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STUDIES ON THE NUCLEAR POLYHEDROSIS OF *PERICALLIA RICINI* F. (LEPIDOPTERA : ARCTIIDAE)

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Studies on the nuclear polyhedrosis of the larvae of *Pericallia ricini* F. (Arctiidae) revealed that the infected larvae exhibited all the typical symptoms of nuclear polyhedrosis. Caterpillars of second, third and fourth instars were highly susceptible, those of the fifth instar moderately so and those of sixth instar highly resistant to the infection. The total haemocyte count decreased in the virus-infected larvae progressively from 48 hours after ingestion of the virus. The polyhedra measured 1284.6 ± 12.48 mμ in diameter. They were completely soluble in weak solutions of NaOH, KOH and Na_2CO_3. The thermal inactivation point of the virus was between 90° and 95°C. Exposure of the polyhedra to direct sunlight for 96 hours substantially reduced its infectivity. But it remained highly infectious after exposure to 35°C in an oven for 96 hours, though in 120 hours it lost its infectivity. It thus appeared that in addition to temperature, perhaps light was also responsible for deactivation of the virus under field conditions. The virus was not infective to four species of alternate caterpillars tested.

INTRODUCTION

The black hairy caterpillar *Pericallia ricini* F. is a polyphagous pest feeding on cultivated crops like cotton, castor, banana, cucurbits, pulses and sesamum. JACOB *et al.*, (1972) reported a nuclear polyhedrosis in this insect. Information gathered on the nature of the pathogen and on the host-pathogen relationships are presented in this paper.

MATERIALS AND METHODS

The larvae used in these studies were reared in the laboratory on castor (*Ricinus communis* L.) leaves. A purified concentrated suspension of polyhedra isolated from the diseased larvae of *P. ricini* and diluted to contain 33×10^7 polyhedra per ml of distilled water and containing 0.1 per cent teepol as wetting agent, formed the infective material. Larval inoculations were done by the spot feeding technique (JACOB, 1972). The polyhedral suspension (5μl) was applied to each spot and the larvae which had consumed the entire leaf area at the spots in 4 to 6 hours, were transferred to fresh uncontaminated foliage individually in sterile plastic cups. Control larvae were fed similarly on spots of distilled water containing 0.1 per cent teepol only.

Susceptibility of the larvae under different instars was assessed as indicated by JACOB & SUBRAMANIAM (1972). Haemocyte counts of the infected larvae were made following the method of SHAPIRO (1967). Statistical 't' analysis was used to compare the differences between means. Dissolution of polyhedra in alkalies was studied by the method of PAWAR & RAMAKRISHNAN (1971). Thermal inactivation point, effect of sunlight on the infectivity and survival of the virus at the highest field temperature were studied as described by LATHIKA & JACOB (1974 c); in the case of survival at the highest field temperature, the polyhedral films were exposed to 35°C in an oven.

Cross transmission to alternate species of lepidopterous larvae was determined by feeding them for 24 hours on their host plant leaves contaminated with the polyhedral suspension.

RESULTS

Symptomatology

The infected second and third instar larvae turned pale 2 to 3 days after ingestion of the virus—a feature not shown by the later instars at this stage. The larvae became lethargic, showed reduced feeding

TABLE 1. Susceptibility of different instars of the larvae of *P. ricini* to infection by NPV.

Instar of larva	No. of larvae inoculated	Incubation period (days)		Per cent larval mortality* due to		Pupation %	Pupal mortality
		Range	Mean	Polyhedrosis	Other causes		
II	50	4–7	5.2	100	—	—	—
III	50	4–8	5.9	92	8	—	—
IV	50	4–10	7.0	92	8	—	—
V	50	6–9	8.2	72	12	16	—
VI	50	8	8.0	8	—	92	—

* There was no mortality due to virus in control.

and finally stopped feeding 2 to 3 days prior to death. Some of the larvae discharged a dark brown fluid through their mouth. In advanced stages of infection the cuticle became very fragile and ruptured readily on touch or by movements, liberating the liquefied body contents containing millions of polyhedra. Death occurred in 4 to 8 days after ingestion of the virus. The cadavers were found either hanging head downwards or lying flat on the leaf or other surfaces.

The body fluid which was clear in the initial stages turned turbid as the infection advanced. Dissection of the infected larvae showed that the fat body was opaque white in appearance.

Larval susceptibility

Results presented in Table 1 show that, as the stage of the larvae at inoculation advanced there was a decrease in the mortality caused by the virus infection and a prolongation of the incubation period. Those larvae which survived the infection when inoculated in the fifth and sixth instars reached the adult stage normally. Thus the 2nd, 3rd and 4th instar larvae showed high susceptibility to the virus infection. Fifth instar also showed fairly high susceptibility with 72 per cent mortality, the sixth instar was however, highly resistant.

Total haemocyte count

Fig. 1 illustrates the changes in the average number of circulating haemocytes (THC) in healthy and virus infected larvae. There was no significant difference between the healthy and virus infected larvae in their THC at 24 hours after inoculation. At all subsequent intervals the diseased larvae had significantly fewer haemocytes. Further, in

TABLE 2. Effect of different alkalies on polyhedra of *P. ricini*.

Time given for dissolution (minutes)	NaOH (%)		KOH (%)		Na_2CO_3 (%)	
	0.1	0.2	0.1	0.2	5.0	10.0
1	+	—	+	—	+	—
2	+	—	+	—	+	—
3	+	—	+	—	+	—
4	+	—	+	—	+	—
5	+	—	+	—	—	—
10	+	—	+	—	—	—
15	—	—	—	—	—	—

\+ Polyhedra present.
— Polyhedra absent.

healthy larvae there was a steady increase in THC with age while in the infected ones there was a steady decrease.

Size and shape of polyhedra

Electron micrograph of polyhedra (Fig. 2) showed that they were irregular in shape and varied considerably in size. The diameter ranged from 943.6 $m\mu$ to 1829 $m\mu$ with an average of 1284.6+12.48 $m\mu$.

Alkali resistance of polyhedra

It may be seen from Table 2 that 0.2 per cent KOH or NaOH dissolved the polyhedra within 2 minutes while 0.1 per cent solution of either alkali required more than 10 minutes to produce the same effect. In solutions of Na_2CO_3 the polyhedra dissolved in 5 minutes in 5 per cent and in 2 minutes in 10 per cent solutions.

Thermal inactivation point of the virus

The results (Table 3) reveal that infectivity of the virus was not affected by exposure to a temperature of up to 70°C for 10 minutes, but the infectivity started declining when the temperature was raised to 80°C and above. The virus did not show

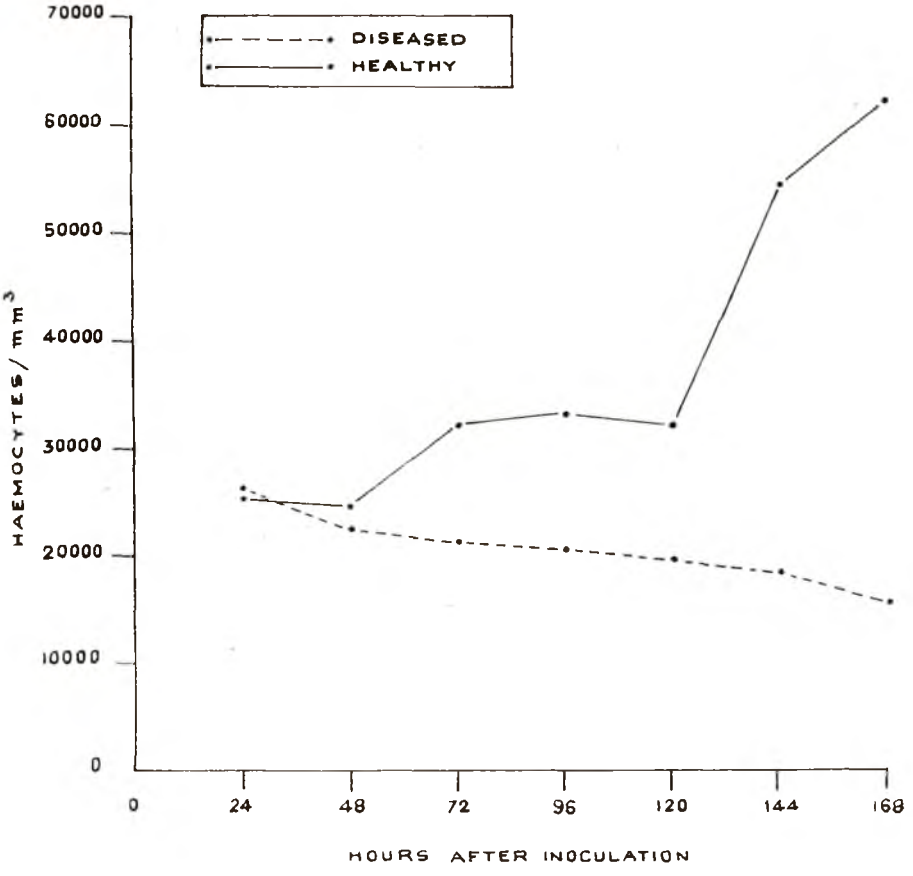

FIG. 1. Average number of circulating haemocytes in healthy and NPV infected larvae of *P. ricini* at different intervals after inoculation.

FIG. 2 Electron micrograph of polyhedra isolated from NPV infected larvae of *Pericallia ricini*. 29675 x.

any infectivity when subjected to a temperature of 95°C. These indicate that the thermal inactivation point (TIP) of the virus lay between 90° and 95°C.

Survival of the virus under the highest field temperature

The data presented in Table 4 show that exposure of the polyhedra to 35°C upto

TABLE 3. Effect of different temperatures on the infectivity of the NPV of *P. ricini* when exposed for 10 minutes.

Temperature °C	No of larvae inoculated	Incubation period in days (Mean)	% Larval mortality due to		Pupation %	Pupal mortality %
			Polyhedrosis	Other causes		
60	50	6.9	100.0	—	—	—
70	50	7.3	100.0	—	—	—
80	50	7.4	84.0	—	16.0	—
90	50	—	32.0	4.0	64.0	—
95	50	—	—	4.0	96.0	—
100	50	—	—	—	100.0	—
Control (without virus)	50	—	—	—	100.0	—
Control (untreated virus)	50	6.0	100.0	—	—	—

TABLE 4. Infectivity of NVP of *P. ricini* exposed to 35°C for different periods.

Duration of exposure to 35°C (hours)	No. of larvae inoculated	Incubation period in days (Mean)	% Larval mortality due to		Pupation %	Pupal mortality %
			Polyhedrosis	Other causes		
12	50	6.1	100	—	—	—
24	50	6.2	96	4	—	—
48	50	6.8	96	4	—	—
72	50	7.8	92	8	—	—
96	50	9.2	88	12	—	—
120	50	10.3	12	4	84	—
Control (without virus)	50	—	—	2	98	—
Control (Untreated virus)	50	6.1	100	—	—	—

96 hours did not significantly affect the infectivity of the virus. But exposure for 120 hours substantially reduced the infectivity and 84 per cent of the larvae pupated normally. There was also a gradual increase in the incubation period of the virus with the increase in the period of exposure to the temperature.

Effect of sunlight on the infectivity of the virus

It is clear from the data presented in Table 5 that the virus remained highly infectious upto 72 hours of exposure to sunlight, though there was a prolongation of the incubation period. The virus however, lost its infectivity with further increase in exposure period. Thus infectivity was reduced to 36% when the polyhedra were exposed to sunlight for 96 hours and it was almost lost after exposure for 120 hours.

Cross infectivity

Results of cross transmission studies reported in Table 6 show that the NPV of *P. ricini* was not infective to any of the 4 species of caterpillars under study.

DISCUSSION

The inverse relationship between larval age and susceptibility to nuclear polyhedrosis infection observed in the present studies has been reported by several earlier workers (TANADA, 1956; MORRIS, 1962; JACOB & SUBRAMANIAM, 1972). It is a case of

TABLE 5. Effect of exposure to sunlight for varying periods on the infectivity of the NPV of *P. ricini*.

Duration of exposure (hours)	No. of larvae inoculated	Incubation period in days (Mean)	% Larval mortality due to		Pupation %	Pupal mortality %
			Polyhedrosis	Other causes		
12	50	6.5	100	—	—	—
24	50	7.0	96	4	—	—
48	50	7.5	96	4	—	—
72	50	9.9	92	8	—	—
96	50	10.3	36	16	48	—
120	50	10.5	8	8	84	—
Control (without virus)	50	—	—	—	100	—
Control (Untreated virus)	50	6.2	98	2	—	—

TABLE 6. Infectivity of NPV of *P. ricini* to different alternate hosts.

Alternate host insect	Stage of larvae at inoculation	No. of larvae tested	% Larval mortality due to		Pupation (%)	Pupal mortality (%)	Infectivity
			Polyhedrosis	Other causes			
Achoea janata	3rd instar	30	0	0	100	0	Nil.
Spodoptera litura	4th instar	30	0	0	100	0	Nil.
Glyphodes marginata	3rd instar	20	0	10	90	0	Nil.
Euproctis fracterna	3rd instar	50	0	5	95	0	Nil.

maturation immunity which according to IGNOFFO (1966 a) is partly due to the normal increase in body weight of the host which might dilute a constant viral dose.

The observation that virus infection causes a decrease in the THC of the infected larvae is in agreement with those in *Heliothis zea* (SHAPIRO *et al.*,1969) *Spodoptera litura* (JACOB, 1972) and *Spodoptera mauritia* (LATHIKA & JACOB, 1974 b). JACOB (1972) attributed this to the destruction of haemocytes and interference in mitotic division of the blood cells by the virus infection. Haemocytes are known to be one of the major sites of infection by NPV.

In common with other polyhedral viruses, the polyhedra of *P. ricini* also dissolve in solutions of NaOH, KOH and Na_2CO_3. It is known that the degree of resistance towards different alkalies varies with polyhedra from different polyhedroses. In its reaction towards NaOH, KOH and Na_2CO_3 the polyhedra of *P. ricini* closely resemble those of *S. mauritia* (LATHIKA & JACOB, 1974 a). These two polyhedra are less resistant to Na_2CO_3 than other reported polyhedroses such as those of *Pterolocera amplicornis* (DAY *et al.*, 1953) and *Diacrisia obliqua* (JACOB & THOMAS, 1974).

The TIP of NPV of *P. ricini* is seen to be between 90° and 95°C. This agrees with those reported for *S. litura* (PAWAR & RAMAKRISHNAN, 1971) and *S. mauritia* (LATHIKA & JACOB, 1974 c). However this exceeds the general 80°C limit reported for other inclusion body viruses (BERGOLD, 1958; AIZAWA, 1963; HUGER, 1963).

It has been reported that higher field temperatures (35°—45°C) may affect viral stability and viral multiplication (BIRD, 1955; THOMPSON, 1959; IGNOFFO, 1966 b). But the present studies show that the NPV of *P. ricini* can withstand continual exposure to 35°C for 96 hours without losing its infectiviry though the virulence started declining on exposure beyond 96 hours. Further, the results presented show that exposure of the polyhedra to direct sunlight for periods up to 72 hours does not affect the viral stability and infectivity. CANTWELL (1967) observed that the NPV of *Trichoplusia ni* is completely inactivated by exposure to direct sunlight for 3 hours. Similarly BULLOCK (1967) also found that *Heliothis* virus applied to cotton foliage loses most of its infectivity after one day and this was attributed partly to the action of ultraviolet rays in the sunlight. LATHIKA & JACOB (1974 c) found that NPV of *S. mauritia* can withstand exposure to sunlight for 72 hours. It thus appears that under the tropical conditions as existing in Kerala, the viruses can withstand exposure to sunlight for longer periods probably due to the difference in the composition of sunlight.

Further, the observation that the virus can withstand exposure to a higher temperature of 35°C for 96 hours while it can stand exposure to sunlight only for 72 hours indicates that under field conditions temperature alone may not be the factor responsible for inactivation of the virus. In a similar study MORRIS (1971) also found that exposure of the NPV of *Lambdina fiscellaria lugubrosa* to 45°C for 200 hours does not affect the final percentage of mortality, but exposure to direct sunlight for 35 hours almost inactivates the virus. Perhaps, temperature along with other factors like ultraviolet radiation may be causing the deactivation.

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