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Inactivation of Nuclear Polyhedrosis Virus (*Baculovirus* Subgroup A) by Monochromatic UV Radiation

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Monochromatic radiation at wavelengths of 290, 300, 310, and 320 nm inactivated occluded nuclear polyhedrosis virus of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough). Data indicate that all of the wavelengths are capable of causing virus inactivation; much greater fluences are needed for virus inactivation as the wavelength increases.

The effectiveness of microbial insecticides is well documented (2). The effects of several environmental factors on the microorganisms have been studied (1, 5, 7–9). The most detrimental factor is reported to be sunlight (10), but few studies have determined the lethality of monochromatic light for microbial insecticides. This paper reports the results of a study on the effects of monochromatic UV light on nuclear polyhedrosis virus of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough).

The UV-B (290 to 320 nm) region of the spectrum (14) was studied. This region of the spectrum was chosen because shorter wavelengths of sunlight do not penetrate to the surface of the earth. The upper limit, 320 nm, was chosen because previous studies (3, 15) have demonstrated that virus activity is not reduced significantly when baculoviruses are exposed to wavelengths longer than 310 nm. Experiments were carried out at 10-nm intervals.

The larvae used in this study were the Goose Lake (GL-1) inbred strain of *O. pseudotsugata*. The larvae were free of endogenous virus (12) and were maintained on a meridic solid diet (13). All insect eggs were surface sterilized with an aqueous solution of sodium hypochlorite (2% Clorox). A purified preparation of the multicapsid *O. pseudotsugata* nuclear polyhedrosis virus was serially diluted to give 14,000 polyhedral inclusion bodies (PIBs) per ml. One milliliter of the PIB suspension was irradiated in quartz cuvettes with a light path length of 1 cm. The PIBs were kept in suspension by bubbling the liquid with sterile moist air. A high-intensity Bausch & Lomb grating monochromator with a 150-W Xenon arc lamp was used as a source of monochromatic light. Fluences at each wavelength were monitored with a calibrated YSI model 65 radiometer. All irradiations were performed at room temperature in the dark or in a laboratory equipped with yellow fluorescent lights. The bioassay method was essentially as described by Martignoni and Iwai (11). Irradiated PIB suspensions were diluted in sterile aqueous Cellosize QP-4400 (0.05%, wt/vol), and 200 μl was dispensed into 35-ml creamer cups containing 15 ml of diet to obtain a concentration of 0.3 to 0.5 PIBs per mm^2 of diet surface. At this PIB concentration, the average mortality of the larvae fed nonirradiated virus was 55%. Only insects that died of nuclear polyhedrosis were used for data analysis. Control groups consisted of larvae fed (i) diet on which nonirradiated virus had been dispensed and (ii) diet on which sterile aqueous Cellosize had been placed. The number of larvae per group was 18 to 25, and two or more groups (replicates) per treatment in a completely randomized experimental design were used. The effects of the irradiation were evaluated on the basis of the percentage of original virus activity remaining after exposure to UV radiation compared with unexposed virus (6). The data were analyzed by Spearman's rank correlation test. Experiments were also carried out to ensure that differences in virus inactivation were not caused by differences in larval susceptibility. The virus was exposed to the different wavelengths at the same fluence. After exposure, the virus was diluted and fed to the larvae as described; the test larvae were from a single batch.

Inactivation of multicapsid *O. pseudotsugata* nuclear polyhedrosis virus by monochromatic UV light was demonstrated by the decreased mortality rate in the groups fed irradiated virus compared with those fed nonirradiated virus (Table 1). The virus activity of the irradiated suspensions decreased, as shown by the decrease in the corresponding percentage of original activity remaining. The data also show that virus inactivation increased with increase in fluence, regardless of the wavelength used. As wavelength was increased, however, the fluence had to be increased to obtain the same degree of inactivation caused by the shorter wavelengths. The Spearman rank correlation coefficient test showed an inverse relationship between fluence and percentage of original activity remaining at each wavelength ($P < 0.05$), indicating that as the dose was increased the degree of virus inactivation was also increased. At a fluence of 1.152×10^3 w/m^2, the percentages of activity remaining after exposure to wavelengths of 290, 300, 310, and 320 nm were 15.4, 28.2, 41.0, and 71.8, respectively. These results, from an experiment with larvae from a single batch, therefore indicate that the differences in percentage of original activity remaining after exposure of the virus to the same fluence were due only to differences in wavelength and not to differences among larval batches.

Since wavelengths shorter than 290 nm do not penetrate to the surface of the earth, the effects seen on *Baculovirus* insecticides when sprayed in the field are due to wavelengths of sunlight that are 290 nm or longer. David and co-workers (3, 4) reported the effects of sunlight and monochromatic radiation (250 to 330 nm) on the granulosis virus of *Pieris brassicae* (Linnaeus). Their findings indicated that shorter wavelengths are more effective in causing inactivation of the granulosis virus.

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TABLE 1. Inactivation of occluded multicapsid nuclear polyhedrosis virus of *O. pseudotsugata* exposed to monochromatic UV radiation

Fluence (W/m^2)	Results (%) at following wavelength (nm)[a]:							
	290		300		310		320	
	Mortality	OAR	Mortality	OAR	Mortality	OAR	Mortality	OAR
0	62.4	100.0	55.4	100.0	56.0	100.0	38.4	100.0
5.760×10^2	42.0	67.3	66.6	(100.0)	63.5	(100.0)	—	—
1.152×10^3	—	—	30.5	55.1	38.0	67.9	39.0	(100.0)
2.304×10^3	29.0	46.5	14.3	25.8	24.5	43.8	35.5	92.4
4.752×10^3	—	—	—	—	10.1	18.0	18.7	48.7
Control (no virus)	0		0		0		0	

[a] Mortality, diet surface treatment bioassay in larvae of *O. pseudotsugata*; OAR, percentage of original virus activity remaining after exposure. Each value is the average of two to four replicates. —, Not tested. Values in parentheses indicate calculated OAR in excess of 100%.

The data presented in this report imply that monochromatic wavelengths between 290 and 320 nm are effective in causing inactivation of multicapsid *O. pseudotsugata* nuclear polyhedrosis virus. Much greater fluences were needed at the longer wavelengths (310 and 320 nm) to cause the same degree of inactivation as that caused by the shorter wavelengths (290 and 300 nm). The variation in the degree of virus inactivation caused by exposure at different radiations but at the same fluence resulted only from wavelength differences and not from differences in susceptibility among batches of experimental larvae.

These findings therefore suggest that, even though the nuclear polyhedrosis viruses are susceptible to a range of radiation, the photon energies associated with the shorter wavelengths of sunlight are sufficient to inactivate the insecticide when applied in the field; that is, even though the relative energy fluence is increased as wavelength is increased, the greater photon energies associated with the shorter wavelengths are capable of causing the inactivation. The potential for synergistic effects of multiple wavelengths cannot be ruled out. Since the virus is composed primarily of DNA and protein and these compounds absorb maximally at approximately 260 and 278 nm, respectively, it is doubtful that wavelengths longer than 400 nm have substantial effects on these agents, although such radiation can be detrimental to more complex systems. The findings reported here are also supported by the results of other investigators (4) who reported that, when crude virus preparations (rather than highly purified suspensions of inclusion bodies) are used in the field, virus activity is better preserved. It is possible that crude virus preparations have longer field life because the impurities partly absorb the short-wave UV radiation, even though longer wavelengths penetrate the impurity shield.

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