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Influence of Larval Age and Dosage of Virus on the Recovery of Occlusion Bodies of the Granulosis Virus of Sugarcane Shoot Borer *Chilo infuscatellus* Snellen

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ABSTRACT

A significant loss in the weight of larvae was observed due to virus infection, which was more pronounced in III, IV and V instars. Yield of occlusion bodies (OB) was low in II instar (59.6 x 10^7/ larva) and high in IV (187.1 x 10^7/ larva) and V (182.7 x 10^7/ larva) instars. Positive correlation existed between larval weight and virus recovery and 10^5 OB was the optimum dose for virus production. Different instars of field collected larvae with virus symptoms yielded 71.6 to 202.1 x 10^7 OB / larva. The yield of OB from male pupa was significantly low (74.7 x 10^7 pupa) compared to female pupa (192.0 x 10^7/ pupa).

KEY WORDS : *Chilo infuscatellus* GV, dosage, larval instar, virus recovery

A granulosis virus was found to infect the sugarcane shoot borer, *Chilo infuscatellus* Snellen (Easwaramoorthy and David, 1979). Further studies showed that the virus may be useful for the control of shoot borer (Easwaramoorthy, 1984) as its application reduced the pest incidence below economic injury level in field trials (Easwaramoorthy and Santhalakshmi, 1989). Mass multiplication of the virus is necessary for taking up large-scale field trials. In this connection, it is essential to know the quantum of virus that can be obtained from host larva of different sizes and also the optimum dose of the virus inoculum to get maximum virus production. The results of the study made on this aspect is presented in this paper.

MATERIALS AND METHODS

The virus inoculum obtained by feeding third instar larvae of shoot borer was purified using alternate cycles of low and high speed centrifugation. Finally, the virus was sedimented by centrifugation at 17,000 rpm for 30 minutes at 5°C in a refrigerated centrifuge.

Healthy larvae of different instars were weighed individually before the inoculation of the virus. The larvae were microfed with 1μl of the virus suspension containing required concentration (10^3 - 10^7 occlusion bodies/larva) using an Agla micrometer syringe. Care was taken to discard those larvae which failed to ingest the entire quantity of the inoculum. The control larvae were fed with an equal quantity of distilled water. The treatments were replicated 3 times with 10 larvae per replication.

The treated larvae were transferred at the rate of three to a plastic box (7.0 cm dia x 7.5 cm ht) provided with filter paper circle at the bottom to absorb excess moisture and three pieces (4-5 cm length) of sugarcane shoot (variety Co 6304) split open at one end. The filter paper and shoot pieces were changed once in 2 days initially and daily when the larvae started dying due to virus infection. The weights of the moribund larvae were recorded individually.

The viral occlusion bodies were recovered by triturating the diseased larvae individually with 5 ml of distilled water and

filtered through double layered muslin cloth and the final volume was adjusted to 10 ml. Appropriate dilutions were made before counting the OB. The OB were counted using a Petroff Hauser and Helber counting chamber with 0.02 mm depth under a phase contrast microscope. The data on recovery of OB were analysed using 'F' test and also correlations were worked out between the weight of larvae and number of OBs recovered.

In another study, the amount of virus recoverable from field collected larvae and pupae was determined. The larvae showing colour change and other symptoms of virus infection were separated instarwise based on head capsule width (Easwaramoorthy, 1984). They were weighed individually and reared on sugarcane shoot bits until they became moribund. The virus was recovered from diseased larvae as described earlier.

The field-collected pupae were separated into apparently healthy and virus-infected ones based on the symptoms described earlier by Easwaramoorthy and Jayaraj (1989). The infected pupae were sexed based on the position of the genital opening (Gupta, 1959) and were weighed individually and virus recovered as described earlier for larvae.

RESULTS AND DISCUSSION

There was significant reduction in the weight of the larvae due to virus infection. The mean initial larval weight of 72.39 mg before treatment decreased to 40.67 mg before death. When the larval instars were considered individually, there was no reduction in weight in second instar, while in other instars, the larval weight decreased significantly due to virus infection (Table 1). The weight reductions were in the order of 41.2 to 46.0 per cent. Reduction in growth of virus infected larvae based on body length have been reported by Adams *et al.* (1968) in *Ceramica picta,* Jacob (1972) in *Spodoptera litura* F. and Rabindra and Subramaniam (1974, 1975) in *Heliothis armigera* (Hbn.) and *Amsacta albistriga* W.

Table 1. Initial and final weights of different larval instars

Instar	Initial weight (mg)	Final weight (mg)
II	24.13	24.80
III	45.53	25.60
IV	96.73	52.27
V	123.20	60.00
Mean	72.39	40.67
		C.D. (P=0.05)
Between initial and final weight		1.88
Between weight x instar		3.76

The recovery of occlusion bodies was significantly high in IV and V instar larvae compared to III (Table 2) and it was three times

Table 2. Recovery of OB from various larval instars of *C. infuscatellus* microfed with GV at five dosages

Instar	No. of OB (X 10^7) recovered at doses of OB					
	10^3	10^4	10^5	10^6	10^7	Mean
II	64.00*	72.00	59.67	57.00	45.33	59.60[a]
III	72.00	73.00	132.60	157.33	140.00	114.99[b]
IV	133.67	155.33	232.01	196.00	218.67	187.14[c]
V	155.33	178.00	223.33	181.33	147.92	182.67[c]
Mean	106.25[a]	119.58[ab]	161.90[c]	147.92[bc]	144.83[bc]	

Figures followed by same letter in the row or column are not significant by F test (P=0.05)

* Interaction not significant.

Table 3. Correlation between weight of larvae and recovery of virus OB

Dosage	'r' value	
	Initial weight	Final weight
10^3	0.7186**	0.4186**
10^4	0.6038**	0.4457*
10^5	0.8157**	0.5699**
10^6	0.6599**	0.5518**
10^7	0.6717**	0.5603**

** Significant at 1% level
* Significant at 5% level

less in second instar larvae. Increase in virus recovery with increase in age of the larvae has been reported in the case of *A. albistriga* (Narayanan *et al.*, 1978) and *S. litura* (Chaudhari and Ramakrishnan, 1979). The virus recovery was low (106.25 x 10^7 OB) at the dosage of 10^3 OB / larva and high (161.9 x 10^7 OB) at 10^5. Further increase in the dosage did not significantly increase the virus recovery.

Significant positive correlations were observed between larval weight and virus recovery. Similar results were reported in the case of *A. albistriga* (Narayanan *et al.*, 1978). The 'r' value between weight of larvae at the time of treatment and virus yield were more stronger (Table 3) than between weight of larvae before death and virus recovery.

Different instars of field collected larvae with virus symptoms yielded 71.57 to 202.14 x 10^7 OB / larva (Table 4). They showed a similar trend as observed in the *per os* tests in the laboratory indicating that the virus recovery is a function of larval weight. There was significant difference between the virus recovery from male and female pupae. The yield of virus from male pupae was comparable with that from second instar larvae, while that from female pupae was comparable with that from fourth and fifth instar larvae. This further confirmed that the virus recovery was dependent on body weight.

When percentage mortality and virus yield are considered together, it is desirable to use fourth instar larvae for mass production of the virus. Though fifth instar larvae also yielded almost same quantum of virus, the percentage mortality of fifth instar larvae was significantly low compared to fourth instar (Easwaramoorthy and Jayaraj, 1990). The study further revealed that a dose of 10^5 OB / larva was optimum for mass multiplication of the virus.

Table 4. Recovery of OB from field-collected larvae and pupae of *C. infuscatellus* showing natural infection

Stage	Mean weight (mg)	No. of OB (x 10^7)
II instar	20.97[a]	71.57[a]
III instar	45.87[b]	119.20[b]
IV instar	72.49[c]	180.40[c]
V instar	95.81[d]	202.14[d]
Male pupa	36.67[ab]	74.71[a]
Female pupa	97.27[d]	192.0[cd]

Figures followed by same letter in a column are not significant by F test (P=0.05)

The virus at 2 x 10^6 OB/ml was found effective when sprayed at fortnightly intervals in the field. At this dose, the total quantity of virus required to spray one hectare of crop works out to 1.50 x 10^{12} OB (250 LE). This can be obtained from 800 diseased fourth or fifth instar larvae.

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