https://doi.org/10.29321/MAJ.10.A03447

Madras agric. J. 61 (10-12) : 957—961 Oct—Dec. 1974.

Studies on the Nuclear Polyhedrosis of *Heliothis armigera* (Hbn.)

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ABSTRACT

Histopathological studies were made in fourth instar larvae of *Heliothis armigera* (Hbn.) inoculated with a dose of 10^6 polyhedra per larva. Histopathological changes were discernible 48 hours after virus treatment. The most susceptible tissues were fat body, hypodermis, tracheal matrix and blood cells. Muscles, nerve tissue foregut, oesophageal valve, connective tissue surrounding the midgut, silk glands and testicular epithelium were also found to be affected by the virus to a lesser extent. Polyhedra could not be observed in the imaginal buds, malpighian tubules and hind gut.

INTRODUCTION

The incidence of a nuclear polyhedrosis in a laboratory culture of *Heliothis armigera* (Hbn.) was reported by Patel *et al.* (1968). Jacob and Subramaniam (1972) described the virus with electron micrographs. In the present investigation, an attempt was made to understand the sites of multiplication of virus in different tissues of the host and also the relative susceptibility of various tissues.

MATERIALS AND METHODS

Fourth instar caterpillars were inoculated with 10^6 polyhedra per larva. Three caterpillars were selected at random from each treated and control groups at 24 hours interval upto 120 hr and killed in hot alcoholic Bouins fixative at 55°C and allowed to soak for 10 minutes. The caterpillars were cut transversely into three portions, transferred to cool alcoholic Bouins fluid and fixed for 24 to 48 hours. The specimens were washed in 70per cent ethyl alcohol to remove the fixative, dehydrated in Ethanol-Butanol series, infiltrated with paraffin for three days and embedded in paraffin according to standard procedures. Sections of 4-6 microns were ctained by a modified azan staining technique after Hamm (1966).

RESULTS AND DISCUSSION

The most susceptible tissues were fat body, hypodermis, tracheal matrix and blood cells. The precise and detailed histopathological changes observed in the infected tissues are described below.

FAT BODY: Initial stages of infection were clearly seen by 48 hr post inocu-

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lation. Some of the nuclei were hypertrophied and deeply stained. In some there was the central condensation of chromatin mass with a clear ring zone around Virogenic stroma of Xeros (1956).

As the infection advanced, the size of the infected nuclei increased considerably as evidenced by diameter measurement (Table 1). By 120 hr after infection almost all the nuclei were packed with

TABLE 1

Post inoculation period in hours	Mean diameter in microns ± S. E.	
	Healthy	Diseased
24	6.85 ± 0.18	8.09 ± 0.19
48	9.87 ± 0.19	11.45 ± 0.30
72	10.01 ± 0.20	18.35 ± 0 36
96	10.35 ± 0.16	22.77 ± 0.39
120	10.12 ± 0.20	23.74 ± 0.45

polyhedra and several nuclei had ruptured liberating the polyhedra (Fig. 1). However, Harpaz and Zlotkin (1965) in their study on the histopathology

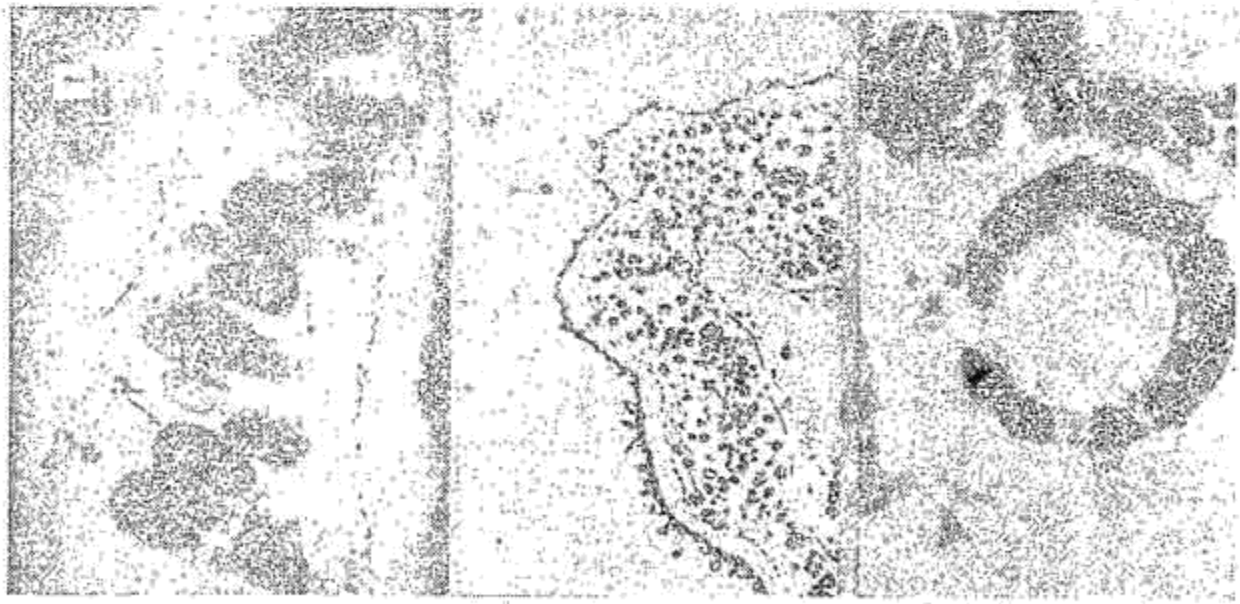

Fig. 1 Fig. 2 Fig. 3

Fig. 1 : Cross-section of infected fat body showing hypertrophied nuclei filled with polyhedra (P) 1500 x

Fig. 2 : Cross-section of hypodermis showing the stratisfied appearance due to virus infection. 300 x

Fig. 3 : Trachea showing infection of matrix cells. 750 x

of *Heliothis peltigera* Schiff observed lack of uniformity in the infection of fat body. Similar observations were made by Jacob (1972) in the nuclear polyhedrosis of *Spodoptera litura* (Fb).

HYPODERMIS: Early signs of infection of hypodermis were discernible 48 hr after ingestion of virus. The thickness of hypodermis increased drastically as infection advanced (Table 2) and the

TABLE 2

Post inoculation period in hours	Average thickness in microns ± S. E.	
	Healthy	Diseased
24	10.39 ± 0.36	11.41 ± 0.30
48	18.12 ± 0.32	29.07 ± 0.45
72	20.33 ± 0.46	52.81 ± 0.78
96	18.95 ± 0.35	64.91 ± 0.76
120	19.50 ± 0.32	78.80 ± 1.24

hypodemis gave a stratified appearance (Fig. 2) with the nuclei filled with polyhedra. In *Hypantria cunea*, Watanabe (1968) observed proliferation of cells in the hypodermis due to virus infection.

TRACHEAL MATRIX: Definite pathological changes were observed in the tracheal matrix only 72 hr after inoculation. There was marked hypertrophy of nuclei and the matrix tissue showing signs of disintegration by 120 hr post inoculation (Fig. 3). Generally tracheal infection is common in nuclear polyhedrosis, but Entwistle and Robertson (1968) reported an unusual case of NPV infection in a hepialid moth wherein the trachea was not infected.

BLOOD CELLS: Polyhedra could be observed in the nuclei of blood cells by 72 hr after ingestion of virus. The number of cells infected increased in the following 24 hr and by 120 hr post inoculation and several cells had ruptured releasing the polyhedra.

OTHER TISSUES: According to Aizawa (1963) and Smith (1967) fat body, hypodermis, tracheal matrix and blood cells were the chief sites of multiplication for the nuclear polyhedrosis viruses as a general rule. However it was not uncommon to notice in the present studies, infection of other tissues and organs like muscles, nerve tissue like neurilemma of nerve cord and brain and ganglion cells, foregut, connective tissues surrounding the mid gut, silk glands and testicular epithelium. Infection of these tissues has been reported by Benz (1963) in *Malacosoma alpicola* Hamm (1968) in *Spodoptera frugiperda* and Mathad *et al.* (1968) in *Trichoplusia ni* (Hubner)

Infection of muscles was noticed from 72 hours after inoculation. By 96 hours, the infection of nuclei was seen in groups either at the periphery of the fibre or deep in between the loosened fibrillae. Some of the cells had started lysing by 120 hours after inoculation and the muscle sheath was loosened from the sarcolemma. Neurilemma of nerve cord and ganglia were found to be infected from 72 hours post inoculation. Infection of brain was noticed at 120 hours after inoculation and the polyhedra filled nuclei were found towards the frontal nerves. The fore gut, silk glands and testicular epithelium contained polyhedra in certain nuclei by 120 hours after ingestion of the virus.

Infection of wing bud, malpighian tubules and hind gut could not be observed in the present studies

ACKNOWLEDGEMENT

The senior author is thankful to the Tamil Nadu Agricultural University for granting permission to publish these findings which formed a part of the M. Sc. (Ag.) dissertion and to the Indian Council of Agricultural Research, New Delhi for awarding Junior Fellowship during the tenure of the course.

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