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Baculovirus Infection of *Oryctes rhinoceros* : Effect of virus on the Total and Differential Haemocyte Counts

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ABSTRACT

In the process of infection by the Baculovirus, the haemocytes of *Oryctes rhinoceros* were found to undergo changes in the total and differential counts. Of the five classes of haemocytes, the granular cells and the plasmatocytes exhibited changes in population levels. In advanced stages of infection, there was selective depletion of plasmatocytes.

KEY WORDS: *Oryctes rhinoceros*, baculovirus, haemocytes, changes.

The vertebrate immune system is known to possess well developed cellular and humoral components. In insects, a primitive form of both the components is found and hence immunity in insects lacks specificity. Different types of blood cells or haemocytes have important roles in the protection of insects against invading microorganisms. In the process of defending the host, the haemocyte populations undergo changes as evidenced by their total and differential counts. In the present study, the effect of baculovirus infection on the total and differential haemocyte counts of *Oryctes rhinoceros* has been investigated.

MATERIALS AND METHODS

Oryctes rhinoceros grubs were collected from the field and reared in the laboratory in an autoclaved mixture of cowdung and sawdust (1 : 1) as described by Bedford (1976). The virus used in this study, *Oryctes baculovirus* strain X, was a kind gift from Dr. P.D. Scotti, DSIR, New Zealand, The viral stock contained 1 x 10^{11} infectious units (IU)/ml.

Healthy third instar grubs of weight 14.5 $\pm$ 1.5g were chosen for all experiments. The virus suspension was diluted with phosphate buffered saline (PBS). For every dilution, 30 grubs were used and the same number of grubs was used for control also. The grubs were infected by dropping 100μl of virus suspension in 5% sucrose solution into their mouths as done by Crawford and Sheehan (1984). After making sure that the suspension had entered into the digestive system, the grubs were released into individual plastic boxes containing cowdung and sawdust. The control grubs received 100μl of PBS only.

Collection of haemolymph

Haemolymph was collected till the 20th day at 3 days interval. Every time, 3 grubs were sacrificed for each virus concentration. After the grubs were rendered motionless by chilling on ice, one of the prolegs was cut and 100μl of the oozing haemolymph was collected in an ice-cold Eppendorf tube and was diluted ten-fold using insect physiological saline (IPS : 8.8g NaCl; 0.2g KCl; 0.3g $CaCl_2$;1 litre distilled water) with 2% acetic acid.

Total haemocyte count (THC) and differential haemocyte count (DHC)

100μl of the diluted haemolymph was immediately placed on the improved Neubauer haemocytometer and total haemocyte count (THC) was made. Differential haemocyte count (DHC) was based on the observation of 200-500 cells by making smears of the haemolymph. These smears were fixed in Bouins fluid and stained with May-Grunwald Giemsa stain (Wago and Kitano, 1985) and observed under a microscope, The key framed by Gupta (1979) was followed for the identification of different haemocyte types.

RESULTS AND DISCUSSION

Total haemocyte count (THC) of normal grubs

All the healthy grubs examined for THC exhibited a gradual increase over a period of

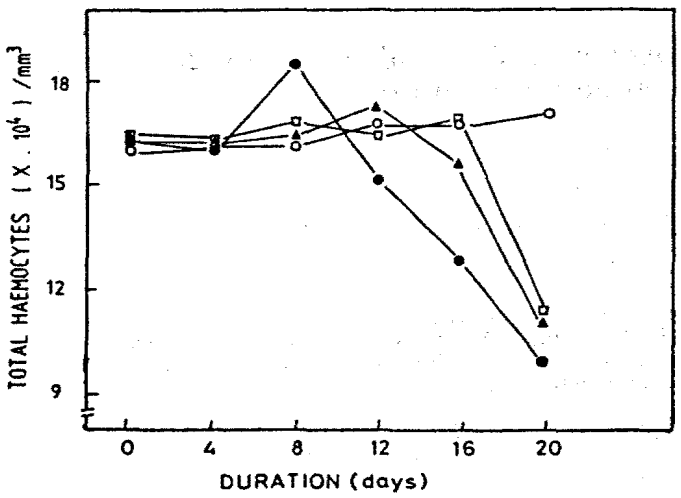

Fig. 1. Effect of different concentrations of virus on the total haemocyte count in the III instar of *O. rhinoceros*
○ — Control
● — 1 × 10^8 IU
▲ — 1 × 10^7 IU
□ — 1 × 10^6 IU

20 days (Fig. 1). The THC which was approximately 1,60,000 cells/mm³ gradually increased to 1,65,000 cells/mm³ on the 20th day. The number of circulating haemocytes decreased at the end of every instar and increased at the onset of the next instar (Wheeler, 1963). In *O. rhinoceros*, the 3rd instar lasts for more than 90 days. The present observations were made only till the 20th day and that explains the slight increase in the THC.

Changes in the Total haemocyte count (THC) due to infection

The baculovirus of *O. rhinoceros* infects the midgut epithelial cells; multiplies and finally invades the haemolymph. In this process, the haemocytes circulating in the haemolymph tend to resist the infection so as to defend the host. In acute stages of infection, the haemocytes may lose their ability to resist and the host dies.

In this investigation, the THC is expressed as cells/mm³ and was generally lower in infected grubs than in healthy ones. On the 8th day of infection, the THC of the grubs which were administered with 1 x 10^8 IU showed a slight increase, which afterwards started declining further and was about 99,000 cells/mm³ on the 20th day. The trend was similar in the case of grubs infected with 1 x 10^7IU and 1 x 10^6 IU, the THC being more on days 12 and 16 respectively, which however declined subsequently. The relative difference in total counts may be due to decrease in viral inoculum and is pronounced 16 days post infection.

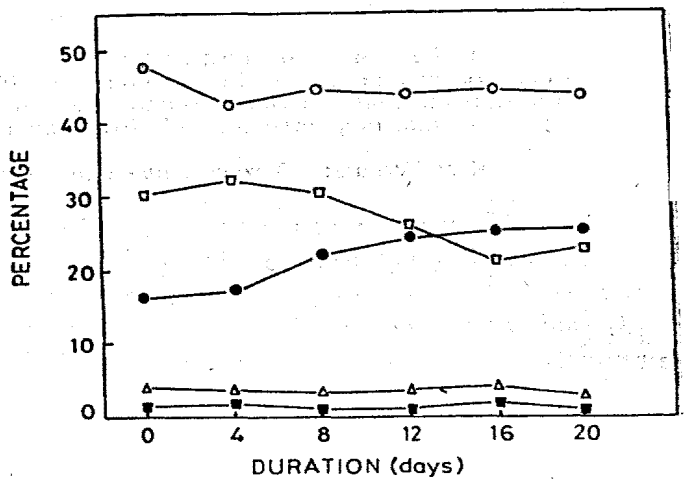

Fig 2. Percentage of different types of haemocytes in the III instar grubs of *O. rhinoceros*
○ — Granular cells
● — Plasmatocytes
△ — Spherule cells
□ — Prohaemocytes
■ — Oenocytoids

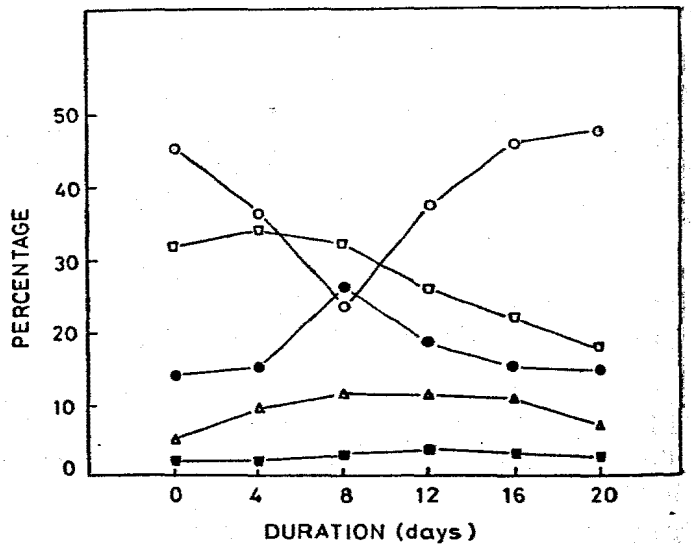

Fig. 3. Effect of viral infection (1 × 10^8 IU) on the proportion of each haemocyte type in the III instar grubs of *O. rhinoceros*
○ — Granular cells
● — Plasmatocytes
△ — Spherule cells
□ — Prohaemocytes
■ — Oenocytoids

Changes in the Differential haemocyte count (DHC) due to infection

Five different types of haemocytes have been distinquished by phase contrast microscopic observations. They were, prohaemocytes (33%), plasmatocytes (13%), granular cells (46%), spherule cells (6%) and oenocytoids (2%) in a normal third instar grub (Fig. 2). The deviation in the DHC due to viral infection with 1 x 10^8 IU is seen from Fig. 3. The granular cells which accounted for nearly 46% of the total haemocyte population were the first type of cells to respond to the infection. Its percentage decreased to 34 and 27 respectively on days 4 and 8. Then it increased upto 35, 45 and 47% on 12, 16 and 20 days respectively after infection.

A possible reason for the increase may be that the prohaemocytes which are the stem cells are initially infected and the granular cells which originated from the infected prohaemocytes might also be affected. This is in accordance with the observation in *Galleria mellonella* by Lea (1986). He reported that the frequency of infection and THC during the course of infection would be consistent with the conclusion that all or most of the granular cells develop from infected precursors; i.e., cells infected at the prohaemocyte stage. Further, autoradiography experiments by injecting virus and tritiated thymidine into *G. mellonella* showed that it required 8—12 days for granular cells to differentiate from prohaemocytes whereas it required only 12—48 hours for plasmatocytes to differentiate.

The plasmatocytes gradually increased to 26% on the 8th day and declined to 19% on the 12th day; further continued to decline to 15% on the 16th day. The increase in the initial stage of infection may be presumably accounted to the differentiation of some of the prohaemocytes into plasmatocytes. The depletion of plasmatocytes after the 8th day may be due to the loss of hypertrophied cells in the haemocoel as observed in the case of *Heliothis virescens* infected by *Campoletis sonorensis* poly DNA virus (Davies *et al.*, 1987) or due to the release of a "plasmatocyte depletion factor" as observed in *G. mellonella* (Chain and Anderson, 1982; 1983).

The prohaemocyte population which accounted for nearly 33% in the control exhibited a slight increase in the infected ones on the 4th day and declined to 25% on the 12th day. The spherule cells (6% in control) exhibited a slight increase on the 8th day (11%) and declined on the 12th day (8%). Since this type of cells does not develop in large number from stem cells and the infected cells do not become hypertrophied, gross differences between the control and experimental ones were not conspicuous. The reduction on the 20th day was due to the loss of spherules from infected spherule cells and so they are difficult to identify.

The oenocytoids (2% in control) were not at all affected. This is because the oenocytoids lack the potential to synthesize DNA and they were not found to divide (Lea and Gilbert, 1966). Furthermore, oenocytoids are not phagocytic and phagocytosis is a prerequisite for infection (Leutenegger, 1967). The grubs infected with 1 x 10^6 and 1 x 10^7 IU also showed the same pattern of changes but the degree of changes were not well pronounced as in the case of 1 x 10^8 IU infection.

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Preliminary Studies on the Pathogenicity of *Metarhizium anisopliae* (Metschn.) Sorokin var. *anisopliae* to Cutworm *Agrotis segetum* (Schiff.)

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ABSTRACT

The entomopathogenic fungus, *Metarhizium anisopliae* (Metschn.) Sorokin var. *anisopliae* was found pathogenic to the larvae of *Agrotis segetum* (Schiff.), *Agrotis ipsilon* (Hufnagel) and *Agrotis spinifera* (Hubn.). It was also found pathogenic to eggs of *A. segetum*. Soil application of the fungus gave 45% mortality of last instar larvae of *A. segetum* at a concentration of 1.2×10^7 spores/g of soil. The fungal spores at 10^7/ml sprayed on *A. segetum* eggs gave 100% mortality. Field application of spores on the eggs proved effective in controlling the pest. The study indicates the scope of using the fungus against the pest in the field.

KEY WORDS: *Metarhizium anisopliae*, pathogenicity, *Agrotis Segetum*

Cutworms are well known pests of potato, oats, tobacco, pulses, cabbage, beet root, groundnut, peas etc. (David and Kumaraswami, 1982). In Gujarat, three cut worm species *viz.*, *Agrotis ipsilon* (Hufnagel), *Agrotis segetum* (Schiff.) and *Agrotis spinifera* (Hubn.) are known to occur. Of these, *A. segetum* is a predominant species. A fungal pathogen, *Metarhizium anisopliae* (Metschn.) Sorokin var. *anisopliae* isolated from the field population of white grubs (Patel *et al.*, 1986) was tested against the cutworms. Pathogenicity of this isolate was tested against the eggs and larvae of *A. segetum* in laboratory as well as in the field. The results obtained are presented in this paper.

MATERIALS AND METHODS

For preliminary pathogenicity testing, twenty five healthy larvae of each species of cutworm were infected by the topical application of the fungus (Thomas, 1974). For testing the efficacy of the fungus against the larvae of *A. segetum*, three kg of sterilized soil was treated with *M. anisopliae* spore suspension prepared in distilled water containing 0.1% Tween-80 solution to have concentrations of 1.2×10^6, 3.6×10^6, 6.0×10^6, 8.4×10^6 and 1.2×10^7 spores/g of soil. Treated soil was transferred into round galvanized iron-sheet cages (30 x 10 cm). In controls, soil was moistened with 0.1% Tween-80 only. Same moisture level was maintained in all the cages. Fifty laboratory-reared final instar larvae of *A. segetum* were released in each cage. They were provided daily with potato

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