Effect of Light Energy on Alkali-Released Virions from Anagraphe falcifera Nucleopolyhedrovirus

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We compared the insecticidal activities of occluded and nonoccluded AfMNPV baculovirus obtained by dissolving the occlusion bodies (OB) with sodium carbonate. Droplet feeding and cotton leaf feeding bioassay techniques were used to determine the dose response against neonate Trichoplusia ni (Hübner) and loss of insecticidal activity when the virus was exposed to simulated sunlight from a xenon light source. Using droplet bioassays to determine a dose response, nonoccluded virus (NOV) was 20 times more active (LC\textsubscript{50} = 4.8 x 10\textsuperscript{5} OB/ml, dissolved) than occluded virus (LC\textsubscript{50} = 9.6 x 10\textsuperscript{6} OB/ml) when the samples remained wet. However, NOV lost activity when air dried before being tested by droplet (LC\textsubscript{50} > 1.0 x 10\textsuperscript{6} OB/ml) or leaf feeding (LC\textsubscript{50} > 3.0 x 10\textsuperscript{6} OB/ml) bioassays. Adding sucrose to NOV prevented the loss of insecticidal activity when samples were dried. The activity of NOV with 2\% sucrose was similar to that of occluded virus samples, with or without sucrose, in both droplet feeding and leaf feeding assays. These results indicate that the OB protected the insecticidal activity of virions from the detrimental effects of drying. The OB also provided some protection from the detrimental effects of simulated sunlight (xenon) exposure. NOV samples exposed to xenon light had significantly greater loss of insecticidal activity than did similar samples of occluded virus. Without advancement in technologies, such as formulations, possible benefits of increased insecticidal activity from the use of nonoccluded virus is probably not sufficient to offset the rapid loss of activity due to drying or light exposure.

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INTRODUCTION

The baculovirus isolated from Anagraphe falcifera (AfMNPV) is a multiply embedded nucleopolyhedrovirus with the potential to become an effective biological pesticide for insect control. This baculovirus has a host range that includes several important lepidopteran pests of cotton and cole crops (Hostetter and Puttler, 1991), including Helicoverpa zeae (Boddie), Spodoptera exigua (Hübner), and Trichoplusia ni (Hübner). Cost of virus production and rapid loss of insecticidal activity when exposed to field conditions are two factors that have limited commercialization of this entomopathogen as a biopesticide.

It has been proposed to reduce production costs by producing nonoccluded virus (NOV) (Hughes and Wood, 1996; Ignoffo et al., 1998). Producing polyhedrin protein for the occlusion body (OB) by in vivo or in vitro systems represents a waste of biological energy and materials that could be directed toward making infective virions. Polyhedra-negative isolates of Autographa californica speyer NPV (AcMNPV) can produce 60\% more infectious virus per unit of larval weight than the wild-type isolate, which produces OBs (Hughes and Wood, 1996). Another possible advantage of NOV could be an increase in initial infective propagules, assuming that the OB plays no role in the infection process. Theoretically, a single virion is sufficient to cause infection of susceptible larvae. Thus, the OB, which contains many virions, as with AfMNPV, represents an inefficient package of infective propagules, assuming that the OB plays no role in the infection process.

Another factor limiting commercialization of AfMNPV is the rapid loss of insecticidal activity when the biopesticide is applied to crops in the field. This loss of

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activity is predominantly the result of exposure to sunlight (Ignoffo and Garcia, 1996). The mechanism that destroys the insecticidal activity of exposed virus is not fully understood, although the loss of activity is generally attributed to a direct effect of ultraviolet light energy on the viral DNA (Ignoffo and Garcia, 1994) or to production of reactive oxygen species. Alternatively, it is possible that insecticidal activity is lost because the protein of the OB may be denatured by light energy such that it does not dissolve sufficiently to release virions in the insect's intestine. In this scenario, the virions may not be able to initiate infection even though they may be viable. Therefore, there may be advantages to predissolving OBs or producing polyhedrin-negative baculovirus for biopesticides.

This research was conducted to compare occluded virus with NOV to determine whether the OB was beneficial or detrimental in terms of insecticidal activity when virus is exposed to light energy.

**MATERIALS AND METHODS**

**Virus**

AfMNPV used in this research is a wild-type virus originally obtained from biosys, Inc. (now Thermo Trilogy Corp., Columbia, MD). AfMNPV was propagated by infecting third instar T. ni and incubating infected larvae at 28°C for 5 days. After incubation, the infected larvae were frozen, collected, homogenized, and filtered through cheesecloth to remove larger insect parts. The filtered preparation was centrifuged at 7000 rpm in a Sorvall RC5C (DuPont) centrifuge for 10 min, the supernatant was discarded, and the pellet containing OBs was resuspended in water. This crude preparation was further purified with a 40, 60, 80% w/v sucrose gradient as follows. Ten milliliters of each sucrose concentration (5°C) was loaded into 50-ml centrifuge tubes with the highest sucrose concentration in the bottom of the tube. Care was taken to avoid mixing the solutions. After chilling, 10 ml of crude virus was pipetted over the sucrose gradient and centrifuged for 10 min in a swinging-bucket clinical centrifuge (International Equipment Co., Needham, MA). After centrifugation, each layer and interface was carefully removed and checked for the presence of OBs. OBs were collected from the 40–60% interface through the 60–80% interface layers, placed in a clean centrifuge tube with DI water, and centrifuged to collect the pellet of purified OBs. The supernatant was discarded and the purified OBs were resuspended in water. OB concentrations were determined by counts with a Bright-line hemacytometer (Hausser Scientific, Horsham, PA). Samples of both crude and purified virus preparations were stored at −20°C.

NOV was prepared for each experiment by dissolving the occlusion protein with sodium carbonate (Na2CO3). The carbonate concentration used to dissolve the OBs was selected based on preliminary experiments using single-dose (5 × 10⁶ OB/ml) droplet assays with crude preparations of virus. Virus was added to provide 5 × 10⁶ OB/ml in concentrations of 0, 0.1, 0.3, or 0.5 % w/v sodium carbonate solutions and mixed for 10 min. These virus samples were diluted 10-fold with a feeding solution for use in a droplet feeding assay (see below) to assess insecticidal activity. Based on the results of this preliminary experiment, the 0.3% w/v concentration of carbonate was selected to provide nonocluded virus throughout the rest of the treatments. The concentrations of virus reported for nonoccluded samples are based on the concentrations of OBs used to prepare the samples. No attempt was made to quantify virions.

**Insect Bioassay Techniques**

For the droplet assay (modified from Hughes and Wood, 1981), a feeding suspension was prepared with known concentrations of virus, and a feeding solution was made with 2% w/v sucrose and 0.1% w/v FD&C Blue 1 dye (Hilton Davis, Cincinnati, OH). For each sample, about 75 small drops (approximately 1 μl each) of the feeding suspension were formed in a 50 × 9-mm plastic petri dish with a Pasteur pipet. Next, about 50 neonate T. ni were placed in each dish to feed from the drops. After about 10 min, fed larvae were identified by the blue color showing through their integument and were selected for transfer to individual diet cups containing 5 ml modified artificial wheat germ diet (Gardiner, 1985). The modified diet did not contain dried cabbage powder but included 1.25 g of cholesterol and 18 ml acetic acid (20%). Thirty fed larvae per virus sample were selected for each treatment and incubated in the dark at 28°C for 7 days. After incubation, the numbers of live and dead larvae were counted for each sample. Larvae that died due to handling were identified because they retained the blue color, and these larvae were excluded from data analysis. Contamination mortality was determined by including a nonvirus control treatment with each experiment.

Virus samples for the droplet assay were prepared in either a wet or dry form. For the simplest wet samples, the virus was mixed at the appropriate concentration in the final feeding solution. Alternatively, for wet samples, a concentrated virus suspension was mixed and 4 ml placed in a petri dish (50 × 9 mm). Concentrated samples were later diluted with 1 ml of 5× concentrated feeding solution to create the final feeding suspensions for assay. Dry samples of virus were made by preparing a concentrated virus sample and applying 100 μl to a 22 × 22-mm glass cover slip for each treatment. Virus samples were air dried for 2 h under room conditions. After drying, each cover slip was placed individually in a 25-ml beaker with 10 ml of
droplet feeding solution. Each sample was mixed with a VirTishear homogenizer (Virtis Co., Gardiner, NY) for about 15 s to remove the virus from the cover slip. After mixing, samples were tested using the droplet feeding assay as described previously.

For the plant assay, cotton plants were grown in 15-cm-diameter pots in a glasshouse for 4 weeks. Five circles (4 cm diameter) for each virus treatment were drawn with a marker on the cotton leaves. A 100-μl sample of virus was spread with a glass rod over the upper leaf surface within the circle and air dried. Plants were then treated with a light exposure (see below). After light treatment, leaf circles were cut from the leaves using a 4-cm circular cutter and placed individually on filter paper (No. 1, 42.5 mm, Whatman) in petri dishes (50 x 9 mm) with about 10 neonate T. ni. Treated leaf disks with larvae were incubated for 24 h at 28°C. After incubation, 6 larvae from each leaf circle (30 larvae for each dose or treatment) were transferred to individual diet cups and incubated for an additional 6 days. After incubation, live and virus-killed larvae were counted for each treatment.

**Additives**

Preliminary assays indicated that air drying of NOV samples on glass cover slips or leaves (techniques used in the light exposure assays) had an adverse effect on insecticidal activity. Therefore, several additives were screened for their ability to prevent the loss of activity of dried samples. OBs were dissolved as previously described and mixed with glycerin, polyethylene glycol (mw = 8000), sucrose, or sodium lignate (PC-1307; Westvaco Corp.) at 0.5% w/v. Samples were dried on coverslips or remained wet and added directly to a feeding solution. Droplet assays were done as described above. The crude virus preparation was diluted to provide final concentrations of 1.0 × 10⁶ OB/ml for occluded samples and 3.0 × 10⁶ OB/ml for the nonoccluded samples in the droplet assay. This experiment was performed three times on separate days for replication. Data were subjected to ANOVA (Proc GLM, SAS 1985) and means were compared using LSD. A subsequent experiment compared rates of sucrose (data not shown) and indicated that results for samples containing 2% sucrose were less variable than results for samples containing other concentrations of sucrose. Based on these results, 2% sucrose samples were included in the following LC₅₀ and light exposure assays.

**LC₅₀ Experiments for Insecticidal Activity**

Insecticidal activity of occluded virus and NOV was determined for purified virus preparations by dose-response experiments using both droplet and plant assays. For the droplet assay, the dose response was determined for six treatments. Treatments included combinations of virus (occluded and nonoccluded) and sample type (wet, dry, and dry with 2% w/v sucrose). All treatments were tested at five virus concentrations made from serial dilutions. Concentrations ranged from 1.23 × 10⁴ to 1.00 × 10⁶ OB/ml, except for wet nonoccluded samples that were tested at 1.23 × 10⁵ to 1.00 × 10⁶ OB/ml. Wet samples were prepared by diluting with feeding solution. For nonoccluded wet samples, the feeding solution contained 0.3% w/v sodium carbonate. Dry samples were made by diluting virus in water for occluded or 0.3% sodium carbonate solution for nonoccluded treatments. Serial dilutions for the dry samples were mixed at 100× the final concentration and dried on cover slips. After drying, samples were mixed with feeding solution as described previously for dry samples.

A dose response was determined by a plant feeding assay for each of four treatments consisting of the combinations of virus (occluded and nonoccluded) and sucrose concentration (0 and 2% w/v). All the virus samples were serially diluted with deionized water (with or without sucrose) for occluded samples and with sodium carbonate solution (with or without sucrose) for nonoccluded samples. Final concentrations ranged from 3.7 × 10⁴ to 3.0 × 10⁶ OB/ml. Samples (100 μl) were pipetted onto marked areas on cotton leaves, spread with a glass rod, allowed to air dry, and assayed as described above. A dose response for each virus sample was determined by probit analysis using Polo-PC (LeOra Software, 1987). The dose-response lines were then used to determine a suitable single dose (LC₅₀) for each treatment to be used in subsequent light exposure experiments.

**Light Exposure**

Occluded and nonoccluded virus samples with and without sucrose were exposed to light energy that simulated natural sunlight (see McGuire et al., 1996). Simulated sunlight (xenon light) was provided by a CPS Suntest machine (Heraeus, Inc.). The Suntest machine was also fitted with a Suncool heat exchanger (Altas, Inc.) set at 5°C to further chill the cooling air passing through the exposure chamber. Samples were exposed at a dial setting of 7.5 for light intensity. [See McGuire et al. (1996) for a description of the light spectrum for this machine.]

Three media (glass cover slips, petri dishes, and cotton leaves) were used in separate experiments for exposing virus to light energy. For each experiment, paired virus samples (xenon light exposed and unexposed) were prepared at a discriminating concentration to provide 80% mortality in the corresponding droplet or plant feeding assay, based on the results of previous dose-response experiments. Virus samples on glass cover slips and petri dishes were exposed for 80 min, and plant leaf samples were exposed for 8 h. A nonvirus control was included in each assay. This pro-
procedure was repeated three times over consecutive days for three replications of each treatment.

For samples dried on glass cover slips, the discriminating doses in the final feeding suspensions were $3 \times 10^6$ OBs/ml for occluded virus and $5 \times 10^6$ OBs/ml for NOV in the final feeding suspensions. A high virus concentration was selected for nonoccluded samples because of their lower activity when dried. For one NOV sample, polyhedron protein was dissolved with sodium carbonate before application to the cover slip. To determine whether light was acting on the OB to reduce insecticidal activity, a second NOV sample was made by dissolving the OB after light exposure by the addition of carbonate with the feeding solution. Dry virus samples were mixed at 100x the final concentration ($3 \times 10^7$ OB/ml for occluded virus and $5 \times 10^8$ OB/ml for NOV). One hundred microliters of each suspension was pipetted individually on to each of two 22-μm glass cover slips and allowed to air dry. After drying, one cover slip for each treatment was placed in the Suntest machine on a water-cooled tray set at 18°C for light exposure. After exposure, the cover slips were placed individually in 25-ml beakers with 10 ml of blue feeding solution, mixed with a VirTishear homogenizer, and included in the droplet feeding assay as described previously.

Closed petri dishes were used to keep the virus samples from drying (= wet samples) during light exposure. The virus concentration tested was $3.0 \times 10^6$ OBs/ml for both occluded and NOV samples. Three samples were compared; occluded virus, occluded virus for light exposure with the polyhedron dissolved after exposure, and nonoccluded virus with the polyhedron dissolved before light exposure. Four milliliters from each sample were placed in two individual 50 × 9-mm petri dishes. One dish for each sample was placed on the cooling tray in the Suntest machine for exposure. The plastic of the dish lid reduced the amount of energy by 35% between 300 and 324 nm (measured by a LiCor 1800 Spectroradiometer; LiCor Corp.) (data not shown). Addition of water to the petri dish did not reduce transmittance of light energy in the UV range recorded. After exposure, 1 ml of concentrated droplet feeding solution was added to each virus sample in the dish to obtain the final feeding suspension. For the sample in which the polyhedron was dissolved after exposure, sodium carbonate was added to the virus sample with the concentrated feeding solution to provide 0.3% w/v in the final virus suspension. Eight treatments were arranged in a $2 \times 2 \times 2$ factorial design, and the procedure was repeated on 3 separate days for replication. Data were analyzed using analysis of variance and the means were separated by the LSMEANS option of the GLM procedure in SAS (1985).

For the plant exposure assay, discriminating doses of occluded and NOV samples were $3.0 \times 10^6$ and $9.0 \times 10^6$ OBs/ml, respectively. Virus-treated leaves were exposed to xenon light by removing the tray from the Suntest machine and placing the plant below the machine. Clear plastic (Tefcel; American Durafilm, Holliston, MA) was placed between the light and the leaves to reduce leaf movement caused by the cooling fan. Previous research demonstrated very little loss of UV with this plastic (McGuire et al., 1996). Leaves were 30–35 cm from the light source and were exposed for 8 h. After exposure, the treated leaf disks were cut out and assayed as described for the plant assay. Again treatments were arranged in a factorial design ($2 \times 2 \times 2$) with virus type (occluded and nonoccluded), sucrose concentration (0 and 2.0% w/v), and light exposures (no exposure and 8 h xenon light exposure) as the main effects. The procedure was repeated over 3 days for three replications and these data were analyzed as before using SAS.

RESULTS

Samples of the virus in concentrations up to 0.5% of sodium carbonate did not lose insecticidal activity in a droplet bioassay. A virus concentration of $5 \times 10^5$ OB/ml provided 99, 99, 97, and 98% mortality in a droplet assay for virus samples with 0, 0.1, 0.3, and 0.5% sodium carbonate, respectively. The final pH of the 0.0, 0.1, 0.3, and 0.5% sodium carbonate solutions was 6.5, 10.7, 10.9, and 11.0, respectively. Microscopic examinations of the samples indicated that the OBs began dissolving in solutions of 0.1% sodium carbonate. In these samples, the OBs appeared larger and had different light refraction properties than OBs in water. In samples with 0.3% sodium carbonate, OBs were no longer visible through the microscope. Based on these results and observations, the 0.3% sodium carbonate concentration was selected to dissolve the OBs for NOV.

Several additives were screened for their ability to protect the insecticidal activity of NOV after drying. Among the additives tested at 0.5% w/v with NOV, only sucrose prevented a loss of insecticidal activity. Insecticidal activity of wet and dry samples of occluded virus were not significantly different ($P = 0.14$) (Table 1). Also, insecticidal activity of wet and dry samples of NOV with sucrose were not significantly different ($P = 0.19$). Dry samples of NOV alone and with polyethylene glycol, lignin, and glycercin had lower insecticidal activity than their corresponding wet sample ($P = 0.004$, 0.0001, 0.0001, and 0.0018, respectively). Dry samples of nonoccluded virus with polyethylene glycol, lignin, and glycercin were not significantly different ($P > 0.05$) from the control, indicating that they had lost all insecticidal activity. As a result of this experiment, samples of occluded and nonoccluded virus containing sucrose were added to subsequent assays to maintain insecticidal activity of nonoccluded virus after drying.
The insecticidal activity of NOV was greater than occluded virus in droplet feeding assays when the samples remained wet. Based on the dose response, the potency of nonoccluded wet virus was 20X the occluded wet virus sample (Table 2). When samples were dried on glass cover slips, NOV samples lost insecticidal activity and a dose response curve was not calculated because the highest concentration (1.0 \times 10^6 OB/ml) provided only 27% mortality. Three samples of occluded virus (wet, dry, and dry with sucrose) were not significantly different, as indicated by their overlapping confidence limits (Table 2). Probit analysis showed that intercepts and slopes among the dry samples of NOV with sucrose and the three occluded virus samples were not significantly different (slope = 1.50, \textit{LC}_{30} = 1.33 \times 10^{5}, \chi^2 = 7.28, df = 6, \text{P} = 0.30). Thus, in the droplet assay for light exposure, NOV samples containing sucrose could be compared with occluded virus samples at the same dose.

Typically, \textit{LC}_{30} values for virus were higher in the plant assay than in the droplet assay. Dose response results for the occluded virus applied to cotton leaves gave an \textit{LC}_{30} of 7.8 \times 10^5 OBs/ml. The nonoccluded virus (no sucrose) did not have sufficient insecticidal activity within the range of concentrations tested for probit analysis to determine a dose response. NOV samples with sucrose retained insecticidal activity. Probit analysis of the occluded virus, occluded virus with sucrose, and NOV with sucrose indicated that the probit lines were not significantly different (\chi^2 = 7.33, df = 4, \text{P} = 0.12). These results support direct single-dose comparisons of the NOV sample containing sucrose to the occluded virus samples with or without sucrose.

In the single-dose light exposure experiment, the NOV sample that stayed wet (enclosed in petri dish) did not lose insecticidal activity, whereas a similar sample (dried on the cover slip) lost all activity (Tables 3 and 4). NOV samples had less insecticidal activity than did samples of occluded virus. When the OBs were dissolved after light exposure (Table 3), the amount of insecticidal activity was similar to that of the occluded virus after light exposure. These data indicate that light exposure did not reduce insecticidal activity by affecting the occlusion body but had a direct

### Table 1

Mean (SD) Percentage Mortality of Neonate \textit{Trichoplusia ni} (Hübner) Exposed to Samples of Nonoccluded (NOV) A/MI-NPV with Additives (0.5% w/v) to Prevent Loss of Insecticidal Activity When Dried

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Virus sample</th>
<th>(X^2)</th>
<th>(\text{LC}_{30}) (OB/ml)</th>
<th>Slope</th>
<th>Lower CI 95%</th>
<th>Upper CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Droplet</td>
<td>Occluded wet</td>
<td>1.6</td>
<td>(9.6 \times 10^4)</td>
<td>1.24</td>
<td>5.8 \times 10^4</td>
<td>1.5 \times 10^5</td>
</tr>
<tr>
<td>Droplet</td>
<td>Occluded dry</td>
<td>0.5</td>
<td>(2.2 \times 10^4)</td>
<td>1.10</td>
<td>9.5 \times 10^4</td>
<td>5.0 \times 10^4</td>
</tr>
<tr>
<td>Droplet</td>
<td>Occluded dry/sucrose</td>
<td>2.7</td>
<td>(1.7 \times 10^4)</td>
<td>1.74</td>
<td>(1.1 \times 10^4)</td>
<td>(2.5 \times 10^4)</td>
</tr>
<tr>
<td>Droplet</td>
<td>NOV wet</td>
<td>1.6</td>
<td>(4.9 \times 10^4)</td>
<td>1.04</td>
<td>(2.4 \times 10^4)</td>
<td>(1.2 \times 10^4)</td>
</tr>
<tr>
<td>Droplet</td>
<td>NOV dry</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Droplet</td>
<td>NOV dry/sucrose</td>
<td>0.1</td>
<td>(1.2 \times 10^4)</td>
<td>1.53</td>
<td>(6.9 \times 10^4)</td>
<td>(1.9 \times 10^4)</td>
</tr>
<tr>
<td>Plant</td>
<td>Occluded</td>
<td>4.7</td>
<td>(7.8 \times 10^4)</td>
<td>1.17</td>
<td>(2.9 \times 10^4)</td>
<td>(4.8 \times 10^4)</td>
</tr>
<tr>
<td>Plant</td>
<td>Occluded/sucrose</td>
<td>7.1</td>
<td>(3.3 \times 10^4)</td>
<td>1.38</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Plant</td>
<td>NOV</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Plant</td>
<td>NOV/sucrose</td>
<td>4.5</td>
<td>(9.8 \times 10^4)</td>
<td>1.04</td>
<td>3.6 \times 10^4</td>
<td>9.8 \times 10^4</td>
</tr>
</tbody>
</table>

Note. na, Insufficient insect mortality to determine a dose response.
Effect of Adding 2% Sucrose on the Loss of Insecticidal Activity for Wet (in a Covered Petri Dish) and Dry (Air Dried in a Glass Cover Slip) Samples of Occluded (LC\textsubscript{50} = 3.0 \times 10^5 OB/ml) and Nonoccluded (NOV; LC\textsubscript{50} Wet = 5.0 \times 10^4 OB/ml; Dry = 3.0 \times 10^5 OB/ml) A\textit{f}MNPV

<table>
<thead>
<tr>
<th>Virus sample</th>
<th>No exposure</th>
<th>80 Min. exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occluded Wet</td>
<td>88.5 (8.7) ab</td>
<td>87.7 (9.5) ab</td>
</tr>
<tr>
<td>Occluded Wet 2% Suc.</td>
<td>92.1 (2.1) a</td>
<td>87.4 (5.2) abc</td>
</tr>
<tr>
<td>Occluded Dry</td>
<td>81.1 (11.7) abc</td>
<td>20.9 (18.0) ef</td>
</tr>
<tr>
<td>Occluded Dry 2% Suc.</td>
<td>88.9 (7.7) a</td>
<td>48.9 (5.1) bcd</td>
</tr>
<tr>
<td>NOV Wet</td>
<td>97.6 (4.1) a</td>
<td>67.8 (12.6) cd</td>
</tr>
<tr>
<td>NOV Wet 2% Suc.</td>
<td>93.3 (11.5) a</td>
<td>60.0 (27.3) d</td>
</tr>
<tr>
<td>NOV Dry</td>
<td>31.1 (2.2) e</td>
<td>2.2 (3.9) f</td>
</tr>
<tr>
<td>NOV Dry 2% Suc.</td>
<td>100.0 (0.0) a</td>
<td>67.8 (23.6) cd</td>
</tr>
</tbody>
</table>

Note. Mortality determined against \textit{Trichoplusia ni} (Hübner) using a droplet feeding assay. Means followed by the same letter are not significantly different; \( P < 0.05, df = 34, MSE = 143.4. \)

effect on the virions. The results indicate that the OB provides protection for the virions exposed to light energy. Sucrose solutions had little effect as a sunscreen, as wet samples with 2% sucrose lost as much activity as wet samples without sucrose (Table 4). Dry samples generally had greater loss of insecticidal activity than wet samples. Also, light-exposed dry samples with no sugar had lower insecticidal activity than similar samples containing sugar (Table 4).

The single-dose plant assay to compare light exposed with nonexposed samples supported the results observed in the droplet feeding assay. Whether exposed or not exposed, NOV without sucrose that was dried on cotton leaves showed no insecticidal activity (<5% mortality; \( H_0: \text{LSmean} = 0, P > 0.50 \)) (Table 5). NOV is more susceptible to degradation by light than occluded virus based on the samples that contained sugar. With no light exposure, both occluded and nonoccluded samples with sugar were relatively close to the expected 80% mortality. After exposure, the activity for the NOV sample was lost (mean = 2.17% mortality; \( H_0: \text{LSmean} = 0, P = 0.74 \)), whereas the occluded sample provided 37% mortality (\( H_0: \text{LSmean} = 0, P = 0.0001 \)).

**Discussion**

Our experiments were designed to compare occluded with NOV for their ability to resist degradation by light energy. Our results show that NOV has a higher insecticidal activity than occluded virus with freshly prepared samples that remained wet. However, insecticidal activity was lost whenever samples of NOV were dried. This loss occurred on both glass and plant leaf surfaces. However, when the NOV remained wet, insecticidal activity was retained at levels near the expected values. These experiments demonstrate that differences in results are often caused by experimental techniques. Previous reports of NOV indicated that much of the insecticidal activity was lost when the OB was dissolved. Shapiro and Ignoffo (1969) used diet overlay bioassays in which the NOV was dried on a diet surface to conclude that most of the insecticidal activity was lost when the OB was dissolved. Their results support our results that show a loss of insecticidal activity for NOV when samples were dried. Perhaps, their samples dried on the diet surface and the drying reduced insecticidal activity. It is apparent that our assay techniques had dramatic consequences on the results for NOV samples.

We modified our droplet feeding assay to prevent samples from drying during light exposure. These data provide strong evidence that the NOV is more susceptible to degradation by light energy than occluded virus. Also, by dissolving the polyhedron protein after light exposure, it was obvious that light exposure did not reduce insecticidal activity by adversely affecting the polyhedron. Occluded virus samples provided similar insecticidal activity whether or not the polyhedron was dissolved after light exposure. If the light exposure affected the polyhedron protein in some way that reduced insecticidal activity, then insecticidal activity should have been regained when the occlusion body was dissolved after light exposure. This response was not observed. Thus, we conclude that the light reduced insecticidal activity by affecting the virions rather than the occlusion protein.

The specific mechanism responsible for the loss of activity of dried virions has yet to be determined. Possible explanations include denatured receptor proteins, disrupted DNA, or ruptured membranes. Also, we were not able to determine whether the damage is the result of loss of water molecules or increased alkalinity during drying. Basic research in this area may provide information useful to support additional development of NOV biopesticides.

Among several additives tried, sucrose was the most consistent in preventing the loss of insecticidal activity of dried samples. Dissolving sucrose in occluded and

**Table 4**

Mortality (SD) of \textit{Trichoplusia ni} (Hübner) Fed Cotton Leaves Treated with Occluded (LC\textsubscript{50} = 3.0 \times 10^5 OB/ml) and Nonoccluded (NOV; LC\textsubscript{50} = 9.0 \times 10^5 OB/ml) A\textit{f}MNPV with and without 2% Sucrose and Exposed to Simulated Sunlight (Xenon) for 8 h

<table>
<thead>
<tr>
<th>Virus sample</th>
<th>No exposure (SD)</th>
<th>8-h exposure (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occluded 2% Suc.</td>
<td>53.3 (5.8) a</td>
<td>37.8 (22.2) c</td>
</tr>
<tr>
<td>NOV 2% Suc.</td>
<td>4.4 (5.1) d</td>
<td>1.1 (2.0) d</td>
</tr>
<tr>
<td>NOV 2% Suc.</td>
<td>72.2 (12.9) b</td>
<td>2.2 (1.9) d</td>
</tr>
</tbody>
</table>

Note. Control mortality was 1.1% in this plant bioassay. Means followed by the same letter are not significantly different; \( P < 0.05, df = 18, \text{MSE} = 107.5. \)
NOV suspensions did not protect insecticidal activity from light degradation. However, when samples were dried before light exposure, those with sucrose did not lose as much activity as similar samples with no sucrose. It is not clear whether the benefit provided by sucrose was due to preventing insecticidal activity loss because of light exposure or drying. We have no evidence to propose a mechanism by which sucrose was able to prevent the loss of insecticidal activity for NOV as it dried. Additional work in this area could provide breakthroughs to improve the physical requirements for improved commercial products with longer storage stability, increased activity, and reduced production costs. Presumably, empirical information such as this may be useful in developing improved formulations of baculovirus biopesticides.

This research identified a problem with using NOV in field situations. NOV lost activity when dried under alkaline conditions in our experiments. This may mimic the situation in the field where occluded virus is applied to cotton, a probable market for this virus. Cotton is known to have alkaline conditions on the leaf surface which may be sufficient to cause OBs to release the virions in the presence of morning dew. As the dew dries during the day, the released virions could lose activity as we observed in our experiments.

There are advantages to occluded virus. First, it seems reasonable to assume that nature has selected for the occluded virus because of ecological benefits. The OB provides a discrete virus particle capable of causing mortality to susceptible hosts. These experiments demonstrate that the OB provides some protection from degradation by light energy and detrimental effects of drying.

In much of the previous research, dissociation of OB was done without determining the insecticidal activity of the final product (Whitt and Manning, 1987; Egawa and Summers, 1972; Nordin and Maddox, 1971). It has been shown that divalent cations have the ability to stabilize the occlusion body (Whitt and Manning, 1988). Although this information may not have a direct effect on the insecticidal activity of the virus, it may indirectly improve the stability of OBs in formulations when applied in the field. Using basic information such as this will contribute to effective development of commercially viable biological pesticides based on microbial agents.

REFERENCES


