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Incidence and transmission of a granulovirus in a large codling moth [*Cydia pomonella* L. (Lepidoptera: Tortricidae)] rearing facility

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Abstract

Incidences of potential per os *Cydia pomonella* granulovirus (CpGV) transmission within a large codling moth colony were identified. CpGV was detected in the water which is used to wash egg sheets. When pre-neonates were extracted from eggs prior to emergence and tested for the presence of CpGV, 40% were found to carry amounts of CpGV detectable by a polymerase chain reaction (PCR) assay, suggesting possible transovarial transmission of the virus. Although symptoms typical of virus infection and larval death were found infrequently within communal rearing trays, the frequency with which CpGV DNA was detected by PCR assays increased from a mean of 31% of 10-day-old larvae to 94% of 25-day-old larvae. CpGV in codling moth cadavers remained virulent after being held at 60 °C for 3 days under conditions similar to the treatment of spent diet at the rearing facility before its disposal. PCR tests of surface samples taken from air filters and rearing rooms of the rearing facility were found to contain CpGV. Bioassays of surface samples from the diet trash bin and a filter through which outside air is passed before entering the rearing chambers resulted in significant codling moth neonate mortality. The virulence of CpGV in dust from the spent diet and the original inadvertent positioning of the diet trash bin directly below one of the air intake ducts are suggested as a possible additional source of CpGV contamination within the facility.
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Keywords: Baculoviridae; *Cydia pomonella*; Polymerase chain reaction; Transovarial transmission; Latent infection

1. Introduction

During periods of peak production, the Okanagan-Kootenay Sterile Insect Release Program (hereafter referred to as SIR) in Osoyoos, British Columbia (BC), Canada produces approximately 15 million codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) per week (personal communication, S. Wood, SIR). Sheets of codling moth eggs are placed egg side down above open trays of fresh moist sawdust-based diet (Brinton et al., 1969). Neonates spin down and enter the diet where they feed communally. After 21 days, when the majority of the larvae are in their last instar, the trays are moved to an emergence room where the codling moth pupate near the surface of the then-hardened diet. Adults are collected and sterilized through γ-irradiation before release into orchards as part of an area-wide codling moth suppression program (Dyck and Gardiner, 1992; Proverbs et al., 1982). Trays of spent diet are moved to a room held at 60 °C for 3 days to kill any remaining larvae before disposal of the diet.

Codling moth are highly susceptible to the codling moth granulovirus (CpGV) which, in per os bioassays, causes high mortality at very low doses (e.g., LD_{50} 3–17 granules/neonate) (Falcon, 1971; Laing and Jaques, 1980). There is evidence that the virus also exists in colonized and wild codling moth populations in a less pathogenic or latent form, and healthy-appearing late instars and adults have been found to contain the virus (Eastwell et al., 1999; Etzel and Falcon, 1976). The codling moth colony at the SIR facility is contaminated with CpGV (Cossentine, personal

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observation) although generally few dead codling moth larvae exhibiting symptoms indicative of viral infection are found in diet trays. To suppress virus in the SIR colony, formaldehyde is included in the diet (Brinton et al., 1969) and general hygiene is carefully exercised in the facility. CpGV has been found associated with moth scales on the surface of colony derived codling moth eggs (Etzel and Falcon, 1976) and both vertical transovum and transovarial transmission of the virus have been hypothesized as means for CpGV transmission (Etzel and Falcon, 1976).

The mechanism by which CpGV is transmitted is of intense interest to rearing programs that rely on the production of large numbers of healthy insects. As a prelude to identifying possible means of suppressing CpGV transmission within the colony we also sought to determine: if virulent virus was being distributed over egg sheets in the egg sheet washing process used in the facility; if emerging neonates are infected; whether the incidence of CpGV in larvae feeding communally in diet trays increases over time resident in the trays; and whether the virus remaining in the used diet remains virulent post heat treatment and is possibly re-entering the facility via the dust generated from the used diet.

2. Materials and methods

2.1. Virus associated with egg sheets—CpGV in egg wash water

In the egg sheet washing procedure used at the SIR rearing facility, each egg sheet (33×137 cm) is cut into six pieces; 81 pieces are placed in a screen holder (book) which allows water to reach both sides of each sheet. Three books of egg sheets are washed together in each of three tubs (90.2 cm long $\times$ 43.2 cm wide $\times$ 38.1 cm deep). The books are washed for 5 min in tap water, 5 min in a 0.16% sodium hypochlorite (NaClO) solution (12% Zep-Bleach Industrial Strength, Manufacturing Co. of Canada, Edmonton), and for 5 min in another basin of tap water. The water in all tubs, with or without NaClO, is changed after every nine books (or three washes).

Sterile-tipped pipetters were used to sample egg sheet wash water during a period of high production in May 2003. Two samples of water, taken from the surface of the first and the last water basin before the first book, and after each set of three books, were placed in sterile microcentrifuge tubes. The study was repeated three times with three different refills of the wash basins. Each sample was tested four times for the presence of the CpGV with a PCR technique (Eastwell et al., 1999). Tap water was used as the control. Briefly, 1 μl of sample was used as a template for PCR with *Taq* polymerase (AmpliTaq: Perkin-Elmer) using primers 'GranL' and 'GranR' to amplify the 207 bp fragment. Reaction conditions consisted of 94 °C for 2 min followed by 35 cycles of: 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s. Followed by 72 °C for 2 min. Reaction products were analyzed by agarose gel electrophoresis. Then 1.5 μl of PCR product was used for the nested PCR with *Taq* polymerase using primers 'sCpGVL' and 'sCpGVR' to amplify. Reaction conditions were the same except 30 cycles were done instead of 35.

To test the virulence of any virus in the sample, 0.2-ml of each sample was placed on the flat surface of pinto-bean based diet (Shorey and Hale, 1965) in each of five, 30 ml plastic cups. The suspension was swirled to cover the entire surface and allowed to dry before five neonate codling moth larvae (SIR, Osoyoos, BC) were placed on the surface of the treated diet in each cup. Sterile distilled water was used as a control. The cups were sealed and incubated at 25 °C and mortality read 7 days later. Since small larvae killed by CpGV frequently disintegrate and are difficult to find in diet, missing larvae in the bioassays were considered dead. The percent of positive PCR tests and mortality was transformed with an arcsine transformation before analyzing the data (ANOVA: SAS, 2000).

2.2. CpGV in developing larvae

Five codling moth egg sheets were obtained from the SIR facility over a period of high production from June to August 2002. For each replication, a single unwashed sheet was cut into eight equal pieces. Two pieces were washed similar to the protocol used at the SIR rearing facility (suspended in and gently manually agitated several times for 5 min in tap water, 5 min in 0.16% NaClO, and 5 min in tap water, each in separate $33.5 \times 29 \times 13.5$ cm plastic tubs) then air-dried The washed egg sheets, as well as two untreated pieces, were placed in separate clean plastic bags containing damp cotton wicks and held at 25 °C until pre-neonate head capsules were visible through the surface of the eggs. Eight (replications 1 and 2) and 17 (replications 3 through 5) pre-emerged codling moth were aseptically extracted from the eggs using insect pins under a microscope and placed in individual sterilized tubes. The remaining eggs were allowed to develop until neonates emerged and a similar number of randomly selected emerged larvae per treatment were placed in individual sterile microfuge tubes. All larvae were tested for the presence of the CpGV using PCR (Eastwell et al., 1999). Each larva was homogenized in 40 μl of 0.1% Triton X-100. To remove the granulin, 10 μl of 0.4 N NaOH was added and the sample heated in a boiling water bath for 6 min, then rapidly chilled on ice. For pre-neonates 20 μl of 0.1% Triton X-100 and 5 μl of 0.4 N NaOH were used. The pH of the resulting suspension was neutralized by the addition of 10 μl of 1 M Tris–HCl, pH 7.6. One microliter was used for PCR as described above. The percentages of larvae yielding positive test results were transformed using an arcsin transformation before data were analyzed (ANOVA: SAS, 2000).

The codling moth larvae reared at the SIR facility develop in trays of diet that are positioned in vertical racks that each holds 3 columns of 25 trays. Seventeen codling moth larvae were aseptically removed from a tray located at the top of a rack containing 10-day-old larvae. Each

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