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Responses of different geographic populations of two potato tuber moth species to genetic variants of *Phthorimaea operculella granulovirus*

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Key words: *Betabaculovirus*, insect virus, bioassay, pathogenicity, viral variant, *Tecia solanivora*, Lepidoptera, Gelechiidae, PhopGV, Baculoviridae

Abstract

Phthorimaea operculella granulovirus (PhopGV) belongs to the genus *Betabaculovirus* of the arthro-pod-infecting Baculoviridae. PhopGV is able to infect several gelechiid species. Among them are the potato tuber moths *Phthorimaea operculella* Zeller and *Tecia solanivora* Povolny (both Lepidoptera: Gelechiidae). In various South American countries, PhopGV-based biopesticides are used to control either *P. operculella* or *T. solanivora*. Many trials have indicated that a particular viral isolate can exhibit very distinct pathogenicity when infecting different host species or different populations of one host species. In this study, we compared host–pathogen interactions using various PhopGV iso-lates and various populations of *P. operculella* and *T. solanivora*. Virus isolates from *P. operculella* were more pathogenic against their original host species than against *T. solanivora*. A PhopGV iso-lated from *T. solanivora* was less efficient against *P. operculella*. In addition, virus isolates differed in pathogenicity toward their hosts (i.e., lethal concentrations of isolates ranged from low to high). Unexpectedly, we also found that host populations of one species from distinct geographic origins did not differ significantly in susceptibility to the same PhopGV isolate. This was the case for both host species and for five PhopGV isolates. Comparative restriction fragment length polymorphism (RFLP) analyses of 11 isolates including those used in bio-assays indicated three main regions of vari-ation in the genome of PhopGV, corresponding to the regions of open reading frame PhopGV046, gene *PhopGV129* (*egt*), and repeat 9 (located between open reading frames PhopGV083 and PhopGV084). Comparison of the nucleotide sequences of the insertions/deletions present in these regions were carried out for the most variable isolate, JLZ9f. The results are discussed in the context of the production and use of PhopGV as a biological agent against these two pest species.

Introduction

Enveloped, rod-shaped viruses belonging to the family Baculoviridae (baculoviruses) infect arthropods (insects and crustaceans), mainly species in the orders Lepidoptera, Hymenoptera, and Diptera (Theilmann et al., 2005). Four genera are presently recognized within the family (Jehle et al., 2006). The genus *Alphabaculovirus* (formerly *Nucleopolyhedrovirus*, NPV) and the genus *Betabaculovirus* (formerly *Granulovirus*, GV) (Fauquet et al., 2005) are the ones counting the most insect virus species (Granados & Federici, 1986). *Betabaculovirus* species only infect Lepidoptera. Numerous baculoviruses cause epizootics among the populations of their insect hosts (Evans, 1986; Fuxa,

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2004) and, thus, members of the group have been considered potentially interesting biological control agents. Since decades, numerous studies have been carried out to develop their use in pest management programs (Yearian & Young, 1982; Huber, 1986; Lacey & Goettel, 1995; Moscardi, 1999). However, just a few of them have been widely used, in spite of social demand and the increasing development of insect resistance to chemical pesticides, boosting the interest for alternative control methods. Various factors can account for this limited use of baculoviruses (Fuxa, 1991; Cunningham, 1995), such as a narrow host range and a low killing speed. For this reason, more work is needed to specify the conditions and limits of their use.

Phthorimaea operculella granulovirus (PhopGV; Reed & Springett, 1971) is largely used for biocontrol purposes (Raman, 1994; Kroschel et al., 1996; Sporleder & Kroschel, 2008; Moura Mascarin et al., 2010. PhopGV was first produced and applied to control the cosmopolitan potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) (Raman, 1994). More recently, PhopGV was also used against the Guatemala potato moth, *Tecia solanivora* Povolny (Lepidoptera: Gelechiidae) (Espinel-Correal et al., 2010), a species which extended its distribution area considerably within the last few decades (Puillandre et al., 2008). *Tecia solanivora* distribution partially overlaps with that of *P. operculella*. In some locations the former displaced the latter, in other locations the species coexist (Dangles et al., 2008) – in large areas of Central and South America, *P. operculella* and *T. solanivora* can be found infecting the same potato stores. Until now, little attention has been paid to the comparative susceptibility of these two species to the same PhopGV isolate and no comparison has been made of the pathogenicity of a given viral isolate among populations of a single host. These two aspects are of major importance if the use of PhopGV is to be promoted. It is essential to determine whether recommended doses give the same level of control independent of the host population considered. Also, in localities where the two pests co-occur, it is essential to identify PhopGV isolates that control both species effectively.

This study compiles 8 years of research in four countries to compare the pathogenicity of viral isolates among different geographic populations of *P. operculella* and *T. solanivora*. The objective was to test the hypothesis that local viruses adapt to local host populations as well as to a new invasive species. To that aim, four laboratory colonies of *P. operculella* and three of *T. solanivora* were set up. To make the results comparable between locations, we applied a biological test that we have previously standardized (Carrera et al., 2008). Aside from the phenotypic comparison, we analyzed viral genomes using restriction fragment length polymorphism (RFLP) to determine whether variation was randomly spread throughout the genome or concentrated in particular regions. Identifying such variable regions could be useful both for faster characterization of new PhopGV isolates and for looking whether sequence variations in determined regions could be related to differences in virus phenotypes. According to this process, we then compared the sequence of the variable regions in two virus isolates showing clear differences in pathogenicity (LC_{50}), one isolate (1346) originating from *P. operculella* and the other (JLZ9f) from *T. solanivora*.

Materials and methods

Insect colonies

Colonies of *P. operculella* and *T. solanivora* were established from larva-infested potato tubers from Cartago, Costa Rica (named Pop-CR and Tsol-CR, respectively), San Gabriel del Carchi, Ecuador (Tsol-EC), Villapinzón, Colombia (Tsol-CO), and Beni Sueif, Egypt (Pop-EG). Rearing protocols were similar to those reported by Gómez-Bonilla et al. (2011) and Espinel-Correal et al. (2012). The insect populations were reared on potato tubers under controlled conditions of 27 ± 2 °C, $60 \pm 10\%$ r.h., and L16:D8 photoperiod.

Virus (PhopGV) isolation and purification

The Tunisian isolate (named 1346) of PhopGV was used as the reference isolate (GenBank: AF499596.1). In addition, four other PhopGV isolates were used for bioassays, namely 1390-2 and 1390-3 (both isolated independently from Peruvian *P. operculella* larvae; Vickers et al., 1991), GV003 (isolated from Colombian *T. solanivora* larva; Espinel-Correal et al., 2010), JLZ9f (isolated from Ecuadorian *T. solanivora* larva), and PhopGVCR1 (isolated from Costa Rica unidentified potato tuber moth (PTM) larva; Gómez-Bonilla et al., 2011). Viruses were amplified on their respective host species through potato surface contamination. Tubers were disinfected with sodium hypochloride, rinsed in distilled water, air-dried, and then contaminated using 100–500 larval equivalents per l (Gómez-Bonilla et al., 2011). Viral occlusion bodies (OBs) were purified on continuous 30–70% (wt/vol) sucrose gradient and quantified as previously described, using the following formula: $6.8 \times 10^8 \times OD_{450} \times$ dilution = number of granules ml^{-1} (Zeddam et al., 2003; Carpio et al., 2012). Purified virus aliquots were stored at -20 °C until use.

Biological assays of virus isolates

Free export or import of some virus isolates was impossible due to local regulations. The same restrictions applied to insect–host populations. Accordingly, the only way to pro-

ceed was to follow a standardized biological test and apply it in local laboratories. Not all virus/host population combinations could be tested (Table 1). Bioassays were carried out on neonate larvae (less than 12 h post-emergence) following a previously published protocol (Espinel-Correal et al., 2010). Briefly, a known amount of OBs was evenly sprayed onto the tuber skin. Dilutions of purified OBs in distilled water were applied homogeneously (1×10^5 to 1×10^{10}) by a pulverization method with a nebulization chamber leading to final concentrations of 0.1–10^4 OBs mm^{-2} on the potato tubers (Carrera et al., 2008). For controls, only distilled water was sprayed. Then, batches of 10–25 potato tuber moth neonates were deposited on each treated tuber (two potato tubers per concentration; larval density was kept under one individual per 2 g of tuber). Finally, larval mortality was recorded for at least five virus doses and a control. At least five independent tests were set up. Total numbers of individuals used in bioassays were 6 975 *P. operculella* (5 875 treated and 1 101 controls) and 2 620 *T. solanivora* (2 149 treated and 471 controls). Dose-response curves based on probit analysis (Finney, 1952) were established by a logistic regression and adjusted by maximum likelihood using Polo Plus software (LeOra Software, 1987). Alternative models (logit and complementary log–log models) were tested and compared using the Akaike information criterion (Akaike, 1980), revealing that the probit model best fit the data sets. Extra-binomial distribution was also checked revealing no overdispersion in the data sets. The software was used to estimate the virus concentration killing 50% of the larvae tested (LC_{50}), the 95% fiducial limits of the LC_{50}, the slope of the concentration-mortality line, and the standard error of the slope. It was also used to compare (at the significance level of 0.05) the respective LC_{50} obtained for different viral isolate/host combinations. Polo Plus was also used to calculate the relative LC_{50} ratios between virus isolates. Ratios with 95% confidence intervals which include 1.0 indicate no significant difference between them (Robertson & Preisler, 1992).

Table 1 Bio-assays carried out during the study testing six *Phthorimaea operculella granulovirus* (PhopGV) isolates against populations of *Phthorimaea operculella* and *Tecia solanivora* of various geographic origin

	Host species					
	P. operculella			*T. solanivora*		
Virus isolate	CR	EG	CO	CR	CO	EQ
1346	+	+		+		+
1390.2	+	+		+		+
1390.3	+	+				
PhopGVCR1	+	+		+		
GV003			+		+	
JLZ9f		+				+

CR, Costa Rica; EG, Egypt; CO, Colombia; EQ, Ecuador.

Molecular characterization of PhopGV isolates

Each virus isolate was amplified on its original host and OBs purified as previously described (Espinel-Correal et al., 2010). An aliquot of each isolate was used for molecular characterization. Viral OBs were dissolved in 0.1 M Na_2CO_3 (pH 11). Viral genomes (DNA) were extracted from OB-released virions using Wizard Genomic DNA Purification kits (Promega, Madison, WI, USA). DNA was digested by *Bam*HI, *Sma*I, *Hind*III, *Nsi*I, *Nru*I, *Nde*I, *Hpa*I, *Dra*III, *Mlu*I, *BstE*II, and *BstAp*I restriction enzymes following manufacturer's recommendations and then electrophoresed on 1% (wt/vol) agarose gel (TAE buffer) along with a molecular weight marker to determine band sizes. Regions exhibiting insertion/deletion features compared to reference isolate 1346 were amplified by PCR using Promega PCR Master Mix. Cycling parameters were 94 °C denaturation (3 min), 35 cycles of 94 °C (45 s), 55 °C (45 s), 72 °C (1 min), followed by a final 72 °C extension (10 min). Primers used were: POGV46F: 5′–AACCCTGAGGATCGCGAAACTAACGAAG–3′ (nt 40500–40527 of the reference isolate 1346); POGV46R: 5′–GTTTCATCGTATGCGCGTCTTTTGGTTC–3′ (nt 41632–410605); POGV84F: 5′–ATGTAGACGCGTCGTTAACCTGGGTGTA–3′ (nt 72075–72102); POGV84R: 5′–ATGAACTGTTAAACGGCTTGAGTGAGCG–3′ (nt 72882–728055); POGV129F: 5′–GCGATGATGAGAATGGGAATGTGAAGAC–3′ (nt 116465–116692); and POGV129R: 5′–TGCCTGCTGTGCTCGACAACAATAGACC–3′ (nt 117401–117374). Amplicons were sequenced in both directions by the dideoxynucleotide chain termination method (Sanger et al., 1977) in an ABI 377 DNA sequencer and sequences were aligned with Clustal X.

Nucleotide sequence data from the PhopGV isolate JLZ9f partially encompassing ORFs PhopGV046, PhopGV083, PhopGV084, and PhopGV129 were deposited in the GenBank under accession numbers JX101761, JX170206, and HQ317403.1, respectively.

Results

Response of different geographic *Phthorimaea operculella* and *Tecia solanivora* populations to the same PhopGV isolates

All data sets discussed below appeared to be homogeneous in the effectivity of the virus against a given host species, allowing further comparisons.

Response from Phthorimaea operculella *populations.* Neonates from two geographically separated

populations (Costa Rica and Egypt) were challenged with three PhopGV isolates from *P. operculella* in standardized bioassays (Table 2). Differences existed in the respective pathogenicity of these isolates, their LC_{50} varying from 1.6 to 17 OB mm^{-2}. *P. operculella* populations from Egypt and Costa Rica responded equally to a same virus isolate (either 1346, 1390-2, or 1390-3).

In conclusion, bioassays showed a remarkable similarity of the LC_{50} calculated for each given viral isolate independently of the geographic origin of the host used in the experiment. This was true for both highly pathogenic (1390-2) and medium pathogenic (1346 and 1390-3) isolates.

Response from Tecia solanivora *populations.* When neonate larvae of *T. solanivora* were challenged either with isolate 1346 or with isolate 1390-2, no significant difference in pathogenicity was noticed between *T. solanivora* populations originating from Costa Rica or Ecuador (Table 2). It thus appeared that the LC_{50} of the isolates tested were constant in *T. solanivora* whatever populations were considered. However, in these cases the slopes were unequal, indicating that the two populations reacted differently, although this could also be due to the higher than usual heterogeneity between replicates.

Comparative pathogenicity of viral isolates JLZ9f and 1346 in *Phthorimaea operculella* and *Tecia solanivora*

The pathogenicity of each isolate appeared to be significantly different for each host species (Table 3). Two well-split groups of virus isolates were found, as the 95% confidence intervals of relative potencies did not include 1; so it was assumed that the LC_{50} for the two species were significantly different (Robertson & Preisler, 1992). The first group (1390-2, PhopGVCR1, and 1346) was more pathogenic for *P. operculella* than for *T. solanovira* larvae, the relative potency ranged from 0.14 to 0.28. For GV003 and JLZ9f, the LC_{50} was lower (i.e., the isolate exhibited higher pathogenicity) in *T. solanivora* than in *P. operculella* larvae. This resulted in relative potencies of 3.55 (GV003) and 2.47 (JLZ9f), which were very different from the results obtained for the other isolates tested. With the exception of PhopGVCR1 (whose original host could have been wrongly identified), all isolates were more pathogenic to their original host than to the secondary one.

Genotypic differences between JLZ9f and 1346

Identification of variable zones in PhopGV genome. Purified genomic DNA of isolates 1346 (reference isolate whose complete sequence has been published) and JLZ9f, individually cut by 11 restriction enzymes, exhibited clear differences in their respective restriction pattern (Figure 1, Table 4). This was also the case for 1390-2 and 1390-3 (data not shown). Using the published nucleotide sequence from isolate 1346, it was found that most of the variation in JLZ9f concentrated within three particular zones rather than being scattered all over the genome. These variable zones were located in gene *129* (as previously reported in Carpio et al., 2012) and open reading frame (ORF) 46 and in the repeated region ORF 9. For isolates 1390.2 and 1390.3, only gene *129* (and not ORFs 46 and 84) differed in size from those of 1346. Because JLZ9f exhibited more differences than the other isolates tested when compared to the reference isolate 1346, it was chosen for a deeper characterization.

Table 2 Medium lethal concentrations (LC_{50}) and fit of probit lines of *Phthorimaea operculella granulovirus* (PhopGV) isolates applied to neonates of distinct geographic populations of *Phthorimaea operculella* and *Tecia solanivora*

					Probit line			
Host	Viral isolate	Host origin (name)	n	LC_{50}	Slope ± SE	χ^2 (d.f.)	P	95% confidence range
P. operculella	1346	Costa Rica (Pop-CR)	347	17.7[1]	0.459 ± 0.082	0.416 (2)	>0.1	6.1–43.0
		Egypt (Pop-EG)	451	16.0[2]	0.598 ± 0.080	0.063 (2)	>0.1	7.0–29.2
	1390-2	Costa Rica (Pop-CR)	382	2.1	0.505 ± 0.050	0.121 (2)	>0.1	1.0–3.7
		Egypt (Pop-EG)	630	1.6	0.504 ± 0.052	0.231 (3)	>0.1	0.7–3.0
	1390-3	Costa Rica (Pop-CR)	413	16.3	0.394 ± 0.038	2.852 (3)	>0.1	8.2–29.7
		Egypt (Pop-EG)	630	14.4[1]	0.417 ± 0.047	0.452 (3)	>0.1	7.1–28.7
T. solanivora	1346	Costa Rica (Tsol-CR)	413	46.2[1]	0.492 ± 0.055	2.290 (3)	>0.1	22.1–90.4
		Ecuador (Tsol-EC)	454	46.7	0.820 ± 0.069	3.291 (7)	>0.1	31.4–71.7
	1390-2	Costa Rica (Tsol-CR)	245	12.6	0.542 ± 0.052	9.667 (3)	<0.05	1.5–44.3
		Ecuador (Tsol-EC)	167	13.0	0.887 ± 0.074	4.29 (1)	<0.05	1.0–30.0

n, number of larvae assayed. The value of LC_{50} is expressed in number of virus occlusion bodies mm^{-2}. Using Polo Plus software (LeOra Software, 1987) 95% confidence ranges were determined.
Recalculated values using raw data from [1]Gómez-Bonilla et al. (2011) and [2]Carrera et al. (2008).

Table 3 Comparison of the medium lethal concentrations (LC_{50}), expressed as number of virus occlusion bodies mm^{-2}, of *Phthorimaea operculella granulovirus* (PhopGV) isolates applied to neonates of *Phthorimaea operculella* (Pop) and *Tecia solanivora* (Tsol)

Viral isolate (original host species)	Host population name	LC_{50}	Relative potency (Pop/Tsol)	95% confidence interval (Pop/Tsol)
1346 (Pop)	Pop-EG	16.0[1]	0.28	0.095–0.845
	Tsol-CR	46.2[2]		
1390-2 (Pop)	Pop-EG	1.6	0.14	0.045–0.422
	Tsol-CR	12.6		
GV003 (Tsol)	Pop-CO	24.7[3]	3.55	1.868–6.728
	Tsol-CO	6.9[3]		
PhopGVCR1 (Tsol)	Pop-CR	17.7[2]	0.25	0.131–0.478
	Tsol-CR	69.1[2]		
	Pop-EG	16.9[2]	0.24	0.119–0.473
	Tsol-CR	69.1		
JLZ9f (Tsol)	Pop-EG	31.8	2.47	1.139–5.361
	Tsol-EC	10.0[4]		

Using Polo Plus software (LeOra Software, 1987) 95% confidence intervals of relative potency were determined. Intervals which include 1 indicate no significant difference between virus isolates (Robertson & Preisler, 1992).
Data from [1]Carrera et al. (2008); [2]Gómez-Bonilla et al. (2011); [3]Espinel-Correal et al. (2010); [4]Carpio et al. (2012).

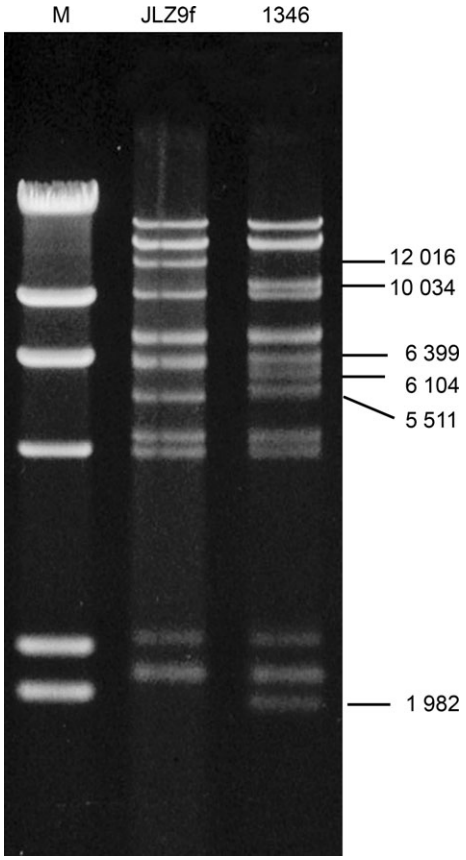

Figure 1 Restriction profiles of the genomes of two PhopGV isolates (JLZ9f and 1346) digested by *Nru*I, and a molecular weight marker (M) with bands of 23130, 9416, 6557, 4361, 2322, and 2027 bp. Molecular weights of bands only present in one of the PhopGV isolates are indicated on the right side.

Sequences of variable zones between JLZ9f and 1346. PCR amplifications of the JLZ9f genome, the most variable of the three isolates, were carried out using primers encompassing zones where variability was detected by RFLP. Sequences of amplicons were compared to those of isolate 1346. Within ORF 46 (located from nt 39303 to 41735 in 1346), the 30-nucleotide sequence GAATCAA CAAATCAGTCTATAGCTCAATCA starting at nt 41525 is repeated three times in a row in 1346 and only twice in JLZ9f (the three pentamers AATCA found in this sequence are underlined). The resulting predicted protein is thus 10 amino acids shorter than the one in 1346. The two deduced proteins align perfectly except at the level of the gap.

At the end of repeat 9 (from nt 72699 to 72829), JLZ9f has a 91 nt extra sequence compared to 1346: AAATACAAAACTTG**AAACAATTATT**AAACTTGAAAC AATTATT*CGTTTATTTTAA*AAATACAAAACTT**AAAC AATTATT***CGTTTATTTTAA***.**

Repeated motifs of this latter sequence are two 13-mers AAATACAAAACTT (underlined), two 11-mers AAACAA TTATT (bold), and two 12-mers CGTTTATTTTAA (italic). The putative protein encoded by the flanking ORF 84, which marginally overlaps with repeat 9, is not affected by the extra sequence present in JLZ9f.

Finally, within gene *129* (on the complementary strand from nt 116818 to 118122), an additional 86 nt sequence was found in JLZ9f, starting at nt 116861, but this sequence is missing in 1346 (Carpio et al., 2012): GTGTTTTCATTTTAAATCGCAACACCCGGTTTGTGT ACCAAATTGCGCGACGATAACTCTTCATTGGCAAA TCGTTTATCACTTGT. This modification in the

Table 4 Comparison of DNA banding patterns obtained for two isolates of *Phthorimaea operculella granulovirus* (PhopGV) after digestion of their genome by 11 restriction endonucleases. 1 = isolate 1346; 2 = isolate JLZ9f; all values in base pairs; FN, no. fragments; =, fragment of same size; '-', fragment not present

	*Sma*I		*Bam*HI		*Hind*III		*Nsi*I		*Nru*I		*Mlu*I		*Dra*III		*Nde*I		*Hpa*I		*Bst*EII		*Bst*ApI	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
A	74610	76250	29906	=	22634	=	23160	=	18349	=	20354	=	19485	=	23302	=	25448	=	23243	=	22736	=
B	26308	=	17766	=	14177	=	19681	=	14772	=	18078	=	16565	=	18456	14500	13198	=	17896	=	18509	=
C	10830	=	11561	=	10705	=	16857	=	14325	=	10707	=	14344	=	18165	=	13025	=	16610	=	14368	=
D	7469	5830	10577	10300	8969	8700	9925	=	10034	12016	10542	=	12533	=	9829	=	11563	=	12351	=	11466	=
E			10129	=	7248	=	6498	=	9333	=	9762	=	12448	=	9010	=	8285	=	12281	=	10606	=
F			8725	=	7126	=	6420	=	7210	=	6309	=	11449	=	8131	=	7597	7800	7668	=	10152	=
G			7792	=	6275	6100	4830	=	6996	=	5749	=	11356	=	6746	6600	6623	=	6590	=	9308	=
H			7789	=	5736	5900	4751	=	6399	6300	4676	=	8409	=	6463	=	4333	=	6218	6400	9297	=
I			6114	6400	4627	=	4439	=	6104	6250	4670	=	4168	4070	5994	=	4077	=	4614	4450	2978	=
J			2901	=	4020	=	4434	=	5511	5450	4381	=	3385	3433	5376	=	4061	=	4267	4325	2770	=
J'																4120						
K			2805	=	3644	=	3020	=	4541	=	4191	=	2494	=	3348	=	4008	=	3470	=	2434	2550
L			943	=	3474	=	2771	2925	4294	=	3620	3410	2096	=	1929	=	3584	=	1984	=	1800	1850
M			870	=	3298	3250	2094	=	2350	=	2953	=			1342	=	3566	3350	1869	=	955	=
N			712	=	3216	=	2084	=	2129	=	2929	=			1126	=	3284	=	166	=	874	=
O			626	=	2476	=	1886	=	2109	=	2848	=					2283	=			579	=
P					2072	=	1536	=	1982	'-'	2513	=					1294	=			265	=
Q					2036	=	1504	=	1067	=	1365	=					1003	=			107	=
R					1449	1500	1488	=	895	=	1174	=					456	=			9	=
S					1441	=	1343	=	627	=	722	=					351	=				
T					1266	=	456	=	180	=	620	=										
U					881	=					579	=										
V					812	=					475	650										
W					791	=																
X					384	=																
Y					324	=																
Z					89	=																
AA					47	=																
FN	4	4	15	15	27	27	20	20	20	19	22	22	12	12	14	15	19	19	14	14	18	18

sequence likely results in a change in the size of the translated protein due to the presence of an alternative stop codon. Sequence comparison indicated that the two variants of ORFs 84 and 129 present in JLZ9f give rise to larger proteins than in the case of 1346.

Discussion

As a recognized biocontrol agent, PhopGV was the object of numerous studies dealing with its structure and histopathology (Lacey et al., 2011), genomic characteristics (Vickers et al., 1991; Léry et al., 1998; Zeddam et al., 1999; Taha et al., 2000), and efficiency to limit host populations (Reed, 1971; Raman, 1994; Kroschel et al., 1996; Sporleder & Kroschel, 2008; Moura Mascarin et al., 2010). PhopGV-based biopesticides are used in different countries, mainly to control *P. operculella* larvae in potato storage. Recent studies carried out in Colombia, Ecuador, and Costa Rica have shown that PhopGV is also effective in controlling *T. solanivora* larvae (Espinel-Correal et al., 2010; Gómez-Bonilla et al., 2011). PhopGV is also pathogenic to the South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Angeles & Alcázar, 1995; Moura Mascarin et al., 2010; Carpio et al., 2012), which causes considerable damage to field- and greenhouse-grown tomatoes in various countries, including some recently invaded areas (Spain, Morocco, and Turkey) (Desneux et al., 2010). However, to our knowledge, the only study dealing with the comparative susceptibility of geographic populations of one of these hosts to PhopGV dates back to the 1980's (Briese & Mende, 1981).

The data presented in this article show that *P. operculella* populations from Costa Rica and Egypt did not differ significantly in their response to the PhopGV isolates tested. The same was found for *T. solanivora* populations from Costa Rica and Ecuador. These results were unexpected because of the large geographic distance separating the insect populations considered. They contrast with those of Briese & Mende (1981), who reported a more than 11-fold difference in susceptibility to a given PhopGV isolate among 16 Australian *P. operculella* populations, but they are in accordance with those from Asser-Kaiser et al. (2007), who found similar LC_{50}'s for susceptible *Cydia pomonella* (L.) populations when challenged with a Mexican isolate of *Cydia pomonella granulovirus* (CpGV-M). CpGV-M-resistant populations exhibited at least 1000-fold higher LC_{50}'s. Our results suggest that with the PhopGV isolates we tested, little variation in pathogenicity is to be expected when applied to populations of *P. operculella* or *T. solanivora*. More work involving other PTM populations and virus isolates is needed to confirm whether a single bioinsecticide formulation would exhibit the same efficacy over a large range of host populations. What could explain the discrepancy between our results and those obtained by Briese & Mende (1981)? The authors indicate that a PhopGV-resistance gene was present in Australian *P. operculella* populations; hence, the variability in susceptibility to PhopGV may be related to variability in presence of this gene among individuals.

That susceptibility to PhopGV of either *P. operculella* or *T. solanivora* did not differ among geographic populations of the pest species is of relevance for biological control purposes, allowing the development of standardized PhopGV-formulations to be used in all PTM-infected localities. Our laboratory data require further validation in potato storages all over PTM distribution areas. Emergence of resistance due to selection by repeated application of a given virus isolate cannot be excluded. Previous works have established that GV-resistant populations of the codling moth, *C. pomonella*, emerged following repetitive treatments using CpGV (Fritsch et al., 2005; Sauphanor et al., 2006). Resistance development in the case of PhopGV, especially considering that a PhopGV-resistance gene was identified in *P. operculella* (Briese & Mende, 1981), is a possibility. CpGV resistance of *C. pomonella* was broken by the use of other isolates (Eberle et al., 2008; Berling et al., 2009). Many PhopGV isolates are presently available (Vickers et al., 1991; Espinel-Correal et al., 2010; Moura Mascarin et al., 2010; Gómez-Bonilla et al., 2011; Carpio et al., 2012) and could represent a valuable resource for preventing resistance. It is thus important to compare their respective pathogenicity as well as to establish their genetic characteristics.

The relative potency of PhopGV toward *P. operculella* and *T. solanivora* larvae depended greatly on the isolates considered. One isolate (1390-2) exhibited a more than seven-fold higher LC_{50} in *P. operculella* than in *T. solanivora*, another (1346) only showed a three-fold increase, and two other isolates (GV003 and JLZ9f) were much more pathogenic to *T. solanivora* than to *P. operculella*. As these two isolates were originally collected from *T. solanivora* larvae found dead in the wild, a possible explanation for the difference in pathogenicity could be a better adaptation of the virus to its original host, as previously suggested (Espinel-Correal et al., 2010). However, this should be verified by testing more isolates. Because of the absence of a suitable artificial diet for the hosts, our bioassays relied on a medical compressor/nebulizer-based device which was standardized to evenly deposit known amounts of the virus onto the tuber skin (Carrera et al., 2008), allowing the comparison of results from different laboratories. This protocol differs from the one used by other authors (Kroschel et al., 1996; Sporleder et al., 2004, 2005, 2007; Sporleder & Kroschel, 2008; Moura Mascarin et al., 2010)

who contaminated tubers by submersion in virus solutions containing a known number of homogenized PhopGV-infected larvae. In the latter case, amounts of OBs retained at the potato surface were not quantified. Thus, it was not pertinent to compare the respective pathogenicity of virus isolates on the basis of LC_{50} values obtained using these different protocols.

With a genome of about 120 kbp (GenBank accession number AF499596.1), complete genome sequencing and analysis of PhopGV isolates would be both costly and time consuming. For this reason, identifying regions of higher variability within the genome of PhopGV could provide a fast and cost-effective way for characterizing the virus isolates. Preliminary assays indicated that three regions of the genome contained most of the variation in RFLP profiles of 11 isolates (data not shown). Those regions encompass part of ORF 46 (encoding for a hypothetical protein of unknown function), repeat 9, and gene *129* (encoding for the enzyme ecdysteroid UDP-glucosyltransferase; O'Reilly & Miller, 1989). Until now, only ORF 84 (Gómez-Bonilla et al., 2011) and gene *129* (Espinel-Correal et al., 2010; Carpio et al., 2012) were identified as variable in some PhopGV isolates, but ORF 46 or repeat 9 were not. Although the 3′ end of ORF 84 slightly overlaps with the 5′ end of repeat 9, the sequence variation we found does not modify the predicted ORF 84 product, which would then be the same for JLZ9f and 1346. A deeper analysis of the sequences published by Gómez-Bonilla et al. (2011) showed a similar pattern for their isolate PhopGV-CR1. Increasing the number of PhopGV isolates tested may lead to the identification of new variable regions, allowing a more precise discrimination between isolates. However, the three regions described here provide a useful tool for genotyping available PhopGV isolates at reasonable cost. Focusing on the screening of the virus variation in these regions allowed discrimination between isolates by sequencing a few PCR products of limited size. The method is simple, fast, and cost effective and might be used to monitor virus isolates used in biocontrol programs. From a practical point of view, results obtained here provide an interesting background for future studies dealing with the development of PhopGV-based biopesticides for the control of lepidopteran (gelechiid) pests.

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