

PN-ABM-567

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 11/4/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C5-144
until Dept 88

Attachment

US-AID GRANT: DPE-5544-G-SS-6031-01

Report Covering half year period until September, 1988

CS-144

A. Training of Mr. Fred Oduol from Kenya.

Dr Naomi Kislev devoted most of her time to the training Mr. F. Oduol on: hygienic handling of insect colonies; isolation of NPV and assay of their virulence on larvae and insect cells in culture; replication of *S. exempta* NPV in cell culture; media preparation for cell culture, and bacteria; maintenance of insect cell cultures. He is now carrying on experiments on inoculation of insect cells with NPV and diseased larval homogenates.

Mr. Oduol participated on the following courses at the Feinberg Graduate School of Weizmann Institute of Science: Electrophoresis and Protein Blotting; Chemistry and Structure of Proteins; Gene Regulation and Expression in Eukaryotic Cells.

B. Scientific Results

We are completing now the biological evaluation of *S. exempta* NPV virulence on *S. littoralis* larvae, and continuing our studies on the replication of *S. exempta* NPV in *S. frugiperda* and *S. littoralis* cells in culture.

In experiments where more than 1000 *S. littoralis* 2nd and 3rd instar larvae were inoculated Per Os, we were unable to demonstrate replication of *S. exempta* NPV or polyhedra (PIB) production.

As inoculum we used two sources: (a) homogenates of diseased frozen *S. exempta* larvae brought from Kenya. Similar diseased larvae were used in Kenya to inoculate *S. exempta* 3rd instar larvae and produced 95-100% mortality within 4-5 days p.i. (b) Sucrose gradient purified polyhedra. (We had demonstrated their virulence by treating with alkali and purifying viruses which were used to challenge cell in culture). Virus

replication and polyhedra formation were observed in *S. frugiperda* cell line, but show only limited replication in *S. littoralis* cell line. In a previous report (Kislev, 1986) we found that *S. littoralis* NPV replicate readily in *S. littoralis* cell line and only limited replication in *S. frugiperda* cell line. Thus we have an indication of progeny virus produced according to its ability to replicate in these cell lines.

S. littoralis larvae were inoculated Per Os at a dose range from 4×10^2 to 5×10^7 PIB per larva. The inoculated larvae were checked daily to adult stage. No viral mortality or sign of viral disease were noticed; and development to adult stage was normal. Percent mortality of inoculated larvae was below or equal to mortality noted in uninoculated control larvae. There was no straight correlation between the concentration of PIB which were used per larva and the number of dead larvae. More than that, we did not find PIB or viral replication in cadavres, (see below).

<u>Experiment</u>	<u>Inoculum Source</u>	<u>Range of PIB</u> per larvae	<u>Percent mortality</u>	
			control	inoculated
1	Purified PIB, SINDO	8×10^3 to 4×10^7	1.4%	0.6%
2	Purified PIB, MAKENDE	4×10^2 to 4×10^7	4.2%	0.5%
3	Purified PIB, GERA	5×10^2 to 5×10^7	2.1%	0%
4*	4 larval homogenates, SINDO	3.5×10^6 to 7×10^6	9.7%	21.7%
5	same as above	1.5×10^2 to 3.5×10^6	5.6%	3%

*High mortality in this experiment stems from less hygienic conditions while conducting the experiment and contamination within the nursery colony. Most cadavres were checked for viral disease but polyhedra and viral replication were not observed; in some cadavres bacterial contamination was noticed; (see below).

During each experiment larvae, as well as pupa and cadavra, were taken out (on 2nd to 18 day p.i.) surface sterilized, homogenized, filter sterilized and/or treated with alkali to liberate viruses from PIB. The homogenates were used to challenge *S. frugiperda* and *S. littoralis* cell lines in culture. The inoculated cells were observed under phase inverted microscope on alternate days until 12 day p.i. We have used 17 *S. littoralis* larvae, 11 pupa and 20 larval cadavra (from the above experiments); no polyhedra were observed in either *S. frugiperda* or *S. littoralis* cell lines. On the other hand, when diseased *S. exempta* larvae (from SINDO, Kenya) were homogenized, treated as mentioned above and used as inoculum to challenge these cell lines, 10 out of 15 larval homogenates were infectious to *S. frugiperda* cell line and many PIB were observed, while only 2 out of 15 were producing PIB in *S. littoralis* cell line.

We concluded that *S. exempta* NPV is not virulent against *S. littoralis* larvae (within the range of concentration of PIB which were used) and cannot be used economically as biological control against this insect pest.

McKinley et al. (1981) reported LC_{50} of 2.9×10^3 PIB for *S. littoralis* NPV and for *S. exempta* NPV LC_{50} of 6.5×10^6 PIB on 1st Instar *S. littoralis* larvae. However when the *S. littoralis* larvae were inoculated by *S. exempta* NPV, the progeny viruses were identified as *S. littoralis* NPV.

In our studies only in one experiment (No. 4) mortality of inoculated *S. littoralis* larvae was higher than that of uninoculated, control larvae. We repeated that experiment and used the same preparation of *S. exempta* larval homogenates as inoculum for another colony of second instar *S. littoralis* larvae, (exp. No. 5). Mortality of the inoculated larvae was lower than the mortality of control larvae; and in both experiments the cadavra homogenates fail to replicate viruses in either *S. frugiperda* or *S. littoralis* cell lines. Our assumption is that in this particular experiment (No.4) we got unhealthy colony of larvae, and mortality was due to other reasons and not correlated to amount of

PIB consumed by the larva. We concluded that *S. exempta* NPV does not replicate readily in *S. littoralis* larvae when inoculated in amount up to 5×10^7 PIB/larvae, otherwise we would be able to detect diseased larvae and progeny viruses by the methods applied in our studies.

Literature

1. Kislev, N. (1986) *J. Invertabr. Pathol.* 47: 369-373
2. McKinley, D.J., Brown, D.A., Payne, C.C. and Harrap, K.A. (1981). *Entomophaga* 26: 79-90