

# POLYHEDRAL VIRUSES INFECTING TWO FOREST INSECT PESTS, *SELIDOSEMA SUAVIS* AND *HELIOTHIS ARMIGERA*

SYD MOORE

Department of Microbiology, University of Otago, Dunedin

and

P. J. ALMA

Forest Research Institute, New Zealand Forest Service, Rotorua

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## ABSTRACT

The inclusion bodies of the cytoplasmic virosis of *Selidosema suavis* (Butler) are equilaterally triangular in profile and have mean vertical height of 1.93  $\mu\text{m}$ , s.e.m. 0.05  $\mu\text{m}$ . The non-occluded virions are 59 nm in diameter.

The inclusion bodies of two isolates of the nucleopolyhedrosis viruses found infecting *Heliothis* (= *Helicoverpa*) *armigera* (Hübner) are square in profile. The dimensions of the polyhedra and the virions of the two New Zealand isolates are given. The epizology of one New Zealand isolate is discussed.

## INTRODUCTION

In 1951-52 and 1956-57 infestations of *S. suavis* resulted in extensive damage to *Pinus radiata* D. Don in Eyrewell State Forest, Canterbury. A disease was observed to infect the caterpillars in 1957. A third outbreak of *S. suavis* started in the spring of 1960 but was controlled by this disease by February 1962. A cytoplasmic polyhedrosis virus was isolated from the diseased *S. suavis* and was diagnosed by the Crop Research Division, DSIR (New Zealand Forest Service 1960-62). Another virus may have been implicated as well; Steinhaus (1960) noted a nucleopolyhedrosis virus from diseased *S. suavis* specimens sent from the Forest Research Institute in 1957. The morphologies of the virions and the inclusion bodies of the cytoplasmic polyhedrosis have never been described and no type material is known to exist. Because of its potential as a biological control agent, further study was considered necessary.

Many species of *Heliothis* are pests; *H. armigera* (the tomato fruitworm) is one of two pest species in New Zealand. Usually it damages tomatoes and corn (maize) but in recent years it has become a significant defoliator of radiata pine seedlings, especially on the central plateau of the North Island. Dead larvae on radiata pine seedlings were found to contain highly refractive inclusions, resembling insect virus polyhedral inclusion bodies. They did not take up Giemsa stain after light heat-fixing, which suggested the cause of death was a nucleopolyhedrosis virus. No other viruses were observed by light or

electron microscopy. A nucleopolyhedrosis disease of *Heliothis* sp. was first described by Mally (1891) from larval *H. armigera* in the United States. It was considered desirable to examine the relationships of size distributions of polyhedra of the New Zealand isolates as geographical isolates of this virus from the United States of America show considerable variation in virulence, which can be related to polyhedra sizes (Shapiro and Ignoffo, 1970). Stairs (1971) states that, despite all the testing of these viruses, little is known about their natural ecology. The study of an epizootic in New Zealand will help to fill this deficiency and may assist future biological control programmes.

This paper examines some morphological features of the cytoplasmic polyhedrosis of *S. suavis* and the nucleopolyhedrosis of *H. armigera*. An epizootic of the nucleopolyhedrosis is described.

## MATERIALS AND METHODS

### *Source and Preparation of Viruses*

Diseased *S. suavis* larvae were collected from exotic conifers near Rotorua. Virus-infected *H. armigera* larvae were collected from radiata pine in Kinleith Forest, Tokoroa and from lupin bushes (*Lupinus arboreus* Sims) in Aupouri State Forest, Northland. The larvae from each locality were macerated in distilled water and allowed to putrefy for 2 days at room temperature.

*S. suavis* macerate was centrifuged at  $1 \times 10^3$  g for 30 minutes to sediment all the polyhedra. The pellet was re-suspended in 1:1 glycerol phosphate buffer pH 7.2 and stored at  $-20^\circ\text{C}$ . The supernatant was again centrifuged at  $5.5 \times 10^4$  g for 1 hr at  $5^\circ\text{C}$  to sediment any possible virions. This pellet was also re-suspended and stored as above. A small portion was re-suspended in distilled water and negatively stained with 2% phosphotungstic acid for examination under the electron microscope.

Polyhedra from part of both *H. armigera* macerates were purified by banding in linear 20-80% v/v glycerol density gradients. The protein from the polyhedra was solubilised by treatment with 0.1M NaCl-0.05M  $\text{Na}_2\text{CO}_3$  at about 5 mg of polyhedra/ml for 15 min at room temperature to release virus particles, which were concentrated by centrifuging at  $1.5 \times 10^4$  g for 30 min at  $5^\circ\text{C}$ . The pellets were re-suspended in distilled water and stained in 1% phosphotungstic acid for electron microscopy.

### *Measurements of Polyhedra*

The dimensions of the polyhedra were determined from the unpurified macerates. To reduce bias the same person measured all the polyhedra (by calibrated ocular micrometer, and with negative phase contrast illumination at 1250 magnifications). A bacterial counting chamber grid was used to provide convenient reference points.

### *H. armigera Epizootic*

Apparently healthy and dead (showing typical symptoms of nucleopolyhedrosis) larvae of *H. armigera* were counted on two-year-old planted stock of radiata pine (approximate height 80 cm) at intervals in December 1969, and January and February 1970 in Kinleith Forest, Tokoroa. The percentage of pine trees showing signs of defoliation was also noted.

## RESULTS AND DISCUSSION

*S. suavis* Cytoplasmic Polyhedrosis

The virus obtained from Rotorua is a cytoplasmic polyhedrosis. Non-occluded virions typical of this virus were readily discernible under the electron microscope (Fig. 1). They are spherical in shape although some are variably subspherical and up to six spikes can be seen projecting from some virions. The mean diameter of 30 spherical virions was found to be 59 nm, well within the usual size range of cytoplasmic polyhedrosis virions (Hosaka and Aizawa, 1964).

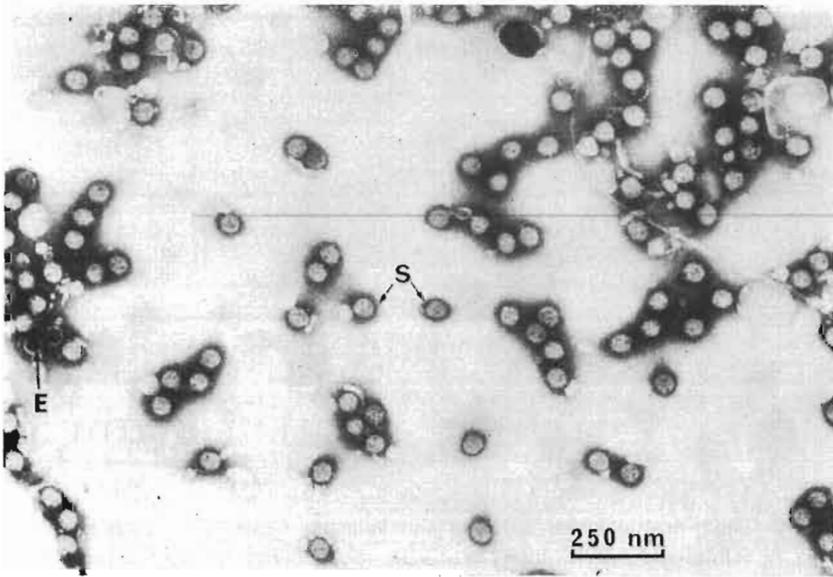


FIG. 1.—An electron micrograph of an impure preparation of non-occluded virions from a cytoplasmic polyhedrosis virus infection of *Selidosema suavis*. E = empty virions, S = spikes projecting from virions.

The polyhedra are equilaterally triangular in profile (Fig. 2) and appear to be tetragonal in shape, which is consistent with the usual range of shapes of this virus type (Smith, 1963). The vertical heights of 100 polyhedra were measured and the mean height was  $1.93 \mu\text{m}$ , standard error  $0.05 \mu\text{m}$ . The height frequency was plotted on probability paper and found to be normal. No other viruses were found infecting *S. suavis* cadavers.

*H. armigera* Nucleopolyhedrosis Virus

## (A) Morphologies:

The polyhedra of the two isolates of nucleopolyhedrosis are square in profile and appear cuboid in shape, which is different from the irregularly-shaped polyhedra found by Bergold and Ripper (1957) from *H. armigera* and by Gregory *et al.* (1969) from *H. zea*.

One hundred polyhedra from each isolate were measured and the mean diameters and standard errors are shown in Table 1. These values fall well outside the range of 0.7 to

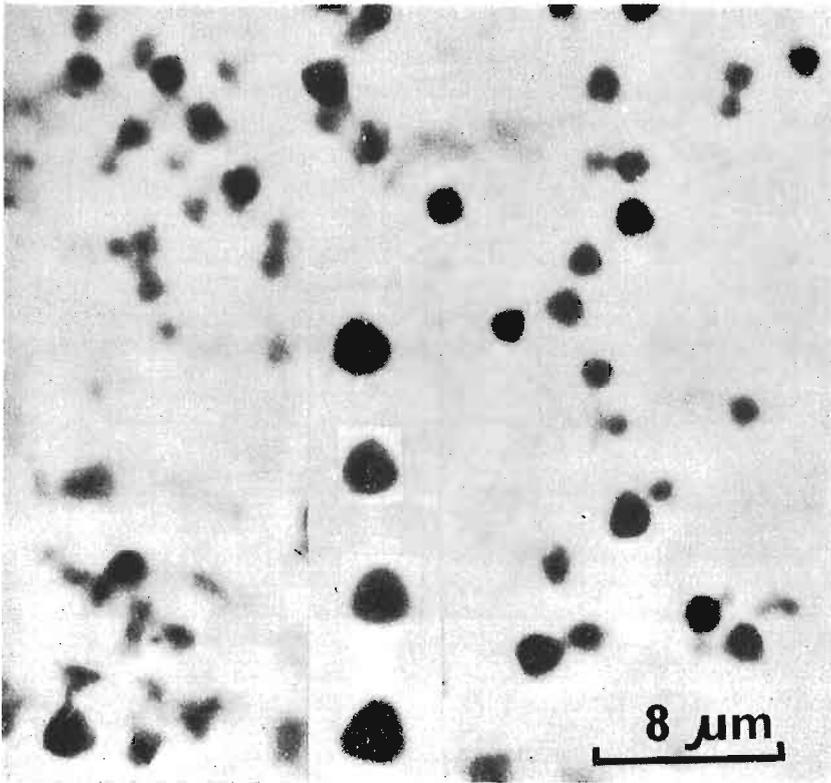


FIG. 2—Polyhedral inclusion bodies of a cytoplasmic polyhedrosis virus infecting *Selidosema suavis*. Insert is a selection of better focused polyhedra.

1.2  $\mu\text{m}$ , majority 1.1  $\mu\text{m}$ , given by Bergold and Ripper (1957). The size frequencies were plotted on probability paper, which demonstrated that the Tokoroa distribution was normal while only the Northland polyhedra smaller than the mean size were normally distributed.

TABLE 1—Sizes of polyhedra and enveloped virions of two New Zealand isolates of a nucleopolyhedrosis infecting larval *Heliothis armigera*.

$\mu\text{m}$  = micrometres, nm = nanometres, n = number of observations,  $\bar{x}$  = mean, s.e. = standard error.

	Polyhedra			Virions					
	n	Diameter ( $\mu\text{m}$ )		n	Length (nm)		n	Width (nm)	
		$\bar{x}$	s.e.		$\bar{x}$	s.e.		$\bar{x}$	s.e.
Northland isolate	100	1.46	0.05	24	273	3	27	69	2
Tokoroa isolate	100	1.81	0.03	65	285	2	73	59	1

The virions released from the polyhedra by alkali treatment are rodlike (Fig. 3). The means and standard errors of the maximum virion lengths and widths from both isolates are given in Table 1. The distributions of virion lengths and widths of both isolates were plotted on probability paper and all were found to be not normally distributed. The differences between the isolates of the means of the lengths and the widths of the virions were found to be highly significant ( $p < 0.01$ ). The two New Zealand isolates are therefore morphologically distinct from each other, and the Tokoroa isolate is unusual in the large polyhedra diameter and small virion width. The mean length and width of virions from both isolates seem to be unusually small when compared to the mean and range of measurements given by Bergold and Ripper (1957) (length  $320 \pm 10 \mu\text{m} \times 90 \pm 10 \mu\text{m}$  width) and by Gregory *et al.* (1969).

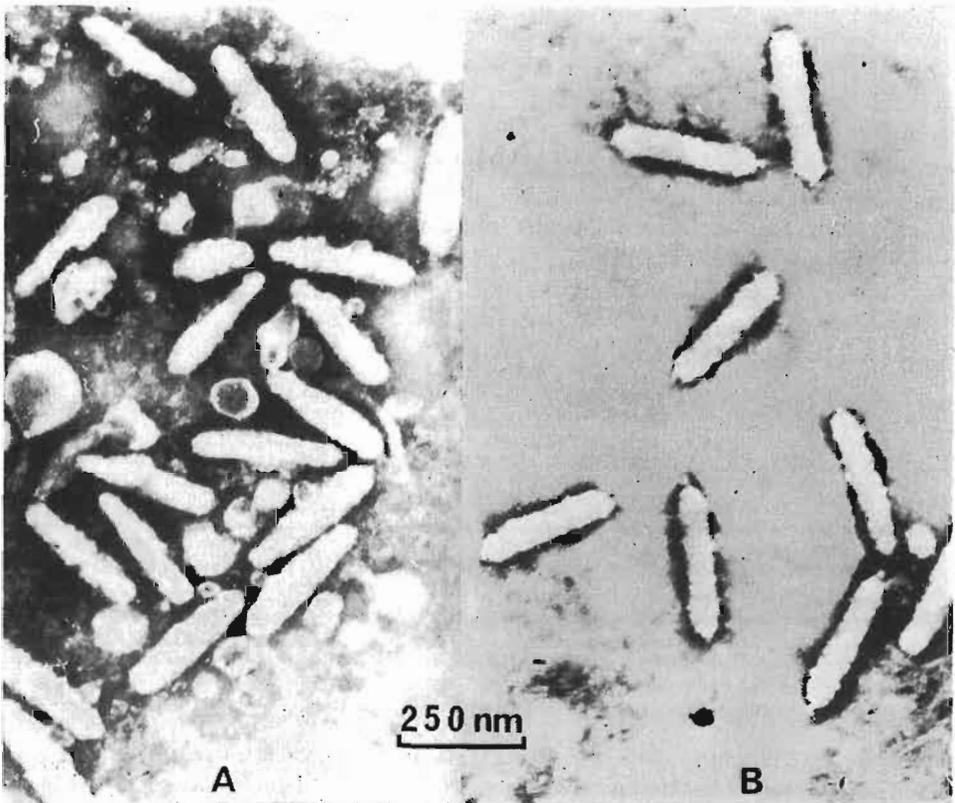


FIG. 3—An electron micrograph of virions, with outer envelopes, of *Heliothis armigera* nucleopolyhedrosis after weak alkali treatment. A. Northland isolate. B. Tokoroa isolate.

(B) Tokoroa Epizootic:

Towards the end of December 1969 the larvae of the second generation of *H. armigera* had defoliated their preferred food plant in the area, *Lotus pedunculatus* Cav., and were beginning to feed on the pine trees (New Zealand Forest Service 1970). The

first signs of the virus infection were also noted at this time. The progress of the epizootic is shown in Figure 4; within 3 weeks the population of *H. armigera* had collapsed.

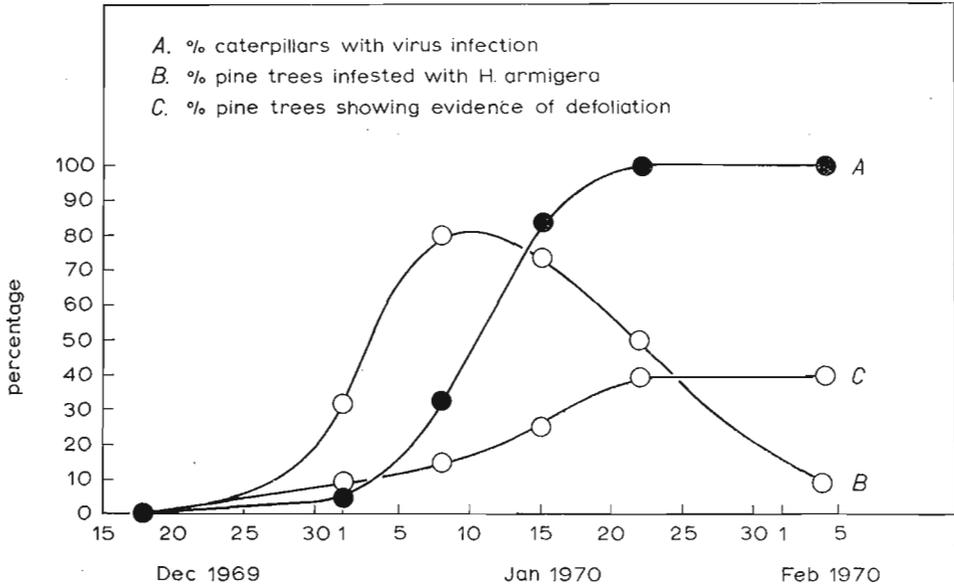


FIG. 4.—The progression of the virus epizootic in a population of *Heliothis armigera* caterpillars (A). Infestation (B) and defoliation (C) of pine trees by the caterpillars is also shown.

The spread of the disease is thought to have been aided by the cannibalistic habits of the caterpillars, which were observed to feed on infected cadavers (Fig. 5). This mode of infection is considered (Tanada, 1963) to be less important than feeding on contaminated food. Typically the caterpillars feed on the soft tissues at the fascicle bases of elongated needles, just behind the terminal bud of the leader. Nearly all dead and healthy larvae were found on and around the terminal buds. It is not known how much this was due to their preference for feeding at this site, or to a tendency for virus-infected insects to climb high in trees, as noted by Graham and Knight (1965). Dispersal of the virus may also have been aided by *Calocoris norvegicus* Gmelin (Hemiptera: Miridae). Nymphs and adults of this species were abundant in the area and were frequently observed with their stylets inserted in dead *H. armigera* larvae. Although they normally feed on the nectar of flowers, the mirids were presumably scavenging nourishment from the decaying cadavers. Many workers have observed the importance of insect scavengers in virus dissemination (Tanada, 1963).

Supplementary observations on larvae remaining on *L. pedunculatus* and feeding on ragwort (*Senecio jacobaea* L.) indicated that a similar pattern of mortality also occurred on these plants.

The movement of the larvae onto the pine trees is reflected in the increasing percentage of trees infested with caterpillars (Fig. 4). This resulted in some defoliation of the leading stems. Fortunately the epizootic rapidly reduced the population and the



FIG. 5—A final instar caterpillar of *Heliothis armigera* feeding on a cadaver of a virus-infected specimen.

percentage of trees showing some signs of defoliation did not rise above 40% (Fig. 4). In a parallel situation without the presence of the virus, *H. armigera* has infested 75% of the trees in a newly planted 1600 ha block, 31% having more than 25% of the foliage removed (Alma, unpublished data). At Tokoroa, serious defoliation of trees was prevented by a naturally occurring virus infection.

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