# LABORATORY BIOASSAY AND HOST RANGE TESTS OF THE GRANULOSIS VIRUS OF *SPODOPTERA LITTORALIS*

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## INTRODUCTION

Few entomopathogenic viruses, which are specific, efficient and safe to non-target organisms have been exploited as pest control agents. Some of these agents (e.g., Baculoviruses) have shown potentiality as an excellent alternative to chemical insecticides in the integrated pest management programmes (IPM) (Elnagar and El-Sheikh, 1989). A member of the family baculoviridae subfamily Eubaculovirinae, is the granulosis virus (GV), (Francki, *et al.*, 1991). This occluded virus was strongly proposed for field application (Tanada, 1964). *S. littoralis* GV was isolated from diseased larvae more than 15 years ago at Bouake in *Cote d'Ivoire* and characterized, in Egypt, through its physical, immunological, and biochemical properties as GV-*Spodoptera* by Aboi-Ela *et al.*, (1994). Although *S. littoralis* NPV, which was isolated in Egypt fourty years ago (Abul-Nasr, 1956), is known by its acute pathogenicity to the 1*st* instar larvae, the granulosis virus acts strongly against later instars (Narayanan, 1985). This fact may be considered in the management of *S. littoralis*. In Egypt, the granulosis virus was neither studied sufficiently, nor infectivity tests were previously conducted. The present work was, thus, designed to evaluate the pathogenicity of the virus and its host range, which are fundamental facts for better understanding of *S. littoralis* GV.

## MATERIALS AND METHODS

The test insect used in the present study was *S. littoralis* (Boisd.) (Noctuidae); the source of its culture was originated from moths collected by a light trap and maintained for several successive generations under laboratory controlled conditions of 25 ± 2°C and 65-70% R.H. Larvae were maintained on a semi-synthetic diet, described by Shorey and Hale (1965), and those for bioassay tests, were maintained on the same diet ingredients excluding the formaldehyde. All the different developmental stages of this test insect were maintained as described by Khamiss (1991).

The granulosis virus used in the present work was available at the Entomovirology Laboratory, Dep. of Econ. Entomol., Fac. of Agric., Cairo Univ.

This virus was originally isolated from *S. littoralis* larvae at Bouake in *Cote d'Ivoire* and purified by P. Monsarrat and F. Baillon (Personal communication).

GV-infected larvae were homogenized in buffer (50mM Tris, 2mM SDS pH 7.8), the homogenate was filtered through several layers of cotton and muslin (Tompkins, 1991). Clarification of the filterate suspension took place by a low centrifugation at 1400 g for 5 min using Beckman J2-21MIE centrifuge, rotor 20 JA. The virus was pelleted from the supernatant by centrifugation at 28000 g for 30 min using the same rotor. The pellet was resuspended in 1ml Tris buffer (50mM pH 7.8) deposited on 30-70% (w/w) continuous sucrose gradient and centrifugated at 55000 g for 20 min using Beckman L7-65 ultracentrifuge, rotor SW 28. The band containing granules was drawn off with a Pasteur pipette, suspended in Tris buffer, and centrifuged at 43000 g for 30 min using Beckman J2-21MIE centrifuge, rotor 20 JA for washing; pelleted granules were then resuspended in Tris buffer. The highly purified viral granules were checked by spectrophotometer DU-70 through 450 nm wave length, and stocked in Tris under -20°C.

Bioassay tests of *S. littoralis* GV :

The highly purified *Sl* GV stock suspension, diluted to 4-5 different concentrations, was tested against the *2nd* (4-5 day-old), *3rd* (6-7 day-old) and *4th* (8-9 day-old) instars of *S. littoralis* larvae pooled out from the virus-free culture. Tested larvae were starved 6-8 hours before exposure to the different virus concentrations.

The lethal concentration (LC) of *S. littoralis* GV was determined for the *2nd* and *4th* larval instars. A plastic plate measuring 18 x 12 x 1 cm, containing 0.5 cm layer of diet was divided by a plastic sheet into 84 squares 1.5 x 1.5 cm each. Each square was dosed with one of the tested concentrations (e.g., 119, 11.9, 1.19, 0.119, 0.0119 OD (1 (Optical density) $OD_{450}$ = 1.48 x $10^{10}$ capsule/ml and 1ml at 1 $OD_{450}$ = 0. 125 mg capsule/ml, (Chang and Tanada, 1978) in 25 µl distillied water dispensed on the diet surface.

The bioassay method described by Elnagar *et al.*, (1983) was used to determine the lethal dose (LD) of *Sl* GV for the *2nd*, *3rd* and *4th* instars. Each virus dose was administered on a disc of diet, 0.4 cm diameter and 0.2 cm thick, placed in a well of a microtiter plate. Five µl of each virus dose (119, 23.8, 11.9, 1.19, 0.119, and 0.0119 OD) was applied on each disc and allowed to air drying before placing the starved larva. Rate of mortality was calculated according to symptoms of viral infection as well as nucleic probe, and corrected according to Abbott's Formula (1925).

Host range of *Sl* GV :

The *Sl* GV was tested against six insect species from Lepidoptera representing different genera from different families : the lesser cotton leafworm, *S. exigua* (Hb.), the black cutworm, *Agrotis ipsilon* (Hfn.) and the maize worn, *Mythimna loreyi* (Dup.) all from Noctuidae, the greater wax moth, *Galleria melonella* (L.) (Pyralidae), the cabbage butterfly, *Artogeia rapae* (L.) (Pieridae), and the pink bollworm, *Pectinophora gossypiella* (S.) (Gelechiidae).

## RESULTS

Obtained results demonstrate that the *2nd* and *4th* instar larvae of *S. littoralis* were susceptible to *Sl* GV and mortality rates among tested larvae, increased with the increase in virus concentration (Table 1). The highest rates of mortalities 96.76% and 91.21% were achieved for the *2nd* and *4th* instar, respectively, at 119 OD/ml. Determination of LC value was based on the percent mortality obtained from the daily record of death. Death due to virus infection occurred during the larval stage. The calculated LC50 for the *2nd* and *4th* instar was 0.052 and 0.813 OD/ml, respectively (Table 1). The time required for initial mortality was 5 and 8 days, for the *2nd* and *4th* instar, respectively, and the last mortalities due to virus treatment were concluded by the *5 4th* and *41st* day. The estimated LC25 and LC90 values, for the above mentioned instars were (0.01 and 1.17 OD/ml) and (0.039 and 288.4 OD/ml), respectively (Table 1). Obtained results proved that the *2nd* instar larvae were more susceptible to virus infection than the *4th* ones, the LC25 value of the latter instar was 3.9 times more than that of the former one. The same trend was observed with the LC50 and LC90, where their values for the *4th* instar, were 15.9 and 246.49 times more than that of the *2nd* one. Concentration - mortality regression line did not indicate any difference, in the slope of the line, between the *2nd* and *4th* instar, which suggests a similar type of response between the two instars to *Sl* GV.

**TABLE (1)**

**Mortality percentage and LC25, LC50 and LC90 values of *Sl* GV versus *S. littoralis* larvae.**

***2nd* instar**

| Replicate No. | % Mortality at indicated virus concentrations (OD) | | | | Estimated LC values | | |
|---|---|---|---|---|---|---|---|
| | 119 | 1.19 | 0.119 | 0.0119 | LC25 | LC50 | LC90 |
| 1 | 41/42* (97.62) | 42/45 (93.33) | 37/54 (68.51) | 16/49 (32.65) | | | |
| 2 | 40/41 (97.56) | 35/38 (92.10) | 38/50 (76) | 16/52 (32.69) | | | |
| 3 | 43/45 (95.55) | 37/44 (84.09) | 33/54 (61.11) | 8 47 (17.02) | | | |
| Total | 124/128 | 114/127 | 108/158 | 41/148 | 0.01 | 0.052 | 1.17 |

y = 30.9 x -1.1.

4*th* instar

| Replicate No. | % Mortality at indicated virus concentrations (OD) | | | | | Estimated LC values | | |
|---|---|---|---|---|---|---|---|---|
| | 119 | 11.9 | 1.19 | 0.119 | 0.0119 | LC25 | LC50 | LC90 |
| 1 | 24/29* (96.55) | 17/23 (73.91) | 29/33 (78.79) | 14/40 (35) | 12 42 (28.57) | | | |
| 2 | 26/30 (90.63) | 21/34 (61.76) | (13/26) (50) | 9 35 (25.71) | 8 28 (28.57) | | | |
| 3 | 26/30 (86.67) | (15/28) (53.57) | 8 31 (25.81) | 7 31 (22.58) | 5 36 (13.89) | | | |
| Total | 83/91 (91.21) | 53/85 (92.35) | 47/90 (52.22) | 30/106 (28.58) | 25/102 (24.51) | 0.039 | 0.8268 | 288.4 |

y = 16.94 X + 0.476;

*No. of virus dead larvae/total number of tested larvae; percentages are between brackets.

Results show that the rate of mortality, among tested larvae increased directly with the increase in virus dose. The time required for initial mortality was 12, 6 and 9 days and all mortalities due to virus treatment occurred during the larval stage and concluded by the 34*th*, 25*th* and 41*th* days for the 2*nd*, 3*rd* and 4*th* instar, respectively, and according to the dose level. Data in Tables (2, 3 and 4) show the estimated LD 25, LD50 and LD90 values of the 2*nd*, 3*rd* and 4*th* instars, respectively. LD25 value for the 4*th* instar was 185 and 160 times more than that of the 2*nd* and 3*rd* instars, respectively. However, the LD25 value of the 3*rd* instar was only 1.15 times that of the 2*nd* one. Also, the LD50 for the 4*th* instar was 38.65 and 24.98 times that of the 2*nd* and 3*rd* instars, respectively, while the LD50 value of the 3*rd* instar was only 1.55 times that of the 2*nd* one. The LC90 of the 4*th* instar was 1.7 and 1.57 times that of the 2*nd* and 3*rd* instars, respectively. The LD90 for the 3*rd* instar was 1.09 times that the 2*nd* one. Analysis of dose - mortality data indicate no difference in the slope of the regression lines of the 2*nd* and 3*rd* instars, while a clear difference in that slope occurred between the 2*nd* and 4*th* instars.

There was an inverse relationship between the virus dose (or concentration) and the time required for 25% mortality (LT25) and 50% mortality (LT50). Results in Tables (5 & 6) indicate that the prolonged duration of larval instars due to GV infection, was more common with the lower infectious doses.

Host range of *S. littoralis* GV :

Results of the host range study are presented in Table (7). A test insect was considered to be a host of *Sl* GV, when the characteristic symptoms of GV disease were observed on the test larva, in addition to the detection of virus in dead larvae. Results in Table (7) show that, *Sl* GV could infect only the 2 insect species belonging to family Noctuidae, *(S. littoralis* and *S. exigua)* using the high concentration 119 OD.

TABLE (II)

Mortality percentage and LD25, LD50 and LD90 values of *Sl* GV versus 2*nd* larval instar of *S. littoralis*.

| Replicate No. | % Mortality at indicated virus doses (OD) | | | | | Estimated LD values | | |
|---|---|---|---|---|---|---|---|---|
| | 119 | 11.9 | 1.19 | 0.119 | 0.0119 | LD25 | LD50 | LD90 |
| 1 | 20/20* (100) | - | 22/39 (56.41) | - | 4 36 (11.11) | | | |
| 2 | 16/19 (84.21) | 9 19 (47.37) | 6 19 (31.57) | 1 9 (11.11) | 1 17 (5.88) | | | |
| 3 | 5 5 (100) | 9 11 (81.82) | 9 12 (75) | 4 8 (50) | 4 10 (40) | | | |
| Total | 41/44 (93.18) | 18/30 (60) | 37/70 (52.86) | 5 17 (29.41) | 9 63 (14.28) | 0.078 | 1.048 | 124.3 |

y = 17.886 X -3.381;

*No. of virus dead larvae/total number of tested larvae; percentages are between brackets.

TABLE (III)

Mortality percentage and LD25, LD50 and LD90 values of *Sl* GV versus 3*rd* larval instar of *S. littoralis*.

| Replicate No. | % Mortality at indicated virus doses (OD) | | | | | Estimated LD values | | |
|---|---|---|---|---|---|---|---|---|
| | 119 | 11.9 | 1.19 | 0.119 | 0.0119 | LD25 | LD50 | LD90 |
| 1 | 28/34* (82.35) | 14/23 (60.86) | 15/33 (45.45) | 6 14 (42.86) | 6 22 (27.27) | | | |
| 2 | 10 10 (100) | 4 6 (66.67) | 3 9 (33.33) | 4 14 (28.57) | 2 8 (25) | | | |
| 3 | 31/32 (96.87) | 19/27 (70.37) | 19/37 (51.53) | 14/40 (35) | 9 38 (24) | | | |
| 4 | 28/26 (96.15) | 13/30 (43.33) | 10 29 (34.48) | 7 22 (31.81) | (6/20) 30% | | | |
| Total | 94/102 (91.36) | 50/86 (53.94) | 47/108 (37.87) | 31/90 (27.78) | 23/88 (18.75) | 0.09 | 1.62 | 135.98 |

y = 16.286 X -3.048;

*No. of virus dead larvae/total number of tested larvae; percentages are between brackets.

TABLE (IV)

Mortality percentage and LD25, LD50 and LD90 values of *Sl* GV versus 4*th* larval instar of *S. littoralis*.

| Replicate No. | % Mortality at indicated virus doses (OD) | | | | | | Estimated LD values | | |
|---|---|---|---|---|---|---|---|---|---|
| | 119 | 23.8 | 11.9 | 1.19 | 0.119 | 0.0119 | LD25 | LD50 | LD90 |
| 1 | 11/11* (100) | 10 13 (76.92) | 10 16 (62.60) | 5 12 (41.67) | 0 | 0 | | | |
| 2 | 9 18 (50) | 10 29 (34.48) | 6 24 (25) | 3 17 (17.65) | 0 | 0 | | | |
| 3 | 13/26 (50) | 19/27 (70.37) | 6 39 (15.85) | 3 46 (6.52) | 0 | 0 | | | |
| Total | 33/55 (60) | 27/70 (38.57) | 22/79 (27.85) | 10 75 (13.33) | 0 | 0 | 14.45 | 40.74 | 214.54 |

y = 11.257 X -11.476;

*No. of virus dead larvae/total number of tested larvae; percentages are between brackets.

TABLE (V)
Calculation of LT25 and LT50 values of *S. littoralis* GV from LD experiments.

| Insect instar | Tested dose (OD) | LT25 (in days) | LT50 (in days) |
|---|---|---|---|
| *2nd* | $5.9 \times 10^{-1}$ | 18.78 | 28.1 |
| | $5.9 \times 10^{-2}$ | 20.53 | 32.27 |
| | $5.9 \times 10^{-3}$* | 35.97 | - |
| *3rd* | $5.9 \times 10^{-1}$ | 10 | 15 |
| | $5.9 \times 10^{-2}$ | 14.71 | 23.1 |
| | $5.9 \times 10^{-3}$* | 16.98 | - |
| *4th* | $5.9 \times 10^{-1}$ | 16 | 26.22 |
| | $1.19 \times 10^{-1}$* | 17.12 | - |

* Rate of mortality did not reach the 50%.

TABLE (VI)
Calculation of LT25 and LT50 values of *S. littoralis* GV from LC experiments.

| Insect instar | Tested Conc. (OD) | LT25 (in days) | LT50 (in days) |
|---|---|---|---|
| *2nd* | 119 | 23.15 | 37.37 |
| | 1.19 | 24.2 | 36.32 |
| | 0.0119* | 48.85 | - |
| *4th* | 119 | 11.46 | 18.12 |
| | 11.9 | 13.02 | 21.46 |
| | 0.0119* | 19 | - |

* Rate of mortality did not reach the 50%.

TABLE (VII)
Susceptibility of different lepidopterous species to infection with *S. littoralis* GV at a dose of 119 DO.

| Family | Test insect species | No. of tested larvae | No. of virus dead larvae | % Mortality response |
|---|---|---|---|---|
| Noctuidae | *S. littoralis* | 145 | 97 | 66.89 |
| | *S. exigua* | 94 | 33 | 35.11 |
| | *Agrotis ipsilon* | 258 | 0 | 0 |
| | *Mythimna loreyi* | 66 | 0 | 0 |
| Pieridae | *Artogeia rapae* | 20 | 0 | 0 |
| Pyralidae | *Galleria mellonella* | 20 | 0 | 0 |
| Gelechiidae | *Pectinophora gossypiella* | 20 | 0 | 0 |

## DISCUSSION

In spite of the late appearance of larval mortality with GV, the early instars were more susceptible to viral infection than the later ones. These results are

parallel to those of Crook and Brown (1982), and Sheppard and Stairs (1977). Based on the LC50 values, the *2nd* instar larvae were more susceptible to *Sl* GV than the *4th* ones, but the LD50 values for the *2nd* and *3rd* instars were similar. The LD50 for *3rd* instar was 1.55 times that of the *2nd* one, while the LD50 for *4th* instar was 38.65 and 24.9 than that of the *2nd* and *3rd* instars, respectively. The LD50 for the *2nd* and *3rd* instars were approximately similar because in the bioassay tests, both the late *2nd* and early *3rd* instar larvae were used. However, a noticeable difference in the LD50 values between the *3rd* and *4th* larval instars were observed. These results agree with those of Easwaramoorthy and Jayaraj (1993), who reported that the LD50 values of the *3rd* and *4th* instar larvae of *Chilo sacchariphagus indicus* GV were 533.3 and 2666.9 IBs/larva, respectively. In this respect, Elnagar *et al.*, (1983) suggested that younger larvae, are likely to be the suitable target for virus treatment where less virus concentration is needed and faster mortality could be attained before serious damage occurs to the crop by older larvae.

In comparison with the NPV's, GV's are generally more selective viruses, (Ignoffo, 1968, Payne *et al.*, 1981 and Groner, 1986). Obtained results also demonstrated that *Sl* GV infected the larvae of the alternative host *S. exigua*, this result agrees with the findings of Smith and Rivers (1956), Ripa *et al.*, 1979), Crook (1986), Zethner and Ogaard (1982) and Easwaramoorthy and Jayaraj (1987) with other GVs. The *Sl* GV failed to cause infection in insect species from different genera belonging to the same family e.g., *A. ipsilon* or *M. loreyi* (Noctuidae). In contrast, *L. pomonella* GV infects insects from different genera in the same family (Tortricidae) (Huber 1978). *Plodia interpunctella* and *Cadra cautella* GV's have a cross transmission among their hosts (Hunter and Hoffman 1972). In most cases in contrast to NPV-induced mortality, mostly in the *1st* and *2nd* instars, GV-treated larvae did not die before the last instar, so a remarkable expanding in the duration of infected larval instars was clearly observed, while those of control had been emerged to adults. Mortality in *H. armigera* due to GV never occurred before the *4th* instar, but mostly just before pupation (Hamm, 1982). Present results on time mortality are in harmony with those obtained by Hamm (1982) and Benz (1963). The GV-infected larvae showed a change into a whitish or brownish colour, particularly on the ventral side, and some of these larvae were larger than the normal ones. It is possible that, *Sl* GV may represent an important complementary agent alongside with *S. littoralis* NPV in the IPM programme of this pest.

## SUMMARY

Standard bioassay studies on *S. littoralis* granulosis virus (*Sl* GV) were carried out in the laboratory to determine values of the lethal concentration (LC) and lethal dose (LD) of virus for the different instars of *S. littoralis* larvae. The LC25, LC50 and LC90 values were determined for both the *2nd* and *4th* larval instar, these values were 0.01, 0.052 & 1.17, and 0.036, 0.831 & 288.4 OD/1.5 $cm^2$

of diet, respectively. The LD50 values were determined for the 2*nd*, 3*rd* and 4*th* larval instar; as $5.2 \times 10^{-3}$, $8.1 \times 10^{-3}$ and $2 \times 10^{-1}$ OD/larva, respectively. Median lethal time, increased with decreased virus dose, and was longer for younger instars than older ones. The host range of *Sl* GV was studied for *Spodoptera exigua, Pectinophora gossypiella, Galleria melonella, Agrotis ipsilon, Artogeia rapae,* and *Mythimna loreyi*. The *Sl* GV infected only *S. exigua* which belongs to the same genus of the homologous host.

## REFERENCES


ABBOTT, W.W. (1925) : Method of computing the effectiveness of an insecticide, *(J. Econ. Entomol., 18 (1) : 265-267).*

ABOL-ELA, S.; G. FEDIERE; A. NOUR-EL DIN; O. KAMISS, and M. SALAH, (1994) : Restriction endonucleases and diagnosis of the granulosis virus isolated from *S. littoralis* (Boisd). in West Africa and multiplied in Egypt. *(Bull. Fac. Agric. Cairo Univ , 45 : 919-932).*

ABUL-NASR. S. (1956) : Polyhedrosis - virus disease on cotton leafworn, *Prodenia litura* F. *(Bull. Soc. ent. Egypt, 40 : 321-332).*

*BENZ, G. (1963) : A nuclear polyhedrosis of Malacosoma alpicola* (Staudinger). *(J. Insect Pathol., 5 : 215-241).*

BOUCIAS. D.G. and G.L. NORDIN, (1977) : Susceptibility of *Hyphantria cunea* to a granulosis virus isolated from *Diacrisia virginica. (J. Invertebr. Pathol., 30 : 377-380).*

CHANGE, P. and Y. TANADA, (1978) : Serological study on the transmission of a granulosis virus of the armyworm, *Pseudaletia unipuncta* (Lepidoptera-Noctuidae), to other lepidopterous species. *(J. Invertebr. Pathol., 31 : 106-117).*

CROOK, N.E. (1986) : Restriction enzyme analysis of granulosis viruses isolated from *Artogeia rapae* and *Pieris brassicae. (J. Gen. Virol., 67 : 781-787).*

CROKK, N.E. and J.D. BROWN, (1982) : Isolation and characterization of a granulosis virus from the tomato moth, *Lacanobia oleracea* and its potential as a control agent. *(J. Invertebr. Pathol., 40 : 221-227).*

EASWARAMOORTHY, S. and S. JAYARAJ, (1987) : Cross infectivity of granulosis viruses infecting *Chilo infuscatellus* Snel. and *C. sacchariphagus indicus* (Kapur). *(J. ent. Res., 11 (2) : 170-174).*

EASWARAMOORTHY, S. and S. JAYARAJ, (1993) : Studies on the granulosis virus of the sugarcane internode borer, *Chilo sacchariphagus indicus* (Kapur). *(Sugarcane 3 : 11-14).*

ELNAGAR, S., and M.A.K. EL-SHEIKH, (1989) : The Baculovirus, as a promising biocontrol element in the pest management of the cotton leafworm in Egypt. *(Proc. Int. DLG-Symposium on Integrated Pest Management in Tropical and Subtropical Cropping Systems, Bad Durkhein, Germany, Feb., 8-15, Vol. II, pp. 669-683).*

ELNAGAR, S., M.F.S. TAWFIK, and T. ABDEL-RAHMAN, (1983) : The susceptibility to nuclear polyhedrosis virus among laboratory population of *Spodoptera littoralis* (Boisd.). *(Z. ang. Ent., 96 : 459-463).*

FRANCKI, R.I.B., C.M.FAUQUET, D.L. KNUDSON, and F. BROWN, (1991) : Classification and nomenclature of viruses, Fifth report of the International Committee on Taxonamy of Viruses. *(Archives of Virology. Supplementum 2. Springer-Verlag. Eds. pp. 452).*

GRONER, A. (1986) : Specificity and Safety of baculoviruses, *(in the Biology of Baculoviruses, Vol. 1, Granados, R.R. and Federici, B.A., Eds., pp. 177-201).*

HAMM, J.J. (1982) : Extension of the host range for a granulosis virus from *Heliothis armigera* from South Africa. *(Environ. Entomol., 11 (1), 159-160).*

HUBER, J. (1978) : About the host spectrum of the codling moth granulosis virus, in Safety Aspects of Baculoviruses as Biological Insecticides, Miltenburger, H.D., Ed., Julich, 75.

HUNTER, D.K. and D.F. HOFFMAN, (1972) : Cross infection of a granulosis virus of the *Cadra cautella* with observations on its ultrastructure in infected cell of *Plodia interpunctella. (J. Invertebr. Pathol., 20: 4-10).*

IGNOFFO, C.M. (1968) : Specificity of insect viruses, *(Bull. Entomol. Soc. Am., 14: 265-276).*

KAMISS, O.A. (1991) : Genetic variations among different geographical isolates of *Spodoptera littoralis* NPV and their stability against pesticide residues. *(M.Sc. Thesis, Fac. of Agric. Cairo University).*

NARAYANAN, K. (1985) : Susceptibility of *Spodoptera litura* (F) to a granulosis virus. *(Current Science, India, 54 (2) : 1288-1289).*

PAYNE, C.C. (1981) : The susceptibility of the pea moth, *Cydia nigricana,* to infection by the granulosis virus of the codling moth, *Cydia pomonella. (J. Invertebr. Pathol., 38 : 71).*

PAYNE, C.C., G.M. TATCHELL, and C.F. WILLIAMS, (1981) : The comparative susceptibilities of *Pieris brassicae* and *P. rapae* to a granulosis virus from *P. brassicae. (J. Invertebr. Pathol., 38, 273).*

RIPA, R., G.M. TATCHELL, and C.C. PAYNE, (1979) : The susceptibilities of *Pieris brassicae* and *P. rapae* to a granulosis virus, in Progress. *(Invertebrate Pathology, Proc. Int. Colloq. Invertebr. Pathol., September 11-17, 1978, 165).*

SHEPPARD, R.F. and G.R. STAIRS, (1977) : Dosage-mortality and time-mortality studies of a granulosis virus in a laboratory strain of the codling moth, *Laspeyresia pomonella. (J. Invertebr. Pathol., 29 (2) : 216-221).*

SHOREY, H.H. and R.L. HALE, (1965) : Mass rearing of the larvae of nine Noctuide species on a simple artificial medium. *(J. Econ. Entomol., 58 : 522-524).*

SMITH, K. and C.F. RIVERS, (1956) : Some viruses affecting insects of economic importance. *(Parasitology, 46 : 235).*

TANADA, Y. (1959) : Synergism between two viruses of the armyworm, *Pseudaletia unipuncta* (Haworth), (Lep., Noctuidae). *(J. Insect. Pathol., 1 : 215).*

TANADA, Y. (1964) : A granulosis virus of the codling moth, *Carpocapsa pomonella* (Linnaeus) (Olethreutidae, Lepidoptera). *(J. Insect Pathol., 6 : 378-380).*

TANADA, Y. and T. HESS, (1991) : Baculoviridae. Granulosis Viruses In "Atlas of Invertebrate Viruses". *(J. R. Adams and J. R. Bonami, pp. 227-257).*

TOMITA, K.O. and T. EBIHARA, (1982) : Cross-transmission of the granulosis virus of the *Hyphantria cunea* Drury (Lepidoptera : Arctiidae), to other lepidopterous insect species, *(Jpn. J. Appl. Entomol. Zool., 26 : 224).*

TOMPKINS, J. (1991) : Purification of invertebrate viruses. In "Atlas of invertebrate viruses" by *(J.R. Adams and J.R. Bonami., pp. 31-40).*

ZETHNER, O. and L. OGAARD, (1982) : Cross-transmission experiments with *Agrotis segetum* granulosis virus, *(Proc. 3rd Int. Col. Invertebr. Pathol., 15th Ann. Meet. Soc. Invertbr., University of Sussex Brighton, U.K.).*