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Granulovirus formulations efficiently protect stored and field potatoes from *Phthorimaea operculella* and *Tecia solanivora* in Costa Rica

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Abstract The control efficiency of a *Phthorimaea operculella* granulovirus isolate from Costa Rica (PhopGV-CR1) against the concurrent insect pests *P. operculella* (Zeller) and *Tecia solanivora* (Povolny) (Lepidoptera: Gelechiidae) was evaluated. In warehouses, the best control efficiency was achieved with a powder formulate applied inside bags, which reduced injury over 70 % compared with the untreated controls. In the field, liquid and powder virus formulations significantly reduced injury between 56.2 and 81.7 % compared with the untreated controls, and were as efficient as chemical treatments. The efficiency of formulations stored at −20 °C for six months (liquid) or at ambient temperature for three months (powder) was maintained, but higher temperatures and/or longer exposure times resulted in loss of pathogenicity. The data presented here favor the inclusion of granulovirus formulations of PhopGV-CR1 in Integrated Pest Management programs against tuberworms in Costa Rica and give clues on storage conditions for the growers in this country.

Keywords *Phthorimaea operculella* · *Tecia solanivora* · Baculoviruses · Granuloviruses · PhopGV · Microbial control

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Introduction

The potato tuberworms, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and *Tecia solanivora* (Povolny) (Lepidoptera: Gelechiidae), overlap spatial and temporally throughout all Central America and in the Northern countries of South America (Rondon 2010; Torres-Leguizamón et al. 2011), and cause the most devastating injuries in potatoes in Latin American field crops (Hilje 1994; Vargas et al. 2004). *P. operculella* female adults lay their eggs throughout the growing season, preferring foliage over tubers (Rondon 2010). When foliage has naturally or artificially senesced and/or tubers are accessible, they deposit eggs in or near the eye buds. The larvae mine leaves, stems, and petioles causing irregular galleries, and excavate tunnels through tubers, causing major yield losses. Infestation of potatoes occurs mainly

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during storage, when tuber quality and quantity is severely affected and losses may be up to 100 %, especially in non-refrigerated systems (Rondon 2010). *T. solanivora* only infests the tubers, causing severe injuries. Upon egg hatching, neonate larvae mine the tubers, which can host several larvae (Hilje 1994), both in the field before harvest, and in the stores (Vargas et al. 2004). Consequently, protection measures against these pests must be taken both in the field before potatoes enter the warehouses and while in storage.

Pre-harvest intervals limit the use of broad spectrum pesticides leading to the highest tuber infestations, where the most economically significant damage typically occurs (Arthurs et al. 2008b). Therefore, alternative options for late-season and post-harvest control that also provide tools for insecticide-resistance management and organic production are needed. Field applications of biopesticide formulations have demonstrated protection of natural enemies while maintaining the ecological balance in the environment (Amonkar et al. 1979). Several baculovirus-based biopesticides have been successfully used against several pests (Berling et al. 2009; Moscardi 1999; Lasa et al. 2007; Szewczyk et al. 2006) and can be incorporated in Integrated Pest Management (IPM) programs given their compatibility with other control methods, including most chemical pesticides (Harper 1986).

The granulovirus of *P. operculella* (PhopGV) has been shown to provide effective control of *P. operculella* or *T. solanivora* in potato crops and, specially, in stored potatoes (Alcázar et al. 1992; Arthurs et al. 2008a, b; Das et al. 1992; Kroschel et al. 1996; Sporleder and Kroschel 2008; Lacey et al. 2010; Lacey and Kroschel 2009; Zeddam et al. 1999). However, its effectiveness against these two species has not been determined in areas where both species coexist. In addition, IPM programs for these pests in Costa Rica have not included granuloviruses because of the lack of indigenous strains, which have not been isolated and characterized until very recently (Gómez-Bonilla et al. 2011). The novel Costa Rican *P. operculella* granulovirus (PhopGV) strains have proved their efficiency under laboratory conditions against the two pests and their inclusion in IPM programs in this country depends on several factors including their field efficiency, impact on non-target species, and affordability (Gómez-Bonilla et al. 2011, 2012).

This paper describes the performance of a highly pathogenic strain from Costa Rica (PhopGV-CR1) under storage and field conditions against concurrent *P. operculella* and *T. solanivora* populations. The paper also addresses the storage conditions for liquid and powder formulations. Since potato growers in Costa Rica have limited resources for long term storage of insecticide formulations, evaluating different temperatures and exposure times is important to determine their affordability.

Materials and methods

Insect rearing

The insect populations of *P. operculella* and *T. solanivora* were used for the production of viral formulations and for analyzing the efficiency of these formulations in laboratory bioassays. Adults from both these species were captured in Cartago (Costa Rica) and reared at 25 °C and a 16:8 light:dark photoperiod at the Research Center Carlos Durán (Cartago, Costa Rica). Larvae were fed on potato tubers previously treated with 0.5 % chlorine solution to remove any potential entomopathogens. Adults were fed honey or 30 % (p/v) sugar.

Viral formulations

Viral formulations were based on a PhopGV strain from Costa Rica, PhopGV-CR1, which has been characterized previously (Gómez-Bonilla et al. 2011). To prepare the viral inocula, two *P. operculella* larval cadavers (one larvae produces ca. 10^9 occlusion bodies—OBs) previously infected with PhopGV-CR1 were macerated with 1 ml Tris–SDS and 3 ml distilled water and mixed. This mixture was applied with a brush on the surface of potatoes that were previously washed thoroughly with soap and water, rinsed in distilled water for several minutes, and air dried. Once dried, 20 neonate larvae were placed on the inoculated potatoes and incubated in plastic boxes at 27 °C for 25 days. Larval cadavers were collected daily from day 15 post-infection on and stored at −20 °C for further use. This procedure was repeated several times to obtain enough inocula for the formulation process.

A liquid formulation was prepared with 20 *T. solanivora* infected larvae (ca. 2×10^{10} OBs) which were homogenized with 5 ml 10 mM Tris with 0.1 % SDS and 5 ml distilled water and added 0.2 % (vol/vol) Tween 20, 0.15 % (wt/vol) sorbic acid, 5 % (vol/vol) glycerol and phosphate-buffered saline (PBS), previously adjusted to pH 6.5 using hydrochloric acid, to reach a total volume of 1 l.

The solid formulation was achieved by adding 1 kg talc to the liquid formulation, air drying it at room temperature for at least 15 days and finally grinding it. A powder formulation with a desiccant like talc is believed to enhance the efficacy of the product (Kroschel and Koch 1996). This formulation was packed in two plastic bags containing 500 g each.

Warehouse assays

Assays were performed over the natural insect populations present in the region. Warehouses with similar environmental conditions that were located 23 km apart in the province of Cartago (Costa Rica), at 2,100 m (warehouse 1) and 2,340 m (warehouse 2) altitude. The former was made of plastic and storage crates rested on the soil whereas the latter was a concrete building. Twenty kilograms of potatoes (var. Floresta) were treated with 100 g of the baculovirus powder formulation. Accumulated experience recommends the exclusive use of powder formulations to avoid the rotting of potatoes associated with liquid formulations (Niño and Notz 2000), and also for the ease of handling and storing the biopesticide (Alcázar et al. 1992). Two types of applications were performed: a traditional dusting application (DA) over potato layers placed in 60 × 20 × 50 cm wooden crates or a bag application (BA) consisting in thoroughly mixing the potatoes with the virus formulation in bags, to achieve a complete coverage of the potato surface, before storing them in similar wooden crates.

BAs were performed with the baculovirus alone (BacB treatment) whereas different treatments were carried out with the DAs: (i) the baculovirus alone (BacD treatment); (ii) the baculovirus and 20 g Vitavax 40 WP (active compounds Carboxyn and Captan) (Proficol S. A., Barranquilla, Colombia), a fungicide (Fun) against *Rhizoctonia solani* and *Fusarium* spp. (BacDFun treatment); and (iii) 100 g Vydate 24 SL (active compound Oxamyl) (Duwest Inc., Guatemala, Guatemala), an insecticide (Che) against moths, and 20 g Vitavax 40 WP (CheFun treatment). Twenty kilograms of potatoes in non-treated crates were used as controls. A group of five crates, each with a BacB, BacD, BacDFun, CheFun or control treatment, constituted a replicate. Crates in each replicate were piled up, stored in diffuse light for four months, and rotated every month. Three replicates were performed and a randomized complete block design was used. Results were expressed as percentage of injured potatoes (those with at least one tunnel) over the total number of potatoes treated.

Pheromone traps for *P. operculella* and *T. solanivora* (one per species per warehouse) were placed and monitored each week. Assays were carried out with the 2008 potato harvest.

Field assays

Assays were carried out in Alvarado (Cartago, Costa Rica) throughout the two seasons in 2009 over natural insect populations. Dry season assays were performed from February to May seeded with a seed stock of the Floresta variety provided by the grower, and protected with insecticides. Humid season assays, from April to July, were carried out in a different field, seeded with the potato seed stock produced in the assayed warehouses. Both fields were divided into 25 m^2 plots, each consisting of five 5 m long rows, separated by 90 cm with 20 potatoes seeded 25 cm apart in each row. A row was kept empty between plots to avoid treatment drift. Four plots, constituting a replicate, were subjected to one of the following treatments: (i) baculovirus formulations alone (Bac), (ii) baculovirus formulations together with insecticide applications (BacChe), (iii) only insecticide formulations (Che), and (iv) non-treated control. The concentrations applied for treatments involving baculoviruses were those recommended by CIP (2000): 250 ml per plot for the liquid formulation and 250 g per plot for the powder formulations. Liquid formulations were sprayed over the lower part of the canopy whereas powder formulations were applied to the soil during seedling and hilling. The treatments were applied as scheduled in Table 1 and arranged in a complete randomize block design to neutralize the edge effect. All plots were treated with several fungicides for protection against several fungal diseases of potatoes (*Rhizoctonia solani* present at seedling and *Phytophthora infestans* during the whole season). The whole assay was replicated six times. The weight of the

Table 1 Weekly schedule of pest control treatments in dry and humid season assays

Week (cultural practice)	Dry season treatments			Humid season treatments		
	Bac	BacChe	Che	Bac	BacChe	Che
1 (Seedling)	+(P)		CHLGR	+(P)		CHLGR
3	+(L)	+(L) and CAR	CAR	+(L)		THO
4 (Hilling)	+(L and P)	+(L and P) and THO	CHLGR, CAR and THO	+(L and P)	+(L and P) THO	CHLGR and THO
5	+(L)	+(L) and CHLSP	CHLSP and DIM	+(L)		–
6	+(L)		–	+(L)	+(L) and CHLSP	CHLSP and DIM
7	+(L)	+(L) and THO	CHLSP and THO	+(L)	+(L) and CHLSP	CHLSP
8	+(L)		–	–	–	–
9	+(L)	+(L) and CHLSP	CHLSP and THO	+(L)	+(L) and CHLSP	CHLSP
10	+(L)		–	+(L)		–
11	+(L)	+(L) and CHLSP	CHLSP	+(L)	+(L) and CHLSP	CHLSP

Three types of treatments were applied: baculovirus alone (Bac), baculovirus and chemical insecticides (BacChe) and chemical insecticides (Che). Two baculovirus formulations, powder (P) or liquid (L), were used singly or combined (L and P) for the Bac and BacChe treatments

CHLGR chlorpyriphos 2.5 GR (Dow Agrosciences, Santiago, Chile) for treatment of moths, *CAR* carbaryl 80 SP (Bayer CropScience, Bogota, Colombia) for *Epitrix* spp. (Coleoptera: Chrysomelidae), *THO* thioxam hydrogen oxalate (Bioamerica S.A., Santiago, Chile) for *L. huidobrensis*, *CHLSP* chlorpyriphos 2.5 SP (Dow Agrosciences, Santiago, Chile) for moths and *Liriomyza huidobrensis* (Diptera: Agromyzidae), *DIM* dimethoathe 40 EC (Basf, Jiutepec, Mexico) for Aphids and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Dosages for all chemical formulations were always those recommended by the supplier

potatoes produced by the four plants placed along 1 m of two of the central rows in each plot was registered at the end of the season (at week 15–16 after seedling) and injury (potatoes with at least one tunnel) calculated as a percentage between the weight of injured potatoes and the total weight. Pheromone traps for *P. operculella* and *T. solanivora* were placed in the four field corners from sowing to harvest and monitored each week.

Laboratory bioassays

Three samples (100 µl) of the virus liquid formulation were stored each at one of the following three temperatures: −20, 4 °C, or room temperature (RT) in amber glasses. A sample (100 g) of the virus powder formulation was stored at RT in darkness. The insecticidal activity of liquid and powder formulation samples was evaluated over the first cultured generations of *T. solanivora* neonate larvae after six and 12 months storage for liquid samples and after three and six months storage for powder samples.

The liquid formulation samples were used at a concentration of 5×10^8 OBs ml^{-1}. Concentrations were determined in 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). The formulations were then applied homogeneously on the 5 cm^2 upper surface of two potato tubers using a nebulizer (Carrera et al. 2008) to reach final concentrations of ca. 500 OBs mm^{-2}. Once dried, 15 first instar *T. solanivora* larvae were placed on the treated surface of each of the two tubers, and incubated at 27 °C for 23 days. Therefore, each replicate of each treatment consisted of 30 larvae. The number of infected larvae present in the surface of the two potatoes, easily recognized from non-infected ones because of their whitish appearance and changed behavior (most come out of the tubers), was recorded daily from day 14 post inoculation onward. Registered cadavers were removed daily. At day 25 post inoculation, potatoes were opened and inspected thoroughly to register cadavers present inside the tubers.

To evaluate the insecticidal activity of the powder formulation samples, doses of 1 g (ca. 2×10^7 OBs),

Table 2 Mean percent injury in potatoes (60 kg per treatment) by *P. operculella* and *T. solanivora* larvae in warehouses 1 and 2 with the following treatments: baculovirus applied inside a bag (BacB), baculovirus dusted over potatoes alone (BacD) or in combination with a fungicide (BacDFun), or a chemical insecticide combined with a fungicide (CheFun)

Treatment	Injury (%) ± SE	
	Warehouse 1*	Warehouse 2*
Control untreated	38.00 ± 4.51a	29.00 ± 4.04a
BacB	6.33 ± 1.76b	8.33 ± 1.20c
BacD	29.00 ± 4.51a	10.67 ± 2.03bc
BacDFun	21.00 ± 9.07ab	19.33 ± 3.53b
CheFun	26.67 ± 1.20a	8.33 ± 0.88bc

The same amount of potatoes was kept untreated as a control

* Mean values followed by different letters are significantly different ($P < 0.05$) for treatment comparisons, which were carried out only within columns

as recommended by CIP (2000), were applied to bags containing two potatoes. Each potato was then infested with ten first instar *T. solanivora* larvae. Two potatoes were used per formulation sample thus making each replicate of each treatment consisting of 20 larvae. Monitoring was done daily as described above for the liquid formulation assays. Fresh liquid and powder formulations were used in bioassays right after production and used as time 0 storage controls. Non-treated potatoes were used as negative controls. Each bioassay with the liquid and powder formulation samples was replicated once.

Statistical analysis

For percent injury data obtained from the warehouse and field assays, a modified Shapiro-Wilks test was applied to check for the normality of the data. Analysis of variance (ANOVA) was used to determine statistical differences between means and the post-hoc Duncan test was employed for multiple comparison between treatments. Larval mortality data obtained with the stored formulations were subjected to analysis of variance (ANOVA) and means were compared (separately for the liquid and the powder formulations) using the Tukey test. Arcsin transformation was used when necessary for not normally distributed data. All analyses were performed using SPSS Statistic ver. 19 (SPSS Inc., Chicago, IL, USA).

Results

Control efficiency of PhopGV-CR1 in warehouses

Control efficiency results were not consistent in the two warehouses. Although better control in both warehouses was achieved when BA was employed, different results were obtained with the other three treatments in the two warehouses (Table 2). In warehouse 1, only the BacB treatment conferred stored potatoes a significant level of protection compared to the non-treated control (ANOVA; $F_{4,10} = 5.38$; $P < 0.05$). In warehouse 2, percent injuries in seed potatoes were significantly lower with all four insecticidal treatments compared to the control (ANOVA; $F_{4,10} = 11.47$; $P < 0.05$). No differences in injury levels were detected between the baculovirus treatment alone and those where the baculovirus was combined with a chemical insecticide and a fungicide or where only a chemical insecticide and a fungicide were applied.

Catches of adults from both species in warehouse 1 increased steadily over the storage period (Fig. 1a). In warehouse 2, however, the two populations behaved differently. *P. operculella* catches were below five adults per trap per week throughout the whole storage period whereas the number of moth catches increased for *T. solanivora* in the first three weeks of storage, remained constant until week 8, decreased until week 12 and they finally increased abruptly from then to the end of the storage period (Fig. 1b).

Control efficiency of PhopGV-CR1 in the field

Injuries in tubers treated with insecticides were significantly reduced with respect to the untreated control tubers: by 56.1, 78.6, and 77.6 % in the dry season assays (ANOVA; $F_{3,20} = 18.30$; $P < 0.001$), and by 61.1, 81.6, and 82.5 % in the humid season assays (ANOVA; $F_{3,20} = 17.35$; $P < 0.001$) after treatment with Bac, BacChe and Che, respectively (Table 3). Injury reduction with the granulovirus formulation (Bac) was not significantly different from that obtained with the granulovirus together with chemical insecticides (BacChe) or with that achieved with only chemical insecticides (Che) in both experimental fields.

Incidence of *P. operculella* was lower than *T. solanivora* during both seasons (Fig. 1). Both

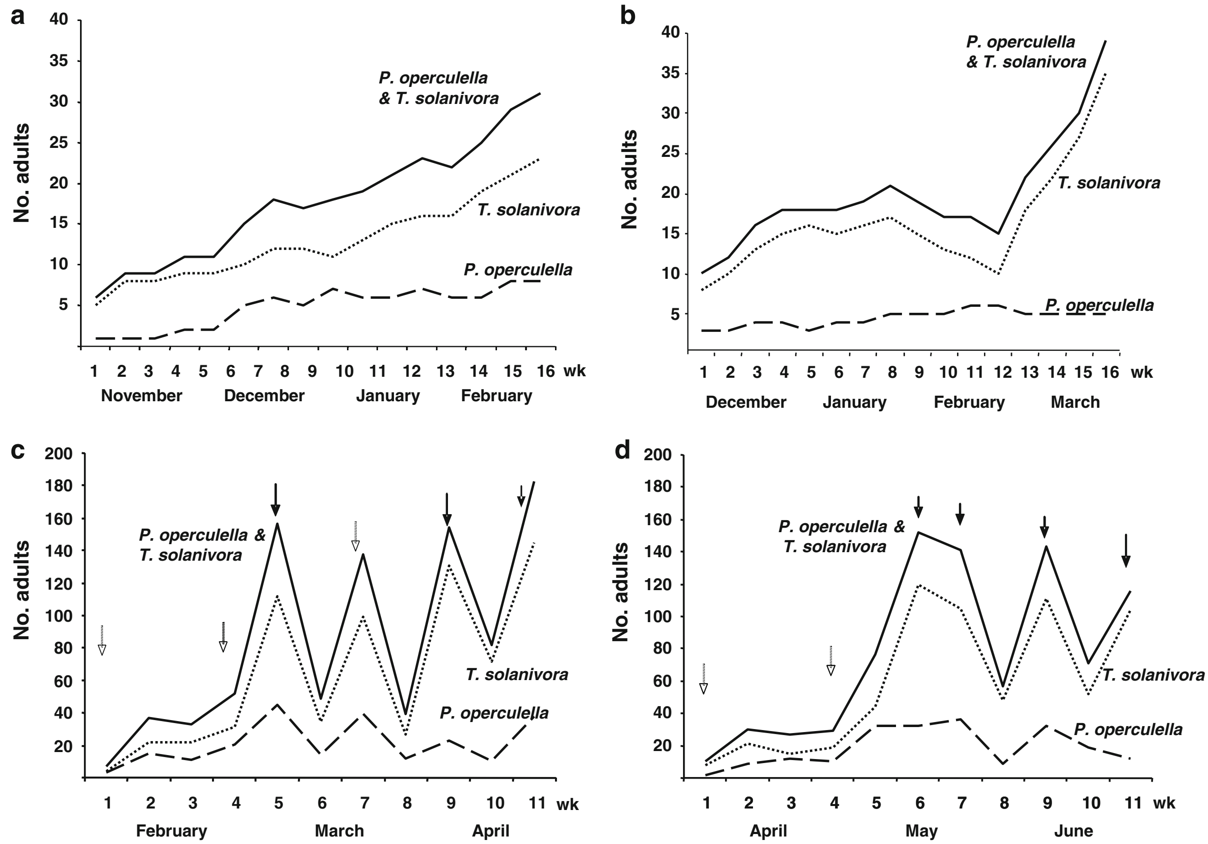

Fig. 1 Changes in the number of male moths of *T. solanivora* (dotted line), *P. operculella* (dashed line) or both (solid line) captured in pheromone traps in warehouses (**a**, **b**) and in potato fields during the dry (**c**) and humid (**d**) seasons. Arrows indicate timing of chemical insecticide applications in plots where a combination of baculovirus and chemicals was applied (dark arrows) or in plots where only chemicals were applied (clear arrows)

populations suffered drastic population decreases upon chemical insecticide applications against moths (Fig. 1).

Efficiency of PhopGV-CR1 powder and liquid formulations after storage

The two factors tested, temperature ($F_{2,9} = 22.91$; $P < 0.001$) and time ($F_{2,9} = 80.96$; $P < 0.001$), affected the efficiency of the liquid formulation both individually and combined as indicated by the significant interaction between them ($F_{4,9} = 9.13$; $P < 0.005$). At −20 °C, samples kept in storage for six months remained as active against *T. solanivora* as fresh formulations (Fig. 2). However, samples stored at 4 °C and RT lost efficiency after only six months and killed 38 and 32 % less *T. solanivora* larvae, respectively, than fresh formulations (Fig. 2). After 12 months, the efficiency significantly decreased for all samples, slightly (16 %) for those kept at −20 °C, and more drastically, by 49 and 87 %, for those kept at 4 °C and RT, respectively (Fig. 2). Powder samples in storage for three months at ambient temperatures remained as active as fresh formulations but their activity decreased significantly by 48.6 % after six months ($F_{2,8} = 8.63$; $P < 0.05$).

Discussion

This paper describes the high degree of protection of stored and field potatoes in Costa Rica provided by PhopGV-based formulations against concurrent populations of the tuberworms, *P. operculella* and *T. solanivora*. Although results were not fully

Table 3 Mean percent injury in potatoes by *P. operculella* and *T. solanivora* larvae in dry and humid season assays treated only with baculovirus formulations (Bac), with the baculovirus formulations and with chemical insecticides (BacChe), or only with chemical insecticides (Che)

Treatment	Injury (%) ± SE	
	Dry season*	Humid season*
Control untreated	19.05 ± 1.62a	14.38 ± 2.23a
Bac	8.37 ± 2.27b	5.59 ± 0.90b
BacChe	4.07 ± 1.43b	2.64 ± 0.95b
Che	4.27 ± 0.97b	2.52 ± 0.71b

Untreated plots were included in the assay as negative controls

* Mean values followed by different letters are significantly different ($P < 0.05$) for treatment comparisons, which were carried out only within columns. The yield from five potato plants was analyzed for each treatment

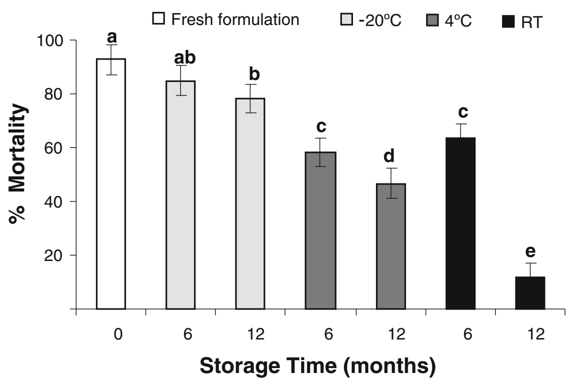

Fig. 2 Mean percentage mortality of *T. solanivora* larvae treated with liquid formulation samples taken after six and 12 months storage at −20, 4 °C and ambient temperatures (RT). Columns headed by different letters are significantly different (Tukey test, $P < 0.05$). Bars indicate SE

consistent in the two warehouses, significant protection was achieved with the BacB treatment in both of them, indicating that the extra step taken to apply the virus inside a bag resulted in higher protection than dusted treatments or the untreated control and may compensate the additional costs. Bag application allowed complete coverage of the whole potato surface, increasing the probability for larvae to ingest a deadly viral dose. Dust applications however, covered mainly the upper and lower potato surfaces with the laterals having much lower chances to receive efficient viral doses. Previous experiences achieving well coverage of potatoes with similar formulations and doses have also succeeded in increasing larval mortality (Alcázar et al. 1992; Das et al. 1992; Niño and Notz 2000; Winters and Fano 1997).

Different results were obtained with the dust applied treatments in the two warehouses, probably because of their different structure. Warehouse 2 represented a greater barrier for moth entry than warehouse 1 where, indeed, the number of moths trapped increased for both species throughout the season, indicating an entrance of moths from outside the warehouse. The number of moths trapped in warehouse 2 increased only in the four months because of the emergence of a second generation of adults from within the warehouse population. Although the use of PhopGV has generally been very successful in stores (Lacey et al. 2010), attacks from new generations have been reported to increase tuber injuries after more than 2–3 months in non-refrigerated storage (Arthurs et al. 2008a; Das et al. 1992). Even so, the use of PhopGV is strongly recommended to prevent the spread of initial infestations or moth establishment within a warehouse (Arthurs et al. 2008a). In Costa Rica, the vast majority of potato growers store their tubers for periods no longer than two or three months at high altitudes (1,800–2,500 m above sea level), where completion of the tuberworm life cycles becomes much slower and pest incidence is considerably lower. In consequence, single applications of granulovirus formulations may be sufficient to protect potatoes during the whole period.

In warehouse 2, baculovirus treatments were at least as efficient in reducing injury as the carbamate oxamyl. Combinations of granuloviruses with pyrethroids (Alcázar et al. 1992), with *Bacillus thuringiensis* and an organophosphate (Moawad et al. 1998a, b), and with an organochlorine (Kurhade and Pokharkar 1997) have given similar results.

Field applications of baculovirus formulations alone or together with an insecticide, protected field potatoes as effectively (reducing potato injuries by 50 and 80 %) as chemical insecticides in both the humid and dry seasons. No significant differences were obtained between treatments in any of the two experimental fields, indicating that PhopGV-CR1 can be used in Costa Rica against these concurrent pests alone, as an alternative to chemicals, or in combination with them. Although the effects of these pesticides on the overall performance of PhopGV have not been examined, most combinations of viruses and chemical pesticides have been found to be compatible (Harper 1986).

The use of PhopGV in potato crops has not traditionally shown results as consistent as in stores (Kroschel et al. 1996; Matthiessen et al. 1978; Reed and Springett 1971) and, as a consequence, it has been much less used in field crops (reviewed in Lacey et al. 2010 and Rondon 2010). However, knowledge on factors influencing field efficiency of baculovirus treatments has increased enormously and, today, application timing or application methods that achieve a good coverage of the host-preferred plant surfaces are of chief consideration. Treatments against tuberworms in potatoes are more efficient when sprayed at dusk over the basal parts of the plant canopy. More importantly, the seed should be well protected at seedling and hilling, when likelihood of tuberworm infestation is highest. In this work, sprayings of the plants were performed with liquid formulations following timing and site recommendations, whereas powder formulations were employed for the seeds.

In this study, the frequency of virus application in the fields was high: weekly liquid formulations and two additional powder formulations at seedling and hilling. These may seem too numerous in comparison with other baculovirus-host systems, but the overlapping host generations, commonly found in tropical regions, demand it. Weekly PhopGV applications are a common practice against *P. operculella* in the Andean region (Wraight et al. 2007). However, such frequent applications are likely to result, eventually, in the evolution of resistance to granuloviruses, as occurred with *Carpocapsa pomonella* granulovirus (CpGV) (Asser-Kaiser et al. 2007). Fortunately, resistance to CpGV has been pushed back with the use of different baculovirus strains (Eberle et al. 2008). A well characterized set of efficient PhopGV strains from Costa Rica and other countries is in store for such a potential outcome (Espinel-Correal et al. 2010; Gómez-Bonilla et al. 2012). This, together with carefully designed IPM programs, can make PhopGV a sustainable control method. Another important aspect of high frequency application is its economic feasibility. For high valuable crops such as potato seeds, the use of weekly baculovirus applications may be profitable, but studies addressing this issue still need to be undertaken.

Tecia solanivora is usually the dominant species throughout the whole season in both storage and in the field. The high efficiency of the viral formulations thus indicates that *T. solanivora* can effectively be controlled by a virus originally isolated from *P. operculella*. Laboratory bioassays had already demonstrated a high pathogenicity of PhopGV-CR1 and other PhopGV strains against their original host, and a rapid adaptation to the alternate host, *T. solanivora* (Espinel-Correal et al. 2010; Gómez-Bonilla et al. 2011, 2012; Zeddam et al. 2003). All such strains have been isolated from regions where both tuberworm species coexist.

The efficiency of these simple PhopGV formulations was maintained only when stored at −20 °C for six months (liquid) or at ambient temperature for three months (powder). In Costa Rica though, most potato growers cannot afford frozen conditions for long term storage of pesticides. Consequently, further development of formulations as well as of methodologies for virus extraction, purification and formulation (Lasa et al. 2008) will need to be undertaken to improve the shelf life of PhopGV-based products under refrigerated or ambient conditions.

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