Evaluation of the codling moth granulovirus and spinosad for codling moth control and impact on non-target species in pear orchards

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Abstract

We compared the efficacy of a commercial preparation of the codling moth, Cydia pomonella L., granulovirus, CpGV (Cyd-X®) and spinosad (Entrust®) at operational rates for codling moth control in 2004 and 2005. Concurrently we monitored the impact of treatments on populations of non-target arthropods. Spinosad was effective at protecting fruit, with <1.6% codling moth injury in experimental plots, compared with up to 37% injury in the untreated plots at harvest. Mid-season outbreaks of the pear psylla, Cacopsylla pyricola Foerster, were also reduced in spinosad plots. Spinosad was safe for several predators, notably the psylla predator Deraeocoris brevis Uhler, but reduced the abundance of hymenopteran parasitoids by 24% and 40% and non-target Diptera by 49% and 35%, respectively in 2004 and 2005. We found no evidence that spinosad disrupted natural control leading to increased densities of secondary pests including aphids and phytophagous mites. CpGV was less effective than spinosad at protecting fruit, with percentage of fruits attacked similar to controls, but killed the majority (67–71%) of neonate coding moth larvae and did not harm non-target species. Additional observations were conducted in commercial orchards (mixed pear and apple) where CpGV and spinosad were used operationally against existing codling moth infestations. In pear, two spray programs applied in replicated 0.4 ha blocks (i.e. CpGV followed by spinosad against the first and second larval generations, respectively and vice versa) reduced fruit injury at harvest and decreased orchard pheromone monitoring trap catches by 74% over two years. In apple, CpGV was less effective at protecting fruit in the first larval generation compared with spinosad, although population suppression was effective early in the season. Spinosad caused no disruptions of beneficial species or secondary pest outbreaks were observed in the commercial orchards. Our results suggest CpGV and spinosad can be effectively used in integrated pest management for codling moth.

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1. Introduction

The codling moth, Cydia pomonella L., is the most serious pest of apple and a significant pest of pear and walnut throughout the world (Barnes, 1991). Neonate larvae bore into the fruit and develop internally. Fifth instar larvae leave fruit in search of cryptic habitats, such as rough bark, in which to spin their cocoons and pupate. In the Pacific Northwest there are two or three generations per year (Beers et al., 1993).

Insecticidal options for codling moth in organic and other orchards adopting low risk or integrated pest management have historically been limited. Most 'soft' control efforts focused around ovicidal oils, removal of infested fruit, and more recently mating disruption (Beers et al., 1993; Calkins and Faust, 2003). The granulovirus of the codling moth (CpGV) has been widely tested in North America (Arthurs et al., 2005; Cossentine and Jensen, 2004; Falcon and Huber, 1991; Jaques et al., 1994; Lacey et al., 2004a; Vail et al., 1991). The virus is sprayed in the orchard as an aqueous suspension to coincide with the hatching of eggs. Neonate larvae ingest occlusion bodies (OB), also called granules, before or during initial entry into fruit.

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Currently three insecticidal formulations of CpGV are registered for commercial use in North America (Lacey et al., 2004b).

Spinosad, comprised of a mixture of spinosyns A and D derived from fermentation of the actinomycete bacterium, Saccharopolyspora spinosa Mertz & Yao, has also recently been registered for orchard use in North America (Doerr et al., 2004; Smirle et al., 2003). Two formulations of spinosad, one approved for organic use, are currently marketed for codling moth control. While the host range of CpGV is limited to codling moth and closely related species (Gröner, 1990), spinosad is effective against tortricid leaf rollers and other orchard Lepidoptera (Doerr et al., 2004; Smirle et al., 2003) and has insecticidal activity within the Diptera, Coleoptera, Thysanoptera, and Hymenoptera (Bret et al., 1997; Dutton et al., 2003; Thompson and Hutchins, 1999).

Although CpGV might be expected to conserve natural enemies and other beneficial organisms in the orchard agroecosystem, a disadvantage of spinosad’s broader host range is the potential to harm beneficial insects, including honey bees and other pollinators (Mayes et al., 2003; Morandin et al., 2005), as well as some predators and parasitoids of arthropod pests (Williams et al., 2003). Disruption of biological control may increase numbers of secondary pests. In orchard tests for codling moth control, Van Steenwyk et al. (2005) documented elimination of the aphid parasitoid Trioxys pallidus Haliday (no other natural enemies were reported) and increased numbers of walnut aphid Chromaphis juglandicola Kaltenbach following spinosad treatment. Elevated numbers of pear rust mite were also reported following spinosad use in pear (Van Steenwyk and Nomoto, 2006).

In integrated pest management (IPM) strategies, natural enemies (i.e. parasites, predators and pathogens) of codling moth and other orchard pests may play significant roles in crop protection (Beers et al., 1993; Cross et al., 1999; Lacey and Shapiro-Ilan, 2005). It is important to ensure that the various components of IPM are compatible. In this paper, we compared the efficacy of CpGV and spinosad at operational rates for codling moth control in an experimental and commercial pear orchard over two years. We also monitored the impact of treatments on beneficial arthropods and secondary pest outbreaks.

2. Materials and methods

2.1. Field site

Orchard tests were conducted in 2004 and 2005 at the USDA-ARS experimental station near Moxee, WA, USA. The test block was a 1 ha Bartlett pear orchard on OHXF 97 rootstock at a planting density of 420 trees/ha. D’Anjou pollinizers on the same rootstock comprised about one out of every nine trees. The orchard was originally planted in 1987 with additional plantings in 1997, 2001 and 2002. The orchard was naturally infested with codling moth. Pheromone-baited BioLure® traps containing a 1 mg codlemone lure (Suterra, Bend, OR) were hung in the canopy to determine Biofix (first male catch) and monitor seasonal flights. Lures and sticky trap liners were changed every 2 weeks. No mating disruption was used. No insecticides were used apart from experimental treatments. Under-tree sprinklers (Nelson low angle impact model L20W) prevented insecticide run-off during irrigation. Conditions were predominantly sunny and dry (<1 cm rainfall) throughout the study period. In 2004 the mean temperatures was 18.4 °C (range 3.3–35.7 °C) during the application period against the first larval generation. During second generation tests, the mean was 21.9 °C (range 8.2–37.4 °C). Temperatures in 2005 were similar, with means of 17.7 °C (range 2.0–35.3 °C) and 21.6 °C (range 7.4–37.0 °C) during the first and second larval generations, respectively.

2.2. Experimental treatments

A commercial preparation of CpGV containing 3 × 10¹³ OB/L ‘Cyd-X®’ (Certis USA, Columbia MD) and spinosad (Entrust® Naturalyte organically approved WP formulation, Dow AgroSciences, Indianapolis IN) were applied separately in a full-season program for codling moth. Application rates were 220 ml/ha (Cyd-X) or 210 g/ha (Entrust). The spreader-sticker NuFilm-17 (Miller Chemicals and Fertilizer Corp.; Hanover, PA) was always included at 440 ml/ha. Treatments were timed to coincide with codling moth egg hatch using Biofix (described above) and a phenology model based on degree day (DD) accumulation (Beers et al., 1993). In 2004 initial applications were made at 282 DD post Biofix (4% egg hatch of the first larval generation) with three further applications at 8 day intervals (336, 461 and 645 DD) to provide residual control until approximately 95% hatch. The second larval generation was treated in the same way, with four applications (1223, 1411, 1535 and 1738 DD). The study was repeated in 2005, with five applications were made in the first (243, 345, 428, 537 and 655 DD) and four in the second (1229, 1453, 1620 and 1771 DD) larval generations.

The study was a randomized complete block design with four (2004) or three (2005) replicate 0.04 ha (16 tree) blocks for each treatment including an untreated control. Replicates were blocked in separate sections of the orchard according to tree age class. Treatments were applied in early morning during calm wind conditions (<0.5 m/s) with a diesel powered 95L capacity airblast sprayer (Hauff Company, Yakima WA) pulled with an all terrain vehicle. The sprayer was calibrated to provide full coverage of foliage and fruit at 935 L/ha at 100kPa. Virus was kept refrigerated and diluted on site. A tarpaulin screen (3 × 9m) held by four people and a buffer tree row between plots were employed to minimize overspray or spray drift between treatments.

2.3. Efficacy of CpGV and spinosad for codling moth

Treatments were assessed mid season (i.e. towards the end or shortly after the first larval generation; starting at
783 and 1012 DD in 2004 and 2005, respectively) and similarly before harvest after the second larval generation (1859 and 1919 DD in 2004 and 2005, respectively). In each time period a minimum of 150 (2004) or 300 (2005) fruit/block were examined in situ for codling moth damage. At harvest, 50 infested fruit from each virus and untreated control block were returned to the laboratory and destructively sampled at 10X to quantify fruit damage and larval mortality inside. The proportion of ‘deep’ larval entries (i.e. ≥6 mm) was also noted as proxy for virus concentration and speed of kill (Arthurs et al., 2005). Exit holes (made by mature larvae leaving fruit) were included in the count of live larvae. Fruit were maintained at 12 °C for a maximum of 2 weeks until processing.

2.4. Sampling non-target arthropods

The abundance of non-target species in experimental blocks was monitored throughout the growing season. Beating tray samples were taken approximately weekly to census benedicts including predatory insects, spiders and parasitoids. Arboreal phytophagous species including pear psylla, Cacoprycilla pyricola Foërster, aphids and thrips were also noted. Arthropods dislodged by beating on two representative branches at shoulder height fell onto a canvas beat tray (45 × 45 cm). To provide a treatment buffer row only the central four trees in each plot were sampled. Samples were taken in the cool of the morning (before 08:00 h) to minimize escape before identification; although mobile species (notably parasitoids) were still probably under-represented for this reason. Parasitoids were collected with an aspirator, stored in 70% alcohol and identified to family. Foliar samples (10 randomly selected shoots/block) were taken bi-weekly to monitor aphid outbreaks within the plots. Sweep net samples were taken every 7–10 days during spraying periods to census leafhoppers and other non-target taxa on the orchard floor. Ten sweeps along a 5 m transect in the central area of each plot were used for collecting samples, which were frozen until processing. Late season populations of resident phytophagous mites were quantified from 100 randomly selected leaves in each treated and untreated plot. Numbers of the eriophyid pear rust mite, Epitrixus pyri Nalepa, were assessed using a leaf brushing machine and the technique described by VanBuskirk et al. (1999).

2.5. Commercial orchard trials

Additional studies were conducted with cooperating growers where formulations of CpGV and spinosad were used operationally. Orchards comprised mixed pear at irregular spaced plantings with under-tree sprinklers (Hood River, OR) and apple cv. Delicious (Mattawa, WA) at planting density of 426 trees/ha and overhead irrigation. In both cases experimental treatments were confined to a 1.6 ha section which had suffered repeated damage from codling moth, identified by the grower based on infestations monitored in previous years. Each 1.6 ha section was divided into four 0.4 ha blocks. Growers applied CpGV or spinosad in adjacent blocks; i.e. two replicate blocks each alternating between treatments, using a tractor-mounted air-blast sprayer calibrated 1870 liter/ha at ≈1550 kPa. Codling moths were monitored with pheromone traps and initial applications started at ≈250 DD (defined previously) with treatments repeated at 7–10 day intervals until ≈90% egg hatch.

In pear (Hood River) there were four (2004) or five (2005) applications of CpGV (Cyd-X at 110–220 ml/ha) or spinosad (Entrust WP® at 160 g/ha) in the first generation, with a further three in the second generation in both years. Because spinosad is restricted for resistance management (630 g/ha/season for Entrust) the test blocks were rotated in the second larval generation, i.e. treatments were spinosad followed by CpGV and vice versa. Neem oil, 1.2%Azadirachtin (Aza-Direct, Gowan Co., Yuma, AZ) + 1% mineral oil was also applied for psylla control in May 2005.

In apple (Mattawa) there were five applications of CpGV (Carpovirusine® formulation at 11/ha, Arvesta Corp., San Fransisco, CA) and spinosad (Success SC® formulation at 438 ml/ha, Dow AgroSciences) in the first generation. Eight applications of CpGV (Carpovirusine at 11/ha or Cyd-X at 220 ml/ha) were required in the second generation due to protracted moth emergence from an adjacent stockpile of wooden fruit bins. Spinosad (Success is limited to 2.12/ha/season) was replaced with five applications of the insect growth regulator methoxyfenozide (Intrepid 2F® at 1.17/l/ha + 0.5–1% oil, Dow AgroScience). At each location all treatment blocks (with the exception of Intrepid which had fewer applications) were sprayed concurrently or within 2 days of each other.

Fruit evaluations for codling moth damage and beating tray samples for non-targets were taken in the commercial orchards as previously described. Beating tray samples from 10–15 trees/block were taken on three occasions, early season (pre-spray), towards the end of the first codling moth larval generation and prior to harvest. In mixed pear (Hood River) due to fruit russetting, pear rust mite populations were also assessed (100 leaves/block from 10 trees) using a leaf brushing technique (VanBuskirk et al., 1999). Green Anjou, the most susceptible variety for both mites and psylla, was selected for non-target assessments. No mite problems were noted in apple (Mattawa).

2.6. Data analysis

Count data for experimental blocks were analyzed using a two-factor (treatment by sample period) repeated measures analysis of variance (ANOVA), including block effects. The abundance of non-target species was compared in three time periods, early, mid and late season, with sampling date the repeated factor. The analyses were done using PROC MIXED (SAS Institute, 2001). Where necessary, data were normalized with log(n+1) transformation.
ANOVA were further separated with simple effects comparisons between pairs of means using the DIFF command and a Bonferroni adjustment for multiple comparisons. For some species, pre-treatments counts were included as covariates, other species had zero or low pre-treatment counts. In the commercial orchards, treatment effects were compared using one and two-way univariate ANOVA with significant F-ratio means separated with Fisher’s LSD. In all analysis, significance values are reported at $P<0.05$.

3. Results

3.1. Efficacy of CpGV and spinosad on codling moth

In both years there were distinctive peak flights for each codling moth generation, (Fig. 1), which tracked the phenology model (Beers et al., 1993). Little fruit damage was caused by first generation codling moth in either year (Table 1). Eggs were observed in plots after the first flight, but newly developing fruit was still hard and apparently difficult for larvae to penetrate. The second flights were larger (Fig. 1) resulting in significant fruit damage in untreated blocks at harvest (Table 1). The increased damage later in the season is illustrated by the significant treatment by generation interaction in both 2004 ($F_{2,6} = 14.5, P < 0.001$) and 2005 ($F_{2,4} = 15.7, P < 0.05$). Spinosad was effective at protecting fruit with $\leq 1.6\%$ codling moth injury in both years, compared with up to $37\%$ injury in the untreated blocks. In both years Cyd-X was significantly less effective than spinosad at protecting fruit, with percent fruit injury at harvest comparable with untreated controls (Table 1). However, dissections revealed most larvae in virus-treated fruit died near the surface in the first stadium, thus reducing the severity of injury. Larval mortality in virus-treated fruit was significantly higher compared with fruit in untreated control plots at harvest; $67.1 \pm 4\%$ versus $20.4 \pm 5\%$ (2004) and $71.3 \pm 2\%$ versus $17.7 \pm 2\%$ (2005), respectively. The percentage of deep entries in virus-treated fruit was reduced compared with untreated fruit where most larvae reached the core to feed on the seeds, i.e. $51 \pm 6$ versus $92 \pm 5 \%$ (2004) and $52 \pm 4$ versus $92 \pm 5 \%$ (2005), respectively ($P<0.05$ in independent samples $t$-tests).

Although fruit evaluations showed the virus was more effective at population suppression than prevention of damage, dissections of late season fruit harvested from the virus blocks suggested possible sub-lethal effects of the virus on larval development. Fig. 2 shows a significantly lower proportion of exit holes recorded from survivors in virus compared with untreated blocks ($P=0.011$, Pearson Chi-Square test on pooled data).

3.2. Effects of spinosad and CpGV on non-target arthropods

In total, 10,443 and 12,109 arthropods were collected in beating trays in 2004 and 2005, respectively. Pear psylla was the most abundant prey associated with populations of several beneficial species. In 2004, most beneficials comprised

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Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean % fruit injury ± SEM$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2004 harvest</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0.40 ± 0.5</td>
</tr>
<tr>
<td>CpGV</td>
<td>0.23 ± 0.2</td>
</tr>
<tr>
<td>Spinosad</td>
<td>0.00 ± 0.0</td>
</tr>
</tbody>
</table>

Data show codling moth fruit injury in replicated 16 tree blocks.

$^a$ Different letters in columns indicate differences ($P<0.05$, 1-way contrasts in PROC GLM).

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Fig. 1. Codling moth pheromone trap counts (Moxee experimental station). Data show average weekly catch from five (2004) or four (2005) traps against accumulated degree days ($°$F) post Biofix.
predatory insects (71.9%) followed by spiders (18.6%) and parasitoids (9.5%), with similar findings in 2005; predatory insects (60.1%), spiders (20.2%) and adult parasitoids (19.7%). Seasonal trends for some of the more commonly recovered species are shown in Fig. 3.

In both years >95% of psylla were adults; nymphs were not readily dislodged. A mid-season peak (first summer-form generation), was not observed in spinosad plots in either year, suggesting reproductive interference (Fig. 3). Repeated measures ANOVA revealed a significant treatment by sample period interaction for psylla in 2004 ($F_{4,16} = 5.5$, $P < 0.01$) and paired comparisons revealed that psylla abundance was reduced during the mid-season in spinosad plots compared with untreated or CpGV-treated plots ($t \geq 3.3; P < 0.05$). Although the treatment by sample period interaction was not statistically significant in 2005 ($F_{4,18} = 2.0$, $P = 0.18$), a similar trend was observed.

*Deracoris brevis* Uhler (Heteroptera: Miridae), was the most abundant beneficial species and comprised 79.4% and 74.7% of all insect predators in 2004 and 2005, respectively. The abundance of *D. brevis* increased mid-season and was not reduced by spinosad treatments in either year. A significant treatment by sampling period interaction in 2004 ($F_{4,12} = 3.7$, $P < 0.05$) was caused by more *D. brevis* in spinosad-plots late season compared with other plots ($t \geq 3.3; P < 0.05$). There was thus circumstantial evidence *D. brevis* was largely responsible for the late season decline in psylla populations. Another psylla predator, *Anthocoris* spp., mainly *Anthocoris tomentosus* Péricart (Heteroptera: Anthocoridae), also increased mid-season. Unlike *D. brevis*, the increased numbers of *Anthocoris* spp. were not observed in spinosad plots in either year, although the relatively small sample size prevented treatment effects from being statistically significant. A large diversity of arboreal spiders was recovered from the foliage in both years; common families included Linyphiidae, Salticidae, Oxyopidae and Clubionidae, although species were not identified. No treatment effects were observed in 2004 although fewer spiders occurred within spinosad-plots in 2005 ($F_{2,7} = 9.5$, $P < 0.05$ and $t \geq 3.4; P < 0.05$ in paired comparisons). Most of this decline occurred late season, i.e. treatment by sampling period interaction approached significance ($F_{4,11} = 2.8$, $P = 0.08$).

Other beneficial species including ladybeetles (Coccinellidae) and lacewings, (*Chrysoperla* spp., *Chrysopa* spp. and *Hemerobius* spp.) were recovered in all treated plots, although generally remained at low populations (<1 per beat tray sample) and no significant effects of treatments were detected. Other predatory Hemiptera including *Geocoris* spp. (Lygaeidae), *Orius* spp. (Anthocoridae), *Cempylomma verbaschi* Meyer (Miriidae) and damsel bugs (Nabidae), as well as hoverflies (Syrphidae), earwigs, *Forficula auricularia* L. and the snake fly Agulla sp. (Neuroptera: Raphidiidae) were also recovered, although these predators were too infrequent to be compared statistically between plots. However, when all these latter groups were pooled in the model as a single group (other predators), no significant effects of treatments were observed.

A total of 319 adult hymenopteran parasitoids among 16 families were collected on beat trays in 2004 and 2005 (Fig. 4). Overall, parasitoid abundance was reduced by 23.9% and 39.2% in spinosad-plots compared with CpGV and untreated plots in 2004 and 2005, respectively. This decline was statistically significant in 2005, illustrated by a treatment by sampling period interaction ($F_{4,8} = 7.6$, $P < 0.01$) and paired comparisons revealed the reduction in parasitoids in spinosad plots occurred mid-season ($t \geq 3.2; P < 0.05$ in paired comparisons). We did not quantify the impact on different parasitoid guilds, although 57% of collected individuals were the encyrtid *Trechmitis insidiosa* Crawford, an important parasitoid of psylla (*Beers et al.*, 1993).

No secondary pest outbreaks occurred in our plots. A mid-season infestation of *Aphis pomi* De Geer was noted in one of the spinosad-plots, but was quickly brought under control by beneficials. Most aphids were alates (overall 74% in 2004) indicating limited reproduction. Other foliar pests including western tentiform leaf miner, *Phyllonycter ecla* Doganlar & Mutuura, stink bugs *Euschistus conspersus* Uhler and *Acrosternum hilare* Say (Hemiptera: Pentatomidae), lygus bug, mainly *Lygus lineolaris* Palisot de Beauvois and the white apple leafhopper *Typhlocyba pomaria* McAtee were found, but remained below damaging thresholds (<1 per beat tray sample) in all plots, and no statistically significant treatment effects were observed for these species. Thrips were a minor pest (<3 per beat tray) post-bloom when treatments were applied, although *Frankliniella occidentalis* Pergande were collected throughout the season. Thrips were maintained at low levels (<1 per beat tray) with spinosad treatments and significant treatment effects were observed in 2004 ($F_{2,7} = 8.1$, $P < 0.05$) caused by fewer thrips within spinosad compared with CpGV or untreated plots mid season ($t \geq 3.0; P < 0.05$ in paired comparisons).
Fig. 3. Pear psylla, *Cacopsylla pyricola*, and common beneficial taxa monitored with beating trays over two years (Bartlett pear, Moxee experiment station). Data show means ± SEM for four (2004) or three (2005) replicate 16 tree blocks. Arrows show timing of spray treatments.
Overall 4660 and 26,007 arthropods were collected in sweep net samples in 2004 and 2005, respectively (in 2004 sampling was more limited around spraying periods). Leafhoppers (Homoptera: Cicadellidae) comprised the majority of epigeal taxa, 49% in 2004 and 88% in 2005. No significant treatment effects were observed for leafhopper abundance in 2004 (all species pooled) or 2005 (major species tested separately were Dikraneura spp. and Ceratagalia spp. or all unidentifiable nymphs pooled). However the abundance of non-target dipterans (representing 47% and 11% of arthropods collected in sweep nets 2004 and 2005, respectively) was reduced by 48.8 ± 3.8% (2004) and 35.3 ± 8.7% (2005) in spinosad-treated compared with other plots (Fig. 5). Repeated measures analysis using pre-treatment covariates and two (2004) or three (2005) sampling periods showed the decline in non-target dipterans in spinosad plots was significant in 2004 ($F_{2,8} = 15.6, P < 0.01$ and $t \geq 4.4, P < 0.01$ in paired comparisons) and 2005 ($F_{2,3} = 13.7, P < 0.05$ and $t \geq 4.4; P < 0.05$). Species mainly comprised fungus gnats (Mycetophilidae) and aphid flies (Chamaemyiidae) but were not further identified.

Late season leaf assessments in both years revealed high populations of pear rust mites, although mite abundance was not increased by spinosad treatments (Table 2). The
Pear mite (PRM), *Epitrimerus pyri*, was evaluated over two years with different methods.

Means in column not significantly different (*P* > 0.05, 1-way ANOVA).

**Table 3**

<table>
<thead>
<tr>
<th>Spray program</th>
<th>Percent fruit injury ± SEM*</th>
<th>Pear cv.</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-season Harvest Mid-season Harvest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPGV/spinosad</td>
<td>Bartlett 8.3 ± 2.2a n/a</td>
<td>1.9 ± 0.5b</td>
<td>1.1 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Spinosad/CpGV</td>
<td>Anjou 6.2 ± 2.0a n/a</td>
<td>0.7 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Spinosad/CpGV</td>
<td>Bartlett 0.9 ± 0.1b</td>
<td>2.4 ± 1.4</td>
<td>0.0 ± 0.0</td>
<td>n/a</td>
</tr>
</tbody>
</table>

The order in which treatments were applied was rotated between the first and second larval generations. n/a, not assessed.

a Applied at 1870 l/ha in two replicate blocks each 0.4 ha. All plots treated with neem oil (AzaDirect) + 1% mineral oil in May 2005.

b A minimum of 300 fruit/block evaluated; different letters indicate significant differences (*P* < 0.05, Fisher’s LSD).

**Table 4**

<table>
<thead>
<tr>
<th>Spray program</th>
<th>Fruit injury and larval mortality %±SEM*</th>
<th>Mid-season</th>
<th>Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury</td>
<td>Mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPGV</td>
<td>3.3 ± 1.6</td>
<td>92.5 ± 7.4</td>
<td>3.5 ± 1.5</td>
</tr>
<tr>
<td>Spinosad/methoxyfenozide</td>
<td>0.8 ± 0.3</td>
<td>80.0 ± 20.0</td>
<td>4.1 ± 1.1</td>
</tr>
</tbody>
</table>

Data show fruit injury and larval mortality in sprayed fruit. Spinosad was replaced with methoxyfenozide (Intrepid 2F® at 1.2 l/ha) in the second generation.

a Applied at 1870 l/ha in two replicate blocks each 0.4 ha.

b A minimum of 450 (mid-season) or 1080 (harvest) fruit/block evaluated; different letters indicate significant differences (*P* < 0.05, independent T-test).

 predatory mite *Typhlodromus* spp. and the mite feeding beetle *Stethorus picipes* Casey (comprising 85% and 47% of all coccinellids observed in 2004 and 2005, respectively) were observed in all experimental plots.

### 3.3. Commercial orchard trials

Fruit evaluations for codling moth control are shown in Tables 3 and 4. Both orchards experienced a significant infestation, coming from adjacent orchard habitat (Hood River) while a large fruit bin pile (Mattawa) also provided a constant source of immigrating moths. In the mixed pear orchard there was a relatively high initial codling moth infestation, with Bartlett the more susceptible variety compared with Anjou (Table 3). Both spray programs (i.e. CpGV followed by spinosad against the first and second generations, respectively, and vice versa) were effective at reducing fruit injury at harvest over two years. Lowest damage occurred when spinosad was applied against the first larval generation. Seasonal pheromone trap catches in the orchard (average of four traps maintained in the same locations) were reduced from a pre-treatment catch of 111.3 in 2003 to 68.8 in 2004 (38% reduction) and 29.3 in 2004 (74% reduction). Larval mortality varied from 63 to 90% in virus-sprayed fruit in the individual blocks. The grower considered the treatments effective and reduced virus application rates to 110 ml/ha during 2005. In apple, spinosad was more effective at protecting fruit in the first larval generation compared with CpGV (Table 4). Nevertheless, examination of infested fruit revealed >92% of larvae in the CpGV plots were killed, indicating effective population suppression early in the season. CpGV was less effective against the second larval generation and killed significantly fewer larvae compared with spinosad/methoxyfenozide, although fruit injury at harvest was similar between blocks treated with either program (Table 4).

In total, 2383 and 1276 arthropods were collected in beating trays from Green Anjou (Hood River) in 2004 and 2005, respectively, with a further 390 from apple in 2004 (Mattawa). Pear psylla was the most common species in pear, with average mid-season counts of 14.5 (2004) and 12.3 (2005) per beat tray sample. *D. brevis* was the most common predator, averaging 3.4 (2004) and 1.6 (2005) individuals per beat tray later in the season. In both years psylla infestations in all blocks declined to low levels (<2 per beat tray) by late season, ostensibly largely due to predation by *D. brevis*. Other predators included *Anthocoris* spp., spiders, coccinellids, lacewings, *Orius* spp., earwigs and also parasitoids; although apart from spiders in 2004 these groups comprised <1 per beat tray sample.

Table 5

<table>
<thead>
<tr>
<th>Spray program</th>
<th>Number PRM/10 leaves ± SEM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal leaf</td>
<td>Spur leaf</td>
</tr>
<tr>
<td>CPGV/spinosad</td>
<td>90.1 ± 22.6</td>
</tr>
<tr>
<td>Spinosad/CpGV</td>
<td>118.0 ± 17.0</td>
</tr>
</tbody>
</table>

Pears were mite (PRM), *Epitrimerus pyri*, was evaluated on Green Anjou (Hood River).

a Applied in 0.4 ha blocks replicated twice.

b 100 leaves/block sampled; means in column not significantly different (*P* > 0.05, 1-way ANOVA).
4. Discussion

4.1. Efficacy of CpGV and spinosad

We noted that CpGV and spinosad controlled codling moth, both in experimental blocks (Table 1) and in commercial orchards faced with economically damaging infestations (Tables 3 and 4). Moth catch data from Hood River suggested two or more years of treatments may be required to bring populations under control. However, increased fruit injury was observed in CpGV-treated plots compared with spinosad (Table 1). This difference is explained by different modes of action. CpGV has no contact activity and must be ingested. The virions (virus DNA) pass through the mid-gut peritrophic membrane, invade and multiply in body tissues including the tracheal matrix and fat body (Federici, 1997). Death occurs in 5–10 days, allowing infected larvae to cause shallow entries or ‘stings’ in fruit. Most orchardists have a low tolerance for damage (typically 1%), although fruit with superficial damage may be suitable for processing for juice or canning. However, CpGV may also cause delayed development or sub-lethal effects (Fig. 2), suggesting CpGV may have a subtle and longer term impact on pest populations. Biache et al. (1998) reported a 25% reduction in adult emergence among codling moth larvae surviving treatment with Carpovirusine. Spinosad is a neurotoxin that demonstrates rapid contact and ingestion activity in insects, causing excitation of the nervous system, leading to cessation of feeding and paralysis (Salgado, 1998). Symptoms of poisoning are consistent with activation of nicotinic acetylcholine receptors, but spined high mortality at the field rate of spinosad for several orchard predators, Anthocoris spp. and parasitoids, although the relative abundance of these groups may have been confounded by the reduced prey (psylla) in spinosad-treated blocks. On the other hand, the small plots might have allowed immigration and the deleterious impact of spinosad treatments (especially for more mobile parasitoids) could be more pronounced in a large orchard. Spinosad also had activity against psylla, thrips and non-target dipterans (Figs. 3 and 5).

Our field data support a series of laboratory bioassays that documented no acute toxicity of a field rate of spinosad for several orchard predators, Chrysoperla carnea, Anthocoris nenorialis F., Galendromus (= Typhlodromus) occidentalis Nesbitt (western predatory mite) and D. brevis (Unruh et al., 2006). However, the field rate of spinosad (and several other nominally more selective new insecticides) caused reduced fecundity in all species. Other sub-lethal effects included reduced egg hatch (C. carnea and D. brevis) and reduced female longevity and offspring survival (A. nemoralis) (Unruh et al., 2006). Two species of parasitoids, including the codling moth larval parasitoid Mastrus ridibundus Gravenhorst, and the European earwig F. auricularia L. also suffered high mortality at the field rate, while both parasitoids also suffered sub-lethal effects at a reduced (10%) rate (Unruh et al., 2006).

A literature survey by Williams et al. (2003) concluded that hymenopteran parasitoids are significantly more susceptible to spinosad than predatory insects. Using the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) criteria for both laboratory and field tests that run from one (harmless) to four (harmful) (Hassan, 1992; Sterk et al., 1999), overall, 71% (42/59) of laboratory studies and 79% (81/103) of field-type studies on predators (27 species) gave a class 1 result with spinosad. Coccinellid species, the antihoroid Orius insidiosus Say, the hemipteran Geocoris punctipes Say and the chrysopids Chrysoperla rufilabris Burmeister and C. carnea Stephens were considered particularly tolerant (Williams et al., 2003). The earwig, Doru taeniatus Dohrn (Cisneros et al., 2002) and some syrphid flies (Michaud, 2003; Torres et al., 1999; Vinuela et al., 2001) were susceptible to field rates of spinosad in laboratory tests. By contrast spinosad showed direct toxicity and sublethal effects (e.g. loss of reproductive capacity) to a wide range of parasitoids (25 species), with 78% (35/45) of laboratory studies and 86% (18/21) of field-type studies returning a moderately harmful or harmful result (Williams et al., 2003).

Despite any harmful effect on parasitoids, we found no evidence that spinosad disrupted natural control leading to outbreaks of secondary pests. High populations of phytophagous mites, predominantly E. pyri, were observed both in small experimental (Moxee) and larger orchard blocks (Hood River), but not exacerbated by spinosad in either case (Tables 2 and 5). Regionally, predatory mites,
chiefly *Typhlodromus* spp., provide effective control of *E. pyri* (Beers et al., 1993) and *Typhlodromus caudiglans*. Schuster was noted from spinosad plots during mite counting. Previous field studies reported operational rates of spinosad had no effect on predatory phytoseiid mites, including *T. pyri* (Miles and Dutton, 2003), although spinosad was reported harmful to adult *Neoseiulus fallacis* Garman (Villanueva and Walgenbach, 2005). However, caution in using spinosad is prudent in commercial orchards where few or no alternative predators are present. As noted earlier, Van Steenwyk et al. (2005) and Van Steenwyk and Nomoto (2006) documented increased numbers of aphids and mites in orchard tests following spinosad use.

5. Conclusions

*CpGV* and spinosad offer new options to manage codling moth in organic or ‘soft’ orchards, although their short residual activity may limit adoption in conventional insecticide programs. The limited protection to fruit also makes *CpGV* less effective under high pest pressure. Our data suggest both products are compatible with integrated pest management, although the use of spinosad should be evaluated carefully during critical periods (e.g. bee pollination) or where conservation of parasitoids is of prime concern. Application timing may help mitigate most negative impacts on beneficials. Nevertheless, because two years is not a long period to detect changes in species abundance or diversity, monitoring and reporting of secondary pest outbreaks would provide a useful method to identify unintended non-target impacts of spinosad applications.

Although spinosad and *CpGV* are useful tools for control of codling moth populations resistant to other pesticides, there is the possibility for development of resistance to both of these agents. Increased tolerance to spinosad has recently been documented among species of leafroller (M. Doerr, pers. comm.) as well as thrips and other populations of Lepidoptera after relatively short term use (Sayyed et al., 2004; Herron and James, 2005). Fritsch et al. (2005) and Sauphanor et al. (2006) also reported development of resistance to *CpGV* in Germany and France in codling moth populations that have received regular virus applications for several years. The alternation of spinosad with *CpGV* and other soft insecticides with different modes of action, and compliance with restricted use policies for spinosad (resistance management) will help maintain the effectiveness of both these products. As pear and apple growers in the United States Pacific Northwest-region adopt more selective interventions for major arthropod pests, including mating disruption for codling moth, there has been an increase in densities of natural enemies in orchards (Gut and Brunner, 1998; Knight et al., 1997; Miliczky et al., 2000). Increased natural enemy density may improve biological control (Knight et al., 1997). The inclusion of both *CpGV* and spinosad warrants consideration in future IPM strategies in tree fruit.

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