PATHOGENICITY OF A GRANULOVIRUS TOWARDS *CHORISTONEURA FUMIFERANA* (LEPIDOPTERA: TORTRICIDAE)

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The spruce budworm *Choristoneura fumiferana* (Clem.) is the most damaging insect of the balsam fir *Abies balsema* (L.) Mill. (Pinaceae) and the white spruce *Picea glauca* (Moench) Voss (Pinaceae) throughout eastern North America. In outbreak conditions, close to 100% tree mortality can occur in untreated mature fir stands (MacLean 1980). *Bacillus thuringiensis* var. *kurstaki* (Bacillaceae) is currently used to reduce spruce budworm damage (Van Frankenhuyzen and Payne 1993). Other possible biological control agents, such as baculoviruses, are also investigated to complement the use of *B. thuringiensis*. Baculoviruses are advantageous because they occur naturally in several insect species and are generally host specific (Federici 1993).

The granulovirus of *C. fumiferana* (*Chfu*GV) is a baculovirus that was first isolated from the spruce budworm (Bird 1959). Knowledge about this baculovirus is still limited and a better understanding of its biological properties, such as viral dose–response of the insect, is needed to evaluate its insecticidal potential. This study reports the pathogenicity of *Chfu*GV by measuring its LD_{50} in *C. fumiferana* larvae.

The *Chfu*GV used in this study was isolated from moribund larvae collected in 1992 from the Gaspé Peninsula within a 50 km radius of Bonaventure (48°15′31′′N, 66°40′29′′E), Québec. Three consecutive *in vivo* passages were done in September of 1996 to produce a sufficient amount of virus for purification and the experiment. The purification technique used was modified from Tompkins (1991). The only change brought to the purification protocol was an additional centrifugation at 300 *g* for 5 min to eliminate the bulk of the insect material after maceration. The concentration of the virus was evaluated with an electron microscope, by comparing the number of viral granules and small latex beads of a known concentration (Agar Scientific, S130-3) that were added to a sample of the viral suspension. Observations confirmed that a stock suspension of pure *Chfu*GV at a concentration of 7.45×10^{11} granules/mL was obtained, free of insect material and other viruses. The virus was stored at 4°C.

Pathogenicity of the *Chfu*GV was approximated in preliminary bioassays, and determined more precisely using a modified diet plug bioassay technique (Kaupp and Ebling 1990; Ebling and Kaupp 1997). Freshly moulted (less than 24 h) healthy fourth-instar larvae were placed in empty Petri dishes and starved for 24 h. They were transferred into 1.9-mL microcentrifuge tubes along with a 3-µg diet plug (McMorran 1965) covered with 2 µL of viral dilutions prepared from the original stock suspension (doses of 1×10^9, 1×10^7, 1×10^5, and 1×10^3 granules/larvae). Three replicates of 60 larvae, for a total of 180 larvae, were used for each viral dilution as well as for the control group, the later receiving 2 µL of distilled water instead of a viral suspension. The diet plug was placed in the lid of the microcentrifuge tube, as budworm larvae are positively photosensitive (Comptois 1988) and also have a tendency to move upwards when

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TABLE 1. Lethal dosages of granulovirus (*Chfu*GV) to fourth-instar (<24 h old) *Choristoneura fumiferana* larvae.

		95% confidence limits	
	Virus granules	Lower	Upper
LD_{10}	6.9×10^2	7.1×10^1	3.4×10^3
LD_{50}	5.7×10^5	2.0×10^5	1.4×10^6
LD_{90}	4.8×10^8	1.6×10^8	2.0×10^9

placed in these tubes. An average ± SD of 70.2 ± 8.0% of the larvae consumed the entire plug within 24 h and were supplied with virus-free artificial diet in the microcentrifuge tube for 12 days. When the additional diet was given, the lid of the tubes was pierced to allow for air exchange and evaporation of excessive moisture from the diet. The diet was changed once, 6 days postinfection.

Data were collected 12 d postinfection, before the appearance of pupae. Three lethal doses (LD_{10}, LD_{50}, LD_{90}) and their confidence limits were calculated using probit analysis (LeOra Software 1994) (Table 1). The observed LD_{50} was 5.7×10^5 granules/larva with 95% confidence intervals for this dose ranging from 2.0×10^5 to 1.4×10^6.

In an effort to diversify budworm management options, the granulovirus should be considered. Similar bioassays conducted with the more commonly used baculovirus, the nucleopolyhedrovirus of *C. fumiferana* (*Cf*MNPV), showed a LD_{50} of 1.1×10^3 polyhedra-inclusion bodies (PIB) for fourth instar budworms (Kaupp and Ebling 1990). This value is significantly lower than the 5.7×10^5 occlusion bodies required to give an LD_{50} with *Chfu*GV. However, differences in viral morphology may explain the divergent results; granuloviruses are characterized by the presence of only one infectious particle per occlusion body, whereas one nucleopolyhedrovirus PIB can commonly have more than a 100 (Adams and McClintock 1991). Therefore, if the LD_{50} values are compared using infectious particles as a measurement unit, both viruses have comparable pathogenic properties. *Chfu*GV is easily produced *in vivo*, and preliminary field results are encouraging (Guertin and Cabana 1998), making the virus an excellent candidate as a biological control agent against the spruce budworm. This study also provides a reference point for granuloviruses of *C. fumiferana,* to which laboratory experiments with other granulovirus strains can be compared.

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