Persistence of a commercial codling moth granulovirus product on apple fruit and foliage

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ABSTRACT

Codling moth, *Cydia pomonella* (L.), larval bioassays were carried out on apples and leaves collected from trees treated with the commercially available codling moth granulovirus, Virosoft CP4®, to estimate the persistence of the product over time. The virus had a significant effect on survival of laboratory derived codling moth larvae placed on apples collected up to five and eight days post-treatment. Larvae died with virus symptoms after feeding on treated foliage and the leaf bioassay was easier to count than the apple bioassay. A combination assay, exposing larvae to leaf discs and fruit may more accurately account for potential exposure of wild neonate codling moth to virus in treated orchards. The addition of fish, soybean or mineral oils to Virosoft CP4® treatments did not significantly increase the efficacy or persistence of the viral insecticide on apples in this study.

Key Words: Virosoft CP4®, leaf discs, *Cydia pomonella*

INTRODUCTION

The codling moth granulovirus (CpGV) (Baculoviridae) is found in wild and colonized codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae) (Tanada 1964; Eastwell *et al.* 1999) which is a major pest of apples and pears throughout most of the temperate world (Cross *et al.* 1999). CpGV is noted for its high virulence when ingested by this host, particularly in the neonate stage (Sheppard and Stairs 1976; Tanada and Leutenegger 1968). Commercial formulations of the virus have been registered for use against the codling moth in Europe since 1988 and in the U.S.A. since 1995. In 2000, Virosoft CP4®, produced by BioTepp Inc., Quebec, became registered for use on apples and pears in Canada.

Commercial formulations of CpGV require application of aqueous suspensions of the virus onto the apples and foliage of treated trees. There is a relatively short time when a wild codling moth can be effectively exposed to CpGV treatment in an orchard. Most wild codling moth eggs are oviposited on leaves (Jackson 1979). Neonates move over leaf surfaces before finding a fruit and chewing through the surface where they remain, feed and develop through to the last instar. Ballard *et al.* (2000) found that CpGV was ingested by codling moth neonates browsing on CpGV treated leaf surfaces, therefore there is the potential for codling moth neonates to encounter and ingest lethal levels of CpGV on both treated foliage and fruit surfaces.

Orchard trials of various commercial preparations of CpGV have generally shown good early suppression of neonate codling moth (Glen and Payne 1984; Jaques *et al*. 1987). However, like other viral insecticides, the CpGV is susceptible to inactivation and dilution due to temperature, exposure to sunlight, and precipitation (Jaques 1975, 1985; Glen and Payne 1984). CpGV has been found to be 50% inactivated in 2-8 d on apples (Glen and Payne 1984; Jaques *et al.* 1987; Arthurs and Lacey 2004).

The goal of this study was to determine

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the persistence of Virosoft CP4® on apples and foliage under the orchard conditions in the interior of British Columbia (BC), Canada. We also evaluated whether various oils, added to the spray mixture, could increase or extend the duration of the Virosoft CP4®'s efficacy under orchard conditions, by possibly improving coverage and increasing the penetration of the virus through the leaf surface.

MATERIALS AND METHODS

All treatments were applied to a high density (1m tree spacing within rows) orchard of MacIntosh apple trees at the Pacific Agri-Food Research Centre, Summerland, BC.

Virulence of Virosoft CP4 over time on apples and leaves. Two blocks of five rows of high density apple trees were treated with Virosoft CP4® at 239 ml/ha (original preparation: $4x10^{13}$ occlusion bodies/946.34 ml) on 12 June, 2003 using an air-blast sprayer set to deliver a volume of 2,347.6 L/ha. The Virosoft CP4® was stored refrigerated. An untreated block of trees separated by $\geq$ 30 trees in the same orchard was used as the control. Two hours after application of the virus, 10 leaves and apples were randomly collected from the treatment and the control. Similar collections were made 1, 4, 6, 8, and 12 days after application of the treatments. The entire trial was replicated on different blocks of trees within the same orchard on 3 July, 2003. Mean ± SE daily temperatures during the orchard collection (12-24 June:17.6 ± 3.0 °C; 3-15 July: 22.5 ± 2.3 °C) and the accumulated daily rain that occurred during the June (0.6 ± 1.1 mm/d) and July replications (0 ± 0 mm/d) were similar to daily temperatures (18.8 ± 4.1 °C) and precipitation (0.7 ± 2.3 mm/day) for this area, from 1 June to 15 July, averaged over a 5 y period (1999 - 2004) (Anon. 2004).

Codling moth neonates used in the assays emerged on the same day that the leaves and apples were collected, from egg sheets obtained from the colony of the Okanagan-Kootenay Sterile Insect Release Program (Osoyoos, BC). Five neonate codling moth were placed on the stem end of each collected apple. The apples were sealed in plastic cups and incubated at 24 °C, 16:8 h L:D before codling moth survival was assessed by cutting open the fruit on days 7 and 14. The total number of live larvae in the apple bioassays was a more accurate assessment of the impact of the virus than percentage mortality as larvae occasionally could not be found. Some neonates naturally move off the apple and die by dessication without feeding in apple assays (Laing and Jaques 1980); others disintegrate beyond recognition due to a viral infection or are difficult to locate.

One disc (1 cm diameter) was cut from each leaf, avoiding the center vein, within an hour of their collection and placed on a layer of stikem (Stikem Special™, Phero-Tech Inc., Delta, BC) in a small plastic petri dish. Ten codling moth neonates were placed on each leaf disc. The discs, with the larvae, were placed in the dark for 60 minutes at 22 ± 1 °C to encourage feeding. Larvae that had consumed leaf material frequently had green coloured alimentary canals, green frass and a portion of the leaf surface was scarred. Five larvae which showed evidence of feeding per leaf were transferred to the surface of a pinto-bean based diet (modified from Shorey and Hale 1965) within individual 30-ml plastic cups. The cups were sealed and incubated at 24 °C, 16:8 h L:D until the number of living larvae was assessed on days 7 and 14. Data were analysed using an ANOVA (SAS 2000) and the means were separated within each day the apples or leaves were collected with individual ANOVAs and Tukey's studentized range test.

Virosoft CP4 + oils. In a separate experiment, four blocks of four rows of high density apple trees were each treated with Virosoft CP4® alone at 239 ml/ha (original preparation: $4x10^{13}$ occlusion bodies/946.34 ml), Virosoft CP4® 239 ml/

ha + mineral oil (Superior 70 oil, United Agri Products, Dorchester, ON) at 2L/ha, Virosoft CP4® 239 ml/ha + fish oil (Crocker's Fish Oil, Inc., Quincy, WA) at 2L /ha or Virosoft CP4® 239 ml/ha + once descummed soybean oil at 2.5 L/ha, 17 June, 2004 using an air-blast sprayer set to deliver a volume of 1,553.6 L/ha. A block of untreated trees separated by >10 trees in the same orchard was used as the control. Ten apples were randomly collected from each treatment and the control at least two hours after application of treatments on day 0, as well as 1, 2, 5, 7, and 10 days post-treatment. The entire trial was replicated on 28 June 2004 on different blocks of trees within the same orchard. Mean ± SE daily temperatures during the orchard collection (17-27 June 2: 22.4 ± 2.5 °C; 28 June to 8 July: 17.3 ± 3.4 °C) and the accumulated rain that occurred during the first (2.1 ± 5.5 mm/day) and second replication (0.8 ± 1.3 mm/day) are similar to mean daily temperatures and precipitation for this area, averaged over 5 y, as recorded above. Bioassays were carried out using codling moth neonates on the collected apples as described above. The number of living larvae were counted seven days post exposure. Data were analysed as described for the previous study.

RESULTS AND DISCUSSION

Efficacy of Virosoft CP4 over time on apples and leaves. The Virosoft CP4® had an overall significant impact on the number of codling moth able to survive the virus treatment, compared to control larvae, when the data were collected both 7 and 14 days post-exposure to field collected apples (7 d: $F_{1, 226} = 77.6$, $P < 0.0001$; 14 d: $F_{1, 226} = 66.7$, $P < 0.0001$). Significantly fewer codling moth larvae survived for seven days in apples from trees treated with the virus, compared to untreated apples, two hours, and 1, 4, and 8 days post-treatment with Virosoft CP4® (Table 1). This significant difference between the number of living larvae in the treated versus control apples was extended to 12 days post-treatment when the data were read on day 14, except in apples collected four days post-treatment (Table 1). The number of live codling moth larvae found both in untreated and treated apples was generally lower over all days, when the assays were read at 14 versus 7 days, due to the difficulty locating larvae in the fruit at the later date. The apples had to be carefully sectioned to find the larvae and long term larval mortality data may have been influenced by decay in the fruit. The disappearance and death of a small number of larvae may be partially due to mortality caused by a colony derived CpGV infection and subsequent disintegration of the dead larvae (personal observation). As this mortality would occur in both treated and control apples any differences in surviving codling moth would be attributable to the treatment. Some additional virus-induced death would be expected to occur between days 7 and 14, however, the poor survivorship of larvae in decaying control apples decreases the value of data obtained at this later date.

Codling moth larvae died after consuming CpGV in leaf disc assays and Virosoft CP4® had a significant impact on the number of codling moth larvae able to survive the virus treatment, compared to control larvae (7 d: $F_{1, 226} = 12.6$, $P < 0.0005$; 14 d: $F_{1, 226} = 7.5$, $P < 0.0007$). Significantly fewer living codling moth larvae were found when larvae fed on leaf discs collected two hours and one day post-treatment from Virosoft CP4® treated trees, compared to discs from untreated leaves (Table 1). Similar results were found when the number of surviving larvae were counted 7 and 14 days post-exposure. The leaf disc bioassay was easier to count than the apple bioassay as more larvae were found in the former.

The difference in the active persistence of the virus determined in the apple and leaf disc bioassays is probably due to neo-

Table 1.
Mean ± SE number of live codling moth larvae found per apple or leaf disc after 7 and 14 days when neonates were placed on Virosoft CP4® treated or control apples or leaf discs. Replicated twice; n = 10 apples or leaves; 5 codling moth larvae per apple and leaf disc.

Days post-treatment	Mean ± SE number of live codling moth larvae per apple and leaf disc			
	Read 7 days post-exposure		Read 14 days post-exposure	
	Virosoft CP4®	Control	Virosoft CP4®	Control
	Apples			
0 (2 h)	0.9 ± 0.2 a [1]	2.4 ± 0.3 b	0.2 ± 0.1 a	1.6 ± 0.3 b
1	0.7 ± 0.2 a	3.1 ± 0.3 b	0.8 ± 0.3 a	1.7 ± 0.3 b
4	1.6 ± 0.2 a	3.1 ± 0.3 b	1.1 ± 0.3 a	1.9 ± 0.4 a
6	2.6 ± 0.3 a	3.2 ± 0.2 a	0.9 ± 0.4 a	3.0 ± 0.3 b
8	2.5 ± 0.2 a	3.3 ± 0.2 b	1.2 ± 0.2 a	2.5 ± 0.2 b
12	2.8 ± 0.3 a	3.4 ± 0.3 a	2.1 ± 0.3 a	3.0 ± 0.3 b
	Leaf discs			
0 (2 h)	2.9 ± 0.3 a	4.3 ± 0.2 b	2.2 ± 0.3 a	3.6 ± 0.2 b
1	4.2 ± 0.2 a	4.8 ± 0.1 b	3.5 ± 0.3 a	4.2 ± 0.2 b
4	4.3 ± 0.2 a	4.6 ± 0.1 a	3.4 ± 0.3 a	4.0 ± 0.2 a
6	4.8 ± 0.1 a	4.8 ± 0.1 a	4.1 ± 0.2 a	3.9 ± 0.2 a
8	4.5 ± 0.2 a	4.5 ± 0.2 a	4.1 ± 0.2 a	3.9 ± 0.3 a
12	4.6 ± 0.2 a	4.6 ± 0.2 a	4.1 ± 0.2 a	3.9 ± 0.2 a

[1] Means within rows and days post-exposure followed by the same letter are not significantly different ($P > 0.05$), determined with Tukey's studentized range test (SAS 2000).

nates feeding more readily and extensively on the surface of the apple than on the foliage. Glen and Clark (1985) found that 90% of codling moth neonates hatching from eggs on leaves spent more than 10 min on leaves before moving to the fruit. Penetration of fruit by codling moth has been recorded to take 1 to 2.5 h (Geier 1963). As the neonates in our study were held for 60 minutes on a leaf disc before transferring them to the diet, each neonate had a realistic, but limited chance to ingest the virus. Ballard *et al.* (2000) did not observe feeding by codling moth neonates on leaf tissue until neonates were left for 15 minutes. It is also possible that more virus is accumulated near the stem and the calyx ends of apples (Arthurs and Lacey 2004) and many of the codling moth neonates choose these areas of the apple to feed and then penetrate. Most wild codling moth would encounter CpGV on both the leaf and apple surfaces, therefore it would be appropriate to improve these assays by exposing neonates to both treated surfaces to obtain a more realistic assessment of potential mortality in the orchard.

Virosoft CP4 + oils. The virus treatments had a significant effect on survival of codling moth larvae ($F_{4, 25} = 32.2$, $P < 0.001$). Significantly fewer living codling moth larvae were found in apples collected two hours, one day, and five days post-treatment from trees treated with Virosoft CP4® alone, or in combination with any of the oils, than in the control apples. In apples collected two, seven and ten days post-treatment, this difference was not significant (Table 2). Although the mean numbers of live codling moth larvae per apple in Virosoft CP4® treatments in combination with an oil was lower on days 0 and 1, the numbers were not significantly lower than those in the Virosoft CP4® treatment alone.

The virulence of the Virosoft CP4® applied in June and early July, under conditions that are typical for the southern

Table 2.
Mean ± SE number of live codling moth larvae per apple after 7 days when neonates were placed on fruit treated with Virosoft CP4® combined with one of three oils, or nothing. Replicated twice; n = 10 apples; 5 codling moth larvae per apple.

Days post-treatment	Mean ± SE number of live codling moth larvae per apple per treatment				
	Virosoft CP4®	Virosoft CP4® + Superior 70 oil	Virosoft CP4® + Fish oil	Virosoft CP4® + Soybean oil	Control
0 (2 h)	1.1 ± 0.4 a [1]	0.6 ± 0.1 a	0.3 ± 0.2 a	0.3 ± 0.2 a	3.9 ± 0.5 b
1	1.2 ± 0.3 a	0.7 ± 0.1 a	1.1 ± 0.3 a	0.7 ± 0.2 a	3.7 ± 0.5 b
2	1.8 ± 0.5 a	1.8 ± 0.5 a	1.1 ± 0.1 a	1.6 ± 1.0 a	3.5 ± 0.1 a
5	2.0 ± 0.7 a	1.6 ± 0 a	2.0 ± 0.2 a	1.9 ± 0.2 a	3.9 ± 0.4 b
7	2.4 ± 0.7 a	1.9 ± 0.5 a	2.2 ± 0.7 a	2.0 ± 0.1 a	3.1 ± 0.9 a
10	2.0 ± 0.2 a	1.9 ± 0 a	2.3 ± 0.4 a	2.0 ± 0.5 a	3.1 ± 0.4 a

[1] Means within rows followed by the same letter are not significantly different (P > 0.05) as determined with Tukey's studentized range test (SAS, 2000).

interior of BC, decreased quickly over time in both trials. In orchard trials in Washington State, Arthurs and Lacey (2000) found a similar decline in activity and estimated a Virosoft CP4® (3.2 oz/ac) half-life on apples to be 3.8 to 8.7 d post June and July treatments, respectively. It is important to recognize that our study was carried out using colony derived larvae and that wild codling moth larvae may feed on treated apple tree leaves and fruit differently under orchard conditions, which may modify the impact of the virus on the host.

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REFERENCES

Anon. 2004. PARC Summerland weather. Http://res2.agr.ca/parc-crapac/summerland/ clim/index_e.htm.

Arthurs, S.P. and L.A. Lacey. 2004. Field evaluation of commercial formulations of the codling moth granulovirus: persistence of activity and success of seasonal applications against natural infestations of codling moth in Pacific Northwest apple orchards. Biological Control 31: 388-397.

Ballard, J., D.J. Ellis and C.C. Payne. 2000. Uptake of granulovirus from the surface of apples and leaves by first instar larvae of the codling moth *Cydia pomonella* L. (Lepidoptera: Olethreutidae). Biocontrol Science and Technology 10: 617-625.

Cross, J.V., M.G. Solomon, D. Chandler, P.J. Jarrett, P.N. Richardson, D. Winstanley, H. Bathon, J. Huber, B. Keller, G.A. Langenbruch and G. Zimmermann. 1999. Review of biocontrol of pests of apples and pears in northern and central Europe: 1. Microbial agents and nematodes. Biocontrol Science and Technology 9: 125-149.

Eastwell, K.C., J.E. Cossentine and M.G. Bernardy. 1999. Characterization of *Cydia pomonella* granulovirus from codling moths in a laboratory colony and in orchards in British Columbia. Annals of Applied Biology 134: 285-291.

Geier, P.W. 1963. The history of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in the Australian Capital Territory. Australian Journal of Zoology 11: 323-367.

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. Entomologia Experimentalis et Applicata 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. Annals of Applied Biology 104: 87-98.

Jackson, D.M. 1979. Codling moth egg distribution on unmanaged apple trees. Annals of the Entomologi-

cal Society of America 72: 361-368.
Jaques, R.P. 1975. Persistence, accumulation and denaturation of nuclear polyhedrosis and granulosis viruses, pp. 90-99. *In* M.D. Summers, R. Engler., L.A. Falcon, and P. Vail (Eds), Baculoviruses for Insect Pest Control: Safety Considerations. American Society for Microbiology, Washington DC.
Jaques, R.P. 1985. Stability of insect viruses in the environment, pp. 285-360. *In* K. Maramorosch and K.E. Sherman (Eds) Viral Insecticides for Biological Control. Academic Press, New York.
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. The Canadian Entomologist 119: 1063-1067.
Laing, D.R. and R.P. Jaques. 1980. Codling moth: techniques for rearing larvae and bioassaying granulosis virus. Journal of Economic Entomology 73: 851-853.
SAS 2000. Proc GLM Users Manual Version 8.1. SAS Institute, Cary, NC.
Sheppard, H. H. and G.R. Stairs. 1976. Effects of dissemination of low dosage levels of a granulosis virus in populations of the codling moth. Journal of Economic Entomology 69: 583-586.
Shorey, H.H. and R.L. Hale. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. Journal of Economic Entomology 58: 522-524.
Tanada, Y. 1964. A granulosis virus of codling moth, *Carpocapsa pomonella* (Linnaeus) (Olethreutidae, Lepidoptera). Journal of Invertebrate Pathology 6: 378-380.
Tanada, Y. and R. Leutenegger. 1968. Histopathology of a granulosis-virus disease of the codling moth, *Carpocapsa pomonella*. Journal of Invertebrate Pathology 10: 39-47.