Evaluation of spray-dried lignin-based formulations and adjuvants as solar protectants for the granulovirus of the codling moth, *Cydia pomonella* (L)

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Abstract

Commercial formulations of the codling moth, *Cydia pomonella* L., granulovirus (CpGV) are limited by their short residual activity under orchard conditions in the Pacific Northwest. We evaluated spray-dried lignin-encapsulated formulations of CpGV for improved solar stability based on laboratory bioassays with a solar simulator and in field tests in an infested apple orchard. In laboratory tests, aqueous lignin formulations containing a high dosage of $3 \times 10^{10}$ occlusion bodies (OB)/L, with and without the additives titanium dioxide (TiO$_2$) and sugar, provided significant solar protection of virus, i.e., mortality of codling moth exposed to lignin formulations that had been irradiated with $9.36 \times 10^6$ joules/m$^2$ was 92–94%, compared with 66–67% from a glycerin-stabilized product (Cyd-X®) or suspension of pure unformulated virus at the same rates. By comparison, a lower dosage of the lignin formulation ($3 \times 10^8$ OB/L) did not provide significant solar protection. Equivalent dosage-dependent patterns in solar protection were observed in further tests with the lignin formulation, when an intermediate ($3 \times 10^9$ OB/L) as well as the low dosage provided no solar protection. Equivalent rates of a blank lignin formulation (containing no virus) did not affect larval mortality, suggesting a protective effect of the lignin on the virus at the high rate.

The use of several spray adjuvants, ‘NuFilm-17®’ and ‘Organic Biolink®’ (sticker-spreaders at 0.06% v/v), ‘Raynox®’ (sunburn protectant at 5% v/v), and ‘Trilogy®’ (neem oil at 1% v/v) did not provide solar protection of a commercial CpGV preparation in laboratory tests. In season long orchard tests (Golden Delicious), the lignin formulation of CpGV applied at $6.57 \times 10^{12}$ OB/ha did not significantly improve control of codling moth or protection of fruit compared with Cyd-X at equivalent rates. Our studies show that lignin-based CpGV formulations provided solar protection at relatively high virus dosages. The testing of lignin formulations containing reduced virus concentrations may allow virus solar protection to be achieved at more economical rates.

Keywords: *Cydia pomonella*; Granulovirus; Apple; Ultraviolet; Adjuvant; Spray-dried microencapsulated formulations; Lignin

1. Introduction

Nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) (Baculoviridae) are important pathogens of a wide range of lepidopterous pests, and several have been developed as microbial pesticides (Federici, 1999). Despite their advantages as relatively environmentally benign agents suitable for use in integrated pest management programs, sensitivity to ultraviolet (UV) radiation, particularly the damaging portion UV-B, range 280–320 nm, remains a major limitation for the commercial development of baculoviruses (Adams and McClintock, 1991; Burges and Jones, 1988; Ignoffo et al., 1989; Jaques, 1985; Jones et al., 1993). A case in point, three formulations of the codling moth, *Cydia pomonella* L., granulovirus (CpGV) have recently become commercially available in North America (Lacey et al., 2004b). The virus is targeted for neonate larvae which ingest occlusion bodies (OB), also called

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granules, before or during initial entry into fruit. One of the major disadvantages of CpGV is its short residual activity under operational conditions (Arthurs et al., 2005; Huber, 1986; Jaques et al., 1987; Keller, 1973; Kienzle et al., 2003; Lacey et al., 2004a), normally requiring reapplication of the virus at 7- to 10-day intervals when codling moth neonates are present in the orchard.

A range of spray adjuvants has been tested with CpGV with the goal of improving virus uptake by larvae and/or increasing persistence of the virus on the surface of foliage or fruit. Substances such as molasses, sucrose, skimmed milk powder, and oxybenzone have been reported to improve CpGV effectiveness slightly, although the rates used are considered relatively high for routine field use (Ballard et al., 2000; Charmillot et al., 1998; Keller, 1973; Krieg et al., 1980).

Recently a spray-dried method to encapsulate viral occlusion bodies with a variety of UV screens (carriers) has been developed. Some of the more effective carriers, such as lignin, were shown to significantly extend residual activity of baculoviruses isolated from Autographa californica Speyer (AcMNPV) and a variant of AcMNPV isolated from Anaglyphia falcifera Kirby (AnalfaMNPV) (=AMNPV) (Behle et al., 2003; McGuire et al., 2001; Tamez-Guerra et al., 2000a) as well as Bacillus thuringiensis (Tamez-Guerra et al., 2000b) during exposure to natural or artificial sunlight.

In the present study, we report on studies evaluating lignin-encapsulated formulations and several spray adjuvants for improved solar protection of CpGV in laboratory tests with a solar simulator and in an infested apple orchard. This is the first report of microencapsulation of CpGV.

2. Materials and methods

2.1. Plant and insect cultures

Codling moth eggs (black head stage ready to hatch) were obtained on wax paper from the colony maintained at the Yakima Agricultural Research Laboratory and reared using the system of Toba and Howell (1991). Fuji apples for laboratory tests were collected from an unsprayed orchard block in September/October 2004 and 2005 at the USDA experimental farm near Moxee WA, and kept in a controlled atmosphere fruit storage chamber (1–2 °C) until use. Apples were 6–7 cm diameter and only those free of pests were selected.

2.2. Virus source

A commercial preparation of CpGV ‘Cyd-X’ (Ceris USA, Columbia MD) containing $3 \times 10^{13}$ OB/L was the virus stock in all tests.

2.3. Laboratory bioassay procedure

Laboratory tests were conducted using the procedure previously described (Lacey and Arthurs, 2005). In short, apples were surface sterilized and sectioned, and the cut surface immediately heat-treated and sealed with wax and foil. The half apple preparation allows an even coverage of virus to be applied over the surface of the fruit and exposed to a controlled dose of irradiation that would not be possible using whole apples. The fruit also maintained viability for neonate codling moth for the duration of the bioassays.

Prepared fruit were sprayed with experimental treatments in a DeVries spray cabinet (DeVries Mfg., Hollandale, MN) using a track-mounted flat fan nozzle (XR TeeJet® 801 VS, Spraying Systems Co., Wheaton, IL) calibrated to deliver 935 L/ha at 206 kPa. After apples had dried, half from each treatment (UV-controls) were individually placed in 0.5 L plastic food containers and immediately infested with five neonates (<2h old) using a fine paintbrush. The remaining half were placed in a reflective cabinet and exposed to UV (300–400 nm) and other wavelengths (visible, 400–800 nm and infrared $\geq$800 nm) with an Atlas Suntest CPS+ solar simulator (Atlas Material Testing Technology LLC, Chicago, IL). Apples were irradiated for 4 h at 765 W/m², providing an accumulated radiant energy of $9.36 \times 10^6$ joules/m² on the shelf where the samples were located, and allowed to cool prior to infestation. All samples were incubated at 25 ± 2 °C, 16:8 L:D for 10 days and then destructively sampled under a dissecting microscope at 10× magnification to quantify fruit damage and larval survivorship. The proportion of ‘deep’ larval entries (i.e., $\geq$6 mm) was also noted; previous studies show depth of entries were a proxy for virus dosage consumed and speed of kill (Arthurs et al., 2005).

2.4. Evaluation of different lignin formulations

Spray-dried virus formulations were prepared with sodium lignin (PC-1307, Westvaco, Charleston Heights, SC) with and without the additives titanium dioxide (TiO₂, Millennium Inorganic Chemicals, Hunt Valley, MD) and sugar (Table 1). Lignin was first mixed in water (10% w/v) for 20 min using a blender (Waring, New Hartford, CT) and the pH of dissolved solution adjusted to 9.0 ± 0.2 with 2% sulfuric acid. TiO₂ and sugar were added as pre-diluted homogeneous suspensions followed by the virus stock, which was first cleaned to remove stabilizing carriers (glycerin) by triple dilution and centrifugation. Glycerin was discarded with the supernatant and the virus retained with the pellet. Calcium chloride (CaCl₂, 10% concentration) was

<table>
<thead>
<tr>
<th>Table 1: Ingredients used to prepare spray-dried lignin formulations of CpGV containing $5 \times 10^9$ OB/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation</strong></td>
</tr>
<tr>
<td>Lignin</td>
</tr>
<tr>
<td>Lignin + sugar</td>
</tr>
<tr>
<td>Lignin + TiO₂</td>
</tr>
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</table>

* Prepared at 5% w/v for spray dryer feed.

b Pure virus, based on expected recovery of OB from Cyd-X product.
blended slowly to all mixtures at a 20% w/w of lignin. A Niro Atomizer Spray Drier (Niro Atomizer Inc., Columbia, MD) was used to spray dry mixtures to form crosslinked microgranules, thus encapsulating viral OBs. Drying conditions were 115–125°C inlet and 65–75°C outlet temperatures, 18–20 ml/min feed rate and 6 kg/cm² air pressure. All spray-dried powders contained a final concentration of 5 × 10⁹ OB/g and 5% moisture content and were refrigerated in sealed plastic bottles until use. Estimates of virus concentration were conservative, assuming no loss of virus during the purification and spray-drying process.

For tests, lignin formulations were diluted and applied to apples at two rates; 3 × 10⁸ and 3 × 10¹⁰ OB/L (2.8 × 10¹¹ and 2.8 × 10¹³ OB/ha, respectively) for bioassay against codling moth neonate using the procedure outlined above. A glycerin-stabilized product (Cyd-X) or suspensions of pure virus were included at the same rates for comparison. Silwet L77 (Silicone-polyether copolymer, Loveland Industries, Inc., Greeley, CO) was always added as a wetting agent at 0.025%. Water + wetting agent was used as a virus-free control. Test suspensions were prepared in 500 ml plastic bottles that were shaken immediately before application. To prevent contamination, the spray apparatus was sterilized with 0.25% NaClO and rinsed with distilled water.

### 2.5. Dosage response with lignin formulation

A further study was conducted to evaluate the relationship between the dosage of virus and protection of the lignin formulation from simulated sunlight as well as quantify any influence of the lignin carrier (excluded in earlier tests). Because TiO₂ and sugar were not effective in the previous study, only the lignin formulation was compared with Cyd-X. Virus formulations were applied at three rates; 3 × 10⁸, 3 × 10⁹, and 3 × 10¹⁰ OB/L, obtained through serial dilution. A blank lignin formulation (spray-dried in the same way but excluding virus) was also tested at the same rates (w/v) as the formulation containing virus. In all other respects, studies were conducted as previously outlined. Ten apple halves were sprayed per treatment and the study was conducted five times.

### 2.6. Evaluation of spray adjuvants

Several spray adjuvants were evaluated as solar protectants when incorporated with the Cyd-X preparation at the time of dilution. The following materials were tested within manufacturers’ recommended rates; the sticker-spreaders ‘NuFilm-17®’ (Miller Corp. Hanover, PA) consisting of 3,4,5-trimethene (96%) and inerts (4%) and ‘Organic BioLink®’ (Westbridge, Vista, CA) consisting of soapbark, alklyphenol, ethoxylate, polysaccharide (10.1%), and inerts (89.9%), both at 0.06% v/v, the apple sunburn protectant ‘Raynox®’ (Pace International; Wapato, WA) primary ingredients water, carnauba wax, organically modified clay, and emulsifiers at 5% v/v, and a fungicide/miticide, ‘Trilogy®’ (Certis USA) a clarified hydrophobic extract of neem oil (70%) and inerts (30%) at 1% v/v. For tests, suspensions of Cyd-X with and without adjuvants added were sprayed on apples at two rates; 3 × 10⁸ and 3 × 10¹⁰ OB/L, for bioassay. In all other respects, studies were conducted as previously outlined. Ten apple halves were sprayed per treatment (virus-free adjuvant only controls were excluded) and the study was conducted five times.

### 2.7. Field study

Orchard tests were conducted in 2005 at the USDA-ARS experimental station near Moxee WA. The test block was a 0.5 ha Golden Delicious cv. Smoothie planted in 1980 on EMLA 7 rootstock at 500 trees/ha that was naturally infested with codling moth. Under-tree sprinklers (Nelson low angle impact model L20W) prevented insecticide run-off. Conditions were predominantly sunny and dry (<1 cm rainfall) throughout the study period, with average daytime highs and nighttime lows of 33.2 and 2.0 °C, respectively (mean 18.0) during the period of the first larval generation and 37 and 7.4 °C, respectively (mean 21.8) for the second larval generation. Two wing-type BioLure® traps baited with red septa lures containing 1 mg codlemone (Suterra, Bend, OR) were hung in the canopy at either end of the block to determine biofix (first consistent catch) and monitor seasonal flight patterns. Lures and inserts were changed every 2–3 weeks.

### 2.8. Field applications

A spray-dried lignin-virus formulation (Table 1) was compared with Cyd-X in single-tree plots against both seasonal codling moth generations. Applications were timed to coincide with the hatching of neonates using an adult biofix and a phenology model based on degree day (DD) accumulation (Beers et al., 1993). Treatments were applied early morning during calm wind conditions (<0.5 m/s) with a motorized backpack airblast sprayer (model SR 420, Stihl, Virginia Beach, VA) calibrated to provide complete coverage of foliage and fruit at 935 L/ha. The spreader/sticker NuFilm-17 was always included at 0.58 L/ha. A tarpaulin screen (3 × 9 m) held by four assistants and a buffer tree were employed to minimize overspray or spray drift. The sprayer was agitated before each tree was sprayed to prevent settling of suspensions and rinsed with distilled water between treatments.

In the first generation, virus treatments were applied at 6.57 × 10¹² OB/ha (standard commercial rate). Initial sprays were made 26 May (226 DD, 2% egg hatch) with three further applications at 14-day intervals (389, 564, and 796 DD). Excepting controls, the experiment was a completely randomized design, with ten replicates. Control trees sprayed with water and blank lignin (8 trees each) were selected from two adjacent rows separated by an untreated buffer row. The study was repeated for the second genera-
tion using the same design, but with one fewer application and reduced rates of virus in half of the plots (i.e., five randomly selected trees received $2.2 \times 10^{12}$ OB/ha). Initial sprays were made 28 July, with two further sprays at 14-day intervals (1205, 1516, and 1786 DD). Larval mortality inside virus-treated fruit was comparatively high ($\geq 88\%$) in the first generation, and it was considered that reduced rates might improve the chance of detecting improved residual control of lignin-virus treatments.

2.9. Field assessments

Evaluations were made at the end of the first (21–22 July; 1056–1075 DD) and second larval generations (15–16 September; 2054–2062 DD). Codling moth injury was visually assessed in situ from a random sample of 50 fruits per tree. Samples of infested fruit (up to 50 per tree depending on availability) were subsequently collected and destructively examined under a dissecting microscope at $\times 10$ magnification. The severity of damage (shallow versus deep entry) and the mortality of larvae inside fruit were noted. Mature larvae that had already left the fruit, indicated by substantial feeding and an open ‘exit hole’ were incorporated in the live sample. Fruit were maintained at 12°C for up to 3 weeks until processing. To census the overwintering population, corrugated cardboard bands were stapled around the bole of treated and control trees and later examined in the laboratory for cocooning larvae. Bands were 8-cm wide and first folded flute-to-flute (single faced size B, Xpedx, Portland, OR) to provide pupation sites between the layers. Bands were placed 30–46 cm above the ground when diapause-destined larvae started exiting unsprayed fruit on 24 August (1771 DD) and were maintained until 3 October (2198 DD), when the majority of the population had exited fruit. Bands were periodically replaced to reduce bird damage.

2.10. Data analysis

Treatment effects were compared using one, two, and three way univariate ANOVA (SPSS, 2003) to establish main and interaction effects. Where appropriate, significant F-ratio means were further separated with Fisher’s LSD for multiple comparisons, at $P < 0.05$. All proportional and count data were normalized via arcsine and log $(n + 1)$, respectively, prior to analysis. In laboratory tests, each test date (i.e., mean of five apple halves) was considered a replicate $(n = 5)$, in field tests each tree was a replicate $(n = 8$ or $10)$.

3. Results

3.1. Evaluation of different lignin formulations

Larvae on untreated control fruit tended to bore towards the center feed on the seeds within and $> 65\%$ of the neonates infested were recovered alive. There were significant differences in larval mortality among the various virus treatments. The majority of virus-killed larvae died as first instars near the surface of the fruit, identifiable by milky-white color and tendency for the epidermis to rupture when prodded. Three-way ANOVA revealed significant main effects of virus formulation, i.e., lignin, glycerin or pure virus ($F_{2,88} = 11.1, \ P < 0.0001$), dosage ($F_{1,88} = 185.6, \ P < 0.0001$) and simulated sunlight exposure ($F_{1,88} = 93.3, \ P < 0.0001$). There were also the following two- and three-way interactions for larval mortality; formulation x dosage ($F_{2,88} = 5.1, \ P < 0.01$), formulation x simulated sunlight ($F_{2,88} = 4.8, \ P < 0.05$), dosage x simulated sunlight ($F_{1,88} = 13.4, \ P < 0.0001$), and formulation x dosage x simulated sunlight ($F_{2,88} = 5.8, \ P < 0.005$). Correspondingly, there were significant main and interaction effects for the proportion of deep entries ($\geq 6\text{mm}$) among virus formulations ($F_{2,88} = 3.3, \ P = 0.04$), dosage ($F_{1,88} = 82.0, \ P < 0.0001$), simulated sunlight ($F_{1,88} = 51.4, \ P < 0.0001$), formulation x simulated sunlight ($F_{2,88} = 5.1, \ P < 0.01$), dosage x simulated sunlight ($F_{1,88} = 4.1, \ P < 0.05$), and formulation x dosage x simulated sunlight ($F_{2,88} = 3.5, \ P < 0.05$). There were no significant effects of experimental treatments on the total number of injuries (shallow + deep) in fruit ($P > 0.15$).

Because data for larval mortality and entries suggested virus formulations responded differently to simulated sunlight exposure according to the dosage applied, separate one-way contrasts were conducted for each dosage and compared to untreated controls to illustrate the nature of these interactions. The lignin-based formulations provided extended simulated sunlight protection (compared with Cyd-X) when applied at the high, but not at the low dosage (Table 2). There were no differences among the lignin formulations in either case, with the exception of TiO$_2$ which was less effective than the sugar at the low dosage. In the non-irradiated fruit (UV-controls), there were no differences among virus formulations ($F_{2,44} = 0.15, \ P = 0.87$). In non-irradiated fruit, mortality was always high at the high dosage (97–99%) and intermediate at the low dosage (62–75%); dosage was significant ($F_{1,44} = 46.9, \ P < 0.0001$). Further means for non-irradiated fruit are not presented.

3.2. Dosage response with lignin formulation

A priori one-way contrasts showed that different dosages of blank lignin carrier had no effect on overall fruit damage by coding moth (shallow stings + deep entries combined), the proportion of deep entries or larval mortality ($P \geq 0.35, \ df = 2, 27$). The blank lignin treatment was therefore dropped from further analysis, and the lignin+ virus formulation was assumed to reflect the combined effect of both agents. A three-way model again revealed significant treatment effects on larval mortality among formulations, i.e., Cyd-X and lignin-virus ($F_{1,48} = 12.8, \ P < 0.0001$), virus dosage ($F_{2,48} = 108.2, \ P < 0.0001$), and simulated sunlight exposure ($F_{1,48} = 148.4, \ P < 0.0001$) with two- and three-way interactions for
formulation × dosage \( F_{2,48} = 9.3, \ P < 0.0001 \), dosage × simulated sunlight \( F_{2,48} = 17.5, \ P < 0.0001 \) and formulation × dosage × simulated sunlight \( F_{2,48} = 8.7, \ P < 0.001 \). There were correspondingly main and interaction effects for the proportion of deep entries \((≥ 6 \text{ mm})\) among dosage \( F_{2,48} = 26.6, \ P < 0.0001 \), simulated sunlight \( F_{1,48} = 87.2, \ P < 0.0001 \), formulation × dosage \( F_{2,48} = 3.3, \ P < 0.05 \), dosage × simulated sunlight \( F_{2,48} = 8.3, \ P < 0.001 \) and formulation × dosage × simulated sunlight \( F_{2,48} = 5.3, \ P < 0.01 \). There were no significant effects of experimental treatments on the total number of injuries (shallow + deep) in fruit \((P > 0.05)\).

As in the previous study, further one-way contrasts showed that lignin-based formulations provided simulated sunlight protection when applied at the high rate \((3 \times 10^{10} \text{OB/L})\), but not the low rate nor in this case intermediate rates (Table 3). As in the previous study there were no differences between virus formulations within the non-irradiated fruit (UV-controls) \( F_{2,48} = 1.1, \ P = 0.3 \) although mortality was again dosage-dependent \( F_{2,48} = 69.5, \ P < 0.0001 \); low (53–55%), medium (90–94%), high (98–100%), and controls (14.8%).

### 3.3. Evaluation of spray adjuvants

Significant differences in larval mortality were explained by virus dosage \( F_{1,80} = 282.9, \ P < 0.0001 \), simulated sunlight exposure \( F_{1,80} = 334.1, \ P < 0.0001 \) and their interaction \( F_{1,80} = 113.3, \ P < 0.0001 \). The main effect of adjuvant type was marginal \( F_{4,80} = 2.8, \ P = 0.03 \), although there were no significant interaction terms for adjuvant type, virus dosage nor simulated sunlight \((P ≥ 0.37)\). The three-way (adjuvant × dosage × sunlight) interaction was marginal \( F_{4,80} = 2.3, \ P = 0.07 \). Equivalent trends were observed for the proportion of deep entries in fruit, although in this case the adjuvant × sunlight interaction approached significance \( F_{4,80} = 2.4, \ P = 0.06 \), but not the three-way \((P = 0.26)\). As in the previous studies there were no differences on the total number of injuries (shallow + deep) in fruit among treatments \((P ≥ 0.13)\).

One-way contrasts were performed to compare the different adjuvants side by side following simulated sunlight exposure (Table 4). No adjuvants provided solar protection compared with Cyd-X alone at either dosage compared to controls. It was noted that the 1% v/v Trilogy reduced virus effectiveness at the high rate; an equivalent statistical trend was observed at the low virus dosage in the absence of UV (i.e., 46% mortality versus ≥56% for other treatments) suggesting a possible antifeedent effect of the neem oil independent of sunlight exposure.

### 3.4. Field studies

Pheromone traps show two distinctive flights (Fig. 1), which closely tracked the phenology model (Beers et al., 1993). The second flight was larger (69% of seasonal catch) and led to more fruit damage (i.e., 34% in control trees versus 6% in the first generation). However, because the majority of trees in the orchard block were not sprayed, infestation levels do not reflect efficacy of virus treatments, but rather indicate the high pressure under which treatments were conducted.

Assessments of the virus trial against both larval generations are summarized in Table 5. There were no differences

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### Table 2

<table>
<thead>
<tr>
<th>Formulation</th>
<th>High dose ((3 \times 10^{10} \text{OB/L}))</th>
<th>Low dose ((3 \times 10^{6} \text{OB/L}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% mortality</td>
<td>% deep entries</td>
</tr>
<tr>
<td>Untreated</td>
<td>34.4c</td>
<td>97.6a</td>
</tr>
<tr>
<td>Pure CpGV</td>
<td>67.2b</td>
<td>67.3b</td>
</tr>
<tr>
<td>Cyd-X</td>
<td>66.8b</td>
<td>65.9b</td>
</tr>
<tr>
<td>Lignin/CpGV</td>
<td>93.6a</td>
<td>29.7c</td>
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<tr>
<td>Lignin/CpGV + sugar</td>
<td>92.8a</td>
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</tr>
<tr>
<td>Lignin/CpGV + TiO₂</td>
<td>92.0a</td>
<td>41.1c</td>
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</table>

Data show average for five replicate tests \((n = 25)\) for fruit sprayed with two rates of virus. Column letters indicate mean separations following significant F-ratio test; Fishers LSD at \(P < 0.05\).

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### Table 3

<table>
<thead>
<tr>
<th>Formulation</th>
<th>High dose ((3 \times 10^{10} \text{OB/L}))</th>
<th>Med. dose ((3 \times 10^{9} \text{OB/L}))</th>
<th>Low dose ((3 \times 10^{6} \text{OB/L}))</th>
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<tr>
<td></td>
<td>% mortality</td>
<td>% deep entries</td>
<td>% mortality</td>
</tr>
<tr>
<td>Untreated</td>
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<td>83.2a</td>
<td>21.4b</td>
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<td>Cyd-X</td>
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<td>62.0a</td>
<td>44.5a</td>
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<tr>
<td>Lignin/CpGV</td>
<td>95.4a</td>
<td>25.6b</td>
<td>41.7a</td>
</tr>
</tbody>
</table>

Data show average for five replicate tests \((n = 25)\) for fruit sprayed with three rates of virus. Column letters indicate mean separations following significant F-ratio test; Fishers LSD at \(P < 0.05\).
Table 4
Mortality and deep entries (≥ 6 mm) of codling moth larvae recovered on half apples previously treated with Cyd-X with and without spray adjuvants and irradiated with 9.36 × 10⁶ joules/m² in a solar simulator.

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>High dose (3 × 10¹⁰ OB/L)</th>
<th>Low dose (3 × 10⁹ OB/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% mortality</td>
<td>% deep entries</td>
</tr>
<tr>
<td>Untreated</td>
<td>29.6c</td>
<td>87.8a</td>
</tr>
<tr>
<td>None</td>
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<td>68.2b</td>
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<td>NuFilm-17</td>
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<td>Trilogy</td>
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<td>72.1b</td>
</tr>
</tbody>
</table>

Data show average for five replicate tests (n = 25) for fruit sprayed with two rates of virus. Column letters indicate mean separations following significant F-ratio test; Fishers LSD at P < 0.05.

Fig. 1. Codling moth pheromone trap data (weekly catch ± SEM) at Moxee experiment station against accumulated degree days (°F) post bloom. Arrows denote timing of virus applications.

in percentage fruit injury; however significantly fewer deep entries occurred in virus treatments compared with controls, in which the majority of larvae reached the core to feed on seeds. More significantly, dissections of fruit revealed the majority of larvae within virus-treated fruit were dead. Mortality rates were higher against the first generation (88–93%) compared with the second (59–71%), which might have been due to hotter and sunnier conditions later in the season. There were no clear benefits from lignin formulation in either case. In the second generation it was noted that reducing the dosage resulted in a significant reduction in efficacy (larval mortality) in the lignin but not the Cyd-X formulation. Assessments of tree bands showed 72–79% fewer overwintering larvae in virus-treated trees (Table 5). There were no differences among virus treatments, although because of the experimental design these bands may have been contaminated with larvae migrating from nearby unsprayed trees.

4. Discussion

Given that the short persistence of CpGV in the field remains an important limitation of commercial formulations (Arthurs et al., 2005; Lacey et al., 2004b), our results showing that lignin-encapsulation provided significant protection from UV radiation were encouraging (Tables 2 and 3). However, it was also noted that lignin was only effective with the high dosage (3 × 10¹⁰ OB/L); which is 4.3-fold higher compared with the ‘standard’ rate of virus currently used in the region. In field tests, the lignin formulation containing the ‘standard’ rate of virus did not improve CpGV effectiveness significantly when applied at 14-day intervals under high pest pressure (Table 5). It was also noted that the additives TiO₂ and sugar failed to improve the effectiveness of our lignin-CpGV formulations (Table 2). TiO₂ prevents sunburn by reflecting light energy, rather than adsorbing as with lignin, and had been shown to improve formulations of NPVs (Bull et al., 1976; Farrar et al., 2003). Sugar has also been noted as a potential larval phagostimulant for CpGV (Ballard et al., 2000) and also to benefit storage stability of AnafaMNPV in spray-dried lignin formulations (Tamez-Guerra et al., 2002).

The reason for the ineffectiveness of the lower dosage lignin formulations is unclear. Lignin is an abundant polymer found in vascular plants that is known to absorb visible wavelengths of light. A water soluble lignin salt was made by chemical modification with an alkaline agent (NaOH). Once dissolved in water the lignin was cross-linked with CaCl₂ which allows the virus to be encapsulated during the drying process in a lignin particle that is insoluble in neutral water.

Table 5
Orchard tests against codling moth (Golden Delicious single-tree plots)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>First generation</th>
<th></th>
<th>Second generation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% fruit injury</td>
<td>% mortality</td>
<td>% deep entries</td>
<td>% fruit damage</td>
</tr>
<tr>
<td>Untreated</td>
<td>6.1</td>
<td>38.5b</td>
<td>77.8a</td>
<td>33.8</td>
</tr>
<tr>
<td>Blank lignin</td>
<td>6.3</td>
<td>36.3b</td>
<td>75.4a</td>
<td>32.1</td>
</tr>
<tr>
<td>Cyd-X</td>
<td>11.1</td>
<td>93.2a</td>
<td>30.7b</td>
<td>26.2</td>
</tr>
<tr>
<td>Lignin/CpGV</td>
<td>9.1</td>
<td>87.8a</td>
<td>22.4b</td>
<td>27.9</td>
</tr>
<tr>
<td>Cyd-X (1/3 rate)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>28.5</td>
</tr>
<tr>
<td>Lignin/CpGV (1/3 rate)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>23.2</td>
</tr>
</tbody>
</table>

Assessments of spray-dried lignin formulations of CpGV were compared to Cyd-X following applications against both larval generations. Column letters indicate mean separations following significant F-ratio test; Fishers LSD at P < 0.05.

a Four applications at 6.57 × 10¹² OB/ha (26 May–7 July).

b Three applications (28 July–25 Aug.), half the trees were sprayed at a reduced rate (2.2 × 10¹² OB/ha).

c Number of diapause-destined larvae captured per band, includes live larvae removed during fruit evaluations.
or acidic water. Although the virions are presumably released in the alkaline gut where the lignin dissolves, a certain amount of virus incorporated into the lignin may be unavailable for the larvae, thus removing the benefits of the formulation at low dosages. Nevertheless, previous studies noted that lignin-encapsulated AnafaMNPV did not lose significant insecticidal activity due to the drying process, when compared with the unformulated virus (Behle et al., 2003; Tamez-Guerra et al., 2002). In the present case a more likely explanation for the reduced efficacy of lignin formulations at lower dosages relates to the amount of lignin that is available for UV protection. At high concentrations, the lignin may serve as a physical barrier by forming several protective layers on the foliar surfaces (lignin deposits were noted at the high but not lower dosages). As well as a barrier, the antioxidant property of lignin has been shown to stabilize and improve the activity of chemical pesticides (DelliColli, 1980). Because in the present studies the volume of lignin was reduced in line with the virus dosage, the protective effects may have been substantially diluted. The testing of high concentrations of lignin containing reduced concentrations of virus would be worthwhile.

The lack of UV protection in the adjuvant tests was disappointing (Table 4); label information for NuFilm-17 and Biolink indicates that they are insecticide extenders which protect against sunlight degradation under the rates tested, while Raynox is marketed as a sunburn protectant for fruit. NuFilm-17 and Raynox also did not extend the persistence of the Carposormin formulation of CpGV in previous orchard trials (Lacey et al., 2004a). Nevertheless, the use of spreader/stickers or other approved materials to improve spray coverage and rainfastness of virus formulations may still be prudent. While increasing the rate at which such adjuvants are applied might provide better UV protection, this practice may not be accepted by growers or regulators. Spray adjuvants may also work less well as virus enhancers if they interfere with feeding (virus uptake) in some way. This was suspected to be the case with Trilogy, as neem oil has antifeedant properties (Isman et al., 1990). The testing of other materials with CpGV, such as stilbene fluorescent brighteners (Hamm, 1999; Farrar et al., 2003; Shapiro and Argauer, 2003) or sprayable particle films (kaolin clays) recently developed for dual use as sunburn protectants and management of a range of orchard pests including codling moth (Unruh et al., 2000) are worthwhile.

Although our laboratory procedure allowed the solar sensitivity of CpGV to be assessed at the fruit surface, the method of assessment is time consuming and requires diligent attention to ensure accuracy. While a 10 day post infestation period was chosen to reduce problems of cannibalism as larvae matured, some of the 21–34% control mortality likely included live but small larvae that were missed during fruit evaluations. As a possible modified approach, which might aid future screening, virus residues could be harvested from fruit after simulated sunlight exposure through washing and incorporated into artificial media and used for bioassay. The amount of infective virus remaining can be estimated by comparing the sample mortality against a standard dilution series of known concentrations. Glen and Payne (1984) used a similar technique in the UK to measure inactivation of CpGV in the field and demonstrated that although infectivity was reduced by half in three days, some activity persisted at least 4–8 weeks after spraying, presumably in UV protected areas such as the calyx of fruit.

In conclusion, extending the persistence of CpGV with spray-dried formulations is possible, although there are still practical limitations to commercial adoption. We noted that high concentrations of lignin quickly settled out of suspension without continuous agitation and on one occasion blocked the nozzle. Under current pesticide regulations, new formulations of existing baculoviruses may require additional regulatory requirements such as re-registration, while any additional costs involved in large-scale spray-drying may also dissuade commercial adoption. The reduced shelf-life stability of spray-dried NPV formulations has also been cited as an issue affecting commercialization (Behle et al., 2003; Tamez-Guerra et al., 2002). The development of a lignin-based adjuvant, which could be tank mixed with virus formulations at the time of application and which would simplify commercial adoption is being investigated at the Crop Bioprotection Research Unit (USDA-ARS-NCAUR).

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