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Infection of *Strategus aloeus* (L.) (Coleoptera: Scarabaeidae) and other Dynastinae with *Baculovirus oryctes*

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Abstract

Adults of *Strategus aloeus* (L.) were shown to be capable of supporting *Baculovirus oryctes* replication. This was confirmed by serology and by restriction endonuclease analysis. Although bioassays were not performed, the quantity of virus produced was similar to that produced in *Oryctes rhinoceros* (L.) and should encourage further trials on the potential of this virus to control *S. aloeus*. *Scapanes australis* (Boisduval) and *Papuana biroi* Endrödi did not support adequate virus replication. Previous findings on the susceptibility of *O. monoceros* (Olivier) were confirmed.

Introduction

Dynastine beetles are widespread agricultural pests in the tropics. Species of *Oryctes*, *Scapanes*, *Strategus* and *Xylotrupes* attack palm trees (Bedford, 1980), while species of *Papuana*, *Prionoryctes* and *Heteronychus* damage the roots of plants, the first two of these three being particularly harmful to tuber crops. Dynastines are frequently difficult to control by chemicals; cultural control methods are often effective, but additional components of integrated control programmes are needed, and pathogens can be useful. The fungus *Metarhizium anisopliae* has been used (Prior & Arura, 1985), but the most spectacular success has been with the virus *Baculovirus* (*Rhabdionvirus*) *oryctes* (Huger, 1966) against *Oryctes rhinoceros* (L.) in the Pacific Ocean islands (Bedford, 1981). The virus has also been used to control *O. monoceros* (Olivier) in the Seychelles (Lomer, 1986*a*).

Information on the genetics of the virus indicates some degree of variation in the virus genome (Crawford *et al.*, 1985; Lomer, 1986*b*). These variants have not yet been bioassayed in insects. Methods for the production of virus in cell culture and storage of infectious inocula at ambient temperatures have been developed (Crawford & Sheehan, 1984).

Attempts to use the virus against *O. monoceros* in the Ivory Coast were unsuccessful (Julia & Mariau, 1976), although work in Tanzania has led to pilot field trials against this species (Paul, 1985). Other pest dynastines that have been tested include *Scapanes australis grossepunctatus* Sternberg from Papua New Guinea (Bedford, 1973; B. D. Gorick & P. F. Entwistle, unpubl. data). Early instar larvae were killed, but the degree of replication in the adult mid-gut was inadequate to permit transmission of the virus. Similar results were obtained in *S. a. salomonensis* Sternberg from the Solomon Islands (R. Macfarlane, pers. comm.). In both cases, the dispersed larval breeding sites make it unlikely that virus epizootics would be initiated.

O. nasicornis (L.), a European dynastine, is also susceptible to the baculovirus (Huger, 1966).

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Materials and methods

The virus isolates used are shown in Table I, and the origins of the beetles used in Table II. A genetic analysis of the virus isolates is available (Lomer, 1986*b*).

TABLE I. Oryctes *virus isolates*

Country of origin	Source (Original source of virus)	Sender
Seychelles	*O. monoceros* adults (Samoa, 1972)	B. Marday
Samoa	*O. rhinoceros* adults (Malaysia, 1970)	I. Aloalii
Philippines/S	*O. rhinoceros* adults (Samoa)	B. Zelazny
Philippines/V, Figao, Albay	*O. rhinoceros* adults (Endemic)	B. Zelazny
Philippines/X, Bugsuk, Palawan	*O. rhinoceros* adults (Endemic)	B. Zelazny
Malaysia (East), Sabah	*O. rhinoceros* adults (Endemic)	S. Shah
PV505	Tissue culture fluid (Philippines, *ca.* 1970)	A. Crawford
Fiji	Infected gut (Samoa, 1972)	B. Macfarlane
India, Kerala	Tissue culture fluid (K. Mohan; endemic)	A. Crawford
Sri Lanka	*O. rhinoceros* larva (Endemic)	P. Kanagaratnam
Indonesia, Bogor, Java	*O. rhinoceros* adults (Endemic)	E. Wikardi
Malaysia (West), Johore	*O. rhinoceros* adults (Endemic)	C. G. Fee
Malaysia (West), Selangor	*O. rhinoceros* adults (Endemic)	C. G. Fee

TABLE II. *Infection of dynastines with* Baculovirus oryctes

Beetle	Origin	Virus	*n*	LT50 (control) (days)	% Inf. *	Virus yield per beetle (mg)
Oryctes monoceros	Seychelles	Mixture	12 (4	40·8 50)	85	0·60
O. rhinoceros	Samoa	Samoan	13 (4	28·9 50)	85	0·73
O. rhinoceros	Philippines	Mixture	10 (10	20·4 48)	90	0·17
O. monoceros	Ivory Coast	Phil/X	10 (5	26·2 45)	70	0·03
O. monoceros	Ivory Coast	Samoan	10	14·4	0	0
O. monoceros	Tanzania	Seychelles	20	N.R.	55	N.R.
O. monoceros	Tanzania	Samoan	20	N.R.	50	N.R.
O. monoceros	Seychelles	Seychelles	17	N.R.	85	N.R.
O. monoceros	Seychelles	Samoan	17	N.R.	85	N.R.
O. monoceros	Kenya	Mixture	5	N.R.	0 (seen in E.M.)	0
Scapanes australis grossepunctatus	Papua New Guinea	Mixture	10 (10	23 25)	20	0
Scapanes australis salomonensis	Solomon Islands	Mixture	7 (3	22 24)	43	0
Papuana biroi	Papua New Guinea	Mixture	10 (5	40 40)	0	0
Strategus aloeus	Costa Rica	Mixture	10 (6	13 30)	63	N.R.
Strategus aloeus	Costa Rica	Mixture	5	15	N.R.	0·12
Strategus aloeus	Costa Rica	Indian	5	19	N.R.	0·04
Strategus aloeus	Costa Rica	Indonesian	5	14	N.R.	0·03

Mixture = All virus strains listed in Table I except Indonesian.
N.R. = Not recorded.
* % infected, by serology.
E.M. = Electron microscopy.

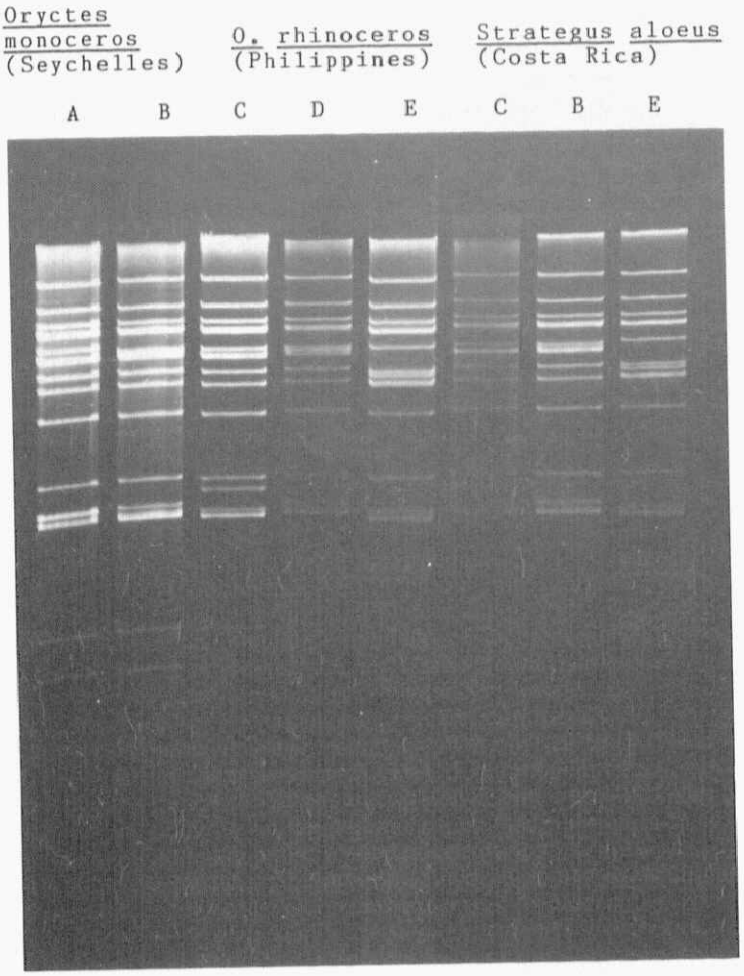

Fig. 1.—*Baculovirus oryctes* DNA restriction endonuclease profiles. A = Samoan virus; B = Mixture of strains excluding C & E; C = Indian virus; D = PV505; E = Indonesian virus. Virus was purified from mid-gut macerates by differential centrifugation followed by banding on sucrose gradients. DNA was extracted from the purified virus by lysis in 2% sodium N-lauroyl sarcosinate and banded on caesium chloride gradients followed by dialysis. The purified DNA was digested with Bam HI and electrophoresed on 0·6% agarose gels.

Beetles were infected by dropping a virus suspension in tissue culture medium (TC100, Gardiner & Stockdale, 1975) or in 10% sucrose onto their mouthparts. *Papuana biroi* Endrödi was infected by immersion into a virus suspension. Infected beetles were kept individually in plastic pots at 24–28°C with a small amount of water until death. The mid-gut was dissected out, macerated and analysed by cross-over electrophoresis against specific anti-*Oryctes* virus serum (Payne *et al.*, 1977). Extracts giving positive reactions were pooled; virus was purified by sucrose gradient centrifugation (Payne, 1974) and the DNA extracted as described by Crawford *et al.* (1985).

Where sufficient DNA was obtained, restriction endonuclease (REN) profiles were run

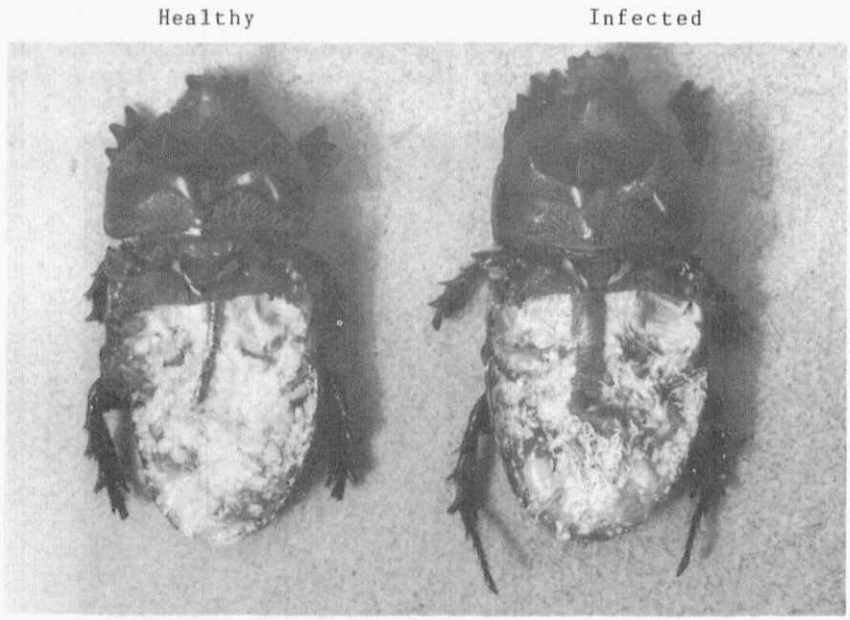

Fig. 2.—Appearance of virus-infected mid-guts of *Strategus aloeus*.

as described by Smith & Summers (1978). Where DNA quantities were too low, the REN-digested DNA was end-labelled with radioactive ^{32}P by the action of the Klenow fragment of DNA PolI (Maniatis *et al.*, 1982), then electrophoresed on 0·6% agarose gels which were dried. X-ray film was exposed to the dried gel and then developed.

The quantity of protein in the purified virus preparation was determined with a Bio-Rad protein determination kit.

Results

The results are summarized in Table II; virus replication sufficient to give cold REN profiles was obtained in *O. rhinoceros*, *O. monoceros* ex Seychelles and *Strategus aloeus* (L.) (Fig. 1). For each species, the REN profile of the progeny virus was the same as that administered. All species showed swelling and whitening of the mid-gut following infection (Fig. 2), as described by Zelazny (1978). Mid-gut macerates reacted to anti-serum, and a virus band was obtained on sucrose gradients. Less replication was observed in *O. monoceros* ex Ivory Coast and *Scapanes australis grossepunctatus* ex Papua New Guinea; end-labelled REN profiles of baculovirus from these beetles, shown in Fig. 3, demonstrate that some replication has occurred. A virus band was observed on sucrose gradients with extracts from *O. monoceros* but not *S. a. grossepunctatus*. No replication was observed in *S. a. salomonensis* ex Solomon Islands nor *P. biroi* ex Papua New Guinea. Positive anti-serum reactions were observed with *O. monoceros*, *O. rhinoceros* and *Strategus aloeus*, some with both subspecies of *Scapanes australis* (Boisduval) and none with *P. biroi*.

The numbers of beetles were not adequate to obtain accurate LT50s, but a marked reduction in lifespan was seen in *Oryctes* spp. and *Strategus aloeus* but not in *Scapanes australis* subspp. or *P. biroi*.

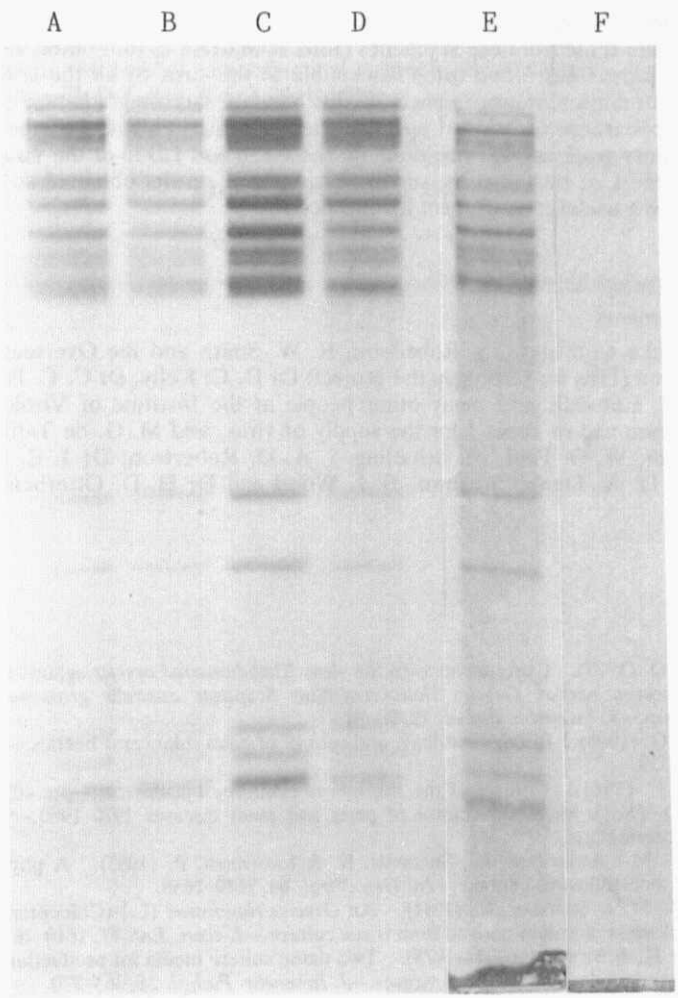

Fig. 3.—*Baculovirus oryctes* DNA restriction endonuclease profiles. A = *Oryctes rhinoceros* ex Philippines infected with Philippines/X virus; B = *O. monoceros* ex Ivory Coast infected with Philippines/X; C = *O. monoceros* ex Seychelles infected with Philippines/X; D = *O. monoceros* ex Seychelles infected with mixture; E = *Scapanes australis grossepunctatus* ex Papua New Guinea infected with mixture; F = *Scapanes australis salomonensis* ex Solomon Islands infected with mixture. DNA was prepared as described in Fig. 1, digested with Hind III, then radioactively labelled with ^{32}P by digestion with Klenow fragment, electrophoresed on 0·6% agarose gels, which were dried and exposed to X-ray film.

Discussion

Previous work on the infection of dynastines with *B. oryctes* has not made full use of the genetic diversity of the virus. The work presented above confirms previous findings on the low susceptibility of *Scapanes australis* subspp. to *Baculovirus* (Bedford, 1973), irrespective of the virus strain used. This is the first report of an attempt to detect virus replication in *P. biroi*; it is not susceptible. Previous findings on the relative susceptibilities of the races

of *O. monoceros* are confirmed, namely that the mainland African races are less susceptible than those from the Seychelles (Julia & Mariau, 1976; Lomer, 1986*a*).

Strategus aloeus was found to be susceptible to the virus by all the criteria employed, i.e. reaction to antiserum, appearance of virus band on sucrose gradients, quantity of virus produced, appearance of mid-gut and retention of genetic identity of the virus. Though more laboratory work needs to be done on the LT50 and LD50 of the virus in this beetle and effectiveness of transmission, on the basis of the results obtained so far, *B. oryctes* might become a useful control agent for this pest.

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