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Host range extension of *Cydia pomonella* granulovirus: adaptation to Oriental Fruit Moth, *Grapholita molesta*

Benoit Graillot · Christine Blachère-López · Samantha Besse ·
Myriam Siegwart · Miguel López-Ferber

Abstract Among various *Cydia pomonella* granulovirus (CpGV) isolates, the Mexican isolate (CpGV-M) has demonstrated a significant ability to reduce damage induced by the oriental fruit moth, *Grapholita molesta* (Busck) (= *Cydia molesta*) in peach crops. To obtain a more efficient virus for *G. molesta* control, an experimental virus population was constructed by mixing various CpGV isolates. This mixture was then selected for replication in a *G. molesta* laboratory colony. After 12 successive passages on this alternative host, the insecticidal efficacy of the virus population had improved. The concentration of virus occlusion bodies required to kill 90 % of neonate larvae was 450-fold lower than that of the original isolate mixture, and 120-fold lower than that of the

CpGV-M isolate alone. Following adaptation to this alternative host, the efficacy against its natural host, the codling moth, *C. pomonella*, was conserved. This mixed isolate population can be produced on *C. pomonella* without loss of efficacy, which is useful from a commercial production perspective. This adapted virus isolate mixture is likely to prove more effective than individual component isolates at controlling *G. molesta*.

Keywords *Grapholita molesta* · *Cydia pomonella* · *Cydia pomonella* granulovirus · Biological insecticide · Host adaptation

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Introduction

The oriental fruit moth, *Grapholita molesta* (Busck) (= *Cydia molesta*), is one of the principal pests of peaches [*Prunus persica* (L.) Batsch], apricots [*Prunus armeniaca* (L.)] and nectarines [*Prunus persica* var. *nucipersica* (Borkh.) C. K. Schneid.]. This pest also causes occasional damage in apples (*Malus domestica* Borkh.), pears (*Pyrus communis* L.), quinces (*Cydonia oblonga* Mill.), plums (*Prunus domestica* L.) and other stone and pome fruits. This pest is widespread in Europe, Asia (China and Japan), in North and South America and in Africa, particularly in Mauritius, Morocco and South Africa. Larvae feed on both foliage and fruits with considerable economic losses (CABI 2016), with over 45 % of fruit infestation in some locations (Pree et al. 1998; Kanga et al. 2003).

Baculoviruses are widely used in pest control (Beas-Catena et al. 2014). Various granuloviruses (genus *Betabaculovirus*) are commercially used for the control of tortricid pests, like *Cydia pomonella* granulovirus (CpGV) for control of the codling moth, *C. pomonella* (Lacey et al. 2005), or gelechiids, like *Phthorimea operculella* granulovirus (PhopGV) for control of the potato tuber moth *Phthorimaea operculella* (Espinell-Correal et al. 2012). As no virus isolate from *G. molesta* has been reported, adaptation of a virus isolated from a related insect species could be an interesting alternative in the development of an effective biological insecticide. This type of adaptation process has been suggested to occur under natural conditions and may explain the efficacy of natural PhopGV isolates against the Guatemalan potato tuber moth, *Tecia solanivora*, some years after this insect invaded potato-growing areas of Venezuela (Niño del Guadron 2000) and Colombia (Espinell-Correal et al. 2010).

The Mexican isolate of CpGV, named CpGV-M, has been tested in laboratory conditions against other phylogenetically related insect species, including *G. molesta* that was considered as partially permissive (Huber 1978), because virus-induced mortality was observed only following inoculation with large numbers of virus occlusion bodies (OBs). Lacey et al. (2005) conducted a comparative analysis of the activity of CpGV-M against *C. pomonella* and *G. molesta* under laboratory conditions. For *G. molesta*, they reported a susceptibility of between 557-fold (LC₅₀) and 589-fold (LC₉₅) lower than observed for *C. pomonella*. In contrast, using a diet surface

contamination technique, Zingg et al. (2012) reported that *G. molesta* was 130-fold less susceptible than *C. pomonella* to the CpGV-M isolate.

Field trials confirmed significant reductions in damage by *G. molesta* when CpGV-M was applied as an insecticide. A reduction in damage of ~20 % was observed in a virus-treated peach plot compared to untreated control plots (Bonhomme et al. 2011). These results led to an extension of the registration of the Carpovirusine™ 2000 (Natural Plant Protection, Pau, France), a CpGV-M based product for the control of *G. molesta* in France. Increasing the pathogenicity, and hence the insecticidal efficacy, of the virus against *G. molesta* by selection in this alternative host would lead to a further reduction of damages and therefore be of interest from a plant protection point of view.

Materials and methods

Insect colonies

Two laboratory colonies of *C. pomonella* were used, a reference colony called CpNPP that was susceptible to the CpGV-M isolate, and a colony resistant to this isolate, named R_{GV}, derived from a natural CpGV-M-resistant population collected in the region of Saint-Andiol (Bouches-du-Rhône, France) and selected for resistance to CpGV-M as described previously (Berling et al. 2009; Graillot et al. 2014). A *G. molesta* colony was also established from individuals collected in 2002 in Italian peach orchards (kindly provided by Fabio Molinari, University of Piacenza, Italy). Insects were reared continuously since then on artificial diet (Heliothis Stonefly diet, Ward's Natural Science, Rochester, NY, USA).

Virus isolates

CpGV-M stock 2020-S1 from Laboratoire de génie de l'environnement industriel (LGEI, Alès, France) was the reference isolate. It is genetically similar to the original Mexican isolate described by Tanada (1964). Most CpGV formulations commercialized in Europe, including Carpovirusine™ 2000 produced by Natural Plant Protection (NPP, Pau, France), are similar to this Mexican isolate. The CpGV isolate NPP-R1 was provided by NPP. This isolate was able to overcome the codling moth resistance to CpGV-M (Berling et al.

2009), and was used to derive the CpGV-R5 isolate characterized by Graillot et al. (2014). The Iranian isolate I12 (Eberle et al. 2008) and English isolate E2 (Crook et al. 1985) were provided by DLR Rheinpfalz as part of the European project CRAFT « Sustain CpGV ».

Preparation of the mixed virus population

In order to provide a maximum genomic diversity, occlusion bodies (OBs) of four virus isolates, CpGV-M, E2, I12, NPP-R1, were mixed in equal proportions. A volume of 50 μl of this mixture at a concentration of 1×10^3 OB μl^{-1} was used to inoculate 24 well plates containing artificial diet. A fourth instar CpNPP larva was then placed in each well to obtain an initial stock of OBs referred to as “virus genotype mixture—passage zero” (vgm-0). These OBs were purified by centrifugation and stored in distilled water at -20°C .

Successive passages on *G. molesta*

Fourteen successive passages (vgm-1–vgm-14) were performed with this virus isolate mixture on *G. molesta* in order to adapt the virus to the new host. For this, 1 g of diet was poured into each well of 24 well plates, then 50 μl of OB suspension at 1×10^3 OB μl^{-1} were deposited onto the diet surface in each well. A single third or fourth instar larva (seven days old) was placed in each well. The plates were then incubated at 25°C for four days. Symptomatic larvae (alive but with clear signs of infection) were collected and placed in groups of three into microfuge tubes and incubated one day more at 25°C , then frozen (-20°C). For OB extraction, larvae were thawed, pooled and homogenized in distilled water, followed by filtration through nylon cloth. The filtrate was centrifuged two times to pellet the OBs. The pellet was resuspended in distilled water and stored at -20°C (Berling et al. 2009). The purified OB concentrations were evaluated after each passage, adjusted to 1×10^3 OB μl^{-1} and used as inoculum for the following passage. A passage of the virus mixture at the twelfth passage (vgm-12) was also carried out on CpNPP larvae (named vgm12-cp). Dead larvae from this passage were processed individually in order to evaluate the virus yield of each larva. All OB titers were determined using a light microscope (Olympus BX41TF) with dark field optics ($\times 600$) and a counting chamber Petroff–Hauser 0.01 mm in depth.

Bioassays

A volume of 200 μl of Stonefly diet was placed in each well of a 96 well plate. A 6 μl volume of OB suspension (or water for controls) was evenly deposited over the flat diet surface of each well. Bioassays were performed each using six OB concentrations of vgm-0, vgm-1, vgm-3, vgm-5, vgm-7, vgm-10, vgm-12, vgm-14 and vgm-12-cp OBs. These were 2–6250 OBs μl^{-1} (fivefold dilutions: 2–10–50–250–1250 and 6250 OB μl^{-1}) using CpNPP larvae, and 10 to 1,000,000 OBs μl^{-1} (10-fold dilutions: 10–100–1000–10,000–100,000 and 1,000,000) using *G. molesta* larvae. Neonate larvae (less than one day old) were then placed in each well and the plates were sealed with ParafilmTM and incubated in a growth chamber at 25°C ($\pm 1^\circ\text{C}$) with a 16:8 h L:D photoperiod and a RH of 60 % (± 10 %). Virus induced mortality was recorded after seven days. When testing on CpNPP insects, 32 larvae were used for each virus concentration and 48 larvae were placed in wells without virus exposition corresponding to 192 infected larvae and 48 non-infected larvae per test. For the test on *G. molesta*, 16 larvae were used for each virus concentration and 24 larvae were placed in wells without virus exposition corresponding to 96 infected larvae and 24 non-infected larvae per test. Larvae dying due to manipulation were excluded from the tests. Each test was repeated three times.

Statistical analysis

Three independent bioassays were set up for each treatment, and their homogeneity verified before pooling the data for probit analysis (Finney 1971) with the POLO + software (LeOra Software, Berkeley, CA, USA) (POLO PC 1987) to deduce the $\text{LC}_{50\text{s}}$ and $\text{LC}_{90\text{s}}$. Comparison between treatments was performed using the multiple treatment comparison on the same program.

DNA extraction and restriction endonuclease (REN) analysis

Viral DNA of CpGV-M, E2, I12, NPP-R1, vgm-12 and vgm-12-cp, was extracted from OBs as described previously (Berling et al. 2009; Graillot et al. 2014). Approximately 500 ng of purified virus DNA was digested with *EcoRI*, *BamHI*, *PstI*, *SalI* (Fisher

Scientific, Illkirch, France) in the supplied buffer at 37 °C for 3 h. The DNA restriction fragments were separated by electrophoresis in 1 % agarose gels in *Tris*-acetate-EDTA buffer at 80 V for approximately 4 h. λ -phage DNA digested with *Hind*III was used as standards for size determination. Fragments were visualized under UV light by ethidium bromide staining. Restriction fragment sizes and DNA quantities were estimated using the Kodak 1D image system (Eastman Kodak Company, Rochester, NY, USA).

PCR analysis

PCR analysis of CpGV-M, CpGV-R5, vgm-12 and five independently reared infected larvae of vgm-12-cp was performed on the basis of the virus *pe-38* gene (Graillot et al. 2016). PCR was performed in a final volume of 50 μ l, containing 2 μ l of each primer (10 pmol μ l⁻¹ MWG-Eurofins), 2 μ l of viral DNA (10 pg), 20 μ l of 2.5 \times PCR Hotmastermix (5PRIME GmbH, Hilden, Germany) and 24 μ l water. Amplification conditions were as follows: a 3 min predenaturation at 94 °C, followed by 35 cycles of 15 s 94 °C; 40 s 65 °C and 1 min 30 s 72 °C. Amplified fragments were separated in a 3 % agarose gel (NOVAGEL GQT, Conda S.A., Torrejon de Ardoz, Madrid, Spain) in Tris Borate EDTA buffer (TBE). Fragments were visualized on a UV transilluminator after ethidium bromide staining.

Results

Pathogenicity for *G. molesta* of virus isolates produced in *G. molesta*

The pathogenicity (*sensu* Thomas and Elkinton 2004) for *G. molesta* (LC₅₀ and LC₉₀) of the initial mixture of OBs, and of the virus populations resulting from the successive passages on *G. molesta* larvae (vgm-1–vgm-14) are presented in Table 1. Both LC₅₀s and LC₉₀s on *G. molesta* larvae decrease from vgm-3. After 12 passages, a plateau was reached representing a relative potency of 53-fold (LC₅₀) to 117-fold (LC₉₀) compared to CpGV-M alone, and no further decreases were observed. The pathogenicity of vgm-12 OBs against *G. molesta* was significantly higher than that of the original mixed isolate OBs (vgm-0) ($\chi^2 = 65.31$, df = 2, P < 0.001).

Table 1 Adaptation of CpGV to *G. molesta*. Variation in the pathogenicity against *G. molesta* during successive passages of an experimental population of CpGV composed of four isolates (named M-R1–E2–I12)

Virus isolate	No. of insects tested	No. of OBs μ l ⁻¹	LC ₅₀ (95 % CI)		Relative potency		LC ₉₀ (95 % CI)		Relative potency	
Mix M-R1–E2–I12	371		3.96×10^3 (208–3.28 $\times 10^3$)		1		2.70×10^5 (3.26 $\times 10^4$ –7.16 $\times 10^5$)		1	
vgm-1	373		8.40×10^3 (4.58 $\times 10^3$ –1.42 $\times 10^4$)		0.48 (0.21–1.09)		1.22×10^5 (6.38 $\times 10^4$ –3.15 $\times 10^5$)		2.21 (0.6–8.15)	
vgm-3	372		1.22×10^4 (7.15 $\times 10^3$ –1.92 $\times 10^4$)		0.36 (0.17–0.8)		9.28×10^4 (5.35 $\times 10^4$ –2.23 $\times 10^5$)		2.77 (0.78–9.84)	
vgm-5	467		2.72×10^3 (1.66 $\times 10^3$ –4.19 $\times 10^3$)		1.46 (0.67–3.91)		2.92×10^4 (1.67 $\times 10^4$ –6.65 $\times 10^4$)		9.25 (2.66–32.15)	
vgm-7	278		2.29×10^3 (303.29–9.14 $\times 10^3$)		1.72 (0.75–3.91)		2.60×10^4 (6.90 $\times 10^3$ –1.06 $\times 10^4$)		10.38 (2.95–36.63)	
vgm-10	424		528.35 (332.02–786.37)		7.51 (3.54–15.93)		3.23×10^3 (1.99 $\times 10^3$ –6.88 $\times 10^3$)		83.34 (24.96–278.27)	
vgm-12	308		73.28 (46.37–114.88)		54.05 (24.69–118.34)		601.44 (339.15–1.40 $\times 10^3$)		448.39 (127.69–1.57 $\times 10^3$)	
vgm-14	550		218.55 (112.55–434.58)		18.12 (7.24–45.37)		2.40×10^3 (1.05 $\times 10^3$ –9.40 $\times 10^3$)		112.54 (25.82–490.56)	
CpGV-M	347		3.70×10^3 (1.97 $\times 10^3$ –6.41 $\times 10^3$)		1.05 (0.46–2.42)		7.01×10^4 (3.55 $\times 10^4$ –1.86 $\times 10^5$)		3.86 (1.03–14.49)	

Probit regressions were fitted using the PoloPlus program (LeOra software 1987). Relative potencies were calculated as the ratios of effective concentrations relative to that of the mixture M-R1–E2–I12. (95 % CI values shown in parentheses)

Pathogenicity for *C. pomonella* larvae of viral isolates from the successive passages in *G. molesta*

Bioassays were performed on a *C. pomonella* colony susceptible to CpGV-M (CpNPP) in order to check if the pathogenicity of the virus isolate mixture against the original host was maintained (Table 2). The lethal concentrations for CpNPP of the OBs produced on *G. molesta* increased slightly, both at the LC₅₀ and LC₉₀ when compared to the original mixture of isolates. Although these differences were statistically significant ($\chi^2 = 130$, df = 14, $P < 0.001$), a two-fold difference is common between CpGV isolates (Reza-panah et al. 2008). The vgm-12 isolate was also tested on *C. pomonella* larvae resistant to CpGV-M (laboratory colony R_{GV}) and was able to kill these insects [LC₅₀ 142.1 (95 % CI 17.6–408.3); LC₉₀ 2.3×10^3 (95 % CI 764.5– 3.2×10^4)], but not as effectively as the CpGV-R5 isolate [LC₅₀ 22.4 (95 % CI 13.7–34.4); LC₉₀ 410.67 (95 % CI 240.2–846.4)] (Graillot et al. 2014) ($\chi^2 = 31.57$, df = 2, $P < 0.001$).

Effect of producing vgm-12 OBs in a susceptible *C. pomonella* colony

In a commercial production process, viral production and insect breeding are easier and more efficient using *C. pomonella* than using *G. molesta*. To examine the feasibility of producing vgm-12 OBs in CpNPP larvae, vgm-12 OBs produced in *C. pomonella* (vgm-12-cp) were subjected to bioassay. The mean (\pm SE) virus yield of vgm-12-cp in CpNPP larvae was 4.40×10^{11} OBg⁻¹ of larva ($\pm 0.23 \times 10^{11}$) corresponding to 2.13×10^{10} OB per larva ($\pm 0.12 \times 10^{11}$). This yield is within the usual range observed for other CpGV isolates (Graillot et al. 2014) and indicates that this virus could be produced using CpNPP larvae. Bioassays were performed using *C. pomonella* and *G. molesta* with vgm-12-cp. The pathogenicity of vgm-12-cp against *G. molesta* [LC₅₀ 297.9 (95 % CI 186.9–476.5); LC₉₀ 2.41×10^3 (95 % CI 1.44×10^3 – 4.99×10^3)] was significantly lower than that of vgm-12 ($\chi^2 = 18.16$, df = 2, $P < 0.001$). However, similar levels of pathogenicity were maintained against CpNPP larvae. Although these differences are statistically significant, they are in the same range as those observed when comparing vgm-12 and vgm-14.

Table 2 Adaptation of CpGV to *G. molesta*. Variation in the pathogenicity against *C. pomonella* during successive passages in *G. molesta* of an experimental population of CpGV composed of four isolates

Viral isolates	No. of insects tested	No. of OBs μl^{-1}		Relative potency		Relative potency	
		LC ₅₀ (95 % CI)		LC ₉₀ (95 % CI)		LC ₅₀ (95 % CI)	
Mix M-R1-E2-I12	553	15.79 (7.78–32.92)		1		103.2 (46–527.52)	1
vgm-1	555	36.75 (11.92–132.14)		0.43 (0.65–0.25)		168.26 (62.53– 5.66×10^3)	0.61 (0.73–0.09)
vgm-3	547	56.25 (41.12–77.90)		0.28 (0.19–0.42)		499.70 (312.5–945.81)	0.21 (0.14–0.55)
vgm-5	559	44.71 (33.08–60.21)		0.35 (0.24–0.55)		335.36 (223.55–573.2)	0.31 (0.21–0.92)
vgm-7	550	30.89 (23.80–39.78)		0.51 (0.33–0.82)		121.78 (87.55–194.08)	0.85 (0.53–2.71)
vgm-10	1017	78.16 (60.87–99.43)		0.20 (0.13–0.33)		797.20 (566.64– 1.22×10^3)	0.13 (0.08–0.43)
vgm-12	498	29.47 (21.09–40.58)		0.54 (0.37–0.81)		238.09 (156.31–416.13)	0.43 (0.29–1.27)
vgm-14	500	86.49 (62.49–118)		0.18 (0.12–0.28)		611.75 (404.79– 1.07×10^3)	0.17 (0.11–0.49)
CpGV-M*	786	13.10 (6.55–23.20)		1.21 (1.19–1.42)		223.10 (110.70–654.18)	0.46 (0.42–0.81)

Probit regressions were fitted using the PoloPlus program (LeOra software 1987). Relative potencies were calculated as the ratios of effective concentrations relative to that of the Mix M-R1-E2-I12 virus. (95 % CI values shown in parentheses)

* Data from Graillot et al. (2016)

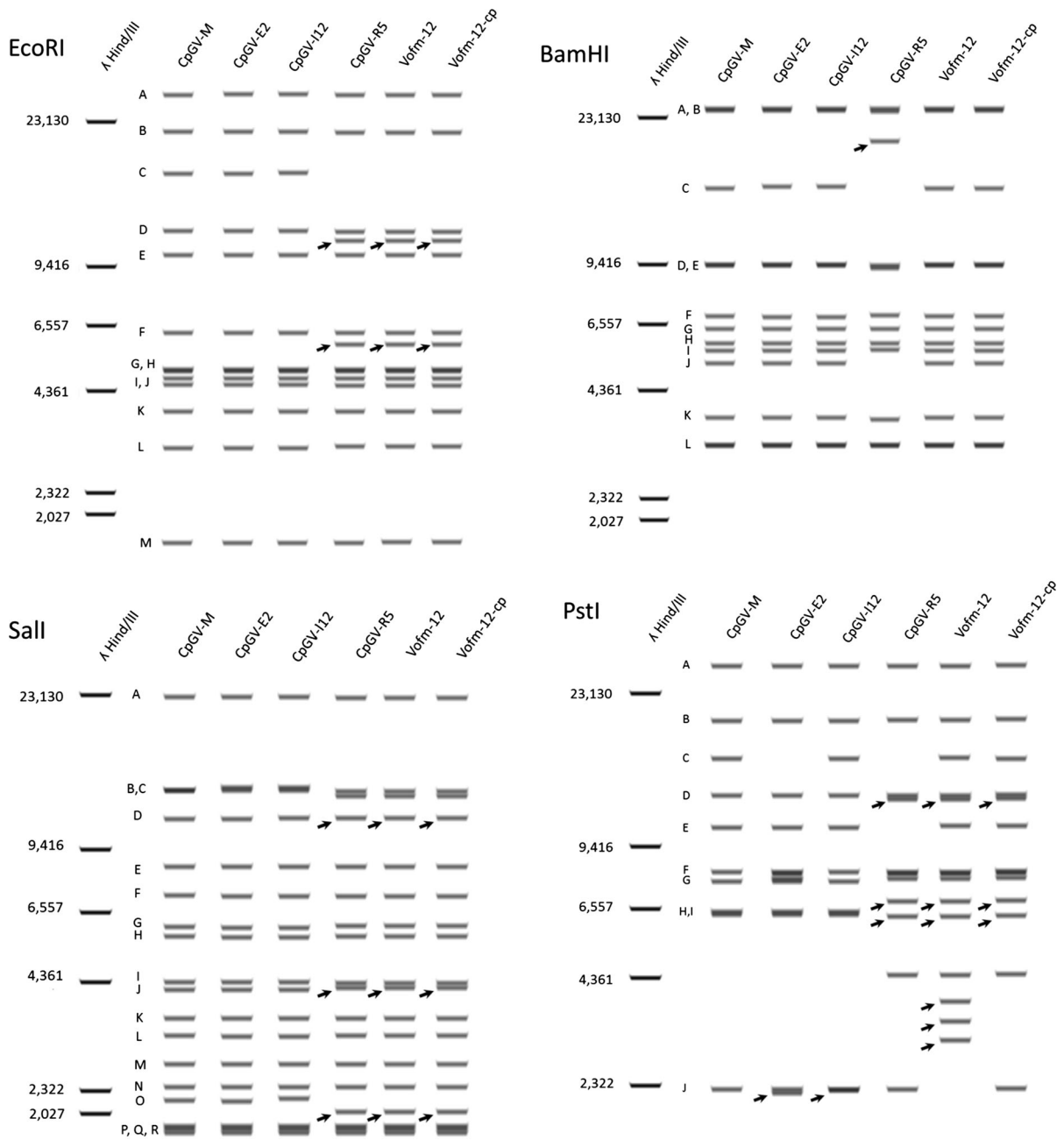


Fig. 1 Schematic REN profiles of the genome of six viral isolates using four restriction endonucleases. *Letters* indicate fragment names. *Arrows* indicate isolate-specific restriction

Characterization of the virus isolates

The REN profiles of vgm-12 and vgm-12-cp were compared to that of the pure virus isolates used to construct the vgm-0 experimental population. REN analysis was performed using *EcoRI*, *BamHI*, *PstI* and

fragments. Lambda-DNA digested with *HindIII* is included for molecular size standard (bp)

SalI. No differences were detected between vgm-12-cp and vgm-12 for these four enzymes. These two isolates presented hybrid profiles between CpGV-M and CpGV-R5. The *BamHI* profile was similar to that of CpGV-M, whereas the *EcoRI* and *SalI* profiles were similar to the corresponding REN profiles of CpGV-

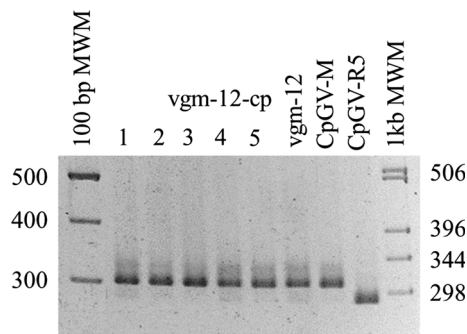


Fig. 2 Variability at the *pe38* virus locus of vgm-12 and vgm-12-cp. Amplification was carried out from vgm-12 and from five individual *C. pomonella* larvae infected with vgm-12 (vgm-12-cp). CpGV-M and CpGV-R5 are included as reference isolates

R5. The *Pst*I profile presented characteristics hybrid between parent viruses (Fig. 1).

The allele composition of the virus populations with respect to *pe38* gene was analyzed using the previously developed PCR approach (Graillot et al. 2016). The CpGV-M allele represented the majority of the vgm-12 population, but a small proportion of the CpGV-R5 *pe38* allele was detected. Analysis of five individual *C. pomonella* larvae infected with vgm-12 (vgm-12-cp 1–5) did not reveal differences with vgm-12 (Fig. 2).

Discussion

Previous studies by Lacey et al. (2005) and Zingg et al. (2012) demonstrated that CpGV-M was able to replicate in *G. molesta*, although the pathogenicity was far lower for this alternative host than for the original host. CpGV isolates present an apparent low genetic variability (Crook et al. 1985). However, the discovery of field insect populations resistant to CpGV-M and the observation that other isolates could overcome this resistance confirmed that such genetic variability exists, at least between isolates (Rezapanah et al. 2008). Different CpGV isolates differed in their ability to replicate in *G. molesta*, suggesting that this virus could be adapted to this host by artificial selection. Indeed, Zingg et al. (2012) obtained a viral isolate of CpGV-M effective against *G. molesta* by selection in the laboratory (CpGV-ABC isolate). They observed a 45-fold higher pathogenicity in terms of the LC₅₀ metric, when compared to CpGV-M on *G.*

molesta while conserving the virus' pathogenicity toward *C. pomonella*.

Adaptation to a new host (new host species or new host genotype), is facilitated by the diversity of the genotypic composition of the colonizing population. Such diversity could result in emergence of new genotypes by recombination. It could also facilitate synergy between virus genotypes resulting in higher pathogenicity for the whole population (López-Ferber et al. 2003). Accordingly, increasing the genetic diversity prior to selection is a common strategy when adapting to a new condition. An experimental virus population was produced by mixing OBs from four virus isolates (CpGV-M, E2, I12 and R1). This mixture was amplified in a fully susceptible host, CpNPP, to produce an experimental population (vgm-0). The vgm-0 mixture presented a lower efficacy against *G. molesta* than the CpGV-M isolate alone at the LC₉₀. The vgm-0 mixture was then subjected to successive passages in *G. molesta*. The response to alternative host was detectable from the second passage until vgm-12. No further increase in pathogenicity was observed after vgm-12.

A 54-fold to 450-fold gain was observed between the original OB mixture and vgm-12 at the LC₅₀ and LC₉₀ levels, respectively. The vgm-12 OBs were also between 50 and 120 fold (at the LC₅₀ and LC₉₀, respectively) more pathogenic than CpGV-M alone against *G. molesta*. Zingg et al. (2012), reported a relative potency of 45 (95% CI 29.5-69.1) of their isolate (CpGV-ABC-V22) at the LC₅₀ level, when compared to CpGV-M in *G. molesta*, but they did not determine the relative potency at the LC₉₀ level. The initial OB mixture presented a reduced LC₉₀ in CpNPP that resulted in significantly different regression slopes compared to CpGV-M alone. Such differences are usually attributed to differences in the mode of action, could be related to the genotypic diversity of the virus mix.

Zingg et al. (2012) also reported that the selected population maintained pathogenicity in *C. pomonella*, the original host species, while our approach ended in a small (two fold) but statistically significant reduction in activity. If this reduction corresponds to a real trend, it could reflect a tradeoff between the ability to replicate in each host. Espinel-Correal et al. (2010) observed that the genotypically homogeneous isolate PhopGV-Col03, that was well adapted to *T. solanivora*, was less efficient on *P. operculella*, whereas

PhopGV-Peru 6, an isolate well adapted to *P. operculella*, lost pathogenicity during serial passage in *T. solanivora*.

Industrial production of a virus insecticide is largely dependent on the ability to mass-rear the host insect. Use of alternative host species, that are easier to rear than the target host is an alternative approach, as long as this technique incurs no appreciable reduction in virus efficacy against the target pest, and no marked changes in the virus genome and resulting phenotype are observed (Chambers 2014). The vgm-12 mixture was produced in CpNPP insects, as this colony is already used for industrial production of CpGV. The production of vgm-12-cp OBs per insect and per gram of insect tissue was found to be similar to those of other CpGV isolates (Eberle et al. 2012; Graillot et al. 2014). No differences in REN profiles were observed between vgm-12 and vgm-12-cp. However, a small decrease in pathogenicity against *G. molesta* after a single passage on *C. pomonella* was detected, suggesting that continuous selection is required to maintain the adaptation to *G. molesta*.

Comparison of the REN profiles of vgm-12 with those of the parental isolates suggested that recombination had occurred among the original isolates, but also that genetic variability was retained after 12 passages in the alternative host. In CpGV-ABC the major genotype is similar to CpGV-M [A type following Eberle et al. (2009)], but about 2 % of a D-type CpGV genome is present (Zingg et al. 2012). An analysis using *pe38* primers that discriminate between CpGV-M and CpGV-R5 (Graillot et al. 2016) was also performed. Interestingly, even though the frequency of *pe38* type CpGV-R5 marker (as determined by PCR) was barely detectable, the vgm-12 isolate had significant pathogenicity to the *C. pomonella* R_{GV} colony that is resistant to CpGV-M, although sixfold lower than that of CpGV-R5. Replication of CpGV genotypes in resistant hosts requires the presence of non-A type *pe38* alleles (Gebhardt et al. 2014). However, in the vgm-12 mixture the low frequency of CpGV-R5 derived alleles cannot explain the high pathogenicity towards the R_{GV} population. Further research is ongoing to clarify this aspect.

In the present study, the main justification for host range extension/modification was to provide a biological insecticide for orchard protection that could complement the existing use of CpGV to control *C. pomonella*. The results obtained in laboratory tests

clearly demonstrated an increase in pathogenicity against *G. molesta* compared to CpGV-M that is already used against this insect, even though its control efficacy did not usually reach the level observed in *C. pomonella*. As previously done with the NPP-R1 isolate (Berling et al. 2009; Graillot et al. 2014), it has been possible to adapt CpGV to a low-permissive host by successive passages. Field trials have to be performed in order to determine whether vgm-12 can improve the control of natural populations of *G. molesta* compared to the existing virus insecticides based on CpGV-M and CpGV-ABC.

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