Relationship between Nuclear Polyhedrosis Virus Susceptibility and Larval Weight in *Heliothis armigera*

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ABSTRACT

The population response of *Heliothis armigera* larvae to nuclear polyhedrosis virus (NPV) was investigated. The virus was introduced by permitting the larvae to feed on lettuce leaves. Median lethal doses (LD_{50}) were determined. The estimated LD_{50} values for the first, second, third, early, and late fourth larval instars were *5*, 141, 1226, 5168 and 24553 polyhedra per larva respectively. In the fifth larval instar a degree of maturation resistance against virus infection was observed. An inverse relationship between mortality and larval weight was detected. Expressing the results in terms of $LD_{50/}$ mg of the larval weight eliminated the observed variation in the susceptibility of larvae. The estimated LD_{50} values for each of the larval instar groups were used to predict the response of the larval population to virus infection. This procedure provided a sound determination of the response pattern, from which 96% of the variation in the larval susceptibility could be described in terms of the log larval weight.

Keywords: Bioassay, *Heliothis armigera*, Viral infection.

INTRODUCTION

The lepidopteran *Heliothis armigera* (Hubner) is one of the world's most harmful pests and has developed widespread severe resistance to chemical insecticides [1]. Increasing awareness of environmental contamination and widespread pest resistance problems pose a severe threat to any management program based on the conventional synthetic insecticides. These pressures are making alternative control agents such as nuclear polyhedrosis virus (NPV) more attractive in the integrated pest management systems [13, 17]. The NPV is an important biological control agent for *Heliothis* species and has been successful in a number of cases [4, *5,* 19, 21]. In theory a virus particle may be capable of infecting an insect host [3]. In practice, however, the probability of infection of the insect host increases with increasing the virus dose, and varies with the genetics of pathogen, type of host, and the environmental conditions [7, 17].

Several authors have reported the decrease in susceptibility to virus infection among older larvae of different insects and the magnitude of these differences is noticeable [5, 7, 12]. Crowding, host plant differences, and temperature are considered to affect the susceptibility of host larvae to infection [17, 20]. However, the effect of any of these factors was negligible as compared with changes of weight occurring during larval growth [8, 11]. For instance the LD_{50} value of the 4th instar *Malacasoma neustiria* larvae when expressed in terms of the polyhedra/mg larval weight was twice of that for the 3rd instar larvae, but related to age the LD_{50} values were, 12320 and 1405 polyhedra/larvae respectively [12]. Similar results have been reported for two other lepidopteran species [2]. Obviously changes in the susceptibility of *H. armigera* larvae to NPV infection during growth period could

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be an important factor of variation in the interactions between virus and larvae under field conditions. The larval weight at the time of infection could be the most critical factor in detemining subsequent mortality [18, 20]. Therefore, a thorough knowledge of the effect of the weight on the susceptibility to NPV infection at population level would be necessary. However, great caution should be applied before the results of laboratory investigations are extrapolated to field conditions because of the biotic and abiotic variability between the two systems [14].

It is reported that dose mortality response of the larvae to virus is a function of the concentration of the virus spray '[5, 18]. In practice the cost of the dissemination of higher dose virus may not be warranted if natural population of larvae is not reduced proportionately. Hence, dose mortality studies with larval population, which is composed of individuals of varying in age, weight and developmental stage, is necessary before the virus is to be considered for wide scale use.

Little has been reported on the susceptibility of larvae at population level. Therefore on the basis of population toxicology, the present study, was undertaken to evaluate the magnitude of the larval susceptibility variation at population level. To predict responses likely to occur in a natural population and to express the results in terms of applicable to field conditions, a suitable approach must be employed [14].

MATERIALS AND METHODS

Rearing techniques: A stock culture of *H. armigera* was established from 185 larvae collected at Nazlou field, Urmia University in 1997. The rearing of *H. armigera* was based on the group rearing method [9]. The culture had been reared for two generations before bioassay studies were initiated. Eggs were stored at 7 ± 2^0C and incubated at room temperature when required.The larvae were separated into different instar groups by splitting the eggs from each female into 4

approximately equal batches. The incubation of each egg batch was then begun 4 days apart. Due to influence of weight on susceptibility, in selection of the larvae, emphasis was made on the weight of the larvae rather than the age, hence, the different weight groups could be categoriezed.

Weighing of larvae: The first instar larvae were handled with a suction device. Large larvae were handled with a fine brush and watch maker forceps. The larvae heavier than 1 mg were weighed on a Mettler balance (Type H31) with nominal accuracy of $\pm$ 0.1 mg. For those lighter than 1 mg, larval weight was estimated by an average of 600 larvae.

Larval instars: Larval instars were determined by measuring head capsule width with using a calibrated ocular micrometer in a binocular dissecting microscope. The mean and range of head capsule width were determined for each of the instar groups.

Feeding chambers: Two sheets of white plastic(5 mm thick and 5 cm x 15 cm) were pressed tightly together, fixed by fusing the edges with a hot needle and perforated by a series of holes drilled right through both sheets. Each hole was about 3/4 of the average leaf area consumed in 24 hours by a given instar. The size of the holes varied for each larval instar. This was determined by a series of preliminary experiments. The diameter of the holes was 4, 5, 10, 15 and 30mm for 1st, 2nd, 3rd, 4th and 5th larval instars respectively. Leaf diaphragms were formed by cutting leaf strips from a young lettuce *(Lactuca sativa. L)* leaf and placing them between the two plastic sheets. Lettuce leaves were easily maintained in turgid condition for the period of the larval feeding. The larvae were confined to the plastic sheets by using a plastic cling wrap.

Virus origin: All nuclear polyhedrosis virus used in these experiments originated from one stock isolate. This was obtained from Mamestrin (MbNPV) which was passaged three times using laboratory reared *H. armigera* larvae. All infected larvae were individually reared on the semi-artificial diet. The dead larvae were diagnosed by mi-

croscopic examination of the stained hemolymph of larvae. The NPV infected larvae were pooled and frozen at about -20°C in several aliquots of 5 ml of distilled water. To provide the stock suspension of virus, the virus infected larvae were crashed with a glass rod in a 50 ml glass tube containing 5 ml of distilled water, and allowed to deteriorate at room temperature. After 6 days the resultant material was blended and siphoned out from the tube with a pipet through cotton wool. The process provided a crude suspension of virus of high but unknown concentration. The concentration of the stock suspension was estimated by counting of polyhedra using a hemacytometer and a phase-contrast microscpe. The concentration of the polyhedra in the stock suspension was 3.16 x 10^8 polyhedra/ml. Stock suspension was stored at about -5°C for immediate bioassays.

Application of virus doses: An Oxford microdispenser was used to dispense different test doses. For the 1st, 2nd, 3rd, 4th and 5th larval instars the appropriate dose was dispensed in 2 μl of the suspension on the center of the leaf diaphragm after assembling the feeding chamber. A control group was made available for all the experiments, for which 2μl of sterile distilled water was dispensed over the leaf.

Dose ranges for each instar group: The various polyhedra inclusion body (PIB) concentrations were made from a crude water suspension of virus containing 3.16 x 10^8 PIBs/ml. Preliminary bioassays indicated that the appropriate number of polyhedra required to infect half of the larval population (LD_{50}) was in the range of 40 to 350 polyhedra/mg for the test insects excepting the 5th instar. The early fifth instar larvae (115± 10.2 mg) showed a degree of resistance to virus infection despite the ingestion of up to 5.5×10^4 polyhedra/larvae. Using these values as a guide, a series of logarithmic dilutions of the stock suspension was made, so that two doses were above the estimated LD_{50} and two below it. Each treatment consisted of 15 larvae. Experiments were replicated three times in three different days. The sterile distilled water treated control group was included in all bioassays.

Larval feeding: Following the application of the dose and assembly to the 1st, 2nd, 3rd, 4th and 5th instar larvae, the plastic sheets were completely wrapped in plastic cling film. A slit was made on the film above each hole and larvae were inserted through into the chamber below. When all larvae were positioned, the entire assembly was covered with a second layer of cling film with its two sides tightly wrapped together. After infection, all the test larvae were kept at 27±2°C under rearing conditions. In all of the different age groups, only larvae which had consumed the entire leaf diaphragm within 24 hours were removed and reared individually on the semi-artificial diet. Those larvae which did not consume the exposed leaf surface within 24 hours or died within the first 24 hours on the semi-artificial diet were excluded from the final results. Mortality was recorded daily, and the experiment continued until all larvae had either died or pupated. All dead larvae were smeared, stained and examined microscopically for the possible presence of the polyhedra.

Statistical analysis: The quantal response analysis was based on the presence or absence of the polyhedra rather than the larval mortality. It is considered that the response is obviously attributable to the virus inoculum across all larval instars. Data were analysed using the probit method [14].

RESULTS

The head capsule width data are presented in Table1. The results of the ratio indicate that the determination of larval instar is possible using head capsule width measurements. The relationship between head capsule width and larval instar could be approximated by the equation:

$Y = \exp(-1.75 + 0.46X)$; $R^2 = 0.99$: (1) in which Y is mean head capsule width and X is larval instar.

Table 1. Head capsule width for first to penultimate *H. armigera* instar groups.

Instar	Number of larvae	Range (mm)	Mean (mm) ± SEM [a]	Ratio n/n-1 [b]
1 st	25	0.23-0.31	0.26 ± 0.003	
2 nd	27	0.41-0.50	0.46 ± 0.005	1.76
3 rd	28	0.62-0.86	0.72 ± 0.02	1.56
4 th	24	0.96-1.62	1.12 ± 0.03	1.55
5 th	25	1.57-1.97	1.75 ± 0.08	1.56

[a] Standard error of mean.
[b] Ratio between means of successive instars.

Table 2. Probit analysis for the mortality of different *H. armigera* instar groups.

Instar group	Chi-square	LD_{50} (number of poly-hedra)	Regression equation
1 st instar	0.58 [a]	5	$Y = 2.66 + 3.04 X$
2 nd instar	6.32 [a]	141	$Y = 1.03 + 1.84 X$
3 rd instar	0.97 [a]	1226	$Y = 1.24 + 1.21 X$
4 th instar < 80 mg	0.26 [a]	5168	$Y = 2.44 + 0.68 X$
4 th instar < 105 mg	2.12 [a]	24553	$Y = 1.00 + 0.91 X$

[a] Chi-square value indicates that the line is significantly well fitted.

The susceptibility of larvae to NPV infection decreased markedly with increasing larval weight. This is illustrated by the numerical results of probit analysis (Table 2). The extent of the decreased in the susceptibility of 5th instar larvae could not be estimated because only 2 out of 45 larvae were infected, despite the ingestion of up to 5.5×10^4 polyhedra/larvae. Using data in table 3, the effect of larval body weight on susceptibility was investigated. To achieve this, the log LD_{50} dose for each instar group was plotted against mean log larval body weight (Fig.1). The relationship was found to be best described by the following equation:

$$\log Y = 1.85 + 1.08 \log X; \quad R^2 = 0.96 \quad :(2)$$

in which, Y is virus dose and X is mean body weight. This equation accounts for 96% of variations in the larval susceptibility to NPV infection expressed as a function of the larval weight. By expressing this result in terms of LD_{50}/mg larval body weight, the same effect can be illustrated in another way as presented in Table (3). This latter procedure eliminates the influence of the weight on susceptibility and when plotted, results in a line with a non-significant slope (Fig.1). It is noted from fig. (1) that there is no significant difference in the susceptibility of the first four instar groups when expressing the results in terms of the polyhedra/mg larval weight. The data in Table 3 were used to provide an expression for predicting larval weight. The results indicated the relationship between the mean weight and correspondig instar group could be best described by the following equation:

$$Y = -40.65 + 25.97X, \quad R^2 = 0.89: (3)$$

where, Y is mean body weight and X the larval instar.

294

Table 3. Calculated number of polyhedra per larvae and per mg larval body weight required to obtain an LD_{50} level for various *H. armigera* instar groups.

Instar group	Mean larval weight (mg) ± [a]SEM	Polyhedra	
		Per larvae	Per mg body weight
1 st instar	0.07 ± 0.01	5	83.4
2 nd instar	3.50 ± 0.03	141	40.32
3 rd instar	16.00 ± 2.20	1226	76.63
4 th instar < 80 mg	70.20 ± 7.25	5168	73.62
4 th instar < 105 mg	96.60 ± 8.2	24553	254.17

[a] Standard error of mean.

DISCUSSION

There is no reason to believe that the dose required to produce infection should be weight related since an infection agent could be able to produce infection regardless of host weight [3]. In *H. armigera* larvae however, there is a steady decrease in susceptibility to virus with age. Even though different doses are capable of causing infection, however more infection occurred in case of larvae receiving higher doses.

The rate of change in the susceptibility of *H. armigera* larvae to NPV infection during larval growth is considered to be important in the interaction between the larvae and virus. Hence, the susceptibility of larvae at different stages of growth encompassing the entire larval weight range were estimated by bioassays. The most striking feature of these trials were the uniform pattern of decreasing the susceptibility to NPV infection over the first four instars followed by a sudden expression of larval resistance in the 5th instar. This phenomenon was encountered in a number of studies [6, 8, 21]. However, the techniques used in those investigations were not appropriate enough for estimating the effects of the larval body weight on host susceptibility to virus infection. Either the dose taken up by each larvae was unknown, and/or ingested over a protracted period, or else the weight of larval age groups was not stated.

To assess the effect of growth accurately, the virus dose must be consumed over a short period, and in amounts known for individual larvae. Larval weight and developmental stage must also be recorded since growth varies with environmental conditions and should be compared in terms of weight rather than age. Some researches with other lepidopteran larvae fulfil these conditions [2, 8, 12], but only one of them used the larval weight range as comparable with the present study [2]. He found a fairly uniform pattern of the weight related decrease in susceptibility with increasing age. However, in the bioassays of the oldest larvae (8 days, 89 mg) it became clear that the slope of the probit line decreases significantly. Therefore, the authors concluded that the flatter slope for the 8 day old larvae may indicate maturation resistance and this resistance develops shortly before or during the prepupation period.

A research with *H. zea* revealed a noticeable decrease in the susceptibility of the older larvae and a sharp decrease in the susceptibility occurred at about 1/3 of the peak larval weight [11]. In the present study, the mean weight and standard error of mean of the early 5th instar *H. armigera* larvae (115± 10.2 mg) was found to occur at about 1/3 of peak larval weight too. This implies that the decrease in susceptibility occurs at the same stage of the larval growth in both *H. zea* and *H. armigera.* The results revealed that the
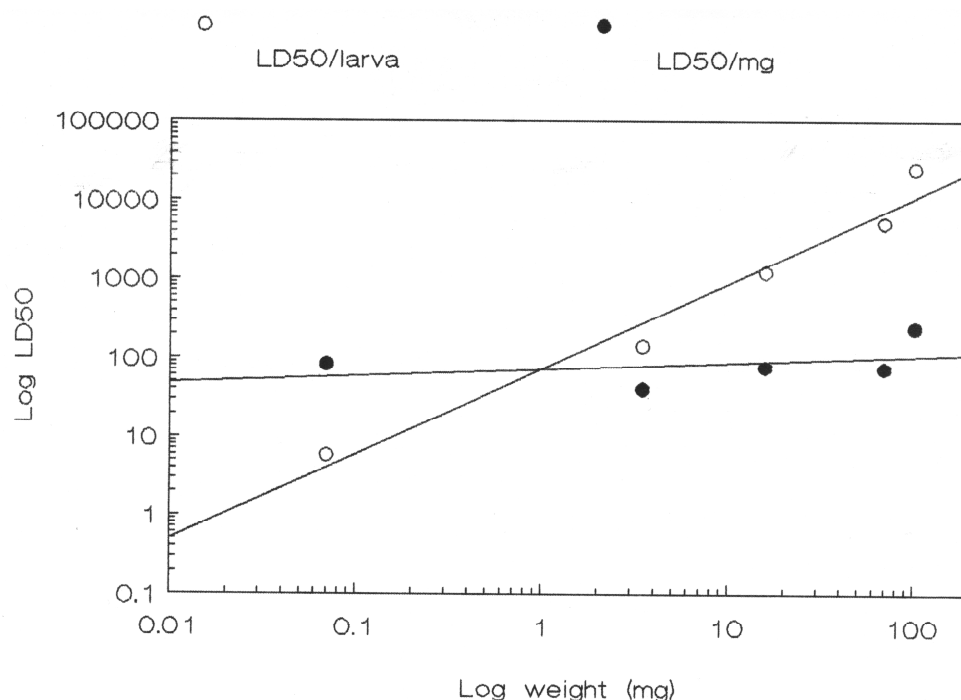
295

Figure 1. Number of polyhedra/larvae and polyhedra/mg of larval weight required to produce an LD$_{50}$ response for various ages and weight of *H. armigera* Larvae.

slope of the fittest lines of the 4th instar larvae is considerably lower than that of the other groups (Table 2). This probably is an indication of the heterogenity of response and it is evident that some larvae were susceptible to low doses of virus indicating that the pattern of the host response is likely to vary markedly within the growth period of each instar. Similar trend has been reported earlier [6].

The physiological basis of the uniform decrease in susceptibility during the larval growth is still unknown, however, some researchers suggested that hormonal effects may cause modification in the cell susceptibility as larvae approach to moult and metamorphosis stage [17, 20]. There is an evidence that β ecdysone may be involved in the variability of the response pattern [20]. Midgut pH, and host plant induced variation in the midgut pH have strong influence on both PIB dissolution rate and virion survival, and on the decrease of the susceptibility of larvae to NPV infection (10, 16). Regardless

of the exact causes of the decrease in susceptibility, NPV spraying program for *H. armigera* control must be considered on the basis of larval weight and physiological age as for practical purposes.

Since direct laboratory studies cannot quantify the dose encountered by larvae in the field, variation in host susceptibility studies due to weight must be carried out. In the absence of specific results, equation (2) could be used to predict the larval susceptibility to NPV infection at a population level, and to provide an insight as to what strategies might maximize the NPV efficiency.

Nuclear polyhedrosis virus must be ingested by the larvae in order to be effective, hence, a good coverage of the plant target is essential. In addition, it is noted that a living organism loses its potency when exposed to sunlight, especially the ultraviolet component. Therefore, repeated applications and much higher doses would be required for the field control of *H. armigera* during an entire cropping cycle. At present NPV is produced

by laborious methods [15, 18] and therefore, for maximum efficiency it is essential to apply the correct amount of virus to achieve an acceptable reduction of larval population.

The results from laboratory tests can be extrapolated to the mortality of larvae under field conditions by providing an appropriate method to predict the value of treatment on the larval population of a mixed weight. The bioassay method, based on the larval population response, could be used for this purpose. Laboratory and field trials with minimum effective application rates, effectiveness of suitable adjuvants and mechanism of maturation resistance remain to be investigated.

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رابطه بین حساسیت به ویروس پولی ئیدر هسته ای و وزن لارو در کرم غوزه

چکیده

حساسیت جمعیت لارو کرم غوزه نسبت به ویروس پلی ئیدر هسته ای برآورد گردید. لاروها ویروس را به همراه برگ کاهو خوردند. دوزهای کشنده پنجاه درصد (LD_{50}) مشخص گردیدند. مقادیر LD_{50} برای لاروهای سنین اول، دوم، سوم، چهارم جوان و مسن به ترتیب ۵، ۱۴۱، ۱۲۲۶، ۵۱۶۸، ۲۴۵۵۳ پلی ئیدر بر هر لارو برآورد شد. لاروهای سن پنجم نسبت به آلودگی با ویروس درجه ای از مقاومت بلوغ را نشان دادند. بین وزن لارو و میزان تلفات لارو رابطه معکوسی مشاهده گردید. با محاسبه مقادیر LD_{50} برحسب میلی گرم وزن بدن، تفاوتهای مشاهده شده در حساسیت لاروهای سنین مختلف از بین رفتند. با استفاده از مقادیر LD_{50} برآورد شده بر هر سن لاروی حساسیت جمعیتی لارو نسبت به ویروس برآورد شد. این عمل روند تغییرات حساسیت لارو را به طور منطقی تعیین کرده و براساس آن می توان ۹۶ درصد تغییرات حساسیت جمعیت لارو را برحسب لگاریتم وزن بدن تعیین و تشریح نمود.