JOURNAL OF APPLIED ENTOMOLOGY

J. Appl. Entomol.

ORIGINAL CONTRIBUTION

Costa Rican soils contain highly insecticidal granulovirus strains against *Phthorimaea operculella* and *Tecia solanivora*

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Keywords
betabaculovirus, bioinsecticide, persistence, PhopGV, potato tuberworm, soil isolates, viral host adaptation

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Received: April 15, 2011; accepted: September 8, 2011.

doi: 10.1111/j.1439-0418.2011.01679.x

Abstract

The aim of this work was to isolate and characterize novel *Phthorimaea operculella* granulovirus (PhopGV) strains from Costa Rican soils. Three novel strains, named PhopGV-CR3, PhopGV-CR4 and PhopGV-CR5, were isolated from three locations in Costa Rica, Alvarado, Zarcero and Abangares, respectively, by means of soaking potato tubers with diluted soil samples. An additional strain, PhopGV-CR2, was identified from diseased larvae from a *Tecia solanivora* laboratory culture. Restriction fragment length polymorphisms obtained for each isolate with six restriction endonucleases (RENs) allowed their identification as four distinct PhopGV strains. Both REN and Polymerase chain reaction analyses indicated the existence of an array of genotypic variants present in all isolates. Bioassays in *P. operculella* and *T. solanivora* showed that PhopGV-CR3 was well adapted to the two coexisting hosts with high levels of pathogenicity against both pest species. The mean lethal dose values of this strain were 2.8 OBs/mm^2 for *P. operculella* and less than 0.5 OBs/mm^2 for *T. solanivora*. We conclude that PhopGV-CR3 shows great promise for soil application against these pests in Costa Rican potato crops.

Introduction

The potato tuberworms, *Phthorimaea operculella* Zeller and *Tecia solanivora* Povolny (Lepidoptera: Gelechiidae), are the most devastating pests of potato in Central America (Vargas et al. 2004; Rondon 2010) and particularly in Costa Rica (Hilje 1994). In Costa Rica, *T. solanivora* is more abundant than *P. operculella*. Larvae of both these species cause serious damage in the field and in storage, where losses may be as high as 100% (Vargas et al. 2004; Rondon 2010). In the field, *P. operculella* females lay their eggs on the plant, usually on foliage throughout the growing season. Hatching larvae mine leaves, stems and petioles causing irregular galleries. Larvae attack tubers by excavating tunnels mainly under storage conditions (Rondon 2010). *T. solanivora* attacks potato tubers exclusively. In the field, females usually lay their eggs at the base of plant stems, and emerging larvae reach the tubers where they dig galleries that result in partial or complete tuber destruction (Niño 2004). Management of *P. operculella* and *T. solanivora* has been traditionally achieved using broad-spectrum chemical insecticides applied at weekly intervals (Bekheit et al. 1997; Niño 2004; Rondon 2010), which has prompted highly resistant biotypes of both tuber moth species and adverse effects on beneficials and the environment (Shelton et al. 1981; Arévalo and Castro 2003; Dogramaci and Tingey 2008).

Baculoviruses (Baculoviridae) are environment-friendly biocontrol agents (BCA) that are compatible with beneficial organisms and that represent a useful

alternative to chemicals for pest control in an increasing number of plant-pest systems (Moscardi 1999; Lasa et al. 2007; Berling et al. 2009). This is due, among other characteristics, to their high persistence in the environment when protected from UV light (Fuxa and Richter 2001; Christian et al. 2006). In this respect, baculoviruses stand out as promising control agents in the context of potato crop protection. They can be applied onto the ground, which shields viral occlusion bodies from UV light and where direct damage occurs on developing tubers. In addition, most baculovirus species have a high degree of intraspecific diversity, which renders them adaptable to unstable habitats like agroecosystems (Cory et al. 1997, 2005; Muñoz and Caballero 2001; Hogdson et al. 2004). Such heterogeneity is manifested, for instance, by the existence of geographical strains and genotypic variants with differing levels of pathogenicity against their hosts (Léry et al. 1998; Hogdson et al. 2004; Cory et al. 2005; Murillo et al. 2006, 2007). Several baculovirus isolates from different regions of the world have been isolated from *P. operculella* or *T. solanivora* (Setiawati et al. 1999; Niño and Notz 2000; Zeddam et al. 2003; Espinel-Correal et al. 2010; Gómez-Bonilla et al. 2011a). However, only a handful of these have proven highly pathogenic against both *P. operculella* and *T. solanivora* (Espinel-Correal et al. 2010; Gómez-Bonilla et al. 2011a,b). All of them belong to the *Betabaculovirus* genus (formerly *Granulovirus* genus or GVs), and all have been isolated from infected larvae. So far, no soil GV isolates of *P. operculella* and *T. solanivora* have been studied. Soil-derived isolates will probably contain higher numbers of soil-adapted genotypes than field-collected isolates from dead insects and may constitute ideal strains for pest control in potato crops.

Baculovirus soil isolates can be obtained by incorporating soil samples into insect-rearing diets (Richards and Christian 1999; Murillo et al. 2006). In this study, we have slightly modified these techniques for isolation of *P. operculella* granuloviruses (PhopGVs) from Costa Rican soil samples with the aim of selecting those with high pathogenicity and virulence against both *T. solanivora* and *P. operculella.*

Materials and Methods

Insect rearing

The populations of *Phthorimaea operculella* and *Tecia solanivora* originally came from Costa Rica and were maintained under constant environmental conditions: 27°C temperature, 60% relative humidity and 16 : 8 (light/dark) photoperiod at the Research Center Carlos Durán (Cartago, Costa Rica). Larvae were fed on potato tubers, which had been previously treated with hypochlorite solution. Adults were fed honey or 30% (wt/vol) sugar.

Collection of virus samples and amplification

Single soil samples taken from depths of 0–30 cm were obtained from four different sites in Costa Rica: Alvarado and Oreamuno (Cartago), Zarcero (Alajuela) and Abangares (Guanacaste), located at 2320, 1870, 2140 and 1100 m altitude, respectively. Alvarado, Oreamuno and Zarcero recorded potato damage by both *P. operculella* and *T. solanivora* during the last decade.

However, given its low altitude, Abangares has not traditionally been a site for the production of potato crops, and no pest outbreaks have been recorded to date for either of the insect species in this region, where new potato varieties better adapted to higher temperatures are now being cultivated.

The sample from Alvarado was 1 kg of vermicompost prepared 2 years before with virgin soil taken from close to a nearby volcano (Irazú). The sample from Oreamuno was 1 kg of soil taken from the edge of a potato field. In this location of intensive agriculture, tuber moths and other pests are controlled with repeated applications of chemical pesticides, and potato crops are rotated with onion, brassica and carrot crops every year. The low soil pH resulting from the use of certain chemical pesticides in this region is corrected by application of quicklime. Samples from Zarcero and Abangares consisted of 1 kg soil from fields that had remained uncultivated for several years. For each soil sample, 100 g of soil was mixed with 100 ml bidistilled water. Five potatoes were then soaked in these suspensions for 30 min, air dried, placed in individual plastic boxes, infested with 20 neonate *T. solanivora* each and incubated at 27°C for 25 days. At least one larva with symptoms of viral infection was obtained from all samples.

An additional virus sample was collected from more than 200 *T. solanivora* larvae with symptoms of granulovirus infection at the insect-rearing facilities of the Research Station Carlos Durán (2440 m altitude) (Cartago) in 2007. As soon as the virus was detected, the chamber was cleaned and quarantined before rearing was resumed.

For amplification of viral isolates, infected larvae from both species were individually homogenized in

1 ml bidistilled water and used to contaminate four tubers at a concentration of 2×10^9 OBs/ml. The OB concentrations were determined with a spectrophotometer and calculated using the method described by Zeddam et al. (2003), based on the equation: OBs/ml = $6.8 \times 10^9 \times$ OD450 $\times$ dilution factor. Each tuber was then infested with 25 neonate larvae and incubated for 25 days. The resulting infected larvae were collected and used again for one further amplification round. Finally, infected larvae were stored at −20°C.

PhopGV-CR1, a Costa Rican isolate that had been characterized previously (Gómez-Bonilla et al. 2011a), was used as a reference. PhopGV-1346 is an isolate from Tunisia kindly provided by Dr. El Bedewi, IPC Egypt, and was multiplied in Egypt for several generations. It is considered the type strain and was used as a reference for biological analyses. PhopGV-4.2 is a purified genotypic variant obtained by plaque purification from the PhopGV-1346 isolate (Vickers et al. 1991; Léry et al. 1998). The entire genome of PhopGV-4.2, of 119217 bp, has been sequenced (GeneBank NC004062) (INRA/CNRS/Université de Montpellier II, Saint Christol les Ales, France) and used as a reference for molecular analysis.

Purification of occlusion bodies (OBs) and titration of OB suspensions

To purify OBs, ca. 50 larval cadavers were collected and homogenized in 0.01 M Tris–HCl pH 7.5 using a Potter-Elvehjem homogenizer (USA) and centrifuged at 664 ***g*** for 5 min at 4°C. The pellet was discarded, and supernatant-containing OBs were centrifuged at 15 000 ***g*** for 20 min, and pellets resuspended in 1 ml bidistilled water and then placed on a discontinuous 30%–70% (w/v) sucrose gradient and centrifuged at 30 000 ***g*** for 120 min. Granules were resuspended in 1× TE buffer (0.1 M Tris–HCl, pH 7.5 and 10 mM EDTA, pH 8.0) and stored at −20°C. OB concentration was estimated using the spectrophotometer-based method described by Zeddam et al. (2003).

DNA extraction and restriction endonuclease (REN) analysis

Purified OB suspensions were incubated with 1/10 vol 2 M Na_2CO_3. DNA was extracted with phenol/chloroform and then precipitated with ethanol, as described by Muñoz et al. (1997). Between 0.5 and 1 μg of viral DNA was incubated with 10 units of restriction enzymes *Sma* I, *Bam*HI, *Hin*dIII, *Nru*I, *Mlu*I, *Nde*I, *Dra*III, *BstE*II, at the conditions specified by the supplier. Reactions were stopped by addition of loading buffer (0.25% bromophenol blue, 40% w/v sucrose in water), loaded in 1% agarose gels with TAE buffer (40 mm Tris–acetate, 1 mM EDTA, pH 8.0) and electrophoresed at 100 V for 2 h. Ethidium bromide-stained gels were then photographed on a UV transilluminator. REN analysis was performed using PhopGV-CR1 as a reference. *In silico* restriction digestion was performed on the PhohpGV-4.2 genotype sequence using the Clone Manager 5 Software (Sci-Ed Central). REN fragments that were not present in all isolates or in PhopGV-4.2 were considered restriction fragment length polymorphisms (RFLPs). Also, RFLPs absent in PhopGV-4.2 were considered novel, and the nomenclature used to designate them was the same as that used previously for another Costa Rican isolate (Gómez-Bonilla et al. 2011a). Specifically, the same RFLP nomenclature of PhopGV-4.2 was employed for the novel restriction fragments migrating immediately below those of PhopGV-4.2, but lower-case letters followed by a number were used instead of the upper-case lettering used for the sequenced genotype REN fragments.

Polymerase chain reaction (PCR)

Amplification of four different PhopGV variable genomic regions (within ORFs 46, 109 and 129 and a region encompassing ORFs 83 and 84) was carried out using different primers (Gómez-Bonilla et al. 2011a). PCRs were carried out in a total volume of 25 μl, containing 0.75–2.5 ng of DNA, 1 pmol of each primer, 3.5 mM $MgCl_2$ and 0.5 vol of a mixture prepared by the supplier containing dNTP's and *Taq* polymerase (Promega, Charbonnieres, France). A sample containing all PCR components except virus DNA was used as a negative control. Amplifications were carried out in a thermocycler under the following conditions: a first cycle of 94°C/4 min, followed by 30 cycles of 94°C/1 min, 50°C/1 min and 72°C/1 min, and a final cycle of 72°C/5 min. The PCR products (amplicons) were subjected to electrophoresis in 2% agarose gels at 150 v for 105 min.

Bioassays

Bioassays to determine the median lethal concentration (LC_{50}) values of all viral isolates on Costa Rican *P. operculella* and *T. solanivora* populations were carried out with neonate larvae (L1). Five different virus concentrations (final concentrations of 0.5, 5,

50, 500 and 5000 OBs/mm^2 on the surface of the potato tuber) were prepared by means of a nebulizer apparatus from purified OB solutions as previously described (Carrera et al. 2008). Two tubers were used for each concentration, twelve larvae were placed on each tuber, and tubers were incubated at 27°C for 25 days. The number of dead and infected (white) larvae, outside the tubers, was recorded daily. At day 25, tubers were opened, and dead or infected larvae remaining inside the galleries were also registered. Each bioassay was replicated four times. Regression lines (relating log-dose and mortality) and LC_{50} values were calculated by Probit analysis (Finney 1971) using Polo PC (LeOra Software, 1987). To compare the LC50 values, the broadly used analysis described by Robertson and Preisler (1992) was followed. When parallelism between regression lines could be assumed to occur, then the relative potencies (LC_{50} ratios) and their corresponding 95% fiducial limits were estimated by constraining the slopes of all response lines to be the same. If the lines could not be considered parallel, the LC50 ratio was calculated and then their 95% confidence limits compared.

Results

Isolation of Costa Rican granuloviruses from soil

GVs were isolated in three of the four soil samples collected. In the samples from Alvarado, Zarcero and Abangares, 47, 4 and 1% of *T. solanivora* larvae present on potatoes contaminated with the diluted soil samples died, respectively. No larvae died on potatoes soaked with the soil sample from Oreamuno. Mortality derived from other entomopathogenic organisms such as fungal spores, nematode larvae or *Bacillus thuringiensis* was not observed in larvae that developed on soil-contaminated diet.

Characterization of Costa Rican granulovirus isolates

The three soil isolates and a fourth one obtained from diseased *T. solanivora* larvae could be differentiated at the molecular level by size differences in genomic restriction fragments (RFLPs) obtained with *Bam*HI, *Nde*I, *Sma*I, *Nru*I, *Mlu*I and *Bst*EII. This allowed their identification as four distinct PhopGV strains (table 1) that were named PhopGV-CR2 for the isolate collected from dead larvae in the Carlos Durán Research Centre, and PhopGV-CR3, PhopGV-CR4 and PhopGV-CR5 for the soil isolates from Alvarado, Zarcero and Abangares, respectively.

Table 1 Restriction fragment length polymorphic (RFLP) fragments observed between the reference genotype PhopGV-4.2 (4.2) and the Costa Rican isolates PhopGV-CR1 (CR1), PhopGV-CR2 (CR2), PhopGV-CR3 (CR3), PhopGV-CR4 (CR4) and PhopGV-CR5 (CR5) with *Bam*HI, *Nde*I, *Sma*I, *Nru*I, *Mlu*I and *Bst*EII

RFLPs (molecular weight)	4.2	CR1	CR2	CR3	CR4	CR5
*Bam*HI-I (6114)	+	+*	+*	+*	+*	+
***Bam*HI-i1**[1] (ca. 6000)	−	+	+	+	+	−
***Nde*I-c1** (ca. 14500)	−	+	−	+*	+	−
*Nde*I-G (6746)	+	−	−	−	−	−
***Nde*I-g1** (ca. 6500)	−	+	+	+	+	+
*Nde*I-I (5994)	+	+	+*	+*	+*	+
***Nde*I-j1** (ca. 5300)	−	+*	−	−	+*	−
*Sma*I-D (7469)	+	+*	+*	+*	−	+*
***Sma*I-d1** (ca. 5500)	−	−	−	+*	−	−
***Nru*I-h1** (ca. 6000)	−	+	−	−	+*	+
*Nru*I-I (6104)	+	−	+	+	+*	−
***Mlu*I-b1** (ca. 5500)	−	+*	+*	+*	+*	+*
*Mlu*I-L (3620)	+	+	−	−	−	−
***Mlu*I-l1** (ca. 3500)	−	−	+	+	+	+
***Bst*EII-g1** (ca. 6400)	−	+	−	−	+*	+
*Bst*EII-H (6218)	+	−	+	+	+*	−
*Bst*EII-I (4614)	+	+	−	−	−	−
***Bst*EII-i1** (ca. 4500)	−	−	+	+	+	+
*Bst*EII-J (4267)	+	+	−	−	−	−
***Bst*EII-j1** (ca. 4300)	−	−	+*	+	+	+

[1]In bold, RFLPs obtained from the Costa Rican isolates that are absent in the reference PhopGV-4.2. These were named using the restriction endonuclease fragment name of PhopGV-4.2 which migrated closest above it in lower case letters followed by a number. Their molecular weight is approximate (ca.). Asterisks indicate fragments present in submolar concentrations.

The novel PhopGV strains could be differentiated between them and from the sequenced genotype (PhopGV-4.2) and the reference strain (PhopGV-CR1) using *Bst*II, except PhopGV-CR2 and PhopGV-CR3, which displayed identical profiles with this enzyme (table 1). Only two fragments allowed their identification as distinct strains: *Nde*I-c1 (fig. 1) and *Sma*I-d1. All four strains shared the following six RFLPs: *Mlu*I-L (3620 bp), *Mlu*I-l1 (3410 bp), *Bst*EII-I (4614 bp), *Bst*EII-i1 (4450 bp), *Bst*EII-J (4267 bp) and *Bst*EII-j1 (4325 bp) (table 1). Submolar concentrations were observed in digestions with several endonucleases for all Costa Rican isolates (fig. 1 and table 1). REN profiles were identical for all isolates following treatment with *Hin*dIII or *Dra*III.

Primers within ORFs 46, 109 and within a region encompassing ORFs 83 and 84 yielded a single amplicon of the same size (1200, 1000 and 250 bp, respectively) for all strains tested, including the reference PhopGV-CR1. Primers within ORF 129 produced a total of six different amplicons in the

Costa Rican strains (fig. 2, table 2). According to Espinel-Correal et al. (2010), each ORF 129 amplicon denotes the presence of a genotypic variant and they designated *egt* region types 1–5 to amplicons of 937, 723, 1023, 569 and 869 bp, respectively. Each PhopGV strain comprised between 2 and 4 of these *egt* region types except for PhopGV-CR5, which had only a type 1 amplicon plus an additional amplicon of 783 bp that had not been described so far and which was designated here as *egt* region type 6 (table 2).

Insecticidal activity of the Costa Rican granulovirus strains

Bioassays in *P. operculella* indicated that the newly isolated Costa Rican PhopGVs, as well as the laboratory isolated strain, PhopGV-CR2, were all as pathogenic as the reference strains PhopGV-1346 and PhopGV-CR1, with LC_{50} values statistically similar to those of these strains (table 3). In *T. solanivora*, isolates PhopGV-CR3 and PhopGV-CR2 killed more than 50% of the larvae at the lowest (0.5 OBs/mm^2) and second lowest (5 OBs/mm^2) concentrations, respectively, indicating a high pathogenicity of both these strains against *T. solanivora*. The LC_{50} values of the rest of the soil isolates, PhopGV-CR4 and PhopGV-CR5, were statistically similar to those of PhopGV-1346 and PhopGV-CR1 (table 3). For all virus treatments in both species, mortality increased with increasing OB concentration. A common slope for concentration–mortality could be fitted for *T. solanivora* but not for *P. operculella*. No virus mortality was registered in mock-infected control insects.

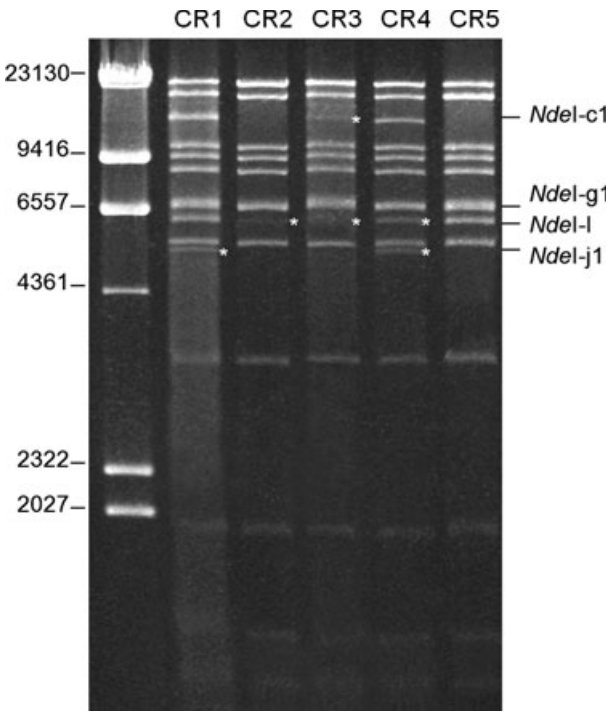

Fig. 1 *Nde*I restriction endonuclease profiles of the genomic DNA of PhopGV-CR1 (CR1), PhopGV-CR2 (CR2), PhopGV-CR3 (CR3), PhopGV-CR4 (CR4) and PhopGV-CR5 (CR5). *Nde*I restriction fragment length polymorphisms are shown at the right of the panel. Asterisks indicate submolar fragments. Reference molecular size fragments are indicated to the left of the panel.

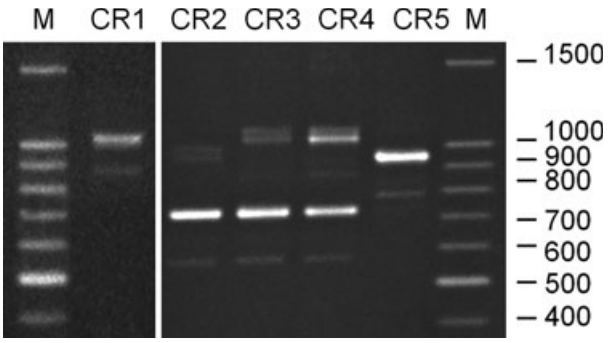

Fig. 2 PCR analysis of ORF 129 from PhopGV-CR1 (CR1), PhopGV-CR2 (CR2), PhopGV-CR3 (CR3), PhopGV-CR4 (CR4) and PhopV-CR5 (CR5). A DNA ladder ranging from 1.5 to 0.1 kb was used as a molecular size marker (M), and its fragment sizes are indicated to the right of the panel.

Discussion

Efficient protection of potato tubers against *P. operculella* and *T. solanivora* in the field may be achieved by soil application of baculovirus-based bioinsecticides (Gómez-Bonilla et al. 2011b). Given that high levels of diversity occur within baculovirus populations, the existence of viral strains or genotypes well adapted to soil habitats and, at the same time, with elevated levels of pathogenicity for both pest species was hypothesized. In this study, three soil PhopGV

Table 2 PCR amplicons (bp) of ORF 129 and their corresponding *egt* region types yielded by PhopGV-CR1 (CR1), PhopGV-CR2 (CR2), PhopGV-CR3 (CR3), PhopGV-CR4 (CR4) and PhopGV-CR5 (CR5)

Amplicon size (bp)	*egt* region type	CR1	CR2	CR3	CR4	CR5
1023	3	+	−	+	+	−
937	1	−	+	−	−	+
869	5	+	−	+	+	−
783	6	−	−	−	−	+
723	2	−	+	+	+	−
569	4	−	+	+	+	−

Table 3 LC_{50} values of viral strains PhopGV-1346 (1346), PhopGVCR-1 (CR1), PhopGVCR-2 (CR2), PhopGVCR-3 (CR3), PhopGVCR-4 (CR4), PhopGV-CR5 (CR5) for *Phthorimaea operculella* and *Tecia solanivora*

Host	Virus strain	Regression line	LC50 (OBs/mm^2)	Relative potency	Fiducial limits	
					Lower	Upper
P. operculella	1346	0.50x + 4.50	7.0	1	–	–
	CR1	0.61x + 4.24	14.0	0.50	0.07	3.32
	CR2	0.28x + 4.60	32.2	0.22	0.04	1.15
	CR3	0.26x + 4.89	9.5	0.73	0.12	4.52
	CR4	0.56x + 4.37	10.5	0.67	0.13	2.58
	CR5	0.35x + 4.58	18.8	0.37	0.07	1.97
T. solanivora	1346	0.44x + 4.41	23.1	1	–	–
	CR1	0.44x + 4.21	65.8	0.35	0.11	1.08
	CR4	0.44x + 4.22	60.6	0.38	0.12	1.15
	CR5	0.44x + 4.62	7.5	3.07	0.98	9.71

strains were identified at the molecular level and their performance as BCAs evaluated in terms of OB pathogenicity.

Several baculovirus strains of *Helicoverpa armigera* single nucleopolyhedrovirus (HaSNPV) or *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) have been previously isolated from soil samples by incorporating soil samples into the artificial diets used to rear insect hosts (Richards and Christian 1999; Murillo et al. 2006). As we lacked artificial diets for *P. operculella* or *T. solanivora*, we had to modify this technique slightly by soaking potato tubers in aqueous soil suspensions. With this technique, we were able to detect PhopGVs in three of the four Costa Rican soil samples analysed, from Alvarado, Zarcero and Abangares.

Overall, 47% of larvae treated with Alvarado vermicompost soil died, indicating a high prevalence of virus in this sample. Considering this soil preparation had not been used for cultivation before, the presence of active virus may seem surprising. However, it was kept next to potato crops and warehouses with stored potatoes, and therefore next to pest populations from which the vermicompost may have acquired the virus naturally. Dispersal of OBs by abiotic agents such as air currents, agronomic practices and rain is crucial for the transition between non-transmissible reservoir populations and ephemeral but transmissible subpopulations (Murillo et al. 2007). The presence of virus in the other two soil samples is more noteworthy. Although GV prevalence in the sample from Zarcero was much lower (4%) than in Alvarado, this sample came from a pasture ground that had remained uncultivated for 10 years, and the closest solanaceum crops were located at a distance of ca. 1 km. The amount of GV OBs in the sample from Abangares was even lower (1%). The lower altitude and higher temperatures of this location are unfavourable conditions for *T. solanivora* or *P. operculella* outbreaks, and potato cultivation was initiated in Abangares only in the last decade using low-altitude varieties. The presence of active GVs in Abangares and Zarcero, although tiny, constitutes further evidence of the long persistence of OBs in the soil in the absence of the insect host, as has been observed previously for other baculoviruses (De Moraes et al. 1999; Murillo et al. 2007). In any case, high persistence in the uppermost soil layers is favoured by the affinity of OBs to soil particles, especially to the clay components, to which most OBs remain strongly bound (Hukuhara and Namura 1972; Jaques 1985; Fuxa and Richter 2001).

The lack of PhopGV strains from Oreamuno, where the incidence of tuberworms is continuous, was unexpected. However, the presence of entomopathogens is sometimes higher in uncultivated than in cultivated soils (Martin and Travers 1989). The presence/absence of 20 RFLP fragments, obtained from six different restriction endonucleases (table 1), allowed differentiation of these strains. Genomic changes such as point mutations, deletions, transpositions or recombinations usually account for the observed polymorphisms (Jehle 1996; Muñoz et al. 1997; Simón et al. 2004). Based on these analyses, each soil isolate was identified as a novel strain (PhopGV-CR3, PhopGV-CR4 and PhopGV-CR5). These three constitute the very first PhopGV strains obtained and identified from soils. An additional strain, PhopGV-CR2, was identified from diseased larvae from a *T. solanivora* colony maintained at the Research Station Carlos Durán.

The high OB pathogenicity values of PhopGV-CR3 against *T. solanivora* suggested that this strain may have originated from a viral population that is well adapted to *T. solanivora*. However, PhopGV-CR3 also showed high pathogenicity against *P. operculella*, indicating that it is adaptated to both coexisting

hosts. Other PhopGV strains have been reported to be well adapted to *T. solanivora*, *P. operculella* or to both host species apparently after having had previous contact with one or both hosts (Espinel-Correal et al. 2010; Gómez-Bonilla et al. 2011a,b). This phenomenon has also been observed in NPVs (Ribeiro et al. 1997; Hogdson et al. 2004) and is probably explained by viral intraspecific heterogeneity, which renders viral populations more adaptable to varying environmental conditions (Muñoz and Caballero 2001), including different hosts or different haplotypes of the same host. In this respect, a recent study revealed a high diversity of *T. solanivora* haplotypes in Central America (Torres-Leguizamón et al. 2011). Indeed, the presence of multiple genotypic variants in a single population may include genotypes more specialized in infecting particular hosts (species and haplotypes), or even coexisting hosts, as occurs with *Pannolis flammea* multiple NPV (Hogdson et al. 2004). Accordingly, all Costa Rican isolates contained mixtures of genotypes, as indicated by the presence of submolar bands in the REN profiles and multiple amplicons in the ORF129 PCR profiles.

A survey of PhopGV isolates recently performed in Colombia collected diseased *T. solanivora* larvae along the invasion pathway of this insect (Espinel-Correal et al. 2010). These isolates contained mixtures of genotypic variants well adapted for replication in both *T. solanivora* and *P. operculella*. All Costa Rican strains shared many of the RFLPs and *egt* region types identified in the Colombian isolates, which strongly suggests that PhopGV-CR2, PhopGV-CR4 and PhopGV-CR5 have the potential to adapt to coexisting hosts when exposed to both of them. In fact, previous studies with the reference Costa Rican strain, PhopGV-CR1, resulted in a fivefold increase in OB pathogenicity against *T. solanivora* after only three passages in this host (Gómez-Bonilla et al. 2011a). Finally, these isolates may also be useful for the control of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), another pest of many solanaceous crops, as reported recently for a Brazilian PhopGV strain (Mascarin et al. 2010). In summary, all novel Costa Rican PhopGV strains seem likely to be good candidates for soil application against *P. operculella* and *T. solanivora*, in particular PhopGV-CR3, as suggested by their soil origin, persistence, potential for host adaptability and high OB pathogenicity.

Acknowledgements

Yannery Gómez-Bonilla was the recipient of scholarships from the Spanish National Institute of Agricultural Research (INIA), the Costa Rican Ministry of Science and Technology (MICIT- CONICIT) and INTA. We are very grateful to Trevor Williams for his help with the English and the editing of this paper as well as for his constant support.

References

Arévalo A, Castro R, 2003. Evaluación post-registro de los insecticidas con licencia de uso para controlar la polilla guatemalteca de la papa *Tecia solanivora* (Povolny, 1973) (Lepidoptera: Gelechiidae) en Colombia. In: Memorias II Taller Nacional, *Tecia solanivora*. CEVIPAPA, Centro Nacional de Productiva, Bogotá, Colombia, 86–89.

Bekheit HKM, Moawad GM, El-Bedawy RA, Mabrouk MA, El-Halim SM, Mahgoub MM, 1997. Control of the potato tuber moth, *Phthorimaea operculella* (Zeller), in potato crop. Egypt. J. Agric. Res. 75, 923–938.

Berling M, Blachere-Lopez C, Soubabere O, Lery X, Bonhomme A, Sauphanor B, Lopez-Ferber M, 2009. *Cydia pomonella* granulovirus genotypes overcome virus resistance in the codling moth and improve virus efficiency by selection against resistant hosts. Appl. Environ. Microbiol. 75, 925–930.

Carrera MV, Zeddam JL, Pollet A, Léry X, López-Ferber M, 2008. Evaluation of *per os* insecticidal activity of baculoviruses by a nebulization method. IOBC/wprs Bull. 31, 40–43.

Christian PD, Richards AR, Williams T, 2006. Differential adsorption of occluded and nonoccluded insect-pathogenic viruses to soil-forming minerals. Appl. Environ. Microbiol. 72, 4648–4652.

Cory JS, Hails RS, Sait SM, 1997. Baculovirus ecology. In: The Baculoviruses. Ed. by Miller LK, Plenum Press, New York, 301–340.

Cory JS, Green BM, Paul RK, Hunter-Fujita F, 2005. Genotypic and phenotypic diversity of a baculovirus population within an individual insect host. J. Invertebr. Pathol. 89, 101–111.

De Moraes RR, Maruniak JE, Funderburk JE, 1999. Methods for detection of *Anticarsia gemmatalis* nucleopolyhedrovirus DNA in soil. Appl. Environ. Microbiol. 65, 2307–2311.

Dogramaci M, Tingey WM, 2008. Comparison of insecticide resistance in a North American field population and a laboratory colony of potato tuberworm (Lepidoptera: Gelechiidae). J. Pest. Sci. 81, 17–22.

Espinel-Correal C, Léry X, Villamizar L, Gómez J, Zeddam JL, Cotes AM, López-Ferber M, 2010. Genetic and biological analysis of Colombian *Phthorimaea operculella g*ranulovirus isolated from *Tecia solanivora* (Lepidoptera: Gelechiidae). Appl. Environ. Microbiol. 76, 7617–7625.

Finney DJ, 1971. Probit analysis. Cambridge University Press, London, UK.

Fuxa JR, Richter AR, 2001. Quantification of soil-to-plant transport of recombinant nucleopolyhedrovirus: effects of soil type and moisture, air currents, and precipitation. Appl. Environ. Microbiol. 67, 5166–5170.

Gómez-Bonilla Y, Léry X, López-Ferber M, Caballero P, Muñoz D, 2011a. Characterization of a Costa Rican granulovirus strain highly pathogenic against its indigenous hosts, *Phthorimaea operculella* and *Tecia solanivora*. Entomol. Exp. Appl. 140, 238–246.

Gómez-Bonilla Y, López-Ferber M, Caballero P, Muñoz D, 2011b. Potato crops in Costa Rica are efficiently protected from *Phthorimaea operculella* and *Tecia solanivora* by an indigenous granulovirus strain. IOBC/wprs Bull. 66, 387–390.

Hilje L, 1994. Caracterización del daño de las polillas de la papa *Tecia solanivora* y *Phthorimaea operculella* (Lepidoptera:Gelechiidae), en Cartago, Costa Rica. Revman. Integr. Plagas 31, 43–46.

Hogdson DJ, Hitchman RB, Vanbergen AJ, Hails RS, Possee RD, Cory JS, 2004. Host ecology determines the relative fitness of virus genotypes in mixed-genotype nucleopolyhedrovirus infections. J. Evol. Biol. 17, 1018–1025.

Hukuhara T, Namura H, 1972. Distribution of a nuclear polyhedrosis virus of the fall webworm, *Hyphantria cunea*, in soil. J. Invertebr. Pathol. 19, 308–316.

Jaques R, 1985. Stability of viruses in the environment. In: Viral insecticides for biological control. Ed. by Sherman KE, Academic Press, Orlando, FL, 285–359.

Jehle JA, 1996. Transmission of insect transposons into baculovirus genomes: an unusual host-pathogen interaction. In: Transgenic organisms-biological and social implications. Ed. by Tomiuk L, Wohrmann K, Sentker A, Birkhauser Verlag, Basel, Switzerland, pp. 81–97.

Lasa R, Caballero P, Williams T, 2007. Juvenile hormone analogs greatly increase the production of a nucleopolyhedrovirus. Biol. Control 41, 389–396.

LeOra Software, 1987. POLO PC, a user guide to Probit or Logit analysis. LeOra Software, Berkeley, CA, USA.

Léry X, Abol-Ela S, Giannotti J, 1998. Genetic heterogeneity of *Phthorimaea operculella* granulovirus: restriction analysis of wild-type isolates and clones obtained *in vitro*. Acta Virol. 42, 13–21.

Martin P, Travers R, 1989. Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 55, 2437–2442.

Mascarin G, Alves S, Rampelotti-Ferreira F, Urbano M, Demétrio C, Delalibera I, 2010. Potential of a granulovirus isolate to control *Phthorimaea operculella* (Lepidoptera: Gelechiidae). Biocontrol 55, 657–671.

Moscardi F, 1999. Assessment of the application of baculoviruses for control of Lepidoptera. Ann. Rev. Entomol. 44, 257–263.

Muñoz D, Caballero P, 2001. Diversidad natural de los baculovirus. In: Los baculovirus y sus aplicaciones como bioinsecticidas en el control biológico de plagas. Ed. by Caballero P, López-Ferber M, Williams T, Phytoma-España, Valencia, Spain, 95–118.

Muñoz D, Vlak JM, Caballero P, 1997. *In vivo* recombination between two strains of the genus nucleopolyhedrovirus in its natural host, *Spodoptera exigua*. Appl. Environ. Microbiol. 63, 3025–3031.

Murillo R, Elvira S, Muñoz D, Williams T, Caballero P, 2006. Genectic and phenotypic variability in *Spodoptera exigua* nucleopolyhedrovirus isolates from greenhouse soils in southern Spain. Biol. Control 38, 157–165.

Murillo R, Muñoz D, Ruíz-Portero MC, Alcázar MD, Belda JE, Williams T, Caballero P, 2007. Abundance and genetic structure of nucleopolyhedrovirus populations in greenhouse substrate reservoirs. Biol. Control 42, 216–225.

Niño L, 2004. Revisión sobre la Polilla de la Papa *Tecia solanivora* en Centro y Suramérica. Rev. Latinoam. Papa. Suppl. 4–21.

Niño L, Notz J, 2000. Patogenicidad de un virus granulosis de la polilla de la papa *Tecia solanivora* (Povolny) 1973 (Lepidoptera: Gelechiidae) en el estado Mérida, Venezuela. Boletín de Entomología Venezolana 15, 39–48.

Ribeiro HCT, Pavan OH, Muotri AR, 1997. Comparative susceptibility of two different hosts to genotypic variants of the *Anticarsia gemmatalis* nuclear polyhedrosis virus. Entomol. Exp. Appl. 83, 233–237.

Richards AR, Christian PD, 1999. A rapid bioassay screen for quantifying nucleopolyhedrovirus (Baculoviridae) in the environment. J. Virol. Methods 82, 63–75.

Robertson JL, Preisler HK, 1992. Pesticide bioassays with arthropods. CRC Press, Boca Raton, USA.

Rondon SL, 2010. The potato tuberworm: a literature review of its biology, ecology, and control. Am. J. Pot. Res. 87, 149–166.

Setiawati W, Soeriaatmadja RE, Rubiati T, Chujoy E, 1999. Control of potato tubermoth (*Phthorimaea operculella*) using an indigenous granulosis virus in Indonesia. Indian J. Crop Sci. 14, 10–16.

Shelton AM, Wyman JA, Mayor AJ, 1981. Effects of commonly used insecticides on the potato tuberworm and its associated parasites and predators in potatoes. J. Econ. Entomol. 74, 161–184.

Simón O, Williams T, López-Ferber M, Caballero P, 2004. Genetic structure of a *Spodoptera frugiperda* nucleopolyhedrovirus population: high prevalence of deletion genotypes. Appl. Environ. Microbiol. 70, 5579–5588.

Torres-Leguizamón M, Dupas S, Dardon D, Gómez Y, Niño L, Carnero A, Padilla A, Merlin I, Fossoud A, Zeddam JL, Lery X, Capdevielle-Dulac C, Dangles O, Silvain JF, 2011. Inferring native range and invasion scenarios with mitochondrial DNA: the case of *T. solanivora* successive north–south step-wise introductions across Central and South America. Biol. Invasions 13, 1505–1519.

Vargas B, Rubio S, López-Ávila A, 2004. Estudios de hábitos y comportamiento de la polilla guatemalteca *Tecia solanivora* (Lepidoptera: Gelechiidae) en papa almacenada. Rev. Colomb. Entomol. 30, 211–217.

Vickers JM, Cory JS, Entwistle PF, 1991. DNA characterization of eight geographic isolates of granulosis virus from the potato tuber moth (*Phthorimaea operculella*) (Lepidoptera, Gelechiidae). J. Invert. Pathol. 57, 334–342.

Zeddam JL, Vásquez-Soberon R, Vargas-Ramos Z, Lagnaoui A, 2003. Cuantificación de la producción viral y tasas de aplicación de un virus de granulosis usado para el control biológico de las polillas de la papa, *Phthorimaea operculella* y *Tecia solanivora* (Lepidoptera: Gelechiidae). Rev. Chil. Entomol. 29, 29–36.

