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Evidence of *Oryctes* Virus in Adult Feces and New Data for Virus Characterization

A virus observed in *Oryctes rhinoceros* (Coleoptera) (A. Huger, *J. Invertebr. Pathol.*, **8**, 38–51, 1966) has been used successfully for biological control of this beetle in the South Pacific area (A. Huger, *Z. Angew. Entomol.*, **72**, 309–319, 1972; B. Zelazny, *J. Invertebr. Pathol.*, **20**, 235–241, 1972; K. J. Marschall, *Nature* (*London*) **225**, 288–289, 1970; C. Hammes, *Cah. ORSTOM Sér. Biol.*, **22**, 52–91, 1974; P. Monsarrat, *Cah. ORSTOM Sér. Biol.*, **22**, 92–111, 1974). Its introduction in Wallis Island reduced by 72–90% the level of damages to coconut palms. A. Huger (*J. Invertebr. Pathol.*, **8**, 38–51, 1966), working on thin sections of infected tissues, reported morphological similarities between the virus and NPV.

In previous papers we have characterized the virus both in its morphology and chemical composition (P. Monsarrat, G. Meynadier, G. Croizier, and C. Vago, *C. R. Acad. Sci. Paris*, **276**, 2077–2080, 1973; P. Monsarrat, J. C. Veyrunes, G. Meynadier, G. Croizier, and C. Vago, *C. R. Acad. Sci. Paris*, **277**, 1413–1415, 1973; J. M. Quiot, P. Monsarrat, G. Meynadier, G. Croizier, and C. Vago, *C. R. Acad. Sci., Paris*, **276**, 3229–3231, 1973; B. Revet, and P. Monsarrat, *C. R. Acad. Sci. Paris*, **278**, 331–334, 1974.) These results and antigenic properties (G. Croizier and P. Monsarrat, *Entomophaga*, **19**, 115–116, 1974) show the close relationship between this virus and baculoviruses.

We have reported viral particles in the feces of infected adults (P. Monsarrat, J. L. Duthoit, and C. Vago, *C. R. Acad. Sci. Paris*, **278**, 3259–3261, 1974). In order to obtain significant amounts of virus we have tried to purify virus from adult feces. To accomplish this, young adults were infected per os by feeding them for 8 days on banana slices contaminated with crude suspensions of infected larvae. The beetles were also contaminated by dipping their head in the suspension. Following this contamination, the beetles were left without any food for 1 month. Then they were put individually in 500-ml beakers containing a 5-mm height of 1/250 M phosphate buffer + 12% sucrose, *p*H 8.5. The feces suspended in the buffer were collected every day. After clearing by centrifugation at 5000*g* for 20 min the supernatant fluids (Fig. 1) were pelleted at 80,000*g* for 90 min. The pellets contained up to 50% of virus particles which were easily purified further by 20–50% sucrose density gradients prepared in the 1/250 M phosphate buffer, *p*H 8.5. The excretion of viruses by infected adults proceeds for 2–3 weeks.

The amount of virus produced by an infected adult in the feces could be estimated roughly up to 0.3 mg/day. These data were obtained by weighing the pellet after clearing and freeze-drying and roughly estimating the percentage of virions it contains by electron microscopy.

This large quantity of virus allowed us to estimate the DNA content of the particle, using the diphenylamine method according to K. W. Giles and A. Myers (*Nature* (*London*), **206**, 93, 1965) and protein content according to the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall (*J. Biol. Chem.*, **193**, 265–275, 1951). We found that the DNA content represents about 16% of the particle weight. This percentage is near the values found for other baculoviruses (P. Wildy, *Monogr. Virol.*, **5**, 32, 1971).

Adults play an important part in the natural dissemination of the virus and suffer from an intestinal disease (A. Huger, *Z. Angew Entomol.*, **72**, 309–319, 1972; J. Monty, Proc. 14th Canberra Congr.

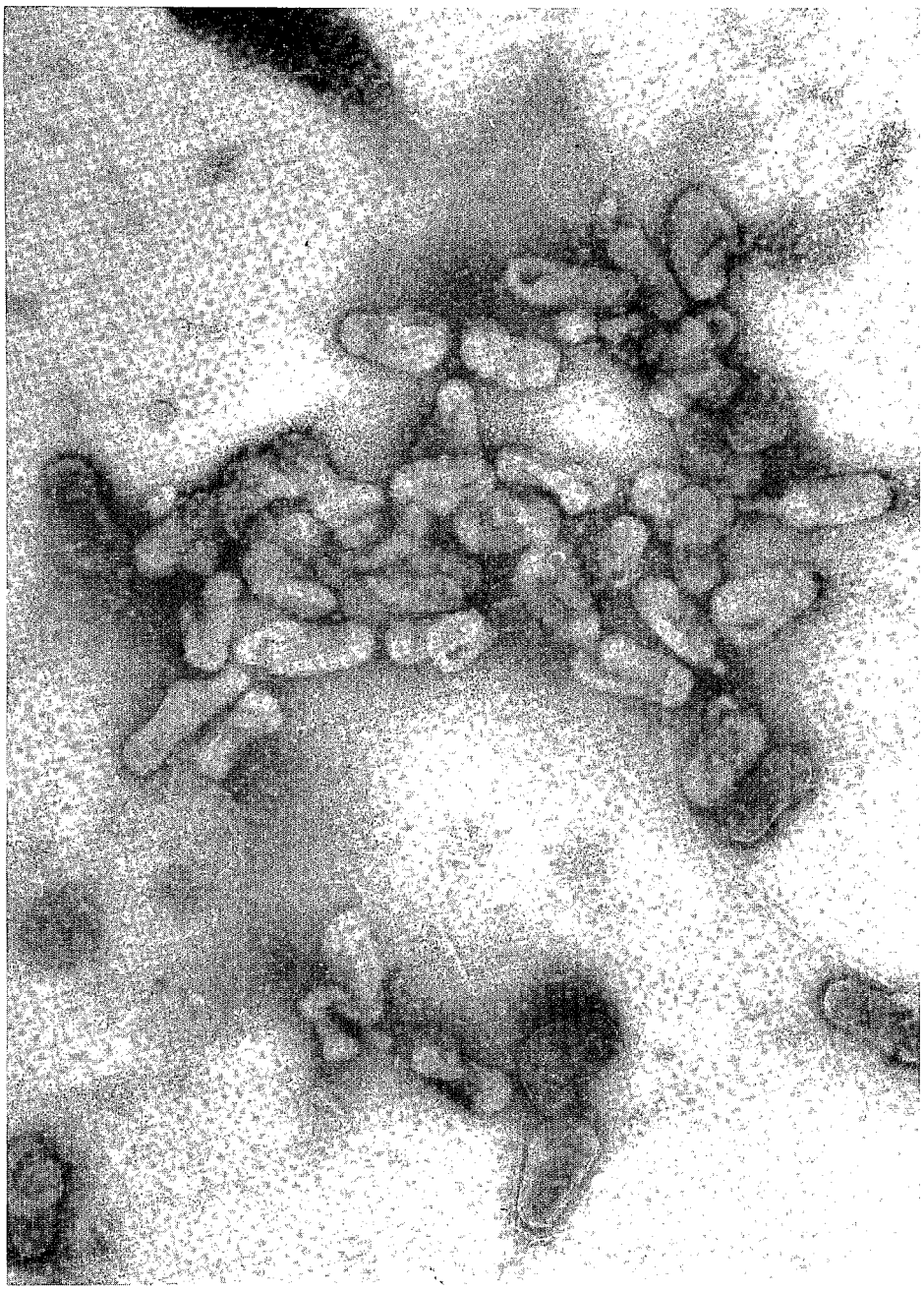

FIG. 1. Electron micrograph of *Oryctes* virus from adult feces after clearing by centrifugation at 5000*g* for 20 min. ×71,200.

Entomol., 1972; B. Zelazny, *J. Invertebr. Pathol.*, **22**, 122–126, 359–363, 1973; C. Hammes, *Cah. ORSTOM Sér. Biol.*, **22**, 52–91, 1974; P. Monsarrat, *Cah ORSTOM Sér. Biol.*, **22**, 92–111, 1974· P. Monsarrat, G. Meynadier, G. Croizier, and

C. Vago, *C. R. Acad. Sci. Paris*, **277**, 1413–1415, 1973). The excretion by adults of great amounts of viruses for a long period of time may partially explain the rapid spreading of the virus noted in Wallis Island, in the South Pacific, by C. Hammes (*Cah. ORSTROM Sér. Biol.*, **22**, 52–91, 1974). Furthermore, the presence of virus particles in spermatids of adults and into chorionated ovocytes (P. Monsarrat, J. L. Duthoit, and C. Vago, *C. R. Acad. Sci. Paris*, **278**, 3259–3261, 1974) allowed us to consider that other pathways of dissemination such as transovarial transmission and contamination of the female by mating may also occur.

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