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Experiments with the Virus *Rhabdionvirus oryctes* against the Coconut Palm Rhinoceros Beetles *Oryctes rhinoceros* and *Scapanes australis grossepunctatus* in New Guinea

G. O. BEDFORD[1,2]

U.N./S.P.C. Rhinoceros Beetle Project, C/o Koronivia Research Station, Nausori, Fiji

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The virus *Rhabdionvirus oryctes,* originally discovered in larvae of the coconut palm dynastid *Oryctes rhinoceros* in Malaysia, is also pathogenic to second- and early third-instar larvae of *Scapanes australis grossepunctatus,* a dynastid pest of palms in New Guinea. Mortality of *Scapanes* larvae occurs within 13 to 15 days of infection. Some older third-instar larvae may be resistant. The possibility of using the virus against *Scapanes* in the field is discussed.

INTRODUCTION

Oryctes rhinoceros is an important pest of coconut palms occurring from India through Southeast Asia, Indonesia, the New Guinea islands, and various islands in the South Pacific. *Scapanes australis,* of which there are four subspecies, is also a serious pest of palms, but it is restricted to Papua-New Guinea and the British Solomon Islands. The nonoccluded virus *Rhabdionvirus oryctes* was discovered in larvae of *Oryctes rhinoceros* in Malaysia, and its description and symptomatology have been given by Hüger (1966). The fat body is the first organ affected, and the disease later invades other tissues. The virus was released in Western Samoa in 1967, where it has become established and has markedly reduced the beetle population, and consequently the damage to palms (Marschall, 1970). Further studies have been carried out on the virus in Samoa by Zelazny (1971). As the virus does not occur in New Guinea, the main aim of the present work was to test it against larvae of the New Guinea rhinoceros beetle, *Scapanes australis grossepunctatus,* to determine whether these larvae were susceptible to it in the same way as *Oryctes,* and also to confirm the effect of the virus on *Oryctes* larvae in New Guinea. Throughout this paper the abbreviations L2 and L3 and used to denote second- and third-instar larvae, respectively.

MATERIALS AND METHODS

Two consignments totaling 23 live *Oryctes* larvae infected with virus were sent from Apia, Western Samoa, for the tests. They were received at Keravat, New Britain, with some 17 of the larvae being freshly dead on arrival, 2 moribund, and 4 living. In the first experiments the virus was obtained from the larvae sent from Apia, but this was later supplemented by virus obtained from artificially infected Keravat larvae. Virus doses were expressed early in the work as the weight of virus

[1] Associated with Biology Department, University of Papua-New Guinea.

[2] Insect Ecologist, U.N.D.P./S.P.C. Regional Project for Research on the Control of the Coconut Palm Rhinoceros Beetle, Apia, Western Samoa.

dead larvae material involved, but later as fractions of one such larva. The virus-infected larvae were homogenized with water in a blendor. Virus material, when not in use, was stored in a freezer below freezing point. Living test larvae were kept individually in half-gallon tins with about 200 g of 1:1 cowdung:sawdust mixture, and the appropriate dose of homogenized virus-infected larvae was added to each tin and mixed into this medium, on which the test larvae then fed.

Results

Experiment 1

Twelve fairly advanced *Scapanes* third-instar larvae were set up individually each with a dose of about 0.3 g of Apia virus-infected larvae and in addition 3.0 g of virus-contaminated medium. Additional doses of virus material were given on later dates. Ten other *Scapanes* L3 were set up as controls without virus. Eight field-collected *Oryctes* L3 were set up with virus, and 7 others without. Certain *Scapanes* larvae accidentally destroyed during the experiment were replaced.

Results for the *Scapanes* larvae with virus are presented in Table 1. Five of the larvae eventually died of the virus, showing typical symptoms. The dead larvae, which lay on the top of the medium, were whitish and flaccid in appearance, with watery skins which turned black and shiny after death. Seven larvae remained unaffected

TABLE 1

Effect of Virus on Twelve Advanced Third-Instar *Scapanes* Larvae

Virus dose	Date dose given	Larvae 1	2	3	4	5	6
0.3 g virus grub	Nov. 12, 1970	Start +[a]	Start +	Start +	Start +	Start +	Start +
1.0 g virus grub	Nov. 24, 1970	+	Destroyed; replacement started +	Destroyed; replacement started +	+	+	+
1.2 g virus grub	Dec. 7, 1970	+	+	+	+	+	+
6.8 g virus grub	Dec. 21, 1970	−	−	Destroyed; replacement started +	−	−	Destroyed; replacement started +
½ virus grub	Jan. 12, 1971	+ Dead L3 Feb. 3, 1971 (virus)	+ Dead L3 Feb. 3, 1971 (virus)	+ Pupa alive Feb. 3, 1971	+ Dead L3 Feb. 12, 1971 (virus)	+ L3 alive March 22, 1971	+ Pupa alive March 22, 1971

Virus dose	Date dose given	Larvae 7	8	9	10	11	12
0.3 g virus grub	Nov. 12, 1970	Start +	Start +	Start +	Start +	Start +	Start +
1.0 g virus grub	Nov. 24, 1970	+	+	+	Destroyed; replacement started +	+	+
1.2 g virus grub	Dec. 7, 1970	+	+	+	+	+	+
6.8 g virus grub	Dec. 21, 1970	Destroyed; replacement started +	−	Destroyed; replacement started +	−	−	−
½ virus grub	Jan. 12, 1971	+ L3 alive March 22, 1971	+ Dead L3 Feb. 12, 1971 (virus)	+ Pupa alive March 22, 1971	+ Dead L3 Jan. 25, 1971 (virus)	− L3 alive March 22, 1971	− L3 alive March 22, 1971

[a] +, Indicates virus dose added to each tin; −, indicates no virus dose given.

and eventually pupated. None of the *Scapanes* larvae without virus died.

Of the 8 *Oryctes* larvae set up with virus, 5 died of virus, one died of green muscardine, *Metarrhizium*, infection, while two pupated and later produced adults. Of the 7 *Oryctes* larvae without virus, 3 survived, 3 died as prepupae, and one died as a pupa. The deaths as prepupae and pupae were probably due to disturbance during checking.

Experiment 2

As only a limited number of *Oryctes* larvae were available when the first experiment was set up, a second experiment was later undertaken with more larvae collected from the field, to confirm the virulence of the first batch of virus received from Apia on November 12, 1970.

Twenty-five *Oryctes* L3 were set up in individual tins on November 23, 1970 with 200 g of medium and 1.0 g of virus larvae also 11.0 g of virus-contaminated medium. On November 7, 1970 a further dose of 0.6 g of virus larvae was given to each. Twenty-one L3 were set up similarly but without virus.

Of the virus fed larvae 14, i.e., 56%, died as larvae with typical symptoms by January 12, 1971. Two died as larvae later, one died as a prepupa, 6 died as pupae, and 2 survived as a prepupa and an emerged adult. Of the larvae without virus, 9 survived as emerged adults and one as a larva, while 3 died as larvae, 4 as prepupae, and 4 as pupae.

Experiment 3

In order to test the virulence against *Scapanes* of Keravat-produced virus material, 19 *Scapanes* L3 and 7 L2 were given on January 13, 1971 a dose of 0.6 g of virus material derived from infected *Oryctes* larvae in Experiment 2. The L3 had been in that stage of development only since mid-December or later. Twenty *Scapanes* L2 and L3 were held as controls without virus. Six *Oryctes* L3 received the same dose of virus as the *Scapanes*.

Most of the *Scapanes* and *Oryctes* larvae, which had been exposed to virus, were dead with typical virus symptoms within 2 weeks. Only two *Scapanes* survived, and these were L2 which had molted to the L3 stage during the period of exposure to the virus. None of the *Scapanes* larvae without virus died.

Experiment 4

To test the virulence of virus material received from Apia on December 14, 1970, 33 field-collected *Oryctes* L3 were distributed into 20 tins, and each tin received about 0.75 of a homogenized virus larva. Six L3 were kept as controls without virus. All of the thirty-three virus-contaminated larvae died with virus symptoms within a month, but none of the larvae without virus died.

Experiment 5

As a test of the virus material produced in *Oryctes* larvae in Experiment 4, nine L2 and two L3 *Scapanes* were distributed in 10 tins on January 28, 1971 and each tin received a dose of 1.8 homogenized virus larvae. Twelve *Scapanes* L2 were distributed in 10 tins without virus. All the larvae exposed to virus died with typical virus symptoms within 2 weeks, except for one L2 which had molted to L3 during this period. None of the larvae without virus died, and all were healthy L3 on March 22, 1971.

Experiment 6

A small experiment was done to investigate the effect of the virus on two other species on New Guinea dynastid larvae. Eight larvae of the elephant beetle, *Xylotrupes gideon ulysses*, (7 L3 and 1 L2), and one larva of *Oryctoderus* sp. were collected from decaying logs at Keravat on November 16, 1970. Four of the *Xylotrupes* L3 and

the one *Oryctoderus* L3 were set up individually and given the following doses of homogenized virus larvae: 0.3 g on November 16, 1970, 1.0 g on November 24, 1970, and 1.2 g on December 7, 1970. Three *Xylotrupes* L3 and the one L2 were kept as controls without virus.

None of the *Xylotrupes* larvae or the single *Oryctoderus* larva showed any adverse effects, and all completed their larval development. The *Xylotrupes* larvae without virus similarly completed their development.

Discussion

The results obtained clearly indicate that second- and early third-instar larvae of *Scapanes australis grossepunctatus* are susceptible to *Rhabdionvirus*.

Although there was no opportunity to conduct experiments to attempt the passage of virus from infected *Scapanes* larvae to healthy *Scapanes* larvae, it should be noted that in experiments 3 and 5, close to 100% mortality occurred in *Scapanes* larvae after 13–15 days of exposure in the food material to virus produced in *Oryctes* larvae. No mortality occurred in *Scapanes* larvae without virus. Zelazny (1971) found that death occurred in *Oryctes rhinoceros* larvae in Samoa 7 to about 22 days after exposure. In Experiment 1, which involved considerably older third-instar *Scapanes* larvae, results were less clear-cut and 58% of the grubs exposed to virus survived without apparent ill effect. It is possible that some older third-instar *Scapanes* larvae may be more resistant to the virus or else some larvae may be intrinsically resistant. Hüger (1966) has shown that larvae of *O. nasicornis* are affected as well as *O. rhinoceros*, and it is of interest to find that larvae of a different genus, *Scapanes*, are also affected by the virus.

In Experiments 3 and 5, the only *Scapanes* larvae to survive exposure to the virus were three L2 which underwent a molt during the period of exposure. Prior to molting, the larvae cease to feed and would not ingest the virus-contaminated food medium. Zelazny (1971) has found that in laboratory conditions in Samoa, some 99% of a given virus dose has broken down after 7 days at room temperature. It is, therefore, possible that by the time the newly emerged L3 resumed feeding, the original virus dose in the medium had broken down, and the larvae thus escaped infection. The small number of *Oryctes* larvae which survived exposure to virus in experiments 1 and 2 may also have survived because they had ceased to feed and were approaching the prepupal stage.

The virulence of the virus against *Oryctes rhinoceros* larvae from the New Guinea population was confirmed in experiments 1, 2, 3, and 4. Mortality which occurred in the controls during the sensitive prepupal and pupal stages was probably caused by disturbance during checking of the tins and to difficulties in molting.

The *Xylotrupes* larvae in the tests did not appear to be susceptible to the virus.

It has been found that adults of *Oryctes rhinoceros* are also susceptible to the virus, the adult life-span being shortened and the fecundity of females markedly reduced (Zelazny, 1971). In fact the adults are the principal agents of spread of the disease. It may be that *Scapanes* may be similarly affected by the virus. If so, this raises the interesting possibility that *Scapanes* adults might be artificially infected, then released to spread the disease among the wild population and into breeding sites, and thus contribute to the amelioration of the *Scapanes* problem.

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