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An RNA Virus in *Autographa californica* Nuclear Polyhedrosis Virus Preparations: Gross Pathology and Infectivity[1,2]

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The pathology and infectivity of an RNA virus infectious to *Trichoplusia ni* larvae was investigated. The enzyme-linked immunosorbent assay (ELISA) and weight depression were used as criteria for virus concentration in larval homogenates and live larvae, respectively. Infected larvae were severely stunted, weighing as little as 13 times less than uninfected individuals of the same age, yet appeared normal morphologically. The virus was found to cause only slight mortality at high concentrations. Infected larvae displayed the pathological stunting response down to a dose of 0.1 ng of virus. Larvae infected with doses 100 times lower did not show the weight response but such inapparent infections were detectable by ELISA. Because of these subtle gross pathological symptoms, particularly at low levels of infection, infected individuals could easily remain undetected in a group-reared colony.

KEY WORDS: *Trichoplusia ni; Autographa californica;* RNA virus; nuclear polyhedrosis virus; enzyme-linked immunosorbent assay (ELISA); infectivity; pathology.

INTRODUCTION

The multicapsid nuclear polyhedrosis virus (Ac*M*NPV) isolated from the alfalfa looper, *Autographa californica* was the first virus of this type demonstrated to have a broad host range (Vail et al., 1971). Since then, numerous investigators have studied Ac*M*NPV for basic research and as a potential microbial control agent. Hess et al. (1977), in investigations based on electron microscopy, described a new icosohedral insect virus in apparent mixed infection with the Ac*M*NPV. Morris et al. (1979) reported on the physicochemical characterization of this RNA virus (TRV) infectious to *Trichoplusia ni* and its apparent similarities to *Nudaurelia capensis* (Tripconey, 1970) and *Antherea eucalypti* (Grace and Mercer, 1965) viruses. Morris et al. (1979) suggested that the propagative host should be analyzed for the presence of extraneous viruses, that such viruses could alter the host range of NPV preparations, and that they could pose potential, although hitherto undocumented, biohazard problems.

The present studies were conducted in 1978–1979 to determine the gross patho-

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logical and biological responses of *T. ni* larvae to TRV infections as well as the quantitative influence of TRV infections on this species. The relationship of TRV dose to viral concentration in infected larvae was also investigated.

METHODS AND MATERIALS

T. ni larvae were reared on the diet described by Henneberry and Kishaba (1966) without formalin. Neonate larvae were used in all tests and were reared individually in 30-ml plastic cups held at 26.7°C. TRV was purified by the methods of Morris et al. (1979). A homogeneous virus preparation obtained from density gradient centrifugation was used as the inoculum. TRV concentration of the resulting suspension was measured (A_{254}) and found to be 0.13 mg/ml. All tests were conducted with dilutions of this suspension in sterile distilled water.

In order to determine the infectivity of TRV to *T. ni.* larvae, concentrations of virus (in 0.1-ml aliquots) ranging from 10,000 to 0.0 ng/cup were layered on the surface of the diet contained in 30-ml plastic cups. Twenty cups/dose were infested with a single neonate larva and incubated for 10 days. On day 10, mortality was recorded, survivors weighed and frozen individually, and later tested for the presence and concentration of TRV using the ELISA.

ELISA tests were performed as described previously (Morris et al., 1982) except that a Gilford Model PR50EIA ELISA processor was utilized with specially designed cuvette strips. Purified anti-TRV gamma globulin (5 μg/ml) was coated on the strips by incubation for 3 hr at 35°C in 0.05 *M* carbonate buffer, *p*H 9.6. The strips were washed three times between steps with 0.1 *M* NaCl, 0.1% Tween-20 in the PR50 and all subsequent incubations were in 200-μl volumes of 0.01 *M* phosphate-buffered saline, *p*H 7.4, 0.05% Tween-20, 2% polyvinylpyrollidone, and 0.2% ovalbumin. Aliquots of test samples (50 μl) were then added and incubated overnight at

TABLE 1

THE INFLUENCE OF VARIOUS TRV CONCENTRATIONS ON *Trichoplusia ni* LARVAL MORTALITY, MEAN LARVAL WEIGHT, PERCENTAGE LARVAL INFECTION, AND CONCENTRATION OF TRV IN LARVAL TISSUES AND FRASS

TRV (ng/cup)	Percentage mortality	$\bar{X}$ Larval wt[a] (mg ± SD)	$\bar{X}$ OD_{405} (± SD) of infected larvae	Percentage TRV infected	$\bar{X}$ μg TRV/ larvae	$\bar{X}$ μg TRV/g infected larvae (± SD)	Estimated μg of TRV/ g pooled frass
10,000	0.0	24.6 ± 20.0 b	0.92 ± 0.52	80	1.02	37 ± 18	13
1,000	0.0	45.5 ± 31.2 b	1.70 ± 0.71	80	5.03	123 ± 131	ND
100	5.3	20.0 ± 9.5 b	1.45 ± 0.72	90	4.00	323 ± 545	22
10	0.0	18.7 ± 12.3 b	2.26 ± 0.63	95	9.73	627 ± 749	ND
1.0	5.3	32.8 ± 23.7 b	1.86 ± 0.81	90	7.91	485 ± 578	20
0.1	0.0	30.9 ± 14.4 b	1.95 ± 0.77	90	8.29	424 ± 645	ND
0.01	0.0	168.4 ± 104.5 a	0.87 ± 0.73	45	2.13	17 ± 27	10
0.001	0.0	222.6 ± 97.0 a	0.40 ± 2.8	20	0.29	4 ± 2	ND
0.0001	0.0	264.3 ± 37.6 a	0.46 ± 0	5	0.34	1 ± 0	<0.1
0.0	5.0	257 ± 48.6 a	0.08 ± 0	0.0	0	0 ± 0	0

Note. n = 20 larvae/dose. ND = not determined.

[a] Means followed by common letters are not significantly different according to Duncans Multiple Range test.

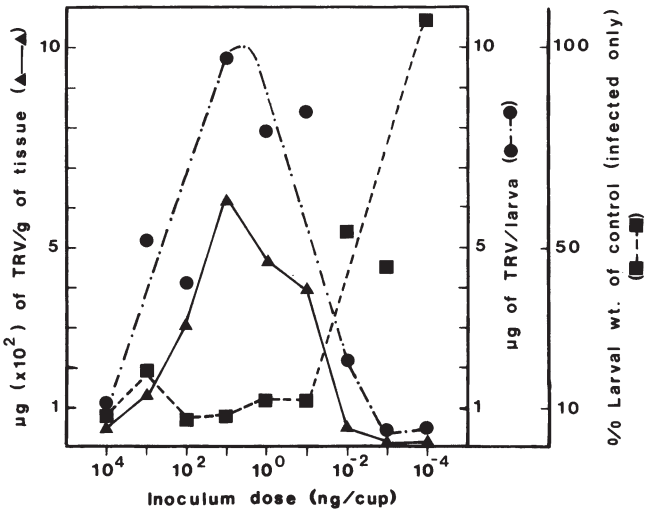

FIG. 1. Effects of TRV dose on virus synthesis and larval weight in infected *Trichoplusia ni* larvae.

4°C. The strips were then washed and treated with alkaline phosphatase-coupled gamma globulin for 3 hr at 35°C. The strips were then developed, after washing, by the addition of 1 mg/ml *p*-nitrophenyl phosphate in 10% diethanolamine, *p*H 9.8, for 1 hr and results were recorded with the PR50 at 405 nm.

Individual larvae (≈0.2 g) were processed for ELISA tests by crushing with disposable applicator sticks in microfuge tubes containing 1 ml of 0.1 *M* tris(hydroxymethyl)-aminomethane (Tris), 0.1 *M* NaCl, 0.01% sodium azide, 0.05% diethyldithiocarbamate at *p*H 7.5, and 0.5 ml of chloroform–butanol (80:20, v/v). The suspensions were emulsified and clarified by centrifugation for 1 min in a Beckman Microfuge prior to addition to ELISA strip wells. In some experiments the virus fraction was concentrated by precipitation with 8% polyethylene glycol (PEG 6000) as described previously (Morris et al., 1982).

RESULTS

TRV caused no appreciable mortality when fed to neonate larvae at dosages as high as 10,000 ng/cup. However, larvae fed dosages as low as 0.1 ng/cup displayed a marked weight reduction (as much as a 13-fold reduction) at 10 days postinoculation when compared to uninoculated larvae (Table 1). The response of larvae to TRV was quite heterogeneous as indicated by the high standard deviations of the larval weights and estimates of virus content in the infected larvae. Maximum synthesis of TRV occurred at dosages between 0.1 and 10 ng/cup as indicated by virus content (Fig. 1) which was near the dose level at which depression of larval weight became significant. At lower dosages (0.001 to 0.0001 ng) of TRV, the larvae failed to show a significant weight response, even though virus could be detected by ELISA in from 5–20% of the inoculated larvae. In such inapparently infected larvae, the content of virus was reduced by as much as 20-fold.

Except for size, the smaller TRV-infected larvae appeared morphologically similar to their healthy counterparts (Fig. 2) and they showed none of the abnormal body proportions associated with some other chronic type infections. Pupation was often delayed in infected larvae and the resulting size of pupae was also reduced. However, this response was not quantified. In addition, considerable quantities of TRV were found in the frass of infected larvae (Table 1).

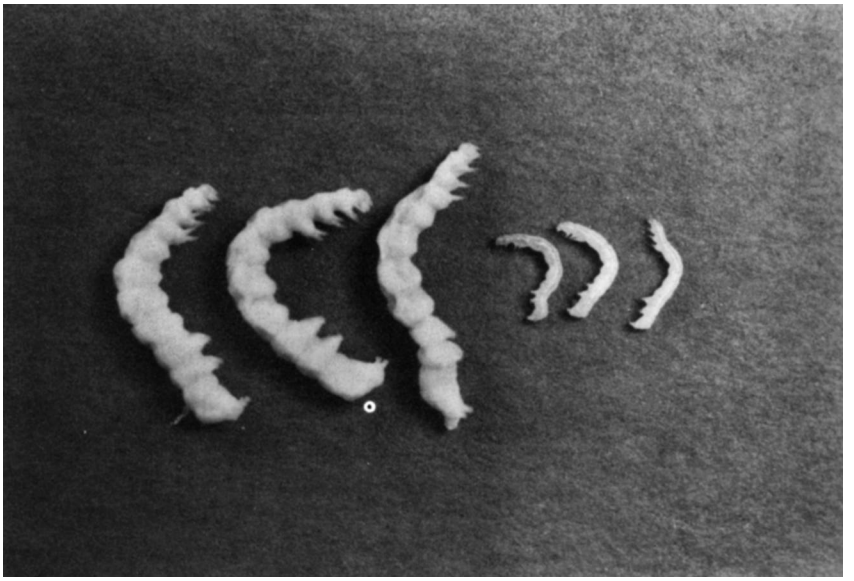

FIG. 2. Ten-day-old healthy (left) and TRV-infected (right) *Trichoplusia ni* larvae.

DISCUSSION

The present studies provide information on biological effects and infectivity of TRV in addition to the biochemical and biophysical data of Morris et al. (1979). The data demonstrated the severe but chronic effects of TRV on neonate *T. ni* larvae. However, even at high doses, TRV did not cause a great amount of mortality through the 10-day test periods. Unlike the symptoms caused by cytoplasmic polyhedrosis virus (Vail et al., 1969) with which *T. ni* larvae express reduced weight, pale color of the integument in the midgut region, and abnormal proportions of head to body length, TRV-infected larvae, although small in comparison to healthy larvae, show no other external symptoms of infection.

These effects were not noted by Morris et al. (1979) probably because older larvae were used and they did not provide enough time for these symptoms to be expressed. A recently described nonoccluded virus of the navel orangeworm, *Amyelois transitella,* does cause severe stunting and disproportionately large head capsules compared to total body size when compared to normal individuals (Kellen and Hoffman, 1981). The symptoms and chronic nature of TRV provide yet another pattern for the nonoccluded virus infections of insects which are in contrast to those reported by Kellen and Hoffman (1981), Harrap et al., (1966), Tripconey (1970), and others. The response of larvae to a TRV concentration of 0.1 ng/cup provides an indication of the infectivity of the virus. The combination of low mortality and subtle symptoms may explain why TRV has been detected and isolated only in the last several years (Morris et al., 1979).

The small size and specific symptoms provide presumptive evidence for TRV infection in *T. ni* larvae. Quantities of TRV are also excreted in the frass of infected larvae and may indicate infection of the gut tissues. This probably provides a mechanism for horizontal transmission of TRV within a colony but in addition also provides a means of detecting TRV when coupled with ELISA without the need for sacrificing larvae. In addition, the delayed and heterogenous development coupled with small pupal size may be an indication

of a TRV-infected colony. The host responses along with ELISA provide several quality control procedures that can be used when *T. ni* larvae are used as propagative hosts for baculoviruses.

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