

A NUCLEAR POLYHEDROSIS VIRUS OF  
*ANTICARSIA GEMMATALIS*:  
I. ULTRASTRUCTURE, REPLICATION,  
AND PATHOGENICITY<sup>1</sup>

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ABSTRACT

A nuclear polyhedrosis virus (NPV) isolated from the velvetbean caterpillar (VBC), *Anticarsia gemmatalis* Hubner, on soybean in Brazil is described. The virus is a multiple embedded virus (MEV) type of the baculovirus group, with symptoms, histology, ultrastructure, and mechanism of replication similar to other reported NPVs. Velvetbean caterpillar larvae are highly susceptible to the NPV. Susceptibility studies were negative for *Trichoplusia ni* (Hubner), *Pseudoplusia includens* (Walker), and *Spodoptera frugiperda* (J. E. Smith); however, *Heliothis zea* (Boddie) was susceptible. The potential role of the NPV in an integrated pest management program for the VBC on soybean is discussed.

The velvetbean caterpillar (VBC), *Anticarsia gemmatalis* Hübner, is a major pest of leguminous crops in the Southeastern United States. The VBC has long been a major pest of velvetbeans in Florida (Watson 1916); with the increased emphasis on soybean production in the state, it has also become the major defoliator of this important crop (Strayer and Greene 1974). The VBC overwinters in south Florida and migrates as far north as North Carolina and west as Texas. It is a key insect pest on soybean in that it initiates the first use of insecticides in the growing season; this destroys native parasites and predators, creating secondary outbreaks of other insect pests (stinkbugs, podworms, foliage feeders) and necessitates further pesticide use.

The entomopathogen *Nomuraea rileyi* (Farlow) Samson is the most significant natural enemy of the VBC in Florida and other southeastern states. Under certain environmental conditions, this fungus can decimate the caterpillar population, causing mortality outbreaks approaching 100% (Watson 1916, Allen et al. 1971). The only other known disease affecting the VBC in the United States is another fungus of the *Entomophthora* group (Carner et al. 1975).

A possible baculovirus of the nuclear polyhedrosis (NPV) type was first reported from the VBC by Steinhaus (1975) from a mixture of disintegrating specimens of *A. gemmatalis* and *Xylomyges* sp. from Peru. Later, Steinhaus and Marsh (1962) diagnosed an NPV from *A. gemmatalis* larvae collected on alfalfa in Peru. An NPV from *A. gemmatalis* was also listed

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in an FAO report on viruses associated with insect pests and disease vectors (Anonymous 1973).

The senior author isolated an NPV from moribund VBC larvae collected on soybean near Campinas, Brazil, in 1972. The virus, which occurs naturally in VBC populations, was found to reach infection levels of 15-20% late in the soybean seasons of 1973 and 1974 (R. N. Williams unpublished). The virus incidence level was reduced in late season because of a dramatic decline in VBC population caused by epizootics of *N. rileyi*.

In a recent report Gatti et al. (1977) noted the occurrence of a nuclear polyhedrosis virus in *A. gemmatalis* from southern Brazil. The authors indicated that the virus was an MEV type NPV. They failed, however, to define the tissue involved or the development of the virus.

In a comparative biochemical study of baculovirus polypeptides of *A. gemmatalis*, *Heliothis zea* (Boddie), *H. armigera* (Hubner), *Rachiplusia ou* (Guenée), *Spodoptera frugiperda* (J. E. Smith), and *Trichoplusia ni* (Hubner), Summers and Smith (1977) showed that the VBC NPV differed significantly from those of the other insect species.

This paper is the first of a series reporting studies conducted since 1973 to evaluate the potential of the NPV as a microbial control agent of the VBC on soybean.

#### METHODS AND MATERIALS

A stock solution of *A. gemmatalis* NPV was prepared by macerating infected late instar larvae in a tissue grinder with distilled water, and then filtering and purifying by differential centrifugation. Virus preparations were standardized on the basis of polyhedral inclusion body (PIB) counts with a Bright-Line hemacytometer.

Five day old larvae used for histological examination were exposed to the virus on a semi-synthetic medium inoculated with  $1.3 \times 10^4$  PIBs/mm<sup>2</sup> of the diet surface (Ignoffo 1965). Histological sections were prepared and stained by the azan technique of Hamm (1966).

Seven day old larvae used for electron microscope studies were exposed as described above and removed at 4, 8, 12, 24, 48, and 72 hr intervals. Infected tissue was fixed by placing sections in 4% glutaraldehyde and .1 M cacodylate buffer overnight at 8°C, post fixing in 1% osmium tetroxide, block staining with aqueous 2% uranyl acetate for 1 hr at room temperature, dehydrating in ethanol, and embedding in Spurr's low viscosity embedding medium, as directed by the manufacturer and using acetone as a transient solvent. Sections of 60-90nm were cut with a diamond knife on a Sorvall Porter-Blum MT-2 ultramicrotome and stained with 5% aqueous uranyl acetate and lead citrate (Reynolds 1963). Electron micrographs were made on a Hitachi HS-9 electron microscope at 75kv.

The susceptibility of VBC, *H. zea*, *T. ni*, *P. includens*, and *S. frugiperda* to NPV was studied by exposing test larvae to diet surfaces contaminated with different rates of PIB/mm<sup>2</sup> and held until death or pupation. In the case of those species showing no susceptibility, additional tests were conducted using virus concentrations of  $1.3 \times 10^4$  PIBs/mm<sup>2</sup>. Two replications of 25 individuals each were made using second instar VBCs and third instar larvae for the other species. Regular semi-synthetic diets for the specific species were used, except that formalin was not included. Previous tests showed some deactivation of the VBC NPV in media with this chemical.

The rate of mortality due to virus infection was determined on a daily and a cumulative basis. The average number of PIBs/larvae, or larval equivalent (LE), was calculated using inclusion body counts from 20 fifth larval instars. After inactive, moribund larvae were triturated, filtered once to remove tissue debris, and serially diluted, PIBs were counted.

## RESULTS

External symptoms of NPV-infected *A. gemmatalis* were similar to those described for other lepidopterous larvae (Drake and McEwen 1959). Tissue involvement differed slightly from that reported in other lepidopterous larvae (Smith 1967) in that epidermal and fat body tissues were the primary sites of infection, with other tissues affected only in the terminal stages of infection. Generally, virus-killed cadavers became deflated with their integument intact. Occasionally, some completely disintegrated, exhibiting the typical characteristics of NPV mortality.

The first evidence of infection was hypertrophy of the nuclei after 24 hr of exposure to the virus. Stained PIBs were first observed in fat body and epidermal cells after 48 hr (Fig. 1). After 5 days both tissues were completely infected, and some mortality occurred. The major portion of larval mortality occurred 6 to 8 days after exposure to the virus.

Electron microscope studies confirmed that the NPV was a multiple embedded virion (MEV) type with a 3 to 1 ratio of multiple to single virions, similar to the VBC NPV reported by Gatti et al. (1977) (Fig. 2). The number of virions per packet ranged from 2 to 9 with the majority ranging from 2 to 4. The mean size and standard deviation of individual free virions was calculated to be  $214 \pm 28$ nm long and  $14 \pm 4.7$ nm in diameter. Both single nucleocapsids and packets of nucleocapsids were surrounded by a typical, double layered envelope.

The polyhedrons varied greatly in size and ranged from triangular to typical polyhedral in form. The nucleoprotein polyhedron was surrounded by a membrane and consisted of a typical linear-patterned protein matrix (Fig. 3).

Virus development was similar to that described for other baculoviruses (Harrap 1972a, 1972b; Hughes 1972; Tanada and Hess 1976; Summers and Arnott 1969). Infection in the midgut resulted in the virions attaching to and penetrating the microvilli (Fig. 4-5) after 4 to 6 hr.

Infected fat body and epidermal cell nuclei showed typical alterations such as chromatin reduction, the appearance of virogenic stroma, and loss of the nucleolus and nucleocapsids associated with the virogenic stroma (Fig. 6). Fibrillar strands were not observed in the nuclei.

Although a few virions were observed in the cytoplasm (Fig. 7), multiplication appeared to be restricted to the nucleus. All cells examined contained free or enveloped single and multiple virions. No evidence was found to support the phenomenon reported by Hughes (1972) and Falcon and Hess (1977) of tissues producing only single or multiple embedded virions. The ratio of 3 multiple to 1 single embedded virion types was the general rule.

*Anticarsia gemmatalis* larvae were highly susceptible to the NPV. Recent studies indicate the LD<sub>50</sub> of 4 day old larvae to be within a range of 0.5-4 PIBs/mm<sup>2</sup> of diet surface area (G. E. Allen unpublished). The dosage

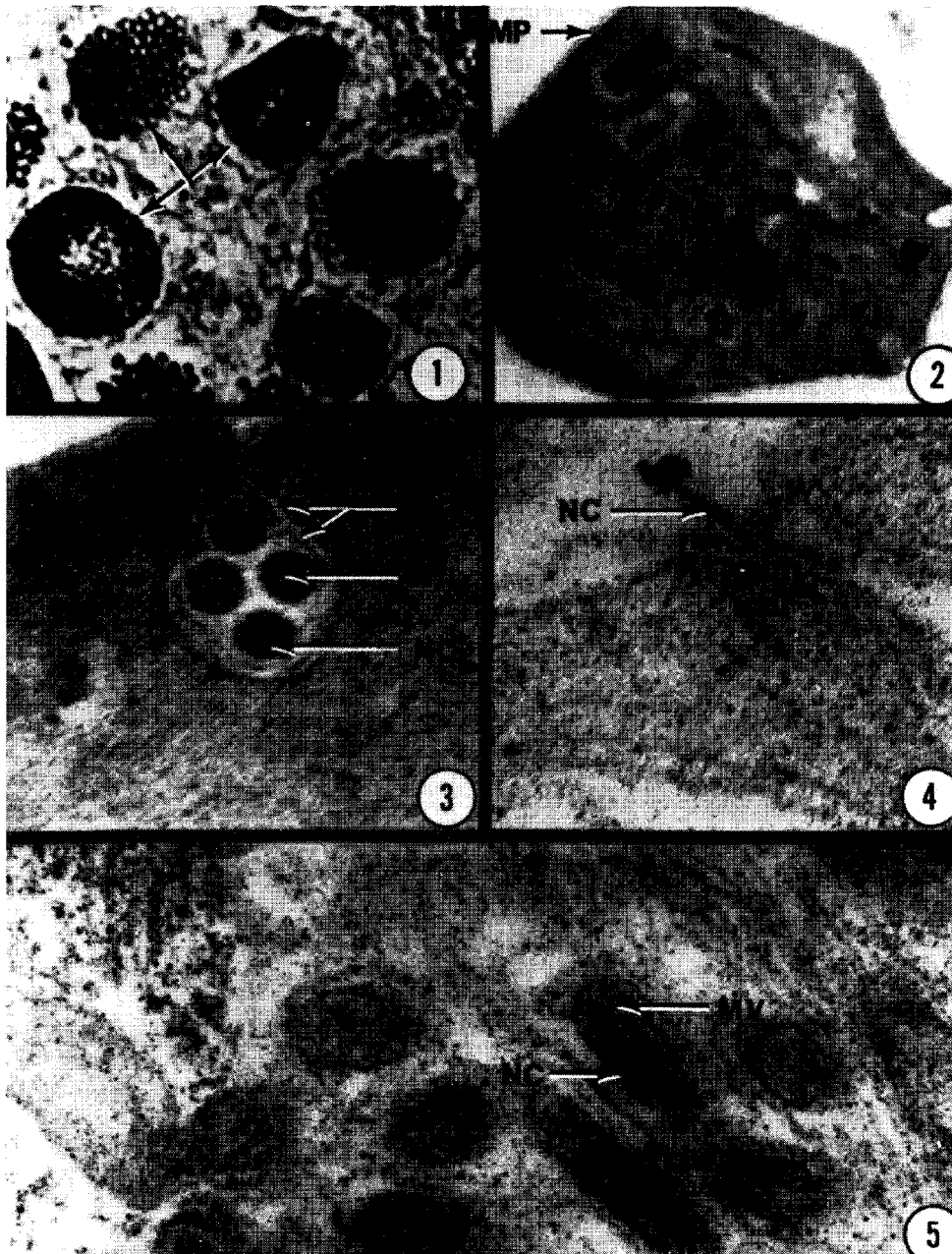


Fig. 1-5. Nuclear polyhedrosis virus from velvetbean caterpillar. Fig. 1. Advanced stage of infection showing nuclei of fat body cells impacted with polyhedral inclusion bodies. 3,700X. Fig. 2. Cross-section of PIB showing multiple and single virions and polyhedral membrane (PM). 42,517X. Fig. 3. Magnified multiple embedded virion showing the double layered envelope (E), nucleocapsid (NC), axial hole within the nucleocapsid (H), and the linear patterned protein matrix of the inclusion body. 230,000X. Fig. 4. Nucleocapsid (NC) attached to a microvilli (MV) cell in the midgut 4 hr. after infection. 171,450X. Fig. 5. Microvilli (MV) infected with a nucleocapsid (NC). 68,580X.

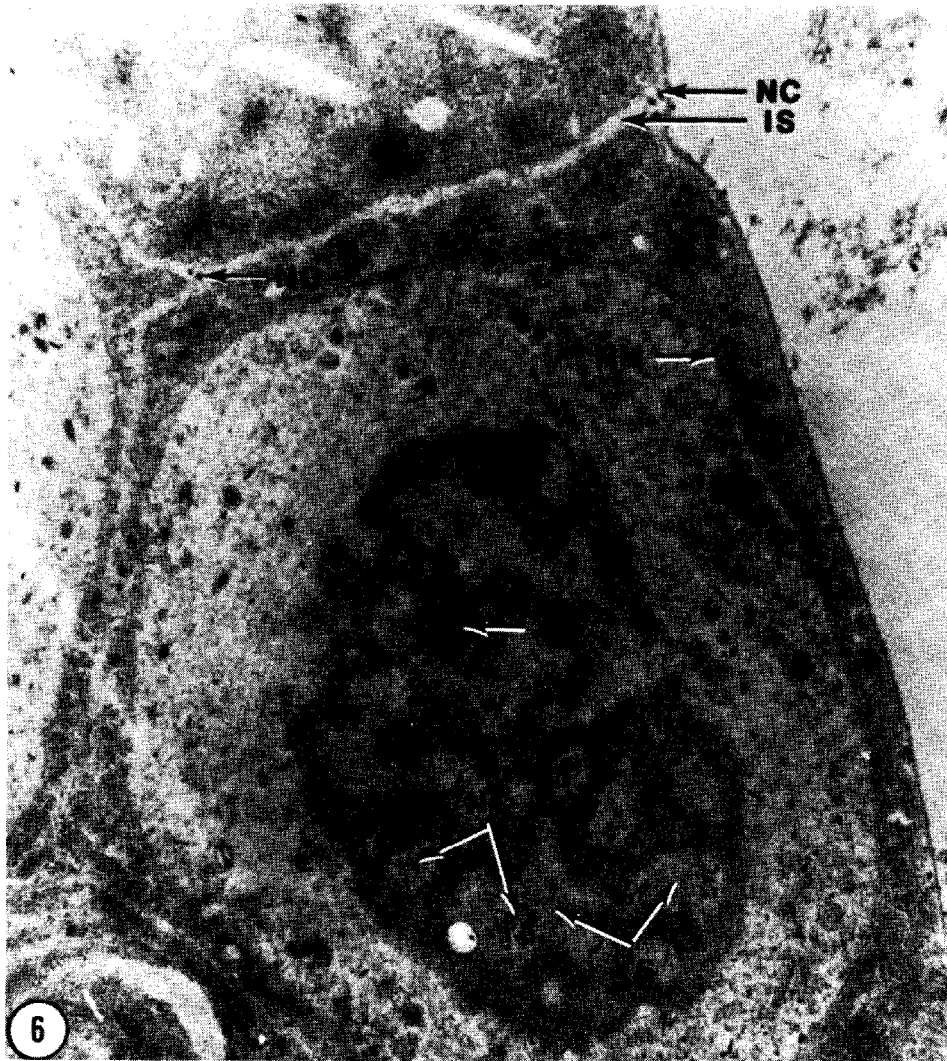


Fig. 6. Fat body cell in the early stage of infection showing hyperthrophied nucleus, nucleocapsids (NC) within the virogenic stroma (VS), chromatin (C), nucleocapsids within the intracellular spaces (IS), and nuclear membrane (NM). 13,297X.

range was based on those used to calculate dosage mortalities for the NPV of *Heliothis zea* (Allen and Ignoffo 1969). Based on larvae of comparable size and age, the VBC NPV appears to be 10 times more virulent than the *Heliothis* NPV. The activity of the virus has been substantiated in field tests using 30, 58, and 115 LE/acre where no differences among treatments were observed (Moscardi 1977).

The only other test species susceptible to the VBC NPV was *H. zea*. Virus from *H. zea* cadavers was harvested, examined by electron microscopy, and used to re-infect VBC larvae in order to determine the causative agent. The VBC is not susceptible to the *Heliothis* SEV NPV, the *Autographa* MEV NPV, or baculoviruses of *T. ni*, *P. includens*, and *S. frugiperda* (G. E. Allen unpublished).

Infected VBC larvae showed a typical loss of appetite after the 4th day,

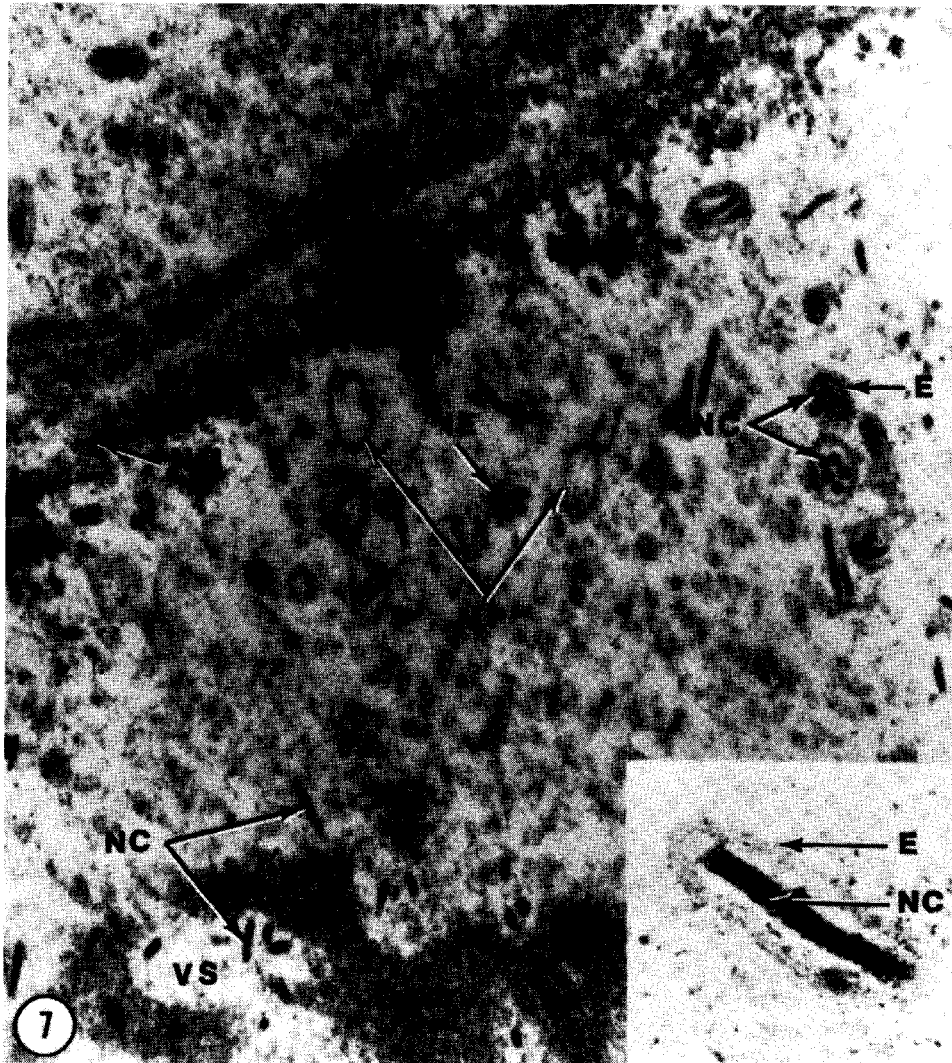


Fig. 7. Infected fat body cell showing developing virions with complete and incomplete envelopes (IE) and free membrane profiles (MP). Insert shows loosely formed double layered envelope (E) surrounding a single nucleocapsid (NC). 130,023X.

resulting in a loss of weight. Control larvae commenced to pupate on the 7th day, whereas those infected with NPV did not pupate at all. Some remained alive until the 10th day, however, and although development time was prolonged, the infected larvae ceased to feed after the 5th day.

The average number of PIBs/larvae (LE) was determined to be  $3 \times 10^8$  PIBs. This number is considerably lower than those reported for other lepidopterous larvae (Ignoffo 1965).

#### DISCUSSION

The symptoms, histology, ultrastructure, and mechanism of replication of the VBC baculovirus appear to be similar to those reported by various authors for other NPVs. The virus is highly virulent when compared with

the *Heliothis* NPV which is not produced commercially, and the *Autographa* now under consideration for federal registration.

Because of this high virulence and the foliage feeding habits of VBC larvae, the virus is considered to have outstanding potential as a microbial control agent in an integrated pest management program currently under development for *A. gemmatalis* in Florida. A key component of the program is the ability to monitor and predict mortality levels of the VBC due to the fungal pathogen *N. rileyi* (Kish and Allen 1977). These authors as well as Allen et al. (1971) have shown that *N. rileyi* epizootics are initiated when populations reach 1 larva/ft of row in soybean. However, because of the 15 to 20 day delay required for the development of *N. rileyi* epizootics capable of reducing larval populations, significant economic damage often occurs. The use of an insecticide during this critical period breaks the developmental cycle of the fungus by eliminating its natural substrate of VBC larvae. On the other hand, the use of low levels of the VBC NPV during this period could possibly suppress VBC populations below the economic threshold while simultaneously permitting the fungus to develop on the remaining population.

The utilization and effectiveness of the VBC baculovirus under field conditions and its compatibility with *N. rileyi* and recommended insecticides will be reported in future papers.

#### LITERATURE CITED

- ALLEN, G. E., G. L. GREENE, AND W. H. WHITCOMB. 1971. An epizootic of *Spicaria rileyi* on the velvetbean caterpillar, *Anticarsia gemmatalis*, in Florida. Fla. Ent. 54:189-91.
- ALLEN, G. E., AND C. M. IGNOFFO. 1969. The nucleopolyhedrosis virus of *Heliothis*: Quantitative in vivo estimates of virulence. J. Invertebr. Pathol. 13:378-81.
- ANONYMOUS. 1973. The uses of viruses for the control of insect pests and disease vectors. Food Agr. Organ. U.N. FAO Agr. Studies, no. 91; World Health Organ. Tech. Rep. Ser., no. 531.
- CARNER, G. R., M. SHEPARD, AND S. G. TURNIPSEED. 1975. Disease incidence in lepidopterous pests of soybeans. J. Georgia Ent. Soc. 10: 99-105.
- DRAKE, E. L., AND F. L. MCEWEN. 1959. Pathology of a nuclear polyhedrosis of the cabbage looper, *Trichoplusia ni* (Hübner). J. Insect Pathol. 1:281-93.
- FALCON, L. A., AND R. T. HESS. 1977. Electron microscope study on the replication of *Autographa* nuclear polyhedrosis virus and *Spodoptera* nuclear polyhedrosis virus in *Spodoptera exigua* J. Invertebr. Pathol. 29:36-43.
- GATTI, I. M., D. M. SILVA, AND I. C. CORSO. 1977. Polyhedrosis occurrence in caterpillars of *Anticarsia gemmatalis* (Hübner, 1818) in the south of Brazil. IRCS Med. Sci.: Cell Membrane Biol; Environ. Biol. Med.; Exp. Animals; Microbiol., Parasitology, Infectious Diseases 5:136.
- HAMM, J. J. 1966. A modified azan staining technique for inclusion body viruses. J. Invertebr. Pathol. 8:125-6.
- HARRAP, K. A. 1972a. The structure of nuclear polyhedrosis viruses. II. The virus particle. Virology 50:124-32.
- HARRAP, K. A. 1972b. The structure of nuclear polyhedrosis viruses. III. The virus particle. Virology 50:133-9.

- HUGES, K. M. 1972. Fine structure and development of two polyhedrosis viruses. *J. Invertebr. Pathol.* 19:198-207.
- IGNOFFO, C. M. 1965. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabricius). I. Virus propagation and its virulence. *J. Invertebr. Pathol.* 7:209-16.
- KISH, L. P., AND G. E. ALLEN. 1977. The biology and ecology of *Nomuraea rileyi* and a program for predicting its incidence on *Anticarsia gemmatalis* in soybean. Univ. Fla. Agr. Exp. Sta. Circ. (In Press).
- MOSCARDI, F. 1977. Control of *Anticarsia gemmatalis* (Hübner) on soybean with a baculovirus and selected insecticides and their effect on natural epizootics of the entomogenous fungus *Nomuraea rileyi* (Farlow) Samson. M. S. thesis, Univ. Fla.
- REYNOLDS, E. S. 1963. The use of lead citrate at high pH as electron opaque stain in electron microscopy. *J. Cell. Biol.* 17:208-13.
- SMITH, K. M. 1967. Insect virology. Academic Press, New York. 256 p.
- STEINHAUS, E. A. 1957. New records of insect-virus diseases. *Hilgardia* 26:417-20.
- STEINHAUS, E. A., AND G. A. MARSH. 1962. Report of diagnoses of diseased insects 1951-1961. *Hilgardia* 33:349-490.
- STRAYER, J., AND G. GREENE. 1974. Soybean insect management. Fla. Coop. Ext. Ser. Circ. 395. 15p.
- SUMMERS, M. D., AND H. J. ARNOTT. 1969. Ultrastructural studies on inclusion formation and virus occlusion in nuclear polyhedrosis and granulosis virus-infected cells of *Trichoplusia ni* (Hübner). *J. Ultrastruct. Res.* 28:462-80.
- SUMMERS, M. D., AND G. E. SMITH. 1977. Structural polypeptides of baculoviruses. *Virology* (In Press).
- TANADA, Y., AND R. T. HESS. 1976. Development of a nuclear polyhedrosis virus in midgut cells and penetration of the virus into the hemocoel of the armyworm, *Pseudaletia unipuncta* J. *Invertebr. Pathol.* 28:67-76.
- WATSON, J. E. 1916. Life-history of the velvetbean caterpillar (*Anticarsia gemmatalis* Hübner). *J. Econ. Ent.* 9:521-8.



