Optimizing Use of Codling Moth Granulovirus: Effects of Application Rate and Spraying Frequency on Control of Codling Moth Larvae in Pacific Northwest Apple Orchards

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ABSTRACT New formulations of the codling moth, *Cydia pomonella* (L.), granulovirus (CpGV) [family *Baculoviridae,* genus *Granulovirus*] are commercially available in North America. In field tests on apple (*Malus* sp. 'Delicious'), we compared different application strategies for CpGV (Cyd-X, Certis USA, Clovis, CA) used in full-season programs against high pest populations. In replicated single tree plots, three rates (0.073, 0.219, and 0.438 liter ha^{-1}) and application intervals (7, 10, and 14 d) killed 81–99% of larvae in fruit and reduced the number of mature larvae recovered in tree bands by 54–98%. Although the proportion of deep entries declined by 77–98%, the amount of fruit injury was not reduced compared with controls. There was a statistical trend between increasing dosage and spray frequency intervals and virus effectiveness, but no interaction between these factors. In a commercial orchard, we assessed a standard (0.219 liter ha^{-1}) and two reduced rates of the virus (0.146 and 0.073 liter ha^{-1}) applied in a weekly spray program in replicated 0.2-ha blocks. In the first generation, fruit injury was reduced in virus-treated compared with three untreated blocks although the decrease was only significant at the standard rate. Mortality rates of larvae (in fruit) were ≥90%, dose dependent, and comparable with rates observed from individual trees sprayed with equivalent treatments in the previous study. Rates of larval mortality declined at all dosages (81–85%) in the first part of the second generation. Most damage and proportionally less mortality occurred in the upper canopy. High pest pressures and untreated blocks contributed to significant damage and the study was terminated early. These data suggest virus programs can be tailored according to the localized pest pressure, but it may not prevent economic damage in high-pressure situations.

KEY WORDS *Cydia pomonella* granulovirus, commercial formulation, inundative biological control, persistence, apple

CODLING MOTH *Cydia pomonella* (L.), remains the principal insect pest of apples, pears, and occasionally walnut throughout the Pacific Northwest (Barnes 1991, Beers et al. 1993). Because most orchardists have a low tolerance for damage (typically 1%), a majority still use routine applications of broad-spectrum insecticides such as Guthion (azinphos-methyl) to maintain this pest at economically acceptable levels. Disadvantages of such practices include the mandatory restricted reentry and preharvest intervals, disruption to beneficial species, and insecticide resistance (Varela et al. 1993, Knight et al. 1994, Dunley and Welter 2000, Sauphanor et al. 2000, Boivin et al. 2001). Moreover, the use of Guthion and other broad-spectrum insecticides in pome fruit may be phased out under the 1996 Food Quality Protection Act (Calkins and Faust 2003).

The reduced reliance on organophosphate and pyrethroid insecticides has promoted less intrusive control tactics for codling moth. One such method, the use of the female sex pheromone to disrupt mating, has become widely adopted in western pome fruit orchards over the last decade (Brunner et al. 2002, Calkins and Faust 2003). Although mating disruption with synthetic lures has benefited from new technology and large-scale demonstration projects, its effectiveness as a pest management tactic declines significantly over smaller scales and at high pest densities (Vickers and Rothschild 1991, Gut and Brunner 1998, Brunner et al. 2002).

Microbial pesticides based on the codling moth granulovirus (CpGV) [family *Baculoviridae*, genus *Granulovirus*] provide growers with an option that would complement mating disruption and other interventions with minimal impact on the environment and beneficial insect species (Gröner 1986, Lacey and Shapiro-Ilan 2003). CpGV is normally applied as an aqueous suspension to coincide with the hatching of codling moth eggs. Neonate larvae ingest occlusion bodies (granules) before or during initial entry into fruit. Granules dissolve in the alkaline midgut releasing the virions, which establish a transient infection

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before invading the tracheal matrix, epidermis, and fat body and other tissues (Huber 1986, Federici 1997, Lacey et al. 2002). In the late stages of infection, further occluded forms of the virus are produced, which cause cell lysis and host death within 5–10 d.

Over the past 30 yr, numerous field trials in Europe (Huber 1986, Pasqualini et al. 1992, Guillon and Biache 1995, Kienzle et al. 2003, Stará and Kocourek 2003) and North America (Jaques et al. 1981, Jaques 1990, Falcon and Huber 1991, Vail et al. 1991, Jaques et al. 1994, Arthurs and Lacey 2004, Lacey et al. 2004a) have demonstrated the potential of CpGV to control codling moth. However, although CpGV has been used by European orchardists for many years (Cross et al. 1999), commercial formulations have only recently become available in North America. In 2003, CpGV was used on an estimated 10,000–12,000 acres in the United States and 200,000 acres in Europe (Lacey et al. 2004b).

Despite these encouraging results, differences in the level of control and frequency of damage are often reported. Additionally, drawbacks of the virus perceived by many growers are the recommendation to make applications at short intervals due to the rapid virus inactivation by solar radiation, the slow speed of kill resulting in the occurrence of codling moth entries or shallow stings in sprayed fruit, and the cost of the virus (Lacey et al. 2000). Because neonate codling moth are only exposed for a short period before entering fruit, the dosage of virus and timing of applications may contribute significantly to the degree of success observed in orchard studies and the economic feasibility of this technology. The objectives of this study were to 1) compare full-season virus programs adopting different application rates and spray intervals in an experimental orchard and 2) assess standard and reduced rates of the virus used in a weekly spray program in a conventionally managed orchard heavily infested with codling moth.

Materials and Methods

Dosage and Spray Interval Studies. This study was conducted in a 0.5-ha block of 6-yr-old Delicious (strain Red Chief on EMLA seven rootstock) located at the USDA–ARS experimental orchard near Moxee, WA. Trees were 2 to 3 m in height and planted at 500 per hectare (3.7 by 5.5-m spacing), excluding Manchurian and Snowdrift crab apple pollenizers. Trees were irrigated weekly using under-tree sprinklers (Nelson rotator model 2000). No insecticides were used apart from virus treatments, and trees were manually thinned after fruit set before codling moth oviposition.

Four Pherocon VI wing-type traps (Trécé Inc., Salinas, CA) baited with red septa lures containing 1 mg codlemone (Suterra, Bend, OR) were hung in the canopy (one per quadrant) to determine biofix (first consistent moth catch) and monitor seasonal flight patterns. Traps were checked weekly after Biofix, and lures and sticky inserts were changed every 2 to 3 wk. Although the plot was naturally infested, to better compare the virus treatments the first codling moth flight was augmented with 400 moths (mixed sex from the YARL laboratory colony) uniformly released at 350 degree-days (DD) (14 June). No mating disruption was used.

Virus Applications. Virus preparations containing 3×10^{13} granules liter^{-1} (Cyd-X, Certis USA, Clovis, CA) were tested throughout the 2004 growing season (2 June–7 September). Different application strategies were compared; treatments were applied in a factorial design with three levels for dose (low, standard, and high; 0.073, 0.219 and 0.438 liter product per hectare) and spray interval (standard and reduced frequency; 7, 10, and 14 d, representing seven, five, and four applications, respectively, per codling moth generation). Dose rates were based on the rate most growers currently use in the region (standard) and the range Cyd-X is labeled for use (1–6 fl. oz. acre^{-1}; 0.07–0.44 liters ha^{-1}). Spray intervals were based on the persistence of single applications of commercial CpGV formulations in the same orchard plot in 2003 (Arthurs and Lacey 2004). The spreader/sticker Nu-Film17 (Miller Chemicals and Fertilizer, Hanover, PA) was included at 0.58 liter ha^{-1}.

Ten trees were randomly selected for treatment with each dose/interval combination. Treatments were applied using a motorized SR 420 backpack air-blast sprayer (Stihl, VA Beach, VA) at a rate of 935 liter dilute spray ha^{-1} (100 gal acre^{-1}). Virus formulations were diluted the morning of use (virus was kept refrigerated before adding to the spray tank), and applications were made before 9 a.m. during calm wind conditions. Sprays were directed to provide complete coverage of foliage and fruit with a tarpaulin screen (3 by 9 m) held by four assistants, and a one or two tree buffer was used to minimize overspray or spray drift. Control trees sprayed with water and Nu-Film17 were selected from a single row upwind of virus treatments.

Timing of treatments to coincide with codling moth egg hatch was determined using biofix (described above) and the Washington State University TFREC (Wenatchee, WA) phenology model based on Fahrenheit DD accumulations (single sine with horizontal cut-offs at 50 and 88°F) monitored at the site (Beers et al. 1993). Initial applications were made at ≈5% egg hatch (all treatments), and treatments were continued until ≈95% egg hatch. The process was repeated for the second generation using the same experimental design. Initial treatments of the first and second codling moth generation were made 2 June (246 DD) and 26 July (1,196 DD or 258 DD past second biofix).

Assessments. Fruit injury (by codling moth) and larval mortality were assessed at the end of the first larval generation (21–23 July/1,172–1,121 DD) and after the second generation near harvest (16–30 September/2,109–2,248 DD). Percentage of fruit injured was assessed in the orchard from a random subsample of 50 fruit per tree. To quantify severity, all codling moth-damaged fruit (up to 30 per experimental tree) were collected and destructively sampled under a dissecting microscope to assess the proportion of deep entries (>6 mm). To quantify larval mortality, the numbers of live larvae and exit holes (indicating a mature larva had already left the fruit) also were noted. The majority (>95%) of virus-killed larvae died as first instars.

To estimate the surviving field population and monitor larval parasitism, corrugated cardboard bands were stapled around the bole of sprayed trees and later examined in the laboratory for cocooning codling moth. Bands, consisting of 8-cm-wide single faced flute size B (Xpedx; Portland, OR), were first folded flute to flute to provide attractive pupation sites between the layers. Bands were placed 30–46 cm above the ground when larvae started exiting unsprayed fruit and were renewed every 2 wk until the majority of larvae had left (first generation 2–26 July/707–1,196 DD; second generation 11 August–4 October/1535–2,285 DD). To reduce the risk of "band contamination" in experimental trees, bands also were placed to capture larvae on unsprayed trees but were not removed. These latter bands generated a larger source of moths in the second flight.

Reduced Rate Study. The study area was a 3.5-ha rectangular block in the middle of a commercial orchard (8.7 ha total) near Zillah, WA. The variety and planting density were the same as the previous study (Delicious at 500 trees per ha), although trees were older (planted 1985) and larger (4–5 m) with a dense upper canopy. The codling moth pressure was high; the grower estimated 15% fruit damage at the previous season's harvest. Trees were chemically thinned at bloom with lime sulfur and Sevin (carbaryl), although workers manually thinned trees between the first and second codling moth generation (finished 15 July) when damaged fruit were preferentially removed.

Four monitoring traps (previously described) were placed high in the canopy equidistant along the buffer row separating the two lines of experimental plots. Traps were checked weekly, and inserts and lures were changed every 1 to 2 wk. No mating disruption was used.

Virus Applications. Individual 0.2-ha plots (eight rows of 12 trees) were marked with colored flags in complete randomized block design and treated weekly with virus (Cyd-X, Certis USA) at the standard (0.219 liter ha^{-1}) and two reduced rates (0.073 and 0.146 liter ha^{-1}). Five replicate plots arranged along two adjacent lines running the entire length of the mid-section of the orchard were sprayed weekly at each dose. The sticker-spreader Nu-Film17 was included at 0.58 liter ha^{-1}. Three additional unsprayed plots located at the top, middle, and bottom of the block served as controls.

Virus applications were made using a tractor-mounted "pul-blast" sprayer (Rear's Mfg. Co., Eugene, OR; capacity 1,135 liter/300 gal) calibrated for 1,060 liter ha^{-1} at 1,550 kPa (225 psi) and 2 mph forward speed. The rear boom was fitted with a belt-driven fan and the upper nozzles were of larger capacity delivering 80% volume to the upper one-third of the canopy. Initial treatments were made 10 May at 205 DD (earlier than the typical 250) and continued for 8 wk (slightly longer than required based on the WSU phenology model due to high pressure and extended flight). The virus trial was continued against the second larval generation; although this time the previously untreated blocks were treated at the standard rate (0.219 liter ha^{-1}) in an attempt to reduce the large infestations. However the study was terminated early (after three further weekly applications starting 16 July at 1,200 DD) due to economic concerns about rising damage levels, especially in the initially untreated blocks. The virus block was sprayed with Guthion (azinphos-methyl) before harvest on 5 and 30 August.

Assessments. Plots were inspected periodically and fruit injury and larval mortality were assessed (on separate sampling days) toward the end of the first codling moth generation (14–15 June/550 DD) and again shortly before the study was terminated (29 July–4 August/1,528–1,673 DD). Injury was assessed in situ from a random subsample of fruit (minimum of 150 per plot), and damaged fruit were later collected (minimum of 50 per plot) to estimate larval mortality in the laboratory, as described previously. Assessments were conducted concurrently in an adjacent section of orchard treated with Guthion. Replicates were five groups of six trees. Damaged fruit were not used to estimate larval mortality because Guthion is a fast acting neurotoxin. Because a majority of codling moth damage was observed high in the canopy, the location of fruit were noted among the low (<1.8 m), middle (1.8–2.5 m), and upper parts (>2.5 m) of the canopy. To minimize influence of immigration or spray drift, only the most central 18 trees (three rows of 6) were used in assessments. Tree bands (as described previously) also were placed on nine of these 18 trees and timed to catch cocooning larvae of the first codling moth generation.

To compare moth activity in plots, passive interception traps (PITs), which were unbaited and caught both codling moth sexes moving locally, were used during the peak of the first (pretreatment) and second (posttreatment) flight period (24 May–7 June/324–483 DD and 14 July–9 August/1,151–1,758 DD). A single PIT, consisting of a 30 by 30-cm clear plastic sheet precoated with high-viscosity STP oil (STP Products Co., Oakland, CA), was hung from a branch in the upper canopy in the center of each plot. Traps were replaced at twice-weekly intervals, and moths were counted in the laboratory but not sexed.

Analysis. All analysis was performed using SPSS 12.0.1 for Windows. Treatment effects were compared using one- and two-way univariate analysis of variance (ANOVA). Significant F-ratio means were further separated with Fisher's least significant difference (LSD) for multiple comparisons at $P < 0.05$. All proportional and count data were normalized via arcsine and $\log(n + 1)$, respectively, before analysis. In the first study (Moxee) each replicate was based on an individual tree ($n = 10$). In the second study (Zillah), replicates were based on 0.2-ha plots ($n = 5$) (virus) and three (untreated).

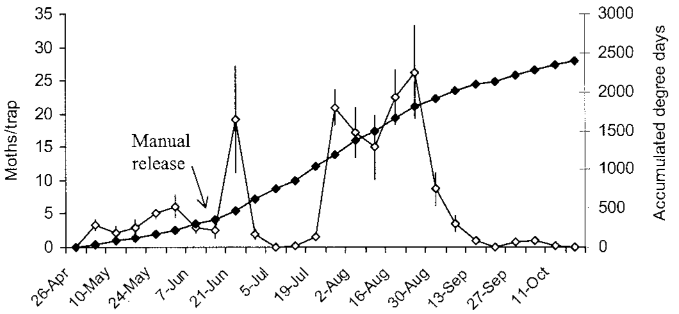

Fig. 1. Codling moth pheromone trap counts (Moxee experimental station). Lines show weekly average ± SEM for four traps (open symbols) against accumulated degree-days postbiofix (filled symbols).

Results

Dosage and Spray Interval Studies. Pheromone trap data are shown in Fig. 1. There were distinctive flights for both generations that closely tracked the Washington State University phenology model (Beers et al. 1993), although the contribution of the manual release of moths also was apparent. The second flight was larger and resulted in higher average fruit injury (48.5 versus 7.4%) and more live larvae (40.8 versus 8.1) in control trees compared with the first larval generation.

Fruit injury assessments after both codling moth generations are summarized in Fig. 2. Because >50% of trees in the block were unsprayed, fruit damage does not reflect efficacy of virus treatments (especially during the second generation) but rather indicate the high pressure under which treatments were conducted. One-way ANOVA (controls included) revealed no significant treatment effects on the percentage of fruit damaged (Fig. 2A). However, all virus-treatments recorded significantly fewer deep entries (>6 mm) compared with controls (Fig. 2B). In general, mean separations show proportionally more deep entries (up to 36%) when fruit were sprayed at less frequent intervals and at lower doses. Among virus-treated trees, two-way ANOVA also revealed no effect

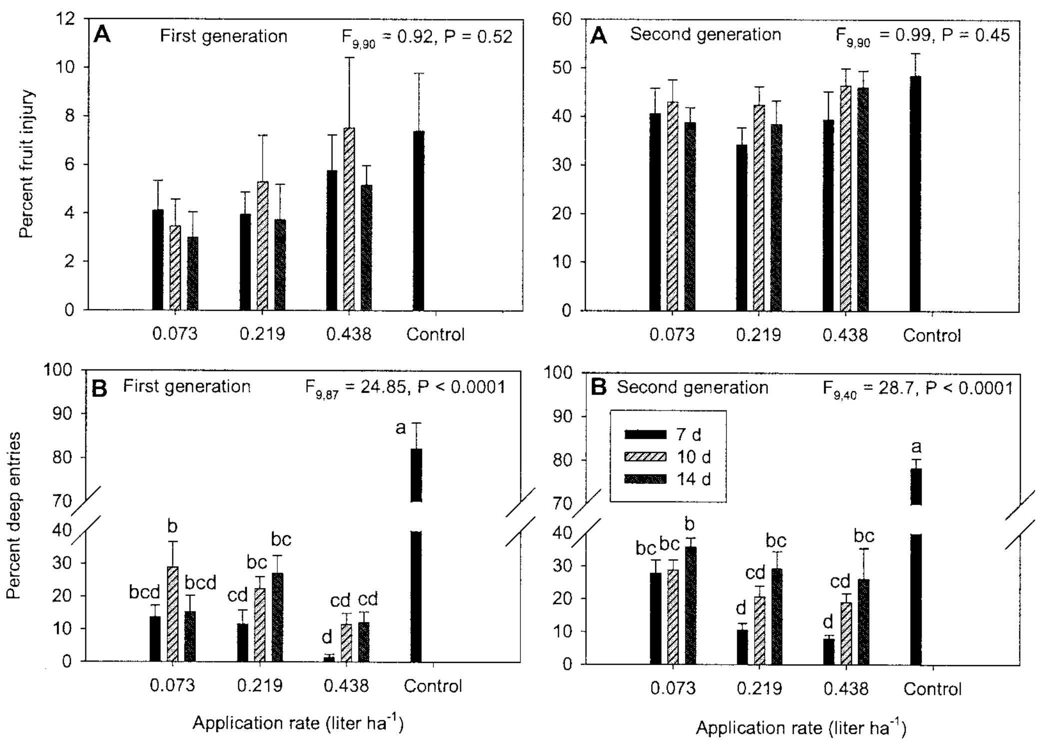

Fig. 2. Percentage of codling moth fruit injury (A) and deep (>6 mm) entries (B) after different treatments of Cyd-X (Moxee experimental station). Bars show average for 10 trees ± SEM for tests against first and second codling moth generations. Letters indicate Fisher's LSD at $P < 0.05$.

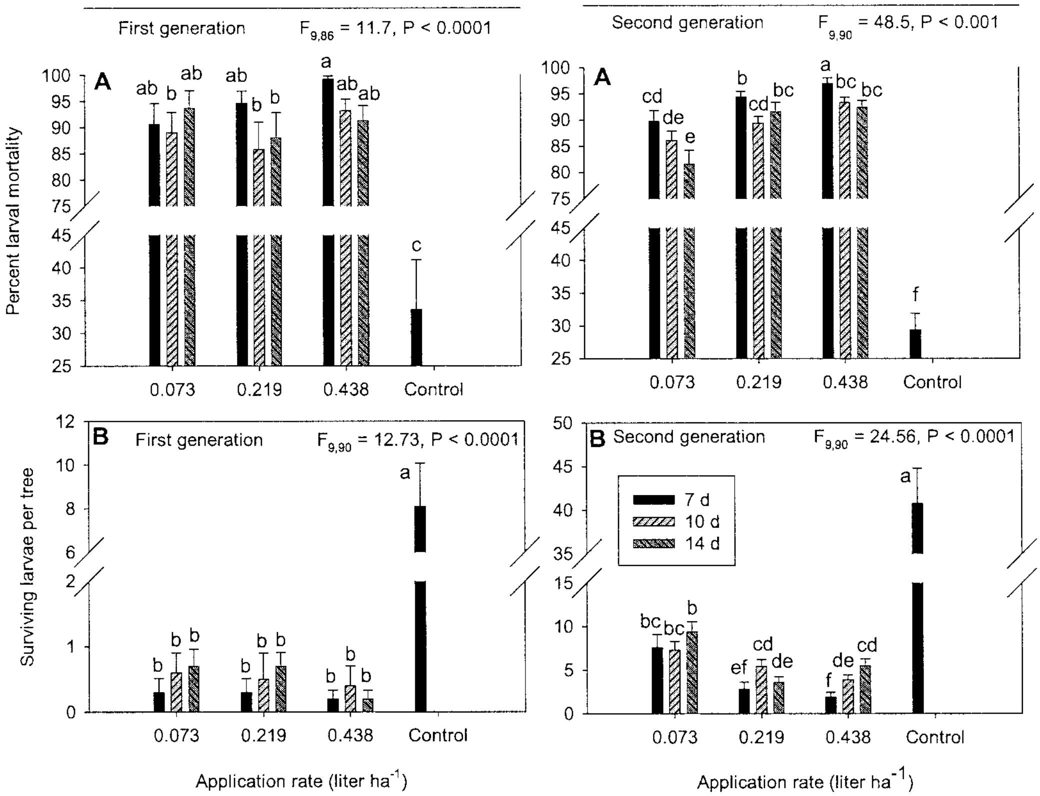

Fig. 3. Percentage of codling moth mortality (A) and number of surviving larvae per tree (B) after different treatments of Cyd-X (Moxee experimental station). Bars show average for 10 trees ± SEM for tests against first and second codling moth generations. Letters indicate Fisher's LSD at $P < 0.05$.

of dosage or application interval on percentage of fruit damage, but both were significant factors for the proportion of deep entries ($F_{2,78} = 6.0, P < 0.005$; $F_{2,35} = 5.8, P < 0.01$ for dosage) and ($F_{2,78} = 5.4, P < 0.01$; $F_{2,35} = 8.2, P < 0.005$ for interval) for the first and second codling moth generations, respectively. There were no significant interaction terms.

Assessments of larval mortality (based on the dissection of infested fruit) and number of live larvae recovered per tree (which included mature larvae captured in tree bands) are summarized in Fig. 3. In both codling moth generations virus treatments resulted in substantially higher (>80%) larval mortality (Fig. 3A) and correspondingly fewer live larvae per tree (Fig. 3B) compared with controls. In the second flight, there was a statistical trend of increasing virus effectiveness associated with higher doses and application frequencies. For example, >97% mortality and less than two larvae per tree were documented at the high rate applied weekly. In the second generation, two-way ANOVA (virus-treated only) also revealed dosage and spray interval were significant factors for both larval mortality ($F_{2,81} = 19.0$; $P < 0.0001$ for dosage) and ($F_{2,81} = 9.8$; $P < 0.0001$ for interval) and number of live larvae recovered per tree ($F_{2,81} = 17.6$; $P < 0.0001$ for dosage) and ($F_{2,81} = 8.9$; $P < 0.0001$ for interval). There were no significant interaction terms.

Differences among virus treatments were less apparent in the first generation, although the sample number was small and test less sensitive. The proportion of deep entries seemed to be a more sensitive measure to compare virus treatments in the first generation.

The egg parasitoid *Ascogaster quadridentata* Wasmael (Hymenoptera: Braconidae) was noted in the first generation recovered in tree bands. Parasitism comprised 21.1% (12 of 57 larvae) in control trees and 8% (two of 25) from a small sample from the virus-treated trees. No parasitism was observed in the larger second larval generation, although internal parasitoids may have entered diapause.

Reduced Rate Study. codling moth pressure in this orchard (especially in the first flight) was very high with 4,456 males caught from the four pheromone traps (Fig. 4). There was a protracted first flight, which extended into the second flight and did not track the Washington State University phenology model (Beers et al. 1993). In addition, a total of 762 moths were caught on PITs. The distribution in catch suggested no initial difference in codling moth pressure between the treatment plots, although untreated blocks contributed more moths in the second flight (Fig. 5A). Similar numbers of moths were caught in a Guthion-treated portion of the orchard compared with virus-treated blocks in the second flight.

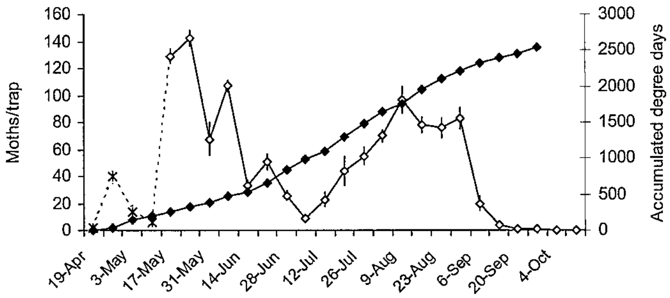

Fig. 4. Codling moth pheromone trap counts in virus-treated blocks (Zillah commercial orchard). Lines show weekly average ± SEM for four traps (open symbols) against accumulated degree-days (filled symbols). A pest consultant provided initial counts from different traps located low in the canopy (dotted line).

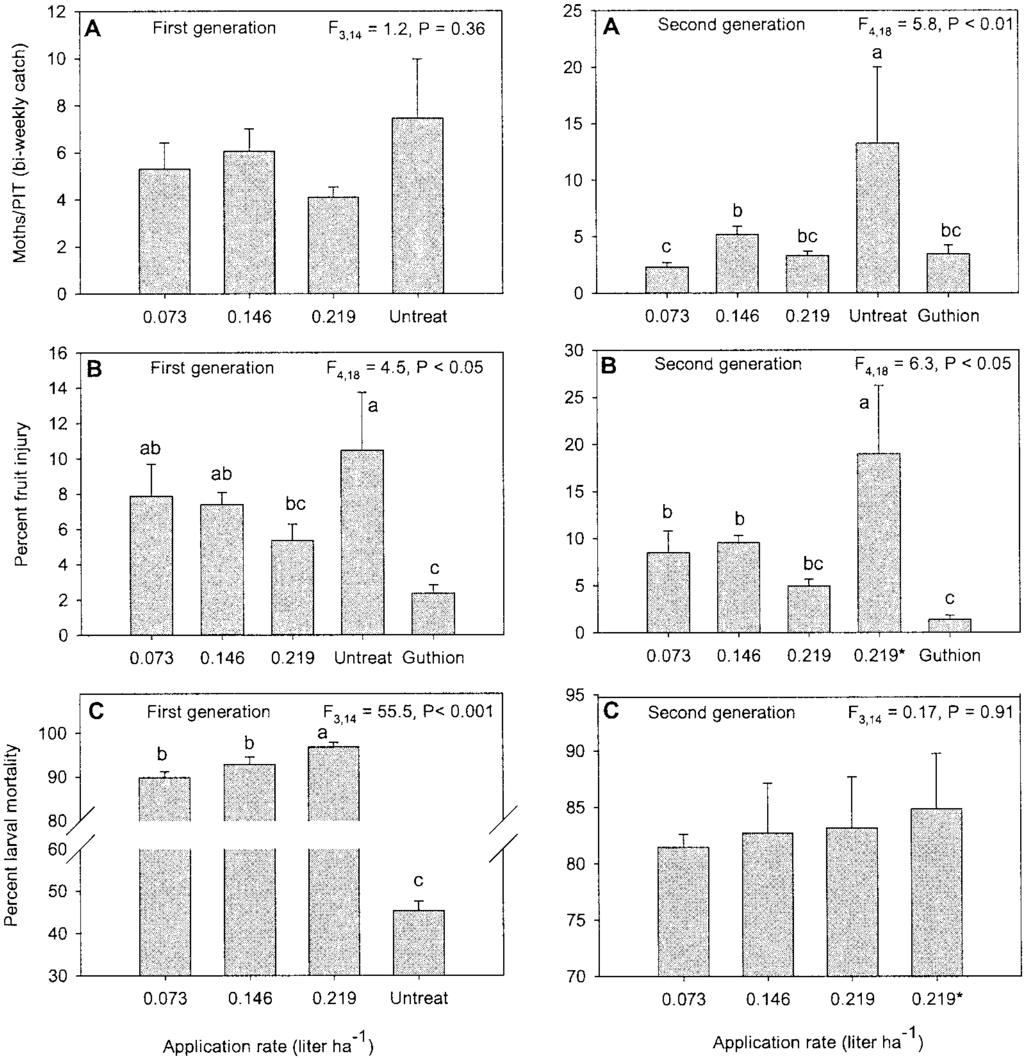

Fig. 5. Operational trial of CpGV (Cyd-X) applied at a standard and two reduce rates in a commercial orchard (Zillah). Bars show moths caught in PITs (A), codling moth fruit damage (B), and larval mortality rate (C) (average ± SEM) for five replicate 0.2-ha blocks in tests against the first and initial part of the second generations. Untreated blocks were sprayed in the second generation (indicated an asterisk). Letters indicate Fisher's LSD at $P < 0.05$.

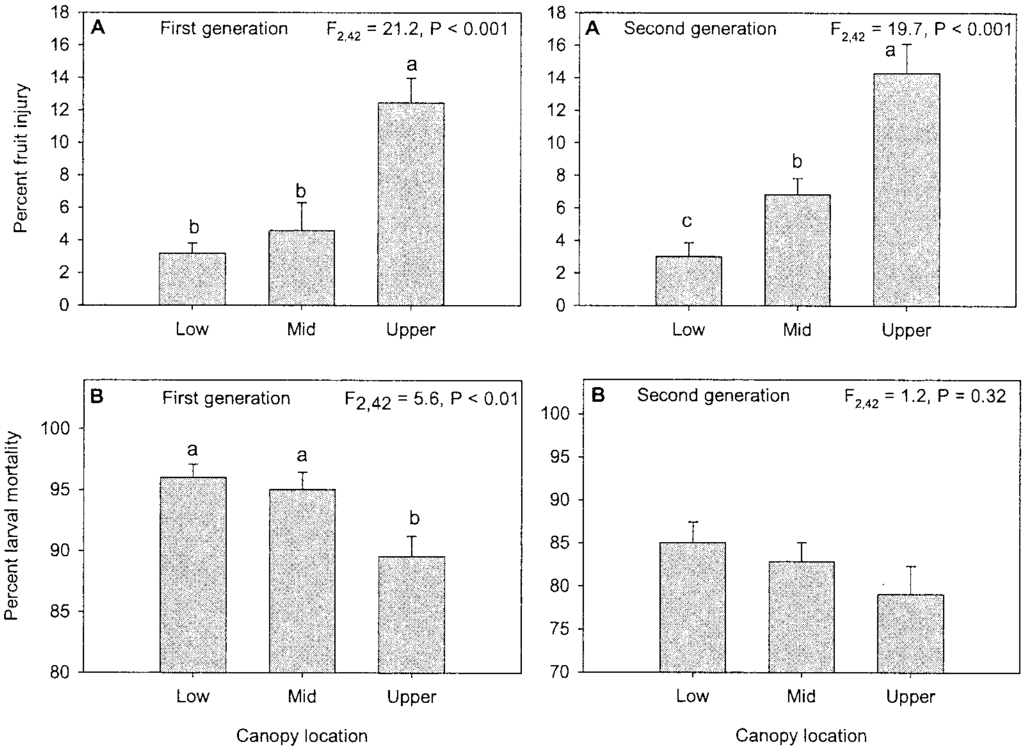

Fig. 6. Effect of canopy location, low (>1.8 m), mid (1.8–2.5 m), and upper (>2.5 m), on fruit damage and larval mortality in 0.2-ha blocks treated with CpGV. Data pooled for three rates (Zillah commercial orchard). Letters indicate Fisher's LSD at $P < 0.05$.

Assessments of fruit injury and larval mortality are summarized in Fig. 5. Unlike the previous study which compared individual trees (Moxee), fruit damage in the 0.2-ha blocks was reduced significantly by virus treatments, compared with the untreated blocks (Fig. 5B). The standard concentration (0.219 liter ha^{-1}) provided more protection compared with the reduced rates. However, damage in all virus plots was high compared with the Guthion-treated block. Mortality rates of first generation larvae were high ($\geq$90%), dose dependent, and comparable (within 2%) to equivalent virus treatments in single-tree plots at Moxee (Fig. 5C). Rates of larval mortality declined in the assessment (early second generation), representing a decrease of 8, 10, and 13%, respectively (with increasing dose) compared with the first generation. Unfortunately, tree bands placed to capture the first codling moth generation were largely destroyed by birds and could not be reliably interpreted.

In virus plots, two-way ANOVA with canopy height and virus dosage as factors revealed no interaction for either fruit injury or larval mortality in either codling moth generations. Data were pooled across virus treatments before one-way ANOVA and mean separations (Fig. 6). Most damage occurred in the upper canopy (where most moth activity and oviposition occurs). It also was interesting to note the trend of reduced efficacy in the upper canopy.

Discussion

In these studies, CpGV applications effectively reduced codling moth populations, both in single tree experimental treatments and used operationally in larger commercial blocks. However efficacy was dependent on both the rate and frequency of application (Figures 3 and 5). Such considerations affect the economic feasibility of using CpGV, especially among conventional (nonorganic) growers that maintain low damage thresholds and have more alternative control measures at their disposal. In practice, the optimal virus application strategy will largely depend on the localized pest pressure. For example, although six to eight treatments per codling moth generation at standard or high rates of virus may be required in high pressure areas, such as border rows, near bin piles or infested adjacent orchards, reduced input strategies may still be acceptable if they prevent outbreaks in low pressure areas such as orchard interiors. Additional virus applications may be required in more southerly latitudes where a significant third codling moth generation is found, whereas fewer may be required in short growing seasons of northern Europe or Canada. For example, in Nova Scotia where there is only one codling moth generation per year, Jaques et al. (1994) reported that only two applications of CpGV were usually needed.

In many organic programs, acceptable control will be a level at which a mating disruption (MD) program continues to be effective. In Switzerland, Charmillot and Pasquier (2002) reported a combination of MD and CpGV was successful, but it took several years to bring codling moth populations back to a very low level, when the virus was applied at 10-d intervals. Gut (1996) classified Washington state apple orchards into four risk classes according to the codling moth pressure and potential for effective MD. For example, very low-risk orchards (<0.1% fruit injury and zero to eight moths per pheromone trap per season) require low inputs (<500 dispersers per ha) and no or one conventional insecticidal cover spray, whereas high-risk orchards (>1.5% injury and >50 moths) require high inputs (1000 dispersers per ha) in addition to four or more cover sprays. Research to interpret monitoring information (e.g., pheromone traps, regular fruit inspections, and packout records) with the control rate required to manage populations (i.e., percentage of mortality) would allow growers to make informed decisions about including CpGV in their spray programs.

CpGV applications primarily work by suppressing codling moth populations and "stings" caused by neonate feeding before death remain a limitation of this approach (Figs. 2 and 5B). Although this study (Fig. 5B) and other studies (Jaques et al. 1987, Jaques 1990, Arthurs and Lacey 2004, Lacey et al. 2004a) show the proportion of damaged fruits and frequency of deep entry damage is reduced by CpGV treatments, it may be necessary to hold codling moth populations at or below economic thresholds. In the operational trial at Zillah, the very high pressure at the start of the season led to unacceptably high fruit damage in virus plots; although moth migrating from control blocks may have contributed additional damage in the second flight. In problem areas, many conventional growers may prefer persistent chemical treatments to protect fruit, especially close to harvest. However, the benefits of CpGV, including no preharvest and restricted entry intervals, improved biological control, worker safety, and slower development of resistance to existing pesticide chemistries warrant consideration in any decision.

Abiotic factors (notably temperature, rainfall, and UV) also reduce the effectiveness of baculoviruses under field conditions (Benz 1987). Treatments in the commercial blocks (Zillah) were slightly less effective (reduced larval mortality) in the second flight (Fig. 5C). This observation is consistent with the increased temperatures and irradiation, especially damaging UV-B (280–320 nm), mid-season. Previous studies using a larval bioassay technique on field-aged residue indicated that larvicidal activity of Cyd-X and two other commercial CpGV formulations declined faster in mid-July (half-life ≈4 d) compared with early June (≈8 d) (Arthurs and Lacey 2004). Increased UV radiation also may have contributed to the reduced larval mortality in the upper canopy (Fig. 6B). Overhead irrigation (used for afternoon cooling during July and August) also may have dislodged residue in the upper canopy. A decline in effectiveness between generations was less apparent at Moxee, although the sample size was small in the first generation.

In the Moxee trial, virus treatments provided better control of larvae than predicated based on previous residual activity bioassays conducted in the same plot (Arthurs and Lacey 2004). In these earlier bioassays, the activity of CpGV formulations declined on average by 59, 68, and 72% (compared with fresh residue) after 7, 10, and 14 d. However, no equivalent decline in effectiveness was observed in Fig. 3A, where mortality rates remained ≥80%. Such a difference may be partly explained by methodology; in the bioassays healthy larvae were placed directly on fruit whereas in the field neonate larvae also may become infected with virus by feeding on contaminated eggshells or foliage before entering fruit. Another factor contributing increased rates of infection within the plot may be autodissemination of the virus by the host. Although we controlled for spray drift (direct contamination) moths flying in the plots may have been contaminated and vectored the virus between adjacent treatments. There is evidence from other host–baculoviruses systems that contaminated adults may transmit virus vertically via contamination of egg masses (Fuxa and Richter 1992, Burden et al. 2002, Fuxa 2004). Birds also may vector baculoviruses through their feces (Hostetter and Biever 1970, Buse 1977). Due to the randomized block design and relatively small plot size, increased infection resulting from autodissemination would have proportionally greater impact in the reduced frequency treatments. Although it is unclear whether virus transmission elevated infection rates locally, it would explain the fairly high rates of failed entries and shallow stings (29–45%) in control trees adjacent in our studies (although other biotic and abiotic mortality factors also may be involved). The possibility for CpGV autodissemination in local populations through the use of attractant traps containing a source of virus will be investigated further. Future work at YARL will also focus on optimizing the product persistence and larval uptake through formulation with phagostimulants and UV screens.

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