Efficacy of Codling Moth Granulovirus: Effect of Adjuvants on Persistence of Activity and Comparison With Other Larvicides in a Pacific Northwest Apple Orchard[1]

Lawrence A. Lacey,[2] Steven Arthurs, Alan Knight, Kimberly Becker and Heather Headrick

Yakima Agricultural Research Laboratory, USDA-ARS, 5230 Konnowac Pass Road, Wapato, WA 98951 USA

J. Entomol. Sci. 39(4): 500-513 (October 2004)

Abstract Control of codling moth, *Cydia pomonella* (L), in conventional orchards has relied heavily on broad spectrum insecticides such as azinphos-methyl (Guthion®, Bayer CropScience, Research Triangle Park, NC). Alternative control options are needed for a variety of reasons, including environmental impact and worker and food safety concerns. Microbial control agents such as the codling moth granulovirus (CpGV) offer alternatives to conventional insecticides for the control of codling moth. Six weekly applications of the label rate (1 L/ha) of the Carpovirusine® formulation of CpGV in an experimental orchard naturally infested with codling moth provided control of first generation codling moth that was comparable to that of larvicidal oil and azinphos-methyl. Although the number of codling moth entries in fruit that were treated with virus alone was similar to that of control trees, the number of deep entries and the number of living codling moth larvae were significantly reduced on CpGV treated fruit. Despite blemishing, virus-treated fruit with minute entries were suitable for consumption or for processing. Studies on the residual activity of Carpovirusine revealed a steady decline in virus activity 1 to 3 d following application. The use of two adjuvants, Nu-Film-17® and Raynox®, did not protect virus from solar inactivation. Among the biological control options available for codling moth, CpGV provides effective and selective control of neonate larvae. Its use *in lieu* of broad spectrum insecticides will contribute significantly to the conservation of other natural enemies in the orchard agroecosystem.

Key Words Codling moth, *Cydia pomonella,* granulovirus, formulation, UV sensitivity

Codling moth, *Cydia pomonella* (L), is a serious worldwide pest of apple and other fruit (Barnes 1991) and the principal pest of apple in the Pacific Northwest of the United States (Beers et al. 1993). Because only minimal codling moth damage is tolerated by conventional orchardists, broad spectrum residual insecticides such as azinphos-methyl (Guthion®, Bayer CropScience, Research Triangle Park, NC) are traditionally used as the first line of defense against this pest. However, control options for codling moth in organic orchards have been limited to oils, trapping, mating disruption, manual removal of infested fruit and the like. The recent registration of three commercial formulations (Carpovirusine®, Calliope, France marketed in USA by Arvesta Corporation, San Francisco, CA; Virosoft CP4®, Biotepp Inc.,

[1]Received 01 June 2004; accepted for publication 30 June 2004.
[2]Email (llacey@yarl.ars.usda.gov).

Charlesbourg, Québec, Canada; Cyd-X®, Certis USA, Columbia, MD) of the codling moth granulovirus (CpGV) in the USA expands the options for control of codling moth in both organic and conventional orchards. CpGV targets neonate larvae and must be ingested before or during entry of larvae into fruit. It is extremely virulent and highly specific, infecting only codling moth and closely-related species (Gröner 1986). Despite considerable earlier research demonstrating its efficacy in the USA and Canada (Falcon et al. 1968, Jaques et al. 1987, Jaques 1990, Jaques et al. 1994), commercial products based on CpGV have not been used extensively in North America prior to the recent registration of the three commercial products. However, commercial CpGV products have been routinely used for control of codling moth in European orchards for several years by organic and conventional growers (Cross et al. 1999). In 2003, CpGV was used on an estimated 4,050 to 4,860 ha in the USA and 81,000 ha in Europe (Lacey et al. 2004).

Concerns expressed by orchardists regarding CpGV include rapid virus inactivation by solar radiation, the necessity for frequent reapplication in some cases and the occurrence of codling moth entries or "stings" in sprayed fruit, particularly during the first generation of codling moth (Lacey et al. 2000, Lacey and Shapiro-Ilan 2003). Codling moth population reduction is generally regarded as the principle benefit of using CpGV. Guillon and Biache (1995) and Charmillot and Pasquier (2002) reported a steady decline in codling moth pressure in French and Swiss orchards, respectively, that have been treated routinely with CpGV for 2 or more years. Our objectives in this study were to determine the persistence of larvicidal activity of the Carpovirusine formulation and UV protective effect of two adjuvants under field conditions. In addition, the efficacy of weekly applications of Carpovirusine throughout the first generation of codling moth was compared with alternative treatments.

Materials and Methods

Field site. Field studies were conducted from 30 May throughout June and into early July 2002 in two adjacent 0.3 ha experimental apple plots located near Zillah, WA. Each plot comprised "Red Delicious" interplanted with "Golden Delicious" as pollinators. Trees were ≈3 m high with a planting density of 360 or 270 trees ha^{-1} in either plot. Trees were maintained according to normal horticultural practices for the region (Ron Britt, pers. comm.) although no insecticides were used in 2002 other than experimental treatments. Both plots were naturally infested with codling moth. In order to estimate the localized population pressure, several different traps were located in one of the orchard plots. Traps consisted of commercially available delta-monitoring traps baited with either sex pheromone lures (2 traps) or pear ester kairomone lures (Knight et al. 2002) attractive to both sexes (6 traps). In addition clear plastic sheets coated with oil and hung in the canopy were used as passive interception traps (9 traps). Climatic conditions (temperature, relative humidity, and solar radiation) were recorded hourly in the orchard using a HOBO Pro Series relative humidity and temperature meter (Onset Computer Corp., Bourne, MA) and a LI-1400 datalogger pyronometer (LI_COR, Inc., Lincoln, NE). Precipitation data for a nearby site were downloaded from the Washington State University Public Agricultural Weather System (PAWS).

Field trial against first moth generation. The efficacy of one CpGV-based product, Carpovirusine (10^{13} occlusion bodies L^{-1}), applied at 1 L ha^{-1} was compared with alternative treatments in one experimental plot (block P). Other treatments com-

prised 1% oil (Orchex®, Calumet Lubricants Company, Indianapolis, IN) at 10 L ha^{-1}, a virus + oil combination (applied at the same rates as the individual treatments) and an industry chemical standard, azinphos-methyl, at label rates (0.56 kg ha^{-1}). Initial treatments were made near the start of egg hatch at 263 DD (Beers et al. 1993), with subsequent treatments applied approximately every 7 d for a total of 6 applications throughout the oviposition period for the first (spring) codling moth generation. Spray dates corresponded to 263, 356, 432, 551, 729, and 809 degree days post biofix taken as the first consistent flight of male codling moth monitored from pheromone-baited traps, to predict peak egg hatch. Developmental rates and thresholds (≈7 to 31°C) followed the Washington State University model (Beers et al. 1993). Azinphos-methyl was applied twice at 21-d intervals on 30 May and 18 June.

All treatments were applied as dilute sprays to single tree plots at a localized application rate of 1,000 1 ha^{-1}. Treatments were made in a complete randomized block design with 10 trees per treatment. All applications were made between 0600 and 1000 (PST) using a 98 L ATV-mounted pull tank sprayer fitted with a 5 h.p. motor and hand-held lance applicator. Sprays were directed to provide complete coverage of foliage and fruit. The cone nozzle in the applicator produced relatively large droplet spectra such that drift between trees was minimal, although a mobile screen held by two assistants was used to help confine treatments for azinphos-methyl application. Conditions remained dry during the applications with wind speeds generally less than 5 kph, although occasional gusts up to 25 kph were noted. All formulations were prepared the morning of use. Virus was kept refrigerated until just before adding to the spray tank.

In another plot (block R), additional treatments of Carpovirusine were applied at 1 L ha^{-1}, including the use of two formulation adjuvants conferring possible protection from solar degradation, the spreader-sticker Nu-Film-17® (Miller Corp., Hanover, PA) at 0.06% v/v and Raynox® (Pace International, Wapato, WA) at 10% v/v. In addition to providing rainfastness, Nu-Film-17 is purported by the manufacturer to shield spray residues from heat and ultraviolet light degradation. Raynox is a sunburn protectant for fruit.

A low dose high frequency Carpovirusine treatment (0.5 L ha^{-1} applied twice each week) which utilized the same quantity of virus per tree per week also was tested. Treatments were made in a complete randomized block design with 10 trees per treatment. Treatments were conducted for 6 weekly applications concurrently and in the same manner as the study previously described, with the exception of the low dose, high frequency treatment which was applied every 3 to 4 d. Control trees in both blocks were treated with water only.

Assessment of CpGV protection of fruit. For treatments in both plots (block P and R), the proportion of fruit damaged and number of live codling moth larvae recovered were assessed shortly before the start of the second codling moth generation (≈1,000 DD). Ten d after the final application (6 d for ½ rate Carpovirusine treatment) a minimum of 100 fruit per tree (1,000 per treatment) were randomly selected and transported to the laboratory for assessments. In the R block, fruit were selected from the northern side of trees to prevent bias resulting from the prior removal of undamaged fruit in the southwest quadrant of each tree (as described below). Among injured fruit, the depth of codling moth entries into fruit was assessed using a modification to a method described by Glen and Payne (1984). Injuries were classified as "minute" (<2 mm), "shallow" (2 to 3 mm) or "deep" (>3 mm).

Assessment of virus residual activity. The decrease in virus activity due to solar

inactivation over 3 d and potential environmental protection offered by the adjuvants Nu-Film-17 and Raynox were assessed for treatments conducted in block R (described above). Because of the more frequent applications, the low rate treatment was excluded from these assessments. The test was conducted three times; following the second, fourth and sixth (final) application. On each of these dates, as soon as the spray application was dry (0-h) and at 6, 30 and 54 h following treatment (representing 1, 2 or 3 full days of sun exposure) 10 fruit per tree (100 per treatment) were transported to the laboratory to bioassay for residual activity. Selected fruit were pest free and removed from the southwest side of trees at various heights to obtain a representative sample with maximum solar exposure for each tree. To protect spray residues fruit were picked by handling only the petiole and placed individually in 90 to 120 ml "Solo" plastic cups on site (depending on the size of apple). Fruit was held in place with a tack inserted through the bottom of each cup. The upper surface of each apple was infested with 3 neonate codling moth (<12 h old) obtained from the colony maintained at the USDA-ARS Yakima Agricultural Research Laboratory on soya-wheat germ-starch artificial diet (Toba and Howell 1991) at 27°C, 40-50% RH, with a 16:8 h L:D photoperiod. Apples were subsequently incubated at 25 ± 1°C for 7 days and destructively sampled to quantify fruit damage and larval survivorship. The size of injuries was not quantified.

Data analysis. Main treatment effects were tested using univariate ANOVA with significant *F*-ratio means separated using Fisher's LSD for multiple comparisons at $P < 0.05$ (SAS Institute 1999). Percentage data (for fruit injury) and count data were normalized via arcsine and $\log_{10}(n + 1)$, respectively, prior to analysis. In the residual activity study, multivariate repeated measures ANOVA (MANOVA) were first used to test for differences in treatment effects according to both exposure period post spraying and test date. Linear regressions were also plotted for each treatment on each date to illustrate the nature of interactions. Each replicate was based on the overall mean for each tree (i.e., 100 fruit in the fruit protection study and 10 in the virus persistence study) for a treatment sample size of 10. In the efficacy study, because codling moth pressures may have been slightly different, statistical comparisons were not made between the P and R plots.

Results

Field trial against first moth generation. Trap catch data for the spring adult generation are shown in Figure 1. Climate data during the study are shown in Figure 2. Conditions remained generally sunny with daytime temperatures up to 35°C and only 14 mm precipitation fell throughout the 6-wk study period. Data on fruit damage (codling moth entries) and presence of live larvae after six successive weekly treatments with virus and oil (alone or combined with virus), virus plus adjuvants, and azinphos-methyl are shown in Table 1. Overall, greater codling moth pressure was observed in the R block. In the P block the lowest fruit injury level (≈3%) was observed in the oil, virus + oil and azinphos-methyl treatments, although only azinphos-methyl was significantly different from the control. The percentage of fruit injured in the virus alone treatment was similar to that in the control treatments in the P block and similar between the controls and the various virus treatments in the R block. No damage was recorded for a small number of trees (0 to 2) in each treatment, although this was not significantly different among treatments.

Although the proportion of fruit injured was not reduced by application of virus,

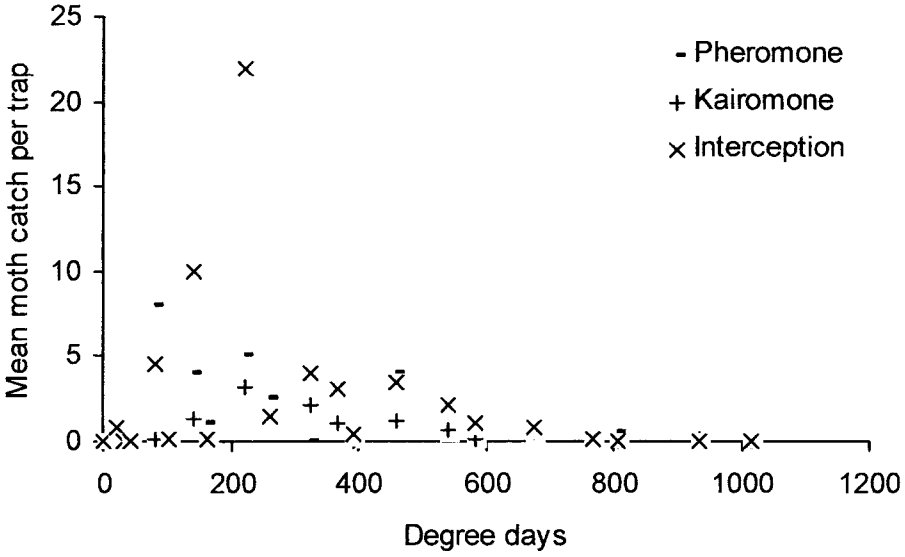

Fig. 1. Number of adult codling moths caught in traps against accumulated degree days post biofix (P-block, Zillah, WA; spring generation 2002). Data are cumulative catches since the preceding catch and represent averages for two sex pheromone (males), six kairomone and nine clear pane (interception) traps (both sexes caught).

there were significantly fewer deep entries in fruit treated with virus (Table 1). Of the codling moth injured fruit in the P block, virus-treated fruit had significantly fewer deep entries (23.9%) compared with azinphos-methyl (68%) or untreated fruit (59%) (F = 3.3; df = 4, 49; P = 0.018). In the R block the proportion of deep entries was also significantly reduced compared with controls in three out of the four virus treatments shown in Table 1. Many of the deep entries (defined as >3 mm) represented larvae boring to the center of the fruit rendering it unmarketable.

All treatments resulted in significantly fewer live larvae per fruit compared with controls on July 12 at the close of the 6-wk study. There were no significant differences in the number of live larvae recovered between treatments within each block. The half rate treatments applied semiweekly (same amount of virus per tree per week) did not provide significantly better protection from codling moth entries than the full rate or virus combined with other materials. However, there were significantly fewer deep entries in this treatment than in the full rate weekly applications of virus alone and no live larvae per 100 fruit were found. The virus plus oil did not provide greater fruit protection or greater population reduction than oil or virus alone. Also the use of Raynox and Nu-Film-17 with Carpovirusine did not result in less fruit damage or fewer live larvae.

Assessment of virus residual activity. Repeated measures MANOVA revealed no significant treatment by test date or treatment by time interactions on the number of injuries per fruit (F = 1.7; df = 6, 70; P = 0.14; F = 1.5; df = 9, 83; P = 0.15,

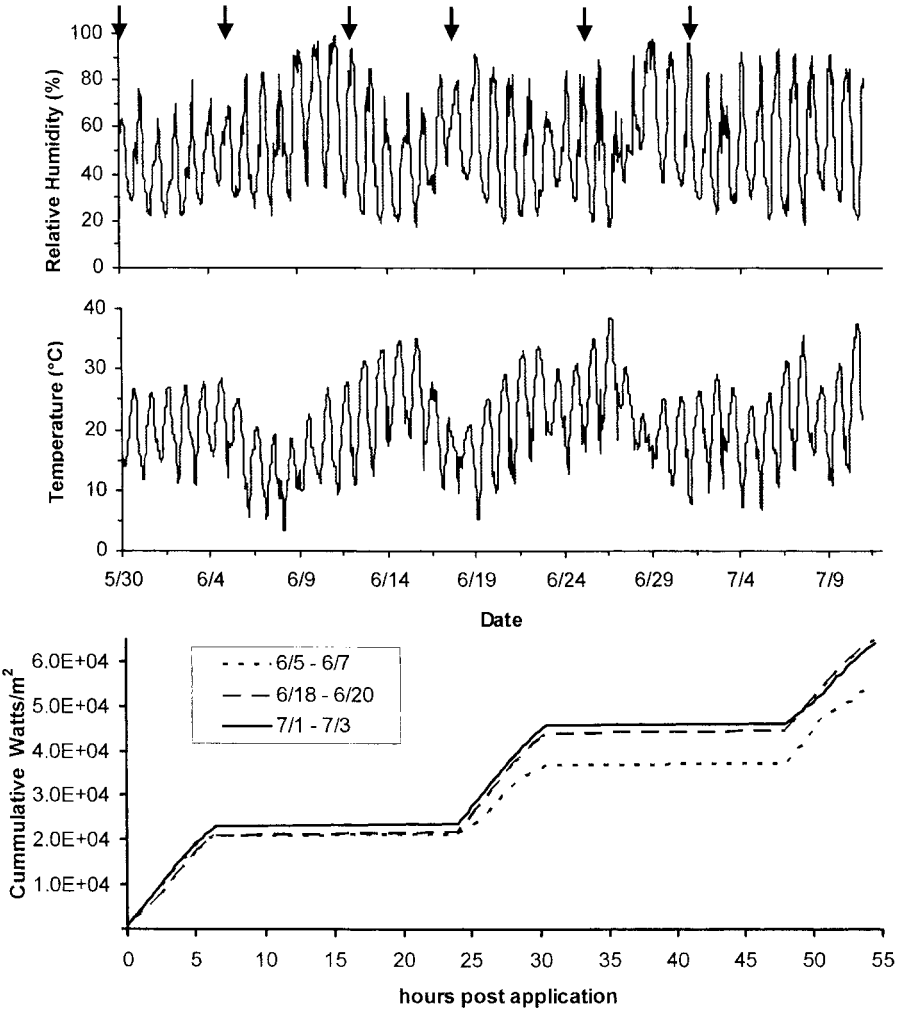

Fig. 2. Climate data in experimental apple plots located at Zillah, WA, June and July 2002. Arrows denote application times for the various treatments (excluding low rate/high frequency virus treatment).

respectively). However, when data were pooled across treatments, there was a gradual increase in the number of injuries per fruit from 1.45 ± 0.04 injuries (5 June), 1.91 ± 0.03 (18 June) and 1.97 ± 0.04 (1 July). Means for each exposure period were pooled for all test dates (Table 2). As observed in the previous study (which reflected codling moth activity in the field), bioassays revealed few differences in the number of injuries occurring between virus-treated and control fruit challenged in the laboratory. However, significantly more injuries were observed in untreated fruit compared with some of the virus treatments on two of the sampling intervals (6 h and 30 h).

Table 1. Fruit damage, depth of entries and presence of live *Cydia pomonella* larvae (±SEM) after six successive weekly treatments with the Carpovirusine formulation of the codling moth granulovirus at 10^{13} granules ha^{-1} wk^{-1} or oil (alone and combined with virus). Azinphos-methyl was applied twice at 21 d intervals

		% Injuries falling among size classifications**			
	Percentage fruit injury	Minute	Shallow	Deep	# Live larvae/ 100 fruit
Block P					
ANOVA (df = 4,49)*	$P = 0.002$	$P = 0.057$	$P = 0.542$	$P = 0.018$	$P < 0.0001$
Control	8.1 ± 2.1ab	17.9 ± 4.4	23.0 ± 6.6	59.1 ± 6.1ab	3.0 ± 0.9a
Carpovirusine	12.2 ± 2.2a	60.8 ± 10.2	15.3 ± 8.7	23.9 ± 5.9c	0.1 ± 0.1b
Carpovirusine + Oil	3.6 ± 1.1bc	49.5 ± 9.9	8.9 ± 6.4	41.5 ± 11.7abc	0.1 ± 0.1b
Oil	3.9 ± 1.4bc	44.7 ± 12.2	26.1 ± 13.3	29.2 ± 11.6bc	0.1 ± 0.1b
Azinphos-methyl	3.3 ± 1.1c	18.8 ± 13.2	12.9 ± 9.2	68.3 ± 13.7a	0.1 ± 0.1b
Block R					
ANOVA (df = 4,49)*	$P = 0.567$	$P = 0.457$	$P = 0.481$	$P = 0.01$	$P < 0.0001$
Control	10.3 ± 2.9	36.7 ± 11.4	10.7 ± 2.9	52.7 ± 10.0a	4.3 ± 1.5a
Carpovirusine	10.5 ± 2.6	55.3 ± 7.0	4.6 ± 2.0	40.2 ± 7.9ab	0.2 ± 0.2b
Carpovirusine half rate	9.2 ± 1.5	74.1 ± 10.0	13.5 ± 6.9	12.4 ± 6.2c	0b
Carpovirusine + Nu-Film17	8.3 ± 1.5	62.3 ± 8.9	15.7 ± 5.5	22.0 ± 6.0bc	0.3 ± 0.3b
Carpovirusine + Raynox	6.2 ± 1.2	68.1 ± 8.6	8.0 ± 5.0	24.0 ± 8.0bc	0.1 ± 0.1b

* Letters within columns indicate treatment differences within each plot following significant *F*-ratio test; Fishers LSD at $P < 0.05$.

** Percentages of each injury level based on size/depth of entry for those fruit with codling moth entries.

Table 2. The number of codling moth injuries per fruit resulting from bioassays conducted on apples picked immediately after application of the Carpovirusine formulation of the codling moth granulovirus (10^{13} granules ha^{-1}) and after 6, 30, and 54 h intervals. Data shown are pooled for three separate tests conducted in the same orchard (applications made 5 June, 18 June and 1 July 2002)

	Injuries/fruit at four intervals after application*,**			
ANOVA (df = 3,116)[b]	0 h $P = 0.85$	6 h $P = 0.04$	30 h $P = 0.04$	54 h $P = 0.95$
Control	1.9 ± 0.08	2.1 ± 0.09a	2.0 ± 0.08a	1.7 ± 0.1
Carpovirusine	1.8 ± 0.1	1.7 ± 0.1b	1.6 ± 0.01b	1.7 ± 0.07
Carpovirusine + Nu-Film 17	1.9 ± 0.1	1.8 ± 0.05ab	1.7 ± 0.08b	1.6 ± 0.07
Carpovirusine + Raynox	1.9 ± 0.1	1.8 ± 0.09ab	1.6 ± 0.01b	1.7 ± 0.08

* Each apple was infested with 3 neonate larvae in the laboratory within 2 hours of removal from the orchard.
** Letters within columns indicate treatment differences following significant *F*-ratio test; Fishers LSD at $P < 0.05$.

To illustrate the effect of virus residue age on mortality of larvae exposed to fruit, linear regressions for each treatment were plotted against time for each sampling date (Fig. 3). Repeated measures MANOVA revealed significant treatment by test date and treatment by time interactions ($F = 3.5$; df = 6, 70; $P < 0.005$; $F = 2.8$; df = 9, 83; $P = 0.01$). The test date by time interaction was not significant ($F = 1.1$; df =6, 31; $P = 0.37$). However, when controls (untreated fruit) were excluded from the same analysis, there were no significant treatment by time interactions for any of the test dates ($F = 1.3$; df = 6, 50; $P = 0.29$; $F = 1.8$; df = 6, 50; $P = 0.11$; $F = 2.1$; df = 6, 50; $P = 0.07$). The treatment by time interaction on each test date is thus best explained by the difference between the slopes of the virus and control treatments. While all virus treatments show an initially depressed but steady increase in larval survivorship over the 3 d, larval survival on untreated fruit (controls) did not similarly increase, although it was consistently higher over the 3 d. Moreover, because the slopes did not vary significantly among the various virus treatments (Fig. 3), there was no clear advantage of using either of the adjuvants tested in combination with Carpovirusine.

Discussion

Although the number of codling moth entries into fruit was not significantly different between virus-treated and control apples, the number of deep entries and the number of live larvae found in the fruit were significantly lower in CpGV-treated fruit (Table 1). Glen and Clark (1985) observed that larvae died, usually in the first stadium, after entering virus-treated fruit. Despite blemishing, CpGV-treated fruit with minute entries are still suitable for consumption or for processing. However, the major benefit observed in our study was the reduction in population density of the moth going into the

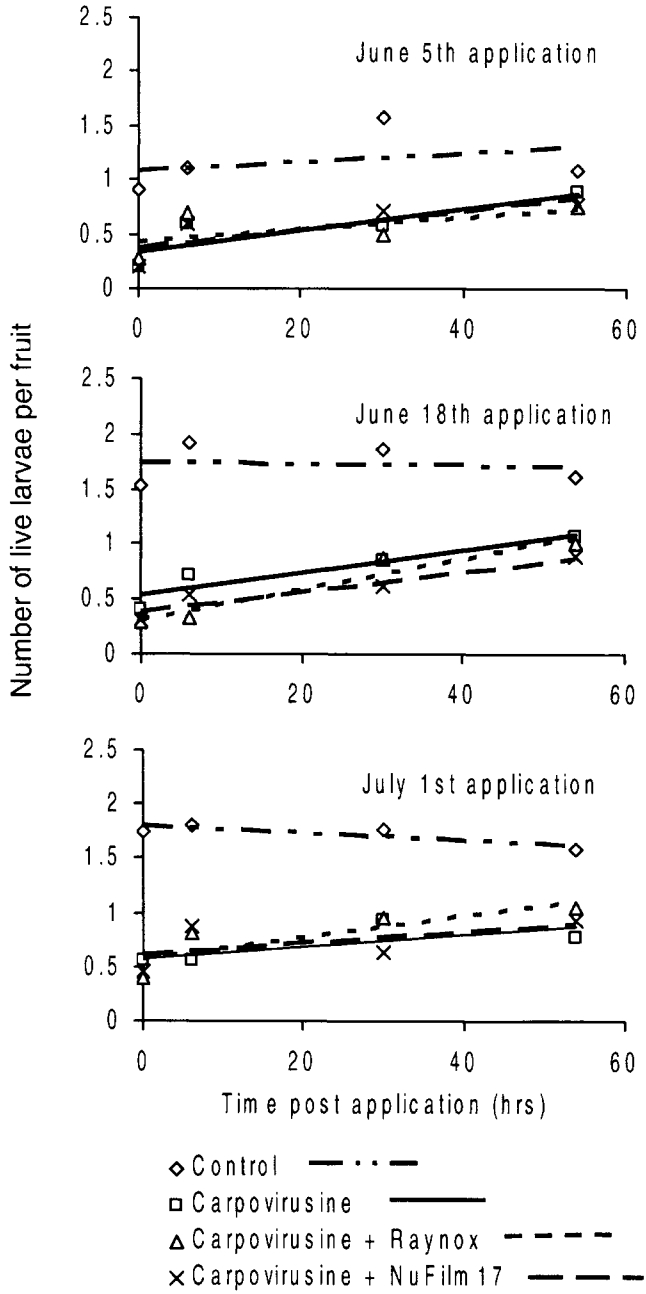

Fig. 3. The number of live codling moth larvae per fruit resulting from bioassays conducted on apples picked immediately after application of the codling moth granulovirus and after 6, 30, and 54 h intervals. Data and linear regression lines are shown for three separate tests conducted in the same orchard.

second generation as illustrated in the final column of Table 1. The use of CpGV in combination with practices already used by organic orchardists, such as the use of oils, Entrust® (spinosad formulation approved for organic orchards, Dow AgroSciences, Indianapolis, IN), and mating disruption could further reduce the moth population. The combination of mating disruption and CpGV applications in European apple orchards has resulted in effective control of codling moth (Miñarro and Dapena 2000, Charmillot and Pasquier 2002). Among the biological control options available for codling moth, CpGV provides effective and selective control of neonate larvae. Its use *in lieu* of broad-spectrum insecticides will contribute significantly to the conservation of other natural enemies in the orchard agroecosystem.

Despite the need for frequent application, the frequency of CpGV application is similar to that of other interventions used by organic orchardists, such as use of oil, when codling moth pressure is moderate to high. Some orchardists have expressed concern that the specificity of CpGV, despite lack of negative impact on natural enemy complexes, does not completely reduce the risk of other lepidopteran pests of tree fruit. Tank mixes that include other viruses and *Bacillus thuringiensis* Berliner have provided control of a broader spectrum of Lepidoptera (Blommers et al. 1987). Alternation of CpGV with Entrust was reported by Arthurs and Lacey (2004) as a strategy used by orchardists for control of codling moth as well as other pest insects.

The ultraviolet light component of sunlight in the 290-320 nm wavelength (UV-B) is harmful to biological systems in general (Diffey 1991) and can rapidly inactivate baculoviruses including CpGV (Keller 1973, Krieg et al. 1981, Fritsch and Huber 1985, Charmillot et al. 1998). The lack of residual activity ostensibly due to solar inactivation requiring frequent applications has been one of the concerns expressed by growers about CpGV. The daily bioassay of CpGV-treated apples for virus persistence in our study showed a steady decline in activity, but did indicate significant persistence of the virus 3 d after application. Results of studies reported by Arthurs and Lacey (2004) corroborate the observed steady decline in virus activity over the 3-d evaluation period for Carpovirusine and two other commercial CpGV products. Despite the significant decline in activity after 1 to 3 days of field exposure to sunlight, Arthurs and Lacey (2004) report evidence of low but significant virus activity as long as 14 d following application. Residual activity of CpGV in our study was comparable to or greater than that reported by other researchers (Keller 1973, Jaques et al. 1987, Jaques 1990). Glen and Payne (1984) observed some virus activity that persisted for 4 to 8 wks after spraying under the field conditions in their study. Glen and Clark (1985) noted that neonate larvae often entered fruit via the calyx, a location where virus would be protected from solar radiation.

Protection of the virus from UV-degradation would decrease the necessity for frequent reapplication. A limited amount of successful research has been conducted on formulation of the virus to improve UV tolerance. Keller (1973) and others reported good protection of the virus with the addition of skim milk powder (1%) to CpGV suspensions. Laboratory studies by Ballard et al. (2000a), using a leaf disc bioassay, demonstrated that 15% cane molasses incorporated within a formulation of purified CpGV significantly reduced the median lethal exposure time to CpGV for neonate larvae, but field trials revealed no significant improvement of CpGV persistence on apple foliage using 10 or 15% molasses formulations. Another field trial by Ballard et al. (2002a) demonstrated that 10% molasses, 10% sorbitol or 0.08% α-farnesene significantly reduced codling moth deep entry damage to fruit when these ingredients

were added to formulations of pure CpGV, but substantial sooty-mold growth was observed on apple foliage treated with formulations containing molasses.

The two adjuvants used in our studies, Raynox and Nu-Film-17, did not significantly protect CpGV from solar inactivation. Burges and Jones (1998) summarize information on a variety of adjuvants that have provided some protection of baculoviruses from UV light. Some of the more effective of these, such as lignin, warrant further investigation to determine their potential for protecting CpGV in apple and pear orchards.

Enhancement of virus activity may reduce the amount of initial damage due to fruit entries by codling moth larvae. This may be accomplished with non-engineered virus by increasing the amount of virus ingested by larvae before they contact the fruit. Neonate larvae have been reported to pick up CpGV before entering fruit (Ballard et al. 2000b). They are also reported to consume apple foliage under controlled conditions (Ballard et al. 2000a, Pszczolkowski et al. 2002a, b). Increased consumption of foliage was reported by Pszczolkowski et al. (2002b) when the leaves were treated with monosodium glutamate (MSG). Phagostimulants such as MSG that could induce consumption of CpGV by neonate larvae on leaves may increase the efficacy of the virus and reduce the degree of fruit damage. A number of other substances reported to increase consumption of virus are reviewed by Burges and Jones (1998) and will be the subject of future investigations with CpGV at our laboratory.

The benefits of utilizing virus compared to that of the broader spectrum azinphos-methyl are less apparent when simply comparing efficacy and labor. The two azinphos-methyl applications resulted in the lowest percentage of fruit injury, but did not provide better codling moth population reduction than that of CpGV alone. Similar results have been reported by Huber and Dickler (1977), Glen and Payne (1984) and Jaques et al. (1994) for CpGV, azinphos-methyl and other organophosphate insecticides. Although labor may be significantly reduced due the lower number of applications and a broader spectrum of tree fruit pests insects are controlled by azinphos-methyl, there are other benefits of virus applications such as little or no nontarget impact and safety considerations for workers and food. Orchards treated with CpGV may be reentered immediately, whereas azinphos-methyl treated orchards have a mandatory 48 to 72 h restricted entry interval and 14 to 21 d pre-harvest interval.

Environmental concerns have been raised regarding several of the insecticides used for control of codling moth, some of which (e.g., azinphos-methyl) have been targeted for discontinued use under the 1996 Food Quality and Protection Act. Also, increasing levels of resistance in codling moth to broad spectrum insecticides including azinphos-methyl have been reported by several researchers in North America and Europe (Knight et al. 1994, Sauphanor et al. 1998, Dunley and Welter 2000, Loriatti et al. 2003). Alternative interventions that control insect pests with minimal environmental impact and are safe for agricultural workers and the food supply are highly desirable. One such method, the use of codling moth female pheromone to disrupt mating, has been used successfully in the Pacific Northwest (Calkins 1998, Brunner et al. 2002), but still relies on initial reduction of the adult moth population in order to be effective (Vickers and Rothschild 1991, Brunner et al. 2002). Microbial control agents, such as CpGV and entomopathogenic nematodes, offer potential for reduction of codling moth (Cross et al. 1999, Lacey et al. 2000, 2004) that would complement mating disruption and other interventions with minimal negative impact on the environment and beneficial insect species. CpGV has also been proposed as a tool

for the management of resistance to conventional insecticides in Europe (Kienzle et al. 2003, Loriatti et al. 2003).

Acknowledgments

We thank Don Thomson and Shigeki Takasaki (Sumitomo, USA) for providing Carpovirusine and literature and our field crew of Mike Young, Angie Bosma, Isaac Bell, Ismene Larios, Jake Marquess, Duane Larsen, Brad Christianson and Chey Temple for their assistance. We appreciate the review of our manuscript by Don Hostetter and David Horton. We are also grateful to Dr. Horton for his advice and assistance with statistical analyses.

References Cited

Arthurs, S. P. and L. A. Lacey. 2004. Field evaluation of commercial formulations of the codling moth granulovirus (CpGV): persistence of activity and success of seasonal applications against natural infestations in the Pacific Northwest. Biol. Contr. 31: 388-397.

Ballard, J., D. J. Ellis and C. C. Payne. 2000a. The role of formulation additives in increasing the potency of *Cydia pomonella* granulovirus for codling moth larvae, in laboratory and field experiments. Biocontr. Sci. Technol. 10: 627-640.

2000b. Uptake of granulovirus from the surface of apples and leaves by first instar larvae of the codling moth *Cydia pomonella* L. (Lepidoptera: Olethreutidae). Biocontr. Sci. Technol. 10: 617-625.

Barnes, M. M. 1991. Tortricids in pome and stone fruits, codling moth occurrence, host race formation, and damage, Pp. 313-327. *In* L. P. S. van der Geest and H. H. Evenhuis (eds.), Tortricid Pests, Their Biology, Natural Enemies and Control. Elsevier Science Publishers B. V., Amsterdam, The Netherlands.

Beers, E. H., J. F. Brunner, M. J. Willett and G. M. Warner. 1993. Orchard Pest Management: A Resource Book for the Pacific Northwest. Good Fruit Grower, Yakima, WA.

Blommers, L., F. Vaal, J. Freriks and H. Helsen. 1987. Three years of specific control of summer fruit tortrix and codling moth on apple in The Netherlands. J. Appl. Entomol. 104: 353-371.

Brunner, J., S. Welter, C. Calkins, R. Hilton, E. Beers, J. Dunley, T. Unruh, A. Knight, R. van Steenwyk and P. van Buskirk. 2002. Mating disruption of codling moth: a perspective from the Western United States. Bull. OILB/SROP 25: 11-19.

Burges, H. D. and K. A. Jones. 1998. Formulation of bacteria, viruses and Protozoa to control insects, Pp. 34-127. *In* H. D. Burges (ed.), Formulation of Microbial Biopesticides. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Calkins, C. O. 1998. Review of the codling moth areawide suppression program in the western United States. J. Agric. Entomol. 15: 327-333.

Charmillot, P. J. and D. Pasquier. 2002. Combinaison de la technique de confusion et du virus de la granulose contre les souches résistantes de carpocapse *Cydia pomonella.* Rev. Suisse Viticul. Arboricul. Horticul. 34: 103-108.

Charmillot, P. J., D. Pasquier and A. Scalco. 1998. Le virus de la granulose du carpocapse *Cydia pomonella:* 2. Efficacité en microparcelles, rémanence et rôle des adjuvants. Rev. Suisse Viticul. Arboricul. Horticul. 30: 61-64.

Cross, J. V., M. G. Solomon, D. Chandler, P. Jarrett, P. N. Richardson, D. Winstanley, H. Bathon, J. Huber, B. Keller, G. A. Langenbruch and G. Zimmermann. 1999. Biocontrol of pests of apples and pears in Northern and Central Europe: 1. Microbial agents and nematodes. Biocontr. Sci. Technol. 9: 125-149.

Diffey, B. L. 1991. Solar ultraviolet radiation effects on biological systems. Review in Physics in Medicine and Biology 36: 299-328.

Dunley, J. E. and S. C. Welter. 2000. Correlated insecticide cross-resistance in azinphos-methyl resistant codling moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 93: 955-962.

Falcon, L. A., W. R. Kane and R. S. Bethell. 1968. Preliminary evaluation of a granulosis virus for control of the codling moth. J. Econ. Entomol. 61: 1208-1213.

Fritsch, E. and J. Huber. 1985. Inaktivierung von Apfelwickler-Crranuloseviren durch UV-Strahlung and Temperatur. Nachrichtenbl. Dtsch. Pflanzenschutzd. 37: 84-88.

Glen, D. M. and J. Clark. 1985. Death of *Cydia pomonella* larvae and damage to apple fruit, after field application of codling moth granulosis virus. Entomol. Exp. Appl. 38: 93-96.

Glen, D. M. and C. C. Payne. 1984. Production and field evaluation of codling moth granulosis virus for control of *Cydia pomonella* in the United Kingdom. Ann. Appl. Biol. 104: 87-98.

Gröner, A. 1986. Specificity and safety of baculoviruses, Pp. 177-202. *In* R. R. Granados and B. A. Federici (eds.), The Biology of Baculoviruses Vol. I, Biological properties and molecular biology. CRC Press, Boca Raton, FL.

Guillon, M. and G. Biache. 1995. IPM strategies for control of codling moth (*Cydia pomonella* L.) (Lepidoptera: Olethreutidae) interest of CmGV for long term biological control of pest complex in orchards. Med. Fac. Landbouww. Univ. Gent. 60 (3a): 695-705.

Huber, J. and E. Dickler. 1977. Codling moth granulosis virus: Its efficiency in the field in comparison with organophosphorus insecticides. J. Econ. Entomol. 70: 557-561.

Jaques, R. P. 1990. Effectiveness of the granulosis virus of the codling moth in orchard trials in Canada. Proc. Fifth Int. Colloq. Invertebr. Pathol. Microbial Contr., Aug. 20-24, Adelaide, Australia, Pp. 428-430.

Jaques, R. P., J. M. Hardman, J. E. Laing, R. F. Smith and E. Bent. 1994. Orchard trials in Canada on control of *Cydia pomonella* (Lep: Tortricidae) by granulosis virus. Entomophaga 39: 281-292.

Jaques, R. P., J. E. Laing, D. R. Laing and D. S. K. Yu. 1987. Effectiveness and persistence of the granulosis virus of the codling moth *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae) on apple. Can. Entomol. 119: 1063-1067.

Keller, S. 1973. Mikrobiologische Bekämpfung des Apfelwicklers [*Laspeyresia pomonella* (L.)] (=*Carpocapsa pomonella*) mit spezifischem Granulosisvirus. Z. Angew. Entomol. 73: 137-181.

Kienzle, J., C. Schulz, C. P. W. Zebitz and J. Huber. 2003. Codling moth granulovirus as a tool for resistance management and area-wide population control. Bull. OILB/SROP 26: 69-74.

Knight, A. L., J. F. Brunner and D. Alston. 1994. Survey of azinphosmethyl resistance in codling moth (Lepidoptera: Tortricidae) in Washington and Utah. J. Econ. Entomol. 87: 285-292.

Knight, A. L., R. P. Potting and D. Light. 2002. Modeling the impact of a sex pheromone/kairomone attracticide for management of codling moth (*Cydia pomonella*). Acta Hort. 584: 215-220.

Krieg, A., A. Gröner, J. Huber and G. Zimmermann. 1981. Inaktivierung von verschiedenen Insektenpathogenen durch ultraviolette Strahlen. Z. Pflanzenkrankh. PflSchutz. 88: 38-48.

Lacey, L. A. and D. I. Shapiro-Ilan. 2003. The potential role for microbial control of orchard insect pests in sustainable agriculture. J. Food Agric. Environ. 1: 326-331.

Lacey, L. A., S. P. Arthurs, D. Thomson, R. Fritts, Jr. and D. Granatstein. 2004. Codling moth granulovirus and insect-specific nematodes for control of codling moth in the Pacific Northwest. Tilth Producers Quarterly 13 (2): 10-12.

Lacey, L. A., A. Knight and J. Huber. 2000. Microbial control of lepidopteran pests of apple orchards, Pp. 557-576. *In* L. A. Lacey and H. K. Kaya (eds.), Field Manual of Techniques in Invertebrate Pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Loriatti, C., J. C. Bouvier, A. Butturini, R. Cornale and R. Tiso. 2003. Carpocapsa: la situazione della resistenza ad azmphos methyl e diflubenzuron in Trentino ed Emilia-Romagna. Inform. Fitopatol. 53: 53-59.

Miñarro, M. and E. Dapena. 2000. Control de *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) con granulovirus y confusión sexual en plantaciones de manzano de Asturias. Bol. San. Veg. Plagas 26: 305-316.

Pszczolkowski, M. A., L. F. Matos, R. Brown and J. J. Brown. 2002a. Feeding and development of *Cydia pomonella* (Lepidoptera: Tortricidae) larvae on apple leaves. Ann. Entomol. Soc. Amer. 95: 603-607.

Pszczolkowski, M. A., L. F. Matos, A. Zahand and J. J. Brown. 2002b. Effect of monosodium glutamate on apple leaf consumption by codling moth larvae. Entomol. Exp. Applic. 103: 91-98.

SAS Institute 1999. SAS-STAT User's Guide, Version 8. Cary, North Carolina: SAS Institute Inc.

Sauphanor, B., J. C. Bouvier and V. Brosse. 1998. Spectrum of insecticide resistance in *Cydia pomonella* (Lepidoptera: Tortricidae) in southeastern France. J. Econ. Entomol. 91: 1225-1231.

Toba, H. H. and J. F. Howell. 1991. An improved system for mass-rearing codling moths. J. Entomol. Soc. Brit. Columbia 88: 22-27.

Vickers, R. A. and G. H. L. Rothschild. 1991. Use of sex pheromones for control of codling moth., Pp. 339-354. *In* L. P. S. van der Geest and H. H. Evenhuis (eds.), Tortricid Pests, Their Biology, Natural Enemies and Control. Elsevier Science Publishers B. V., Amsterdam, The Netherlands.