Relative Virulence of Polyhedrosis Viruses and
Host-Resistance in the Silkworm, *Bombyx mori* L.
(Lepidoptera : Bombycidae)¹

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From the studies made on the host and virus relationships among various strains of the silkworm, it has been demonstrated that the two cytoplasmic-polyhedrosis viruses, forming the tetragonal shaped and the hexagonal shaped polyhedra, are equal in virulence. On the other hand, the cytoplasmic-polyhedrosis virus seemed to be more infectious toward the silkworm than the nuclear-polyhedrosis virus. General existence of a moderate but significant relationship between the interstrain susceptibilities to nuclear- and cytoplasmic-polyhedrosis viruses indicated that the mechanisms of resistance of the host to both the virus infections are similar to a greater extent.

INTRODUCTION

The virulence of viruses from different insect species has been quantitatively studied (STAIRS, 1964), and the comparison of the virulence of viruses from the hosts which were geographically entirely isolated has been made (OSSOWSKI, 1960; DAVID and GARDINER, 1965). STEINHAUS and MARTIGNONI (1962) pointed out the definition of virulence as the ability of a microorganism to invade and injure the tissues of its host or the relative capacity of a microorganism to overcome the body defences of the host. However, there are many practical problems concerned with the measurement of the virulence of viruses. In the interaction of a virus with its host, both members of this complex have an important role in determining the outcome which is a measure of the virulence of the virus, and each in turn is subject to factors in the microenvironment in which this contact occurs.

On the other hand, it has been ascertained that there is a wide range of susceptibility to peroral infection with cytoplasmic-polyhedrosis virus among various silkworm strains (ARUGA and WATANABE, 1964). Therefore, when the incidence or mortality of a population from an infectious disease is used as an index of the degree of virulence of the infection, the host-resistance may be one of the important factors affecting the measurement of the virulence. Hence, when the virulence of viruses to the silkworm is compared, it is desirable that the experiment should be planned to use the various silkworm strains with different sus-

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ceptibility to each of the virus.

This study was undertaken to gain some information about the relative virulence of nuclear- and cytoplasmic-polyhedrosis viruses by comparing relationship between the viruses and host, using several silkworm strains as host materials.

MATERIALS AND METHODS

The third-instar larvae of 12 silkworm strains and the fourth-instar larvae of 14 strains were used in this study. Thoroughly washed suspensions of nuclear polyhedra, tetragonal (TC) and hexagonal (HC) shaped cytoplasmic polyhedra were standardized to contain $1 \times 10^{8.5}$ polyhedra/ml respectively. Each stock was serially diluted to obtain another five virus concentrations; 10^8 , $10^{7.5}$, 10^7 , $10^{6.5}$ and 10^6 polyhedra per ml.

The virus suspension of each concentration was smeared on mulberry leaves and fed to the silkworm larvae for 20 hours. All larvae were reared at 25°C. On the sixth day after each virus administration, the tested larvae were dissected and the number infected with each polyhedrosis was ascertained from microscopical observation of the tissue smear. All data were transformed and analyzed by the probit method (FINNEY, 1952).

RESULTS AND DISCUSSION

Relative virulence of two cytoplasmic-polyhedrosis viruses

Interstrain comparisons based on the median infective concentration (IC_{50}) for

Fig. 1. Relationship among susceptibilities of various silkworm strains to two cytoplasmic-polyhedrosis viruses, forming tetragonal shaped (TC) and hexagonal shaped (HC) polyhedra. Strains plotted in this figure and log IC_{50} values have been stated in Table 1. Tested larvae were in the third-instar and reared in summer, 1963.

two cytoplasmic-polyhedrosis viruses, TC and HC, were carried out with the third-instar larvae reared in summer, obtaining the results shown in Table 1.

Table 1. SUSCEPTIBILITIES OF THE THIRD-INSTAR LARVAE TO NUCLEAR- AND CYTOPLASMIC-POLYHEDROSIS VIRUSES

Silkworm strains	Peroral inoculation of								
	TC-virus ^a			HC-virus ^b			N-virus ^c		
	n ^d	log IC ₅₀ ^e	S.E. ^f	n	log IC ₅₀	S.E.	n	log IC ₅₀	S.E.
N 1	74	5.45	.142	74	5.45	.165	100	6.90	.183
NBC-10	72	5.80	.126	73	6.05	.113	99	6.80	.098
Shuka	74	6.10	.096	71	6.65	.108	100	6.85	.086
Okusa	74	6.25	.084	76	6.60	.122	100	7.60	.162
SN-7	125	6.75	.125	125	7.00	.224	150	6.40	.187
C 124	125	6.85	.280	125	6.10	.196	150	6.35	.097
Hosho	75	6.85	.108	73	7.00	.094	100	7.05	.126
SC-7	125	6.90	.366	125	7.00	.238	150	6.40	.322
OCC-9	75	6.95	.244	74	7.50	.132	99	7.85	.143
N 124	75	7.70	.324	72	7.80	.187	100	7.50	.176
Daizo	125	8.45	.232	125	8.50	.146	149	8.90	.166
NBN-10	72	8.60	.227	69	8.25	.162	100	7.55	.094
Mean log IC ₅₀		6.89			6.99			7.18	

^a Cytoplasmic-polyhedrosis virus forming tetragonal polyhedra; ^b Cytoplasmic-polyhedrosis virus forming hexagonal polyhedra; ^c Nuclear-polyhedrosis virus; ^d Total number of tested larvae; ^e Infective concentration caused 50 per cent infection (number of polyhedra per 1 ml); ^f Standard error of log IC₅₀.

Larvae were reared in summer, 1963.

Log IC₅₀ values for TC virus varied from 5.45 to 8.60 (6.89 as mean value) for different strains, *i.e.*, the strain with highest value was about 1,000 times more resistant than the most susceptible strain. The same interstrain variation of log IC₅₀ was also observed in the case of HC-virus infection; log IC₅₀ values varying from 5.45 to 8.50 (6.99 as mean value).

In order to clarify further relationships among the virus virulence under investigation, IC₅₀ values for TC-virus of tested strains were plotted against the value of each strain for the HC-virus (Fig. 1). As shown in Fig. 1, a linear relationship for log IC₅₀ values was observed between TC- and HC-viruses and the susceptibility of each strain to both the viruses noted to be highly correlated ($r = +0.928$, $p < 0.01$). The foregoing results of Table 1 and Fig. 1 indicate that the two cytoplasmic-polyhedrosis viruses, TC- and HC-viruses, are equally virulent and the host mechanisms of resistance to both the viruses are almost similar.

Interstrain susceptibilities to nuclear- and cytoplasmic-polyhedrosis viruses

Interstrain comparison of log IC₅₀ value for nuclear-polyhedrosis virus was made with the third-instar larvae reared in summer. As shown in Table 1, the values

varied from 6.35 to 8.90 (7.18 as mean value). Comparison between the mean log IC_{50} value for the nuclear-polyhedrosis virus and that for TC-virus or HC-virus could serve as an estimate of their relative virulences. From the foregoing results, it is appeared that the two cytoplasmic-polyhedrosis viruses are more infectious toward the silkworm than the nuclear-polyhedrosis virus.

Since the virus-host relationships may be altered by host physiology and environment, in the next series of experiments, the fourth-instar larvae of another 14 strains were reared in fall and used for similar analysis of interstrain susceptibilities to the cytoplasmic- and nuclear-polyhedrosis viruses (Table 2). Log IC_{50}

Table 2. SUSCEPTIBILITIES OF THE FOURTH-INSTAR LARVAE TO NUCLEAR- AND CYTOPLASMIC-POLYHEDROSIS VIRUSES

Silkworm strains	Peroral inoculation of					
	TC-virus			N-virus		
	n	log IC_{50}	S.E.	n	log IC_{50}	S.E.
Cambodge	100	6.55	.119	100	7.75	.077
OCC-9	100	6.60	.134	100	8.95	.212
C 4 (<i>lem</i>)	76	7.20	.142	80	6.75	.168
C 4 (+)	100	7.25	.245	100	7.05	.200
Shungetsu	100	7.30	.091	100	8.20	.307
Daizo × Cambodge	100	7.55	.085	100	8.35	.098
C 108	100	7.90	.075	100	8.00	.126
Daizo (<i>lem</i>)	80	8.00	.192	80	9.25	.316
C 108 × Daizo	100	8.05	.191	100	8.70	.224
Cambodge × Daizo	100	8.55	.305	100	9.05	.224
C 108 (<i>lem</i>)	100	8.60	.154	100	8.05	.111
C 108 (+)	98	8.70	.111	100	9.25	.248
Daizo (+)	100	8.75	.366	100	9.55	.285
Daizo	100	8.95	.268	100	9.30	.193
Mean log IC_{50}		7.85			8.44	

Larvae were reared in fall, 1963.

values of the fourth-instar larvae for TC-virus varied from 6.55 to 8.95 (7.85 as mean value) with strains and a large interstrain difference in susceptibility was observed. On the other hand, interstrain susceptibility to nuclear-polyhedrosis virus varied from 6.75 to 9.55 (8.44 as mean value) for log IC_{50} . From the results of Tables 1 and 2, as far as mean IC_{50} value is concerned, it may be concluded that the cytoplasmic-polyhedrosis virus is more infectious to the silkworm than the nuclear-polyhedrosis virus.

However, since the IC_{50} values in this study were stated as the number of polyhedra per 1 ml, it may not be emphasized here that the cytoplasmic-poly-

hedrosis virus is truly more virulent than the nuclear-polyhedrosis virus. Because, it is known that the polyhedra vary in size and in the number of virus particles which they contain, and thus counting the polyhedra of both the viruses is likely to give a different estimate in the number of particles present. But when the log IC_{50} values of various silkworm strains for the TC-virus were plotted against their values for the nuclear-polyhedrosis virus, a linear virus-host relationship was observed and it clearly indicated the relative virulence between both the viruses (Figs. 2 and 3).

Fig. 2. Relationship among susceptibilities of various silkworm strains to cytoplasmic-polyhedrosis virus (TC-virus) and nuclear-polyhedrosis virus (N-virus). Strains plotted in this figure and their log IC_{50} values have been stated in Table 1. Tested larvae were in the third-instar and reared in summer, 1963.

Fig. 3. Relationship among susceptibilities of various silkworm strains to cytoplasmic-polyhedrosis virus (TC-virus) and nuclear-polyhedrosis virus (N-virus). Strains plotted in this figure and their log IC_{50} values have been stated in Table 2. Tested larvae were in the fourth-instar and reared in fall, 1963.

Susceptibility of each silkworm strain to both the viruses in the third-instar is significantly correlated ($r = +0.569$, $p < 0.05$). Furthermore, in the case of the fourth-instar larvae, a moderately linear relationship was recognizable between the two values of log IC_{50} for nuclear- and cytoplasmic-polyhedrosis viruses in each of the strain ($r = +0.591$, $p < 0.05$). Hence, it may be interpreted that the mechanism of the host resistance to peroral infection with each of the viruses is partly common.

Nuclear- and cytoplasmic-polyhedrosis viruses differ in their route of infection to susceptible tissues. In the infection of nuclear-polyhedrosis virus the midgut is not susceptible and the virus, when ingested, must pass through it to contact with the susceptible tissues (epidermis, fat body, blood, etc.). On the other hand, steps of the infection with cytoplasmic-polyhedrosis comprises the entering of the virus into the lumen of the gut, the adsorption of virus particle to the cell wall of

midgut and the penetration of the particle into the cell interior where the virus finally replicates. Therefore, if the above mentioned interpretation holds correct, it would appear that the inactivation of virus in the alimentary tract (AIZAWA, 1953, 1962) and the inhibitory mechanism of the midgut cells against virus adsorption are greatly responsible for the host resistance to infection with both the nuclear- and cytoplasmic-polyhedrosis viruses.

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