia, the infection of *Oryctes* virus is common on adults and less frequent on larvae (Ramle *et al.*, 2002). Infected adult has a swollen gut and an infected larva becomes transparent when viewed against light. Three strains of virus has been identified as strains A, B and C (Figure 1). Strain A is commonly found in many localities, strain B is found in Selangor and Perak, while strain C is found only in Sabah (*Figure 2*).

IMPACT OF VIRUS ON LARVAE AND ADULTS OF *Oryctes*

Strain B is the most virulent compared to strains A or C. It caused 65% mortality on the larvae and 86.7% on the adults.

IMPACT OF VIRUS RELEASED IN THE FIELD

The strain B virus was released into the field by the capture, infect and release technique. The levels of virus infection increased from 37.5% to 100% as early as four months after introduction, and it was maintained at high levels for two years (*Table 1*). As the infection level increased, the virus spread from the released point to

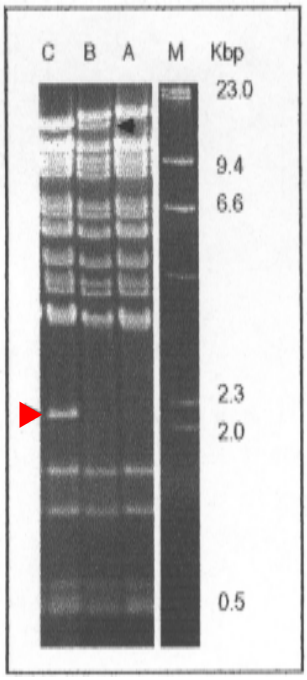

Figure 1. DNA profiles for strain virus A, B and C. Arrows showing the extra band that is used to identify strain B and C from strain A, M marker.

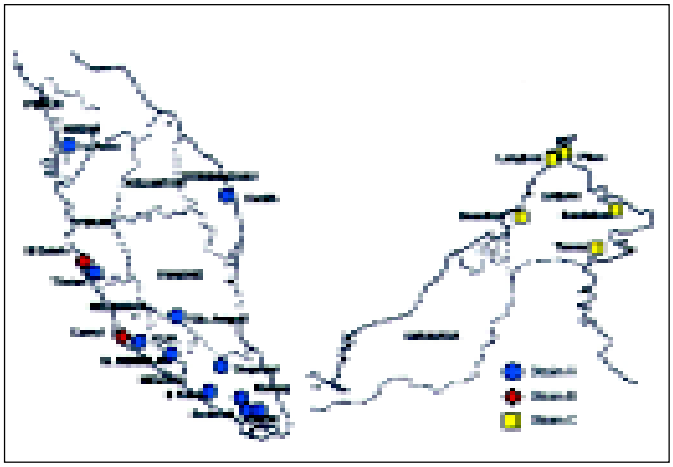

Figure 2. Map showing the geographical distribution of virus strains in Malaysia.

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TABLE 1. LEVELS OF VIRUS INFECTION IN EACH REGION AT THE RELEASE OVER TIME

Release regions	Virus infection (%) over time (month after release)						
	0	2	4	6	11	15	24
Inner		68a	100a	90a	100a	100a	100a
Middle	37	83a	100a	100a	100a	92a	67a
Outer		70a	100a	93a	100a	100a	93a
Control*		Na	66b	Na	100a	92a	80a

Notes: Means in column with same letter are not significantly different (P>0.05).
* Control plots is 3 km away from the release point.

surrounding areas. Three months after introduction, the DNA fragments of the released virus were detected at the released point. After 15 months, it spread to 2-3 km away from the released point (*Figure 3*). The damage on palms decreased over time, from an average of 11.3% at three months after introduction (MAI) to 3.4% at 24 MAI (*Table 2*).

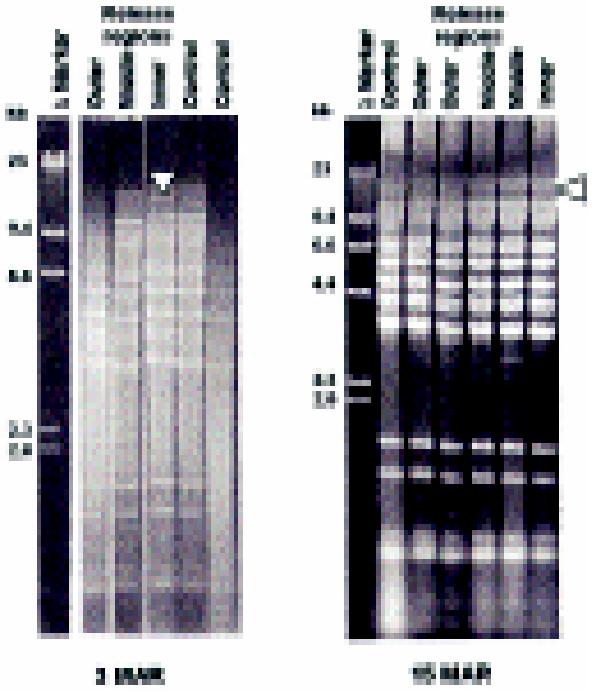

Figure 3. The establishment of released strain B virus in the release area. MAR= month after release. Arrows showing the band that is used to identify strain B.

TABLE 2. LEVELS OF FROND DAMAGE AT THE RELEASE SITE OVER TIME

Release regions	Frond damage (%) over time (month after release)		
	3	15	24
Inner	16.2b	5.1a	2.8a
Middle	9.4a	4.3a	4.2a
Outer	8.0a	2.6a	3.1a
Average	11.2	4.0	3.4

Note: Means in column with same letter are not significantly different (P>0.05).

THE INTRODUCTION OF VIRUS IN THE FIELD

Activities involved in the field introduction of virus could be divided into three phases: pre-release site assessment, time of release, and post-release monitoring and impact assessment.

Pre-Release Site Assessment

1. Identification of existing type of virus.

Recognition of the developmental stages of *Oryctes* is important, especially on the sex of the adult. Adults caught from traps were placed individually in vials to avoid cross contamination. The samples were dissected for the gut. The visual appearances of healthy and infected guts were recorded (*Figure 4*). The guts were then extracted to get sufficient amount of virus DNA for strain identification,

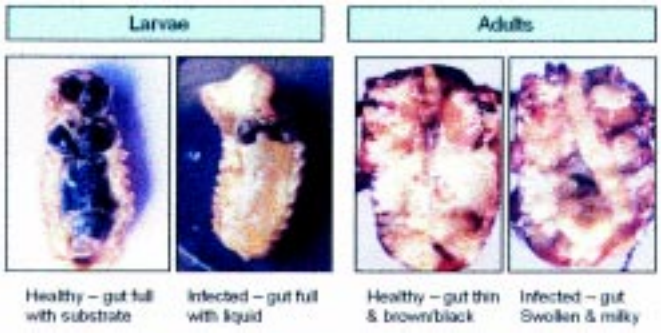

Figure 4. The virus infection symptoms on larvae and adults of Oryctes.

by restricting the DNA using the endonuclease enzyme, *HindIII* .

2. Determination of virus infection on immature and adults of Oryctes.

The guts were extracted by a quick and robust method of DNA extraction. The presence of virus in the early stage of virus infection was determined by the polymerase chain reaction technique using the virus specific primers (*Figure 5*).

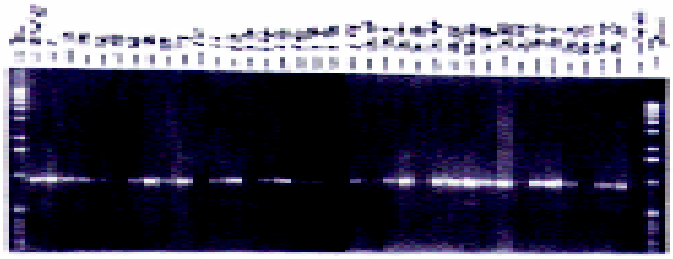

Figure 5. PCR results showing the presence of Oryctes *virus on infected guts of rhinoceros beetles. Arrow showing a single vital band at size 950pb.*

3. Assessment of palm damages.

Palms were inspected for *Oryctes* attack. This included fresh frond damage, number of frond damage, number of little leaves and the total number of fronds.

4. Collection of young healthy adults for viral dissemination.

Young healthy adults could be collected by trapping or from breeding sites.

Time of Release

5. Inoculation of young adults and field release.

The young healthy adults were kept without food for a day, then inoculated by dropping the virus solution onto the mouthparts of the beetles (*Figure 6*). The infected beetles were then released to the field.

Post-Release Monitoring and Impact Assessment

6. Determination of adult population by trapping method.

Population of adults was determined by trapping for three to four consecutive days. The captured adults were placed individually as in step 1.

7. Determination of virus infection on immature and adults.

The infections of virus were determined from immature and adults. The presence of virus in the early stage of infection was determined by polymerase chain reaction technique using the virus specific primers as in step 2.

8. Assessment of palm damage.

Levels of palm damage for post-release were assessed as in step 3.

9. Determination of spread of released virus.

The guts of adults were dissected for DNA analysis. The DNA samples were then used for strain identification as in step 1.

Figure 6. Inoculation of adults with virus solution.

CONCLUSION

The strain B virus killed both larvae and adults of *Oryctes*. In the field, virus infection increased and maintained at high levels, causing reduction in palm damage. The virus was species specific infecting the *Oryctes* beetle. Therefore, it is safe to the environment and non-target organisms. The technique to introduce the virus into the field is relatively simple. Successful control of *Oryctes* by virus could reduce the use of pesticides.

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