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Characterization of a Costa Rican granulovirus strain highly pathogenic against its indigenous hosts, *Phthorimaea operculella* and *Tecia solanivora*

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Abstract

A granulovirus isolate collected from diseased *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) larvae in Costa Rican potato [*Solanum tuberosum* L. (Solanaceae)] crops was characterized at the molecular and biological level. Restriction endonuclease analysis identified this isolate as a novel *P. operculella* granulovirus (PhopGV) (Baculoviridae: *Betabaculovirus*) strain and was designated as PhopGV-CR1. In addition, PCR amplification of four specific variable genomic regions yielded multiple amplicons for two open reading frames, revealing the presence of different genotypic variants within the virus population. Biologically, PhopGV-CR1 was highly pathogenic for its two indigenous hosts, although significant differences of up to four-fold were detected against *P. operculella* [LD_{50} = 17.9 occlusion bodies (OBs) mm^{-2}] and *Tecia solanivora* (Povolny) (Lepidoptera: Gelechiidae) (LD_{50} = 69.1 OBs mm^{-2}). The two *P. operculella* colonies, from Costa Rica and France, were equally susceptible to PhopGV-CR1. Serial passage of PhopGV-CR1 over four generations in *T. solanivora* increased its pathogenicity by five-fold in three generations, suggesting an ongoing adaptation to its alternate host.

Introduction

Two of the most important pests of potatoes [*Solanum tuberosum* L. (Solanaceae)] in Central America and the northwestern countries of South America are *Phthorimaea operculella* (Zeller) and *Tecia solanivora* (Povolny) (both Lepidoptera: Gelechiidae). *Phthorimaea operculella*, the potato tuberworm, is a cosmopolitan species (Figure 1) and the most damaging pest of potatoes in tropical and subtropical regions (Rondon, 2010). *Tecia solanivora*, originated from Central America, has rapidly established in various Iberoamerican countries in the last 20 years (Figure 1) and has become a major potato pest in the Andean region (Pollet et al., 2003). In 2000, this pest was reported to have invaded the Canary Islands (CAB International, 2000). The mining larvae of both these species cause severe damage to tubers in the field and in storage, where losses may account for up to 100% under non-refrigerated conditions (von Arx et al., 1987; Raman et al., 1987; Niño, 2004). Tuberworm control with chemical pesticides during the last 50 years has caused the development of multiple resistances to several organophosphorous and synthetic pyrethroids (Shelton et al., 1981; Arévalo & Castro, 2003; Dogramaci & Tingey, 2008; Saour, 2008). All this boosted the development of alternative control methods for these pests that should also protect their natural enemies (Amonkar et al., 1979; Kroschel & Koch, 1996; Kurhade & Pokharkar, 1997; Symington, 2003; Lacey et al., 2010).

Integrated pest management (IPM) programs for *P. operculella* and *T. solanivora* have been developed and implemented in various countries (von Arx et al., 1987;

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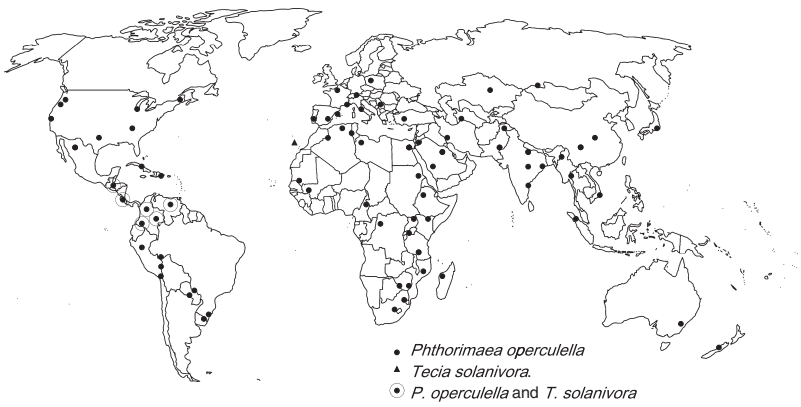

Figure 1 Distribution map of *Phthorimaea operculella* and *Tecia solanivora.*

Chandel & Chandla, 2005; Kroschel & Lacey, 2008; Zeddam et al., 2008). They are based on a handful of similar practices (Das et al., 1992), all with the *P. operculella* granulovirus (PhopGV) (Baculoviridae: *Betabaculovirus*) as their main component. Various geographical isolates of PhopGV have proven their efficiency as biopesticides under rustic storage conditions, as well as during field outbreaks in several countries, e.g., Peru, Colombia, Bolivia, Ecuador, Venezuela, USA, Tunisia, India, Yemen, and Australia (Reed & Springett, 1971; Raman et al., 1987; Kroschel et al., 1996; Lagnaoui et al., 1996; Setiawati et al., 1999; Lacey et al., 2010). The different PhopGV isolates show distinct activity against alternate hosts (Zeddam et al., 1994, 2003; Pokharkar & Kurhade, 1999; Villamizar et al., 2005).

In spite of the existence of a wide array of efficient PhopGV strains, the search for novel, indigenous isolates is always desirable because they are better adapted than foreign ones to their natural environment (Cory et al., 2005). In addition, novel strains may become excellent tools for managing insect resistance, as has occurred with an Iranian strain of *Cydia pomonella* granulovirus (CpGV), highly infectious to the codling moth, *C. pomonella* (L.), populations from Europe that had developed resistance against the traditionally commercialized CpGV strain from Mexico (Eberle et al., 2008). Finally, novel strains with efficient insecticidal properties against a certain complex of hosts may offer a better alternative to chemicals than those specialized in a single host (Zeddam et al., 2003; Moura Mascarin et al., 2010). In Costa Rica, such an isolate would be ideal to simultaneously control *P. operculella* and *T. solanivora*, whose populations overlap spatially and temporally in Zarcero and Cartago, the two Costarican regions where potato is mostly cultivated (Anonymous, 2010).

The aim of this work was to characterize novel PhopGV strains from Costa Rica with efficient insecticidal activity against both *P. operculella* and *T. solanivora* that will allow their inclusion in IPM programs in the short- or medium-term.

Materials and methods

Insect rearing

Two *P. operculella* laboratory colonies were reared. The first, *P. operculella*-FR, originated from an Egyptian population (Taha et al., 1999), was kindly provided by Dr. Abol-Ela (Faculty of Agriculture, Cairo, Egypt) and has been maintained at the Institut de Recherche pour le Developpement (IRD) laboratory (St. Christol-lès-Alès, France) since 1999. The other colony, *P. operculella*-CR, originated from the Research Center Carlos Durán (9°56′N, 83°48′W) (Cartago, Costa Rica) and was reared in the same research center since 2006. Both colonies were maintained under constant environmental conditions: 27 °C, 60% r.h., and L16:D8 h photoperiod. The colony of *T. solanivora* was established from a natural population collected in Oreamuno (10°00′N, 83°50′W) (Cartago, Costa Rica) in 2006 and reared under similar environmental conditions in the Research Center Carlos Durán (9°56′N, 83°48′W) (Cartago, Costa Rica).

The procedure followed to rear both insect colonies was based on that described by Angeles & Alcázar (1995) with slight modifications. Basically, larvae were fed on potato tubers, which were previously treated with chlorine solution to kill potential contaminating fungi or bacteria. Adults were fed a 30% (wt/vol) solution of honey or sugar. Females laid their eggs on filter papers which were collected every 24–48 h, incubated in a chamber at 27 °C until they darkened, and then placed on potato tubers. Under these conditions, the entire life cycle of both insect species varied between 4 and 5 weeks.

Viruses

The Costa Rican strain (PhopGV-CR1) of the *P. operculella* granulovirus was isolated from field-collected larval cadavers in Alvarado (9°56′N, 83°48′W) (Cartago, Costa Rica) in 2006, amplified on *P. operculella*-FR, and re-amplified in the same host colony to obtain enough viral inoculum for the whole study. Infected larvae were homogenized in distilled water and this suspension was spread onto potato surfaces at a concentration of ca. 100–500 larval equivalents l^{-1}. Between 15 and 20 neonate larvae were then placed on each potato and these were incubated for 3–4 weeks at 27 °C, after which time the infected larvae were collected and used as inoculum for another passage in larvae using the same procedure. The other isolates used in this work were (voucher specimens are stored at the IRD): (1) PhopGV-1346 from Tunisia (kindly provided by Dr. El Bedewi, International Potato Center, Cairo, Egypt, and multiplied during several years in Egypt), (2) PhopGV-1390.9 from Kayra, Peru (kindly provided by Dr. J. Cory, Oxford, UK) (Vickers et al., 1991), and (3) PhopGV-4.2, a clone of the PhopGV-1346 isolate (Vickers et al., 1991; Léry et al., 1998), whose genome has been completely sequenced (GenBank NC004062) (INRA/CNRS/Université de Montpellier II, Saint Christol-lès-Alès, France). All three reference isolates were amplified in 100 *P. operculella*-FR neonates as described above.

To purify occlusion bodies (OBs), ca. 50 larval cadavers were collected and homogenized in 10 ml 0.01 M Tris-HCl pH 7.5 using a Potter-Elvehjem homogenizer (Bellco Glass, Vineland, NJ, USA) and centrifuged at 664 ***g*** for 5 min at 4 °C. Supernatants were centrifuged at 20 000 ***g*** for 20 min, pellets resuspended in 1 ml bidistilled H_2O, and then placed on a continuous 30–70% (wt/vol) sucrose gradient and centrifuged at 20 000 ***g*** for 20 min. Occlusion bodies were collected with a Pasteur pipette, resuspended in 1 ml 1× TE buffer (0.1 M Tris-HCl, pH 7.5, and 10 mM EDTA, pH 8.0), centrifuged at 20 000 ***g*** for 20 min, and stored at −20 °C. Occlusion body concentration was determined with a spectrophotometer and calculated using the following formula: $6.8 \times 10^8 \times OD_{450} \times$ dilution = number of granules ml^{-1}. A linear regression between absorbance and OB concentration was determined at $\lambda = 450$ nm with 1 OD_{450} for 6.8×10^8 OBs ml^{-1} (Zeddam et al., 2003).

DNA extraction and restriction endonuclease analysis

Purified OB suspensions were incubated with 25 µl of 2 M Na_2CO_3 for 5 min, and DNA was extracted using a phenol/chloroform/isoamyl alcohol protocol and then precipitated with ethanol, as described in previous works (Muñoz et al., 1998). Between 0.25 and 0.5 µg of viral DNA was incubated with 10 units of each of these restriction enzymes: *Sma*I, *Bam*HI, *Hin*dIII, *Nru*I, *Mlu*I, *Hpa*I, *Nsi*I, *Nde*I, *Dra*III, *Bst*EII, and *Bst*ApI (Promega, Charbonnières-les-Bains, France) at the conditions specified by the supplier, according to the results obtained previously (Vickers et al., 1991; Léry et al., 1998). After addition of loading buffer (0.25% bromophenol blue, 40% wt/vol sucrose in water), samples were loaded in 1% (wt/vol) agarose gels with TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) and subjected to electrophoresis at 80 V. Ethidium bromide stained gels were then photographed on a UV transilluminator. The restriction endonuclease fragment molecular weights were determined by comparison with the corresponding sequenced PhopGV-4.2

Table 1 Forward and reverse primers used for PCR amplification of the different PhopGV isolates

Primer	Sequence (5′–3′)	AT (°C)	PhopGV ORF	CpGV ORF
Forward: 83-1	ATGTAGACGCGTCGTTAACCTGGGTGTA	63	83	ODV 25
Reverse: 83-2	ATGAACTGTTAAACGGCTTGAGTGAGCG			
Forward: 84-1bis	CCGCGCCGATTACCAACAGCAGCACTAT	63	84	ORF 92
Reverse: 84-2	CCTTGTAGCGTAACACTGTTTGTGTCTC			
Forward: 109-1	CGGTAGACGTGTAGATAATGTGCGCGTT	64	109	Lef 9
Reverse: 109-2	TCATCAATGATACCATAAGGCCCGGGCG			
Forward: 129-1	GCGATGATGAGAATGGGAATGTGAAGAC	64	129	EGT
Reverse: 129-2	TGCCTGCTGTGCTCGACAACAATAGACC			
Forward: Po 1	GAGATTAGACGAGTTCATCCAGAC	70	1	Granuline
Reverse: Po2	TTGTTGTCGCTTTGGAGCTAGTAC			
Forward: Po 5	CTGTCAGGACGTTCTTTGATTACT	68	ORF 87	Lef 4
Reverse: Po6	CTGCTATACGCGTACATGTCACCA			

AT, annealing temperature; ORF, open reading frame; ODV, occlusion derived virion; Lef, late expression factor; EGT, ecdysteroid UDP-glucosyltransferase.

fragments and the lambda DNA marker fragments. Marker fragments for the PhopGV-CR1 isolate were labeled using the same letter as the closest larger fragment of PhopGV-1346 in lower case, and with a sub index in cases in which marker fragments of different sizes shared the same letter.

Analysis of DNA by PCR

Previous restriction fragment length polymorphism (RFLP) analysis on the genetic diversity of PhopGV isolates originating from various countries allowed to determine four regions of high genomic sequence variability (variable regions), affecting open reading frames (ORFs) 46, 84, 109, and 129 (Léry et al., 2005). A fifth region, affecting ORFs 90 and 91, was recently found in isolates coming from Colombia (Léry et al., 2008). Primers encompassing these variable regions were designed using the complete PhopGV sequence (NC004062) (Table 1). In addition, primers for *lef-4* and granulin genes were included as controls (Table 1).

PCR reactions were carried out in a total volume of 25 μl, containing 10–100 ng of DNA, 1 pmol of each primer, 3.5 mM $MgCl_2$, and 12.5 μl of a mixture prepared by the supplier containing dNTPs and *Taq* polymerase (Promega). Amplifications were carried out in a thermocycler under the following conditions: a first cycle of 94 °C per 4 min, continued by 30 cycles of 94, 50, and 72 °C min^{-1} and a final cycle of 72 °C per 5 min. The PCR products (amplicons) were electrophoresed in 2% wt/vol agarose gels at 150 V. The PhopGV-1346 strain was used in all PCR reactions as a reference.

Bioassays

The bioassays to determine the mean lethal concentration at which 50% of the test organisms die (LC_{50}), an expression of pathogenicity, were carried out with neonate larvae (L1) as described by Espinel-Correal et al. (2010). To assess the LC_{50} of PhopGV-CR1 in *P. operculella*-FR, *P. operculella*-CR, and in *T. solanivora*, the viral inoculum was previously amplified in each host colony. Six viral concentrations were prepared (10^5–10^{10} OBs ml^{-1} in 2 ml of water) and applied homogeneously on the surface of the potato tuber using a nebulizer (Carrera et al., 2008). Two tubers of ca. 5 cm diameter were used for each concentration and 10 neonate larvae were placed on each. For each concentration, 3–5 replicates were arranged. The final concentrations applied on the potato surface were 0.1, 1, 10, 100, 1 000, and 10 000 OBs mm^{-2}. As most larvae leave the tubers before dying, the numbers of dead and infected larvae were recorded daily for 3 weeks to allow pupation. At the end of the experiment, tubers were opened to register dead and infected larvae remaining inside the galleries as well as the infected pupae. Corpses were dissected and microscopy preparations were performed to confirm the presence of OBs. Mortality results were subjected to probit analysis (Finney, 1971) using the POLO-PC program (Le Ora Software, 1987).

Successive passages of occlusion bodies in vivo

Occlusion bodies obtained from all *P. operculella* larvae in the first bioassay were pooled, amplified separately in *P. operculella*-CR and *T. solanivora*, and designated passage zero. These OBs were used as inocula to infect five groups of 60 larvae. Dead larvae were pooled to obtain enough inoculum (designated passage I) for the subsequent passage (II), and the same procedure was followed for two further passages (III and IV).

To assess the pathogenicity of PhopGV-CR1 throughout four generations of larvae, a bioassay identical to that described above was performed with five viral concentrations: 5×10^5, 5×10^6, 5×10^7, 5×10^8, and 5×10^9 OBs ml^{-1}. PhopGV-1346 was used as a reference. This bioassay was replicated three times.

Results

Molecular characterization of PhopGV-CR1

PhopGV-CR1 genomic profiles obtained with 10 restriction endonucleases and by PCR with four sets of primers were compared with those of PhopGV-1346, PhopGV-4.2, and PhopGV-1390.9. Restriction endonuclease profiles produced with *Nde*I were unique for the three isolates compared with five RFLPs, allowing profile discrimination (Table 2). With *Bam*HI (Figure 2) and *Bst*EII, PhopGV-CR1 showed a novel RFLP that distinguished this isolate from PhopGV-4.2 and PhopGV-1390.9, which were identical. *Hpa*I, *Mlu*I, and *Nsi*I did not differentiate PhopGV-CR1 from PhopGV-4.2, but they were useful enzymes to tell apart PhopGV-4.2 from PhopGV-1390.9. With *Nru*I, PhopGV-CR1 and PhopGV-1390.9 showed identical profiles, but they differed by one fragment from that of PhopGV-4.2 (Table 2). Finally, no polymorphisms were observed between restriction endonuclease profiles produced by *Sma*I, *Hin*dIII, or *Dra*III. Submolar fragments were observed in both field isolates, PhopGV-CR1 and PhopGV-1390.9, with several enzymes (Figure 2; Table 2).

By PCR, the set of primers encompassing ORF-84 generated a PhopGV-CR1 profile that differed from that of the reference strains, PhopGV-1346 and PhopGV-1390.9 (Table 3). Using the set of primers for ORF129, PhopGV-CR1 appears similar to PhopGV-1 390.9, both differing from PhopGV-1346 by the presence of amplicons of 1 023 and 869 bp, and the absence of one amplicon of 723 bp (Table 3).

Table 2 Polymorphic fragments present in the restriction endonuclease (REN) profiles of PhopGV-CR1 and PhopGV-1390.9 with the RENs *Bam*HI, *Nru*I, *Mlu*I, *Hpa*I, *Nsi*I, *Nde*I, and *Bst*EII in comparison with the cloned genotype PhopGV-4.2

REN	PhopGV-4.2	PhopGV-CR1	PhopGV-1390.9
*Bam*HI	I (6114)	+[1]	+
	–	i1 (ca. 6000)	–
*Hpa*I	–	–	g1[1] (ca. 5000)
	–	–	m1[1] (ca. 3400)
*Nde*I	–	c1 (ca. 14 500)	–
	G (6746)	–	–
	–	g1 (ca. 6500)	–
	I (5994)	+	–
	–	j1 (ca. 5800)[1]	–
*Bst*EII	–	g1 (6400)	–
*Mlu*I	–	–	l1 (3500)
*Nru*I	I (6104)	–	–
	–	+	h1 (6300)
*Nsi*I	–	–	i1 (2600)

Approximate molecular weight (in base pairs) is indicated in parentheses.
+/–, presence/absence of fragment.
[1]Submolar fragment.

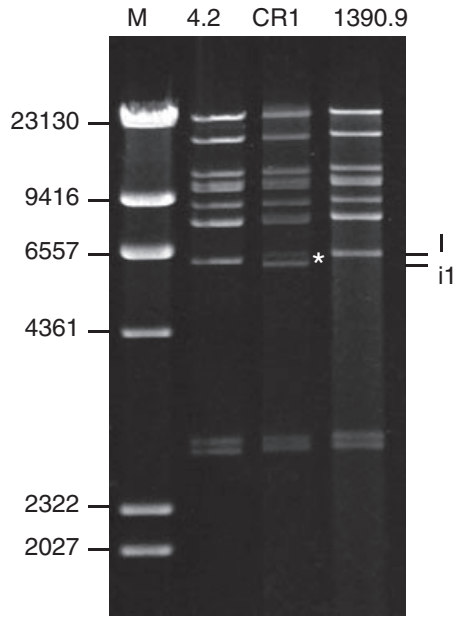

Figure 2 *Bam*HI restriction endonuclease profiles of the genomic DNA extracted from PhopGV-4.2 (4.2), PhopGV-CR1 (CR1), and PhopGV-1390.9 (1390.9). Restriction fragment polymorphic fragments *Bam*HI-I (I) and *Bam*HI-i1 (i1) are indicated to the right of the picture. The asterisk points out the submolar fragment I in CR1. Lambda DNA digested with *Hin*dIII was used as molecular weight marker (M) and its fragment sizes are included on the left of the picture.

Table 3 PCR amplicon sizes (bp) of genomic regions ORF129 and ORF84 amplified for PhopGV-1346, PhopGV-1390.9, and PhopGV-CR1

ORF of region amplified (gene function)	PhopGV-1346	PhopGV-1390.9	PhopGV-CR1
ORF129 (*egt*)	–	1023	+
	937	+	–
	–	869	+
	723	–	–
ORF84	–	–	330
	241	+	+

+/–, presence/absence of amplicon.
ORF, open reading frame; *egt*, ecdysteroid UDP-glucosyltransferase.

A single amplicon was amplified with the primer sets ORF1, ORF83, ORF87, and ORF109, with identical size for the three isolates analyzed. These amplicons were not further analyzed.

Pathogenicity of PhopGV-CR1

The pathogenicity of PhopGV-CR1 was assayed on indigenous populations of its two natural host, *P. operculella* and *T. solanivora*. In addition, two *P. operculella* populations were tested to detect potential differences in susceptibility to the virus by geographically distant host biotypes.

The dose–mortality responses of PhopGV-CR1 for the two *P. operculella* populations and for *T. solanivora* were fitted with a common slope; the interaction between host populations and log (virus dose) was not significant ($\chi^2 = 3.82$, d.f. = 2, P = 0.15).

The pathogenicity (expressed as LC_{50}) of PhopGV-CR1 for the two *P. operculella* biotypes is not significantly different, indicating that this PhopGV strain is equally pathogenic against host populations as distant as those from Costa Rica and Egypt (Table 4). However, the pathogenicity of PhopGV-CR1 for *T. solanivora* was four-fold lower than for *P. operculella* from France, as indicated by the 95% confidence intervals of the relative potency, which did not include 1 (Robertson & Preisler, 1992). The relative potency is the ratio between the LC_{50}s of the reference treatment (PhopGV-CR1 for *P. operculella*-FR in this particular assay) and each of the other LC_{50}s.

Pathogenicity of PhopGV-CR1 upon serial passage in *Phthorimaea operculella* and *Tecia solanivora*

The pathogenicity of PhopGV-CR1 was significantly enhanced at the second and third passages in *P. operculella* (by six-fold) and *T. solanivora* (by five-fold), respectively, as indicated by the potency 95% confidence limits, which

Table 4 LC_{50} values of PhopGV-CR1 obtained from two colonies of *Phthorimaea operculella* and the Costa Rican population of *Tecia solanivora*

Insect population	Regression line	LC_{50} (OBs mm^{-2})	Relative potency	95% confidence interval
P. operculella-FR	0.48x + 4.54	17.0	1	–
P. operculella-CR	0.48x + 4.53	17.9	0.95	0.42–2.15
T. solanivora	0.48x + 4.24	69.1	0.25	0.10–0.59

OB, occlusion body.

were above 1 (Robertson & Preisler, 1992). LD_{50} values did not vary thereafter upon serial passage (Table 5), indicating that the virus got quickly adapted to its host. Moreover, the final pathogenicity of the viruses, once adapted to one or the other host, was not statistically different for any of the virus isolates tested (Table 5). The pathogenicity of PhopGV-1346 increased, by 7.5- and 13.1-fold, at the second passage in *P. operculella* and *T. solanivora*, respectively (Table 5).

Discussion

The present study describes the molecular and biological characterization of a granulovirus (GV) isolated from *P. operculella* in Costa Rica and named PhopGV-CR1. Restriction endonuclease analysis showed a high similarity of PhopGV-CR1 to reference strains, revealing that the novel Costa Rican isolate is a geographical strain of this viral species, namely PhopGV. Several studies have shown that variation among restriction patterns of PhopGV isolates collected in very distant locations (Australia, Peru, Yemen, Indonesia) is limited, although at least several of these virus populations have probably been isolated for quite long periods. The reason for such a strong conservation of restriction sites in viral sequences is unclear, but this fact is not unique among GVs (Zeddam et al., 1999).

The presence of submolar fragments in several restriction endonuclease profiles and the existence of multiple fragment amplification with the same set of primers strongly suggest the presence of different genotypes within PhopGV-CR1. Genotypic heterogeneity within the same virus strain has also been observed for other PhopGV isolates (Léry et al., 1998), and is very common among other GV and nucleopolyhedrovirus (NPV) populations (Smith & Crook, 1988; Caballero et al., 1992; Burden et al., 2006; Figueiredo et al., 2009). This denotes the existence of functional diversity among PhopGV genotypes. Indeed, purified genotypes with significantly different pathogenicity, virulence, or OB yield to that of the wild-type mixture have been described (Muñoz et al., 2000; Hodgson et al., 2001;

Table 5 LC_{50} values of PhopGV-1346 and PhopGV-CR1 obtained from *Phthorimaea operculella* (Po) and *Tecia solanivora* (Ts) upon serial passage

Virus	Host	Passage	Regression line	LC_{50} (OBs mm^{-2})	Relative potency	95% confidence interval
1346	Po	I	0.56x + 4.26	21.1	1	–
		II	0.56x + 4.35	2.8	7.54	2.54–22.58
		III	0.56x + 4.58	3.0	7.09	2.39–21.58
	Ts	I	0.48x + 4.18	47.7	1	–
		II	0.48x + 4.73	3.6	13.09	4.75–38.20
		III	0.48x + 5.85	2.0	23.38	5.32–110.69
CR1	Po	I	0.45x + 4.42	18.8	1	–
		II	0.45x + 4.78	3.0	6.28	2.43–16.94
		III	0.45x + 4.75	3.6	5.19	1.86–15.15
		IV	0.45x + 4.74	3.7	5.13	1.55–17.87
	Ts	I	0.50x + 4.27	28.9	1	–
		II	0.50x + 4.41	14.6	1.97	0.79–4.98
		III	0.50x + 4.63	5.4	5.35	2.00–14.71
		IV	0.50x + 4.65	4.9	5.91	2.22–16.29

OB, occlusion body.

Simón et al., 2008). This constitutes evidence of the importance of genetic diversity in the capacity of a pathogen to adapt to its environment, in particular to its hosts.

Adaptation of a virus isolate to different host colonies was tested using the same bioassay methodology. The LC_{50} of PhopGV-CR1 was determined in two populations of *P. operculella* from France and Costa Rica, and also in a Costa Rican colony of the alternate host, *T. solanivora*. The pathogenicity of PhopGV-CR1 in its homologous host is similar to that obtained with the reference strain, PhopGV-1346, its purified clone PhopGV-4.2, and two isolates from Colombia and Peru, and lower than that of two other Colombian isolates (Espinel-Correal et al., 2010). Comparison of these values with those of strains from Yemen (Kroschel et al., 1996) or Indonesia (Zeddam et al., 1999) is difficult because of the different methodologies employed for the bioassays.

The pathogenicity of PhopGV-CR1 was four times lower for its heterologous host, *T. solanivora*. Similarly, for Colombian *T. solanivora*, a non-adapted virus strain is less efficient (Espinel-Correal et al., 2010). Larvae of *T. solanivora* have been found infected by baculoviruses naturally (Zeddam et al., 2003; Niño, 2004; Villamizar et al., 2005). The viruses they contain appeared to be related to PhopGV. However, the process of adaptation was not explored, and the efficacy of these isolates in the control of potato tuber moths was not indicated.

Granulovirus strains with good insecticidal performance are desirable for an efficient control of both pests. This may be achieved, for example, by thoroughly screening isolates from various geographical regions. Indeed, three Colombian PhopGV strains, recently isolated by Espinel-Correal et al. (2010), have been found to be up to 50-fold more pathogenic than the Costa Rican or the Peruvian PhopGV strains, showing LC_{50} values as low as 1.16 OBs mm^{-2}. These highly pathogenic strains were isolated from *T. solanivora* in regions where both hosts coexist, whereas the Peruvian strain did not have previous contact with this host. *Tecia solanivora*'s origin has been traced back to Central America. It is thus likely that virus populations infecting potato tuber moths in this region, like PhopGV-CR1, had previously been in contact with both host species, and retain the ability for quick adaptation to their hosts. Increasing pathogenicity of the virus adaptation to its host has been observed to occur upon serial passage of genotypically heterogeneous virus populations (Kolodny-Hirsch & Van Beek, 1997; Berling et al., 2009). Both the known genome plasticity of the baculoviruses and the genotypic heterogeneity of baculovirus populations play a role in this adaptation. As the PhopGV-CR1 strain was composed of an array of distinct genotypes, quick adaptation to its natural hosts was suspected. To demonstrate this, PhopGV-CR1 was passaged serially for four host generations, and indeed its pathogenicity significantly increased, reaching LC_{50}s as low as 3.0 OBs mm^{-2} for *P. operculella* and 5.4 OBs mm^{-2} for *T. solanivora*. These values are strikingly similar to some of the field-adapted Colombian PhopGV strains (Espinel-Correal et al., 2010). Likewise, 15-fold increased pathogenicity was registered for AcMNPV against *Plutella xylostella* (L.) when passaged 20 times in this host (Kolodny-Hirsch & Van Beek, 1997). More recently, a similar observation was made on the CpGV NPP-R1 strain that was passaged four times in a colony of *C. pomonella* resistant to a commercialized CpGV strain from Mexico (Berling et al., 2009). In this case, genotype selection accounted, at least partially, for host adaptation, as the proportion of a genotype similar to the one dominant in the Mexican CpGV strain was sharply reduced in this strain after only four serial passages (Berling et al., 2009). It is likely that adaptation of PhopGV-CR1 to *T. solanivora* can also be explained by genotype selection, but molecular characterization of the genotypes composing the PhopGV-CR1 strain from the first and fourth serial passage in *P. operculella* and *T. solanivora* is needed to confirm this hypothesis. Surprisingly, the reference strain, PhopGV-1346 also got quickly adapted upon contact with the Costa Rican populations of both the original and the heterologous hosts. This constitutes further evidence of the ability of this virus to adapt to its hosts. In addition, the fact that PhopGV-1346 shows LC_{50} values similar to those of the *T. solanivora* field-adapted Colombian strains seems to invalidate a link between pathogenicity and a 86 bp genomic insertion in the *egt* gene (observed as a 1 023 bp fragment by PCR). This insertion occurs in the Colombian isolates (Espinel-Correal et al., 2010) and also in PhopGV-CR1, but is absent in the PhopGV-1346 strain.

Preliminary results on the efficiency of PhopGV-CR1 have been obtained under both field and storage conditions (Gómez-Bonilla et al., 2011a,b). In the field, the virus reduced damage between 50 and 80% compared with the untreated controls, whereas over 70% damage reduction was obtained in stored potatoes. These data favor the inclusion of PhopGV-CR1 formulations in IPM programs.

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