FINAL PROJECT REPORT

Project Title: Optimizing the use of the codling moth (CM) granulovirus
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OBJECTIVES:

1. Determine the lowest dosage of CpGV that will provide effective control of codling moth larvae.
2. Determine optimal intervals for spray application.
3. Continue to assess the shelf life of commercial formulations at various temperatures.
4. Investigate the potential of several adjuvants for protecting CpGV from solar degradation.

SIGNIFICANT FINDINGS:

- Season-long treatments of CpGV (Cyd-X) at 3 rates (1, 3 and 6 oz acre) and 3 application intervals (7, 10 and 14 days) resulted in significantly fewer deep entries and surviving larvae but did not reduce the proportion of fruit damaged by codling moth.
- There was a significant trend of fewer deep entries and higher larval mortality rates with increasing rate of CpGV and shorter application interval.
- In replicated ½ acre plots, CpGV provided > 90% larval mortality at 1, 2 and 3 oz/acre, but was not as effective as Guthion in protecting fruit.
- The efficacy of 3 commercial CpGV formulations were significantly reduced (52-77%) by exposure to UV light (9.36×10^6 joules/m^2) in a solar simulator.
- Bioassay procedures to screen adjuvants providing possible UV protection of CpGV formulations were developed.
- The Cyd-X and Virosoft formulations of CpGV maintained larvicidal activity after storage at 2 and 25˚C for over 132 weeks, but activity was sharply reduced after storage at 35º for 16 and 40 weeks, respectively.
- Although lignin encapsulation provided significant protection of CpGV exposed to simulated sunlight in laboratory studies, under field conditions it did not.

METHODS

Optimal spray strategies (Objective 1 and 2)

Assessment of full-season virus programs adopting different application rates and spray intervals in an experimental orchard.

This study was conducted at the USDA experimental orchard near Moxee, WA. Virus applications were made to individual trees (Red Chief) using a Stihl SR420 backpack airblast sprayer with a large tarpaulin and a one-tree buffer used to confine treatments. Virus treatments (Cyd-X, Certis, USA) were applied in a factorial design with three levels for dose (1, 3 and 6 oz/acre) and application interval (7, 10 and 14 days). Dose rates covered the range labeled for use and intervals were based on persistence of treatments observed in 2003 (Arthurs and Lacey, 2004). For each treatment ten randomly selected trees were sprayed at a 100 gal./acre plus Nufilm17 at 8oz/acre. Control trees were sprayed with Nufilm17 plus water. Initial virus treatments were made at 5% egg hatch and continued until ≈ 95% (Beers et al. 1993). CM injury was assessed from 50 fruit per tree at the end of the first and second generations. Damaged fruit was removed to the laboratory to assess both larval mortality and proportion of deep entries (> ¼ inch depth). Cardboard bands placed around trees captured surviving larvae.

Comparison of different rates of virus applied weekly to Guthion in a conventionally managed orchard heavily infested with CM.
This study was conducted within a 21 acre Delicious orchard near Zillah, WA. Virus applications were made using a 300 gal. tractor-mounted 'pull blast' sprayer. Individual ½ acre plots were marked out and treated in complete randomized block design with 3 rates of Cyd-X (1, 2 or 3 oz/acre). Five replicate blocks were sprayed at each dose @ 110 gal./acre plus NuFilm17 (8oz/acre) weekly throughout the season, with initial treatments made at 5% egg hatch. Three untreated areas served as controls. For the assessments, fruit injury was assessed from the central area of each plot and from adjacent areas treated with Guthion (azinphos-methyl). At the end of the first CM generation, 100 damaged fruit per plot were taken to the laboratory to assess larval mortality. Clear sticky 'interception traps' hung in the canopy at each plot's center were used to compared moth activity in the 2nd flight.

RESULTS AND DISCUSSION

Optimal spray strategy - study 1. Figure 1 shows fruit injury, proportion of deep entries and larval mortality for each virus treatment at harvest. Virus applications did not reduce fruit damaged by CM, but the majority of damage was in the form of shallow stings (< ¼") and larval mortality was high (>80% in all treatments). There was a statistical trend of fewer deep entries and higher larval mortality rates with increasing rate of CpGV and shorter application interval. Rates of larval mortality were supported by the number of larvae captured in tree bands (data not shown).

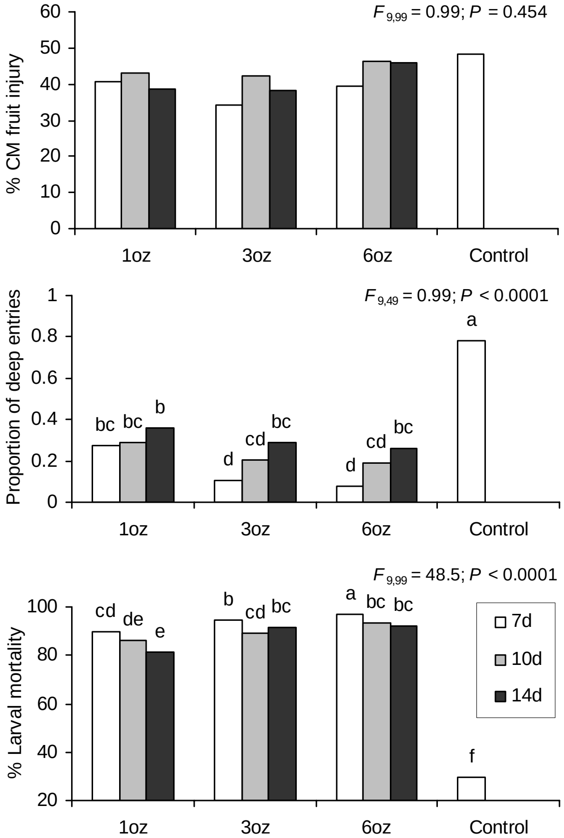
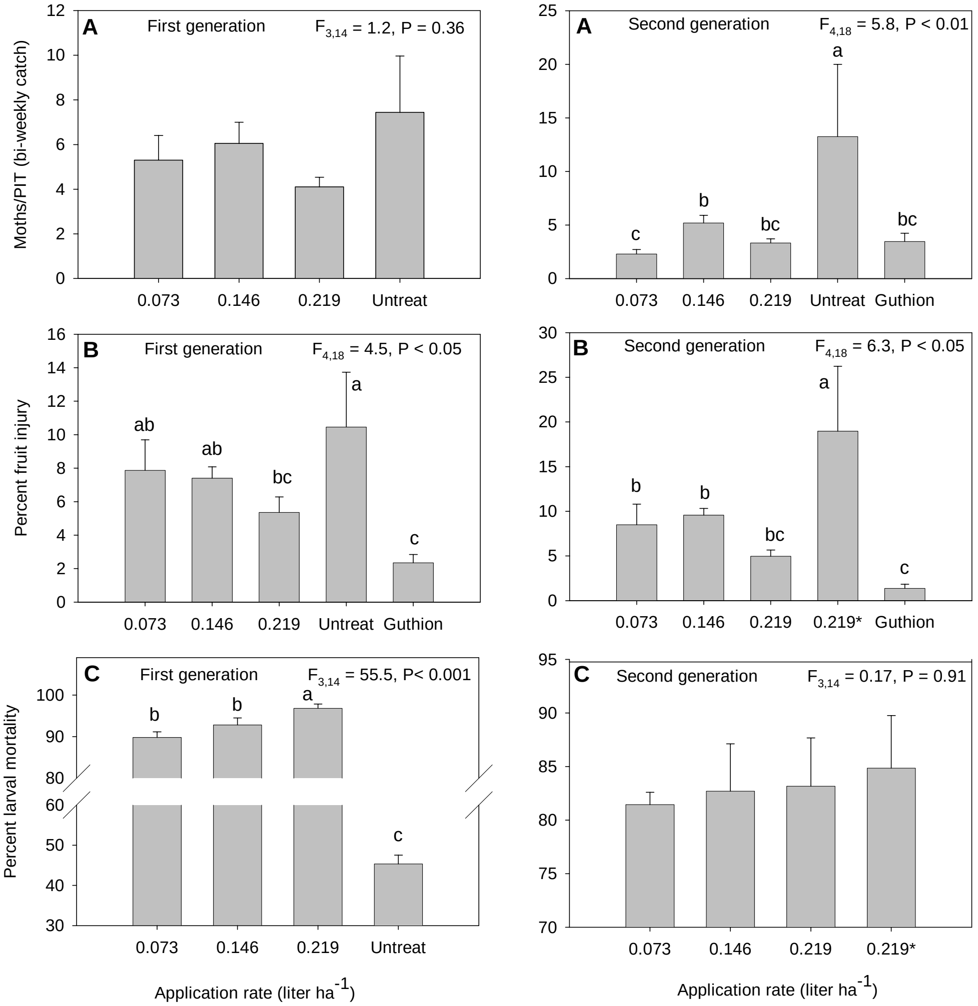


Figure.1. Fruit damage, deep entries and CM mortality following different treatments of Cyd-X in individual tree plots (data for 2nd generation). Letters indicate Fishers LSD at $P < 0.05$.

Optimal spray strategy. Figure 2 compares fruit injury, larval mortality and moth activity following 6 weekly applications of Cyd-X together with Guthion and untreated areas. There was less CM damage in virus plots compared with untreated areas, but more compared with Guthion-treated areas. Most damage was observed higher in the canopy (data not shown). Rates of CM mortality in virus-treated plots were similar to those observed in individual trees sprayed with equivalent rates of virus in the previous study. Data from interception traps showed fewer moths in virus-treated and Guthion-treated plots compared with untreated areas. Despite this, the virus study was terminated before harvest when fruit damage approached 10%. The heavy infestation (pheromone-baited traps averaged 70 moths/week) and untreated areas may have contributed to the high damage level.

Figure 2. Fruit damage, CM mortality and passive interception trap catches following different treatments of Cyd-X in ½ acre blocks in a 21A commercial orchard. Letters indicate Fishers LSD at $P < 0.05$.



Data from the experimental and commercial orchard provides information on the effectiveness of different virus programs against codling moth. The dosage and application frequency of virus that provides acceptable control (in many organic programs this will be a level at which a mating disruption program continues to be effective) will depend largely on the localized pressure of codling moth. Correlating moth counts from monitoring traps with the level of control required will allow growers to make informed decisions about including codling moth virus into their spray programs. **Storage studies.** 2003-2005. The Cyd-X and Virosoft formulations have maintained their larvicidal activity when bioassayed at 100,000 fold dilution after storage of product for 132 weeks at 2 and 25°C using bioassays techniques developed by Lacey et al. (2002). A significant decline in larvicidal activity was obsevered for Cyd-X and Virosoft after storage at 35ºC for 16 and 40 weeks, respectively. The larvicidal activity for the Carpovirusine formulation declined considerably at the highest temperature but maintained activity at 2˚C for 116 weeks (Table 1). Despite prolonged maintenance of larvicidal activity of the Cyd-X and Virosoft products after storage at 25°C, we recommend refrigeration of all products until they are used.

Table 1. Storage of codling moth granulovirus. Number of weeks stored at 2, 25, or 35°C with formulation producing $\geq$95% mortality in neonate codling moth larvae (10^{-5} dilution of product).

Temperature (°C)	number weeks stored with larvicidal activity maintained		
	Cyd-X	Virosoft	Carpovirusine
2	140+	132+	116
25	140+	132+	2
35	16	40	2

+ = end point not yet reached

UV protection studies. (Objective 4)

Because exposure to solar radiation limits the activity of CpGV in the Pacific Northwest, we established a bioassay system in the laboratory to assess various adjuvants for UV protection (see Lacey and Arthurs, 2005). In the procedure apples are sterilized, halved and the open end sealed using molten wax, aluminum foil and glue in preparation for virus treatments. Apples are treated in a DeVries spray cabinet which is calibrated to deliver specific quantities of experimental virus formulations (standard and high dilution) and then subsequently exposed to a controlled dose of UV and other wavelengths of light equivalent to 9.36×10^6 joules/m^2 in a solar simulator (Atlas) for 4 hours. Treated and control apples are then challenged with neonate codling moth larvae from a lab colony. Resulting fruit damage and larval mortality are measured in order to compare the activity of the virus treatments and hence quantify the most effective sunscreens for CpGV. The same approaches without the solar exposure can be used to test other adjuvants conferring possible enhancements to virus formulations, such as phagostimulants, rain-fasteners etc.

2004. In preliminary tests, we assessed virus degradation of 3 commercial formulations without additional adjuvants. The results showed a severe decline in activity resulting from the UV exposure (Table 2). These data suggest that efforts to identify sunscreens and other adjuvants that will be effective for conditions in the Pacific Northwest are worthwhile.

Table 2. Mean CM mortality on apples treated with standard rate of CpGV (1000-fold dilution) and exposed to 9.36×10^6 joules/m^2 simulated sunlight plus controls.

	Virosoft	Carpovirusine	Cyd-X
UV	29.7	20.4	46.8
No UV	95.1	90.2	98. 2
% reduction	*68.8*	*77.4*	*52.3*

2005. We evaluated lignin-encapsulated formulations of CpGV and use of various sun blocks for improved ultraviolet (UV) protection based on laboratory bioassays with a solar simulator and in field tests in an infested apple orchard. In laboratory tests spray-dried lignin-based formulations with and without the additives titanium dioxide (TiO_2) and sugar provided extended UV-protection of virus when applied at a high dosage of 3×10^{10} OBs/L (i.e. 92-94% control compared with 66-67% from a commercial glycerin-stabilized or unformulated product) but not at a lower dosage containing 3×10^8 OB/L (Table 3). Equivalent dosage-dependent patterns in solar protection was observed in further tests with the lignin only formulation, when an intermediate dosage (3×10^9 OB/L) was also found to be ineffective. The dosage of a blank lignin formulation did not affect larval mortality, suggesting that the UV protection at the high dosage reflected the combined effect of lignin and virus (Table 4).

Table 3. Percentage mortality and deep entries (≥ 6 mm) of codling moth larvae recovered on half apples previously treated with unformulated, glycerin-stabilized and experimental spray-dried lignin formulations of CpGV and irradiated with a solar simulator. Data show average for five replicate tests (n = 25) for fruit sprayed with two rates of virus.

Formulation	High dose (3×10^{10} OB/L)		Low dose (3×10^{8} OB/L)	
	% mortality	% deep entries	% mortality	% deep entries
Untreated	34.4c	97.6a	34.4c	97.6a
Unformulated	67.2b	67.3b	39.2bc	86.1ab
Cyd-X	66.8b	65.9b	55.0ab	78.8b
Lignin	93.6a	29.7c	52.8ab	75.8b
Lignin + sugar	92.8a	28.5c	58.0a	79.4b
Lignin + TiO_2	92.0a	41.1c	42.4bc	81.3b

Column letters indicate mean significant differences using Fishers LSD at $P < 0.05$.

Table 4. Percentage mortality and deep entries (≥ 6 mm) of codling moth larvae recovered on half apples previously treated with glycerin-stabilized and experimental spray-dried lignin formulations of CpGV and irradiated with a solar simulator. Data show average for five replicate tests (n = 25) for fruit sprayed with three rates of virus

Formulation	High dose (3×10^{10} OB/L)		Med. dose (3×10^{9} OB/L)		Low dose (3×10^{8} OB/L)	
	% mortality	% deep entries	% mortality	% deep entries	% mortality	% deep entries
Untreated	21.4c	83.2a	21.4b	83.2	21.4	83.2
Cyd-X	55.7b	62.0a	44.5a	73.2	37.8	61.9
Lignin	95.4a	25.6b	41.7a	73.5	37.3	70.6

Column letters indicate mean significant differences using Fishers LSD at $P < 0.05$.

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 protect a commercial CpGV preparation from solar inactivation in laboratory tests (Table 5). 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 reduce fruit injury compared with a commercial preparation (Cyd-X) at equivalent rates (Table 6). Our studies show that lignin-encapsulated CpGV formulation provides solar protection but only at relatively high dosages. The testing of high concentrations of carrier containing reduced virus concentrations of virus would be worthwhile.

Table 5. Percentage mortality and deep entries (≥ 6 mm) of codling moth larvae recovered on half apples previously treated with CpGV (Cyd-X) with and without spray adjuvants and irradiated with a solar simulator. Data show average for five replicate tests (n = 25) for fruit sprayed with two rates of virus.

	High dose (3×10^{10} OB/L)		Low dose (3×10^{8} OB/L)	
Adjuvant	% mortality	% deep entries	% mortality	% deep entries
Untreated	29.6c	87.8a	29.6	87.8
None	58.8a	68.2b	38.9	89.3
NuFilm-17	58.4a	60.6b	44.8	86.2
Biolink	58.4a	75.3ab	37.3	87.9
Raynox	52.8ab	74.4ab	35.0	89.8
Trilogy	42.4bc	72.1b	39.2	85.2

Column letters indicate mean significant differences using Fishers LSD at P < 0.05.

Table 6. Orchard tests with CpGV against codling moth. Assessments of spray-dried lignin formulations were compared to Cyd-X following 4 applications at 6.57×10^{12} OB/ha (3 oz/ac) against the 1st generation and 3 applications against 2nd generation (½ of trees were sprayed at a reduced rate of 2.2×10^{12} OB/ha= 1oz/ac).

Formulation	First generation			Second generation			
	% fruit damage	% mortality	% deep entries	% fruit damage	% mortality	% deep entries	Tree bands[1]
Untreated	6.1	38.5b	77.8a	33.8	27.4c	80.8a	84.1a
Blank Lignin	6.3	36.3b	75.4a	32.1	17.8c	86.1a	65.4a
Cyd-X	11.1	93.2a	30.7b	26.2	64.6ab	61.0b	17.4b
Lignin GV	9.1	87.8a	22.4b	27.9	71.4a	59.9b	22.8b
Cyd-X (½)	-	-	-	28.5	65.7ab	66.9b	23.6b
LigninGV(½)	-	-	-	23.2	58.6b	58.8b	18.6b

Column letters indicate mean significant differences using Fishers LSD at P < 0.05.

[1] Bands captured diapause-destined larvae, number includes any live larvae removed during fruit evaluations.

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