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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 3×10^{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 9.36×10^6 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 (3×10^8 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 (3×10^9 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 6.57×10^{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.
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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 *Anagrapha falcifera* Kirby (AnafaMNPV) (=AfMNPV) (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®' (Certis USA, Columbia MD) containing 3×10^{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® 8001 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 (<2 h 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 $\geqslant$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/m^2, providing an accumulated radiant energy of 9.36×10^6 joules/m^2 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., $\geqslant$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 (TiO_2, 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. TiO_2 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 ($CaCl_2$, 10% concentration) was

Table 1
Ingredients used to prepare spray-dried lignin formulations of CpGV containing 5×10^9 OB/g

Formulation[a]	Lignin, g (ml of 10% solution)	Sugar (g)	TiO_2 (g)	$CaCl_2$ (g)	CpGV[b] (9.96 OB/ml)
Lignin	20 (200)			4	3
Lignin + sugar	15 (150)	6		3	3
Lignin + TiO_2	15 (150)		6	3	3

[a] Prepared at 5% w/v for spray dryer feed.
[b] Pure virus, based on expected recovery of OB from Cyd-X product.