THE PROPAGATION OF THE ARMYWORM *SPODOPTERA EXEMPTA* (WALK.) (LEP., NOCTUIDAE) IN MASS PRODUCTION OF THE NUCLEAR POLYHEDROSIS VIRUS FOR PEST CONTROL

BY

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This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with my approval as University Supervisor.

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These studies were aimed at propagating the armyworm Spodoptera exempta (Walk.) on a semi-synthetic diet for production of its nuclear polyhedrosis virus for subsequent use in the control of natural populations in field outbreaks of this notorious pest.

The armyworm, S. exempta was reared from a virus-free culture using eleven different artificial diets prepared with grass meal as the main constituent. Diets were also prepared from soybean meal, wheat germ and yeast. The eleven diets on which trials were carried out gave significant differences in larval growth rates, duration of pupation and pupal weights, rates of adult emergence, female fecundity, and variation in female fecundity from generation to generation.

Newly emerged adults were placed in mating cages, five pairs of males and females per cage. Egg oviposition began on the third day post-emergence. Adults were kept for 10 days (one week from the time of first oviposition) and then discarded, and new adults introduced into the mating cages. Eggs were collected, sterilized, incubated, and the first instar larvae introduced into the semi-synthetic diets in threes or fours.

Four methods were tested for suitability for mass production of S. exempta NPV: spraying of PIB suspension onto surface of semi-synthetic diet, incorporation of PIBs into the diet, force-feeding larvae on small quantities of PIB suspension and infection through natural diet dipped into a PIB suspension. In order to provide a base for comparison of the effectiveness of the four infection methods, the LT50s were calculated. The LT50 values (and upper and lower
fiducial limits) were: dipping, 146.6 hrs. (142.2 and 151.1 hrs); spraying, 141.4 hrs (137.4 and 145.2 hrs); incorporation into diet, 198.2 hrs (194.2 and 201.8 hrs) and force-feeding, 139.0 hrs. (135.9 and 142.1 hrs). The fiducial limits were calculated at 95% probability.

In tests on optimum stage for larval infection, it was determined that the third larval instar (LT₅₀ 128.8 hrs, fiducial limits 122.4 and 135.7 hrs) would be the optimum stage for infection in mass virus production. Earlier instars gave pre-mature larval mortality and later instars showed maturity resistance to infection.

Tests aimed at investigating the optimum period of controlled feeding showed that the larval mortality rate depended on the length of time in which the larvae had been allowed to feed on the infective diet initially. Fastest mortality occurred in larvae exposed to infective diet for 96 hrs (LT₅₀ 93.1 hrs with 88.1 and 98.4 as upper and lower fiducial limits). Larvae exposed to the infective diet for 24 hrs had an LT₅₀ of 137.4 hrs. (fiducial limits 130.6 hrs and 144.6 hrs). The predominant larval age at the LT₅₀ was fifth instar.

Tests were also carried out on infectivity of S. exempta NPV to third instar larvae. A probit analysis on the results gave an LD₅₀ value of 48.4 PIBs/larva (lower and upper fiducial limits 39.2 and 59.4 PIBs/larva), and an LT₅₀ that varied from 146.2 hrs to 221.3 hrs, depending on the dosage. Thus an LT₅₀ for larvae treated at 320 PIBs/larva was 182.0 hrs.
Records were kept on prepupal and pupal mortality in insects treated at varying concentrations as larvae, on adult emergence in surviving pupae and on the effect of sub-lethal dosages on female fecundity. The rate of emergence in pupating insects was inversely proportional to the level of PIB dose fed to larvae. Further, there was no significant difference in the number of eggs oviposited by females infected as larvae at different PIB doses and control insects.

These investigations opened up possibilities of rearing armyworm on a simple diet with milled good quality grass as the main diet constituent. The use of semi-synthetic diets based mainly on natural materials would cut costs in mass rearing of hosts in insect virus production. Further, results showed lack of significant difference in the fecundity of adults reared from the natural diet, the grass meal diet and the diet based on yeast and wheat germ.

Insect viruses/properties that would make them ideal for the control of some insect pests, such as high virulence to the hosts and high specificity. Yet a major obstacle in the widespread use of insect viruses has been the economical and satisfactory means of production of such viruses. The factors that should be taken into consideration in virus production were therefore investigated in this project. Spraying a polyhedral inclusion body (PIB) suspension onto the surface of the semi-synthetic diet was found to have advantages over incorporation of PIBs into the diet, feeding larvae on natural diet dipped into a PIB suspension, or micro-feeding test larvae.

Infection of S. exempta with equivalent doses of NPV at the six larval instars showed a clear variation in their susceptibility to NPV infection. The LT<sub>50</sub> value for 2nd larval instars infected at
1.6 x 10^5 PIB/ml was 102.8 hrs (97.4 hrs and 108.5 hrs lower and upper fiducial limits respectively). Fifth larval instars infected at the same PIB level had an LT_{50} value of 190.5 hrs (182.4 hrs lower and 199.1 hrs upper fiducial limits). In order to compromise larval age larval susceptibility and optimum period for exposure of larvae to infective diet, it was shown that third instar larvae exposed to diet infected at 1.6 x 10^5 PIBs/ml and allowed to feed continuously for 24 hours, and subsequently transferred to virus free diet resulted in the highest cadaval weight, 173.3 mg (+ 32.9 mg SEM), and gave the optimum polyhedral development and therefore the highest number of PIBs per larva (1.1 x 10^{12}).

The potential activity of S. exempta NPV in pest control was indicated by the quantitative in vivo estimates of the virulence of the nucleopolyhedrosis virus to its host. Low LD values obtained for 7 day old larvae were indicative of the high virulence of S. exempta NPV to the larvae. Data on post-larval mortality showed that such mortality is quite substantial and can account for a high level of cumulative mortality, especially in larvae infected at a low PIB dosage. There was no significant difference between mean number of eggs oviposited by adults surviving from larvae treated at all levels with control adults, at \( p = 0.2 \). The results obtained from this project indicate the possibility of propagating larvae on a simple diet, and which can be subsequently used for the production of viruses.