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Insect Pathogens and Entomopathogenic Nematodes

Biological Control in IPM Systems

Edited by:
Ralf-Udo Ehlers, Neil Crickmore, Juerg Enkerli, Itamar Glazer, Martin Kirchmair, Miguel Lopez-Ferber, Sigrid Neuhauser, Hermann Strasser, Cezary Tkaczuk, Michael Traugott

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Virus

Virus Session 1: PTM granuloviruses

Virus Session 2: CpGV granuoviruses

Virus Session 3: Nucleopolyhedroseviruses

Insect pathogens and entomopathogenic nematodes
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Stored potatoes in Costa Rica are efficiently protected from *Phthorimaea operculella* and *Tecia solanivora* with an indigenous granulovirus strain

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Abstract: The control efficiency of a *Phthorimaea operculella granulovirus* isolate from Costa Rica (PhopGV-CR1) against the insect pests *P. operculella* and *Tecia solanivora* under storage and field conditions was evaluated. The virus reduced damage by over 70% compared with the untreated controls. These data favour the inclusion of PhopGV-CR1 formulations in IPM programs.

Key words: granulovirus, microbial control, postharvest control, *Tecia solanivora*, *Phthorimaea operculella*

Introduction

Potato tubers constitute one of the key resources of human diet in the world, especially in Central and South America, the geographical origin of this commodity. The number of production cycles greatly varies between regions, from a single per year up to four cycles under optimal conditions. The tubers can be stored without major processing in warehouses, which need only to be dry and dark, without needs of temperature or humidity control. This traditional simple storage allows tuber conservation until required for consumption or processing.

Harvest storage constitutes an attraction point for pests, mainly rodents and insects. In potatoes, important postharvest losses are related to the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) (Zeller), infestations. *P. operculella* is widely distributed all around the world in temperate to warm regions. It attacks the aerial parts of the potato plant in the field, but does not cause damage on the quantity or the quality of tuber production. However, in warehouses it feeds on tubers, rendering them unsuitable for consumption. In recent years, a second insect, *Tecia solanivora* (Lepidoptera: Gelechiidae) (Povolny), originating from Guatemala, started to colonize new areas from Costa Rica in 1970, towards southern countries. Since 1999 it has been observed in the Canary Islands (Torres-Leguizamón et al., 2011). *T. solanivora* larvae feed only on the potato tubers. It can infest tubers in the field which, upon arriving to the warehouses constitute the inoculum for a wide scale infestation that can result in a complete loss (Rondon 2010). Therefore, alternative options for postharvest control that also provide tools for insecticide-resistance management and organic production are needed.

Successful IPM programs against the tuber moths have lately included granulovirus-based formulations for storage use (Arthurs et al.; 2008; Zeddam et al., 2003). However, in Costa Rica, IPM programs for these pests have not yet included granuloviruses (GVs) given the lack of indigenous strains, which have not been isolated and characterized until only very recently (Gómez-Bonilla et al., submitted). The novel Costa Rican *P. operculella* GV (PhopGV) strains have proved their efficiency under laboratory conditions against the two pests. Their inclusion in IPM programs in this country only depends on determining their efficiency. This paper describes the performance of a highly pathogenic strain from Costa Rica (PhopGV-CR1) under storage conditions.

Material and methods

Insect rearing

The insect populations of *P. operculella* and *T. solanivora* originally came from Costa Rica and were maintained at 25ºC and a 16:8 light:dark photoperiod at the Research Center Carlos Duran (Cartago, Costa Rica). Larvae were fed on potato tubers previously treated with 0.5% chlorine solution. Adults were fed honey or 30% (p/v) sugar.

Viral formulations

Viral formulations were based on the Costa Rican PhopGV-CR1 strain, previously characterized (Gómez-Bonilla et al., submitted). Twenty *P. operculella* infected larvae (2.13 x 10^{10} OBs) were homogenized and added: 0.2% Tween 20, 1 l water and 1kg talc. The resulting mixture was air dried at room temperature for at least 15 days, grinded and packed in two plastic bags containing 500g each.

Efficiency assays

Warehouses were located 23km apart in the province of Cartago (Costa Rica) at 2100m (warehouse 1) and 2340m (warehouse 2) of altitude. The former was made of plastic, and storage boxes rested on the soil whereas the latter was a concrete building. Twenty kg of potatoes (var. Floresta) were treated with 100g of the baculovirus powder formulation. Two types of applications were performed: a traditional dusting application (DA) over potato layers placed in storage boxes, or a bag application (BA) consisting in thoroughly mixing the potatoes with the virus formulation in bags before storing them in similar boxes. BA applications were performed with the baculovirus formulation alone (BacB treatment) whereas different treatments were performed with the DA application: i) the baculovirus alone (BacD treatment); ii) the baculovirus and 20g of the fungicide Vitavax 40 WP (BacD+F treatment); and iii) 100g of the insecticide Vydate 24 SL and 20g Vitavax 40 WP (F+I). Groups of five boxes were piled up, stored in diffuse light for four months and rotated every month. Three replicates were carried out and non-treated boxes were used as controls. A randomized complete blocks design was used. Results were expressed as percentage of damaged potatoes (those with at least one tunnel) over the total number of potatoes. Pheromone traps for both moths were placed and monitored each week. Assays were carried out with the 2008 potato harvest.

A modified Shapiro-Wilks test was applied to check for the normality of the data and the analysis of the variance (ANOVA), and the Duncan test (SAS software package, SAS Institute Inc., USA) was applied to determine statistical differences.

Table 1. Percent damage by *P. operculella* and *T. solanivora* in warehouses 1 and 2.

Treatment	Damage (%) ± S.E.	
	Warehouse 1	Warehouse 2
Control untreated	38.00 ± 4.51 a	29.00 ± 4.04 a
BacB	6.33 ± 1.76 b	8.33 ± 1.20 c
BacD	29.00 ± 4.51 a	10.67 ± 2.03 b c
BacD+F	21.00 ± 9.07 a b	19.33 ± 3.53 b
F+I	26.67 ± 1.20 a	8.33 ± 0.88 b c

Results and discussion

In warehouse 1, the BacB treatment resulted in significantly lowest damage than any treatment applied as DA or the non-treated control (Table 1). In warehouse 2, percent damage in seed potatoes was significantly lower in all four treatments compared to the control (Table 1). The best control in both warehouses was achieved when BA was applied, indicating that the extra step taken to apply the virus inside a bag results in better potato coverage as has occurred in other instances (Alcázar et al., 1993; Niño de Guadrón and Notz, 2000). The added operational step might be worth the extra cost.

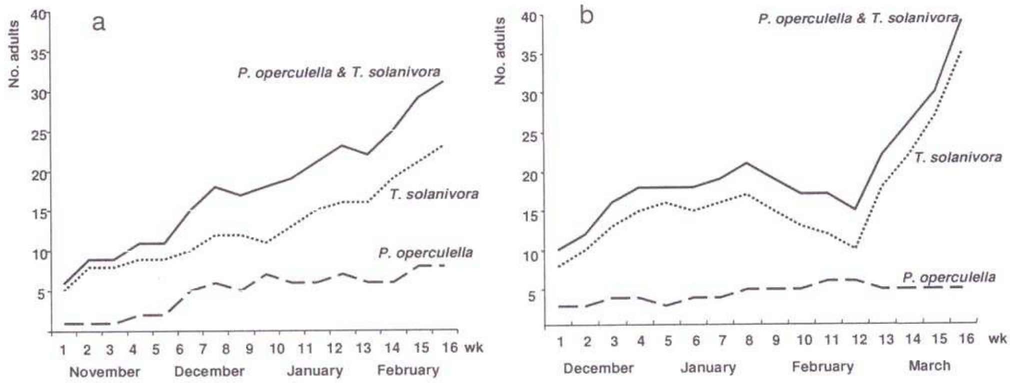

Figure 1. Number of adults of *T. solanivora* and *P. operculella* captured in pheromone traps in warehouses 1 (a) and 2 (b) during the 2008-2009 period.

Different results were obtained with the other three treatments in the two warehouses, probably because of their different structure. The concrete-made warehouse 2 represents a greater barrier for moth entry than warehouse 1, as demonstrated by moth catches (Fig. 1). In warehouse 2, *T. solanivora* populations increased during the last month most probably because of the emergence of adults from the second generation. In any case, the level of protection after 2-3 months storage has been reported to decrease dramatically in other instances (Das et al., 1992; Arthurs et al., 2008). *T. solanivora* was the dominant species throughout the whole season in this study (Fig. 1) and in previous recordings in this region

(Gómez-Bonilla & Guzmán, 1997), indicating that *T. solanivora* can effectively be controlled by PhopGV-CR1 as occurs with other PhopGV isolates elsewhere (Espinel-Correal et al., 2010; Zeddam et al., 2003). In conclusion, it seems that PhopGV-CR1 offers an efficient alternative to control *T. solanivora* and *P. operculella* in the warehouses of this region.

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Insect pathogens and entomopathogenic nematodes
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Potato crops in Costa Rica are efficiently protected from *Phthorimaea operculella* and *Tecia solanivora* by an indigenous granulovirus strain

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Abstract: The control efficiency of a *Phthorimaea operculella* granulovirus isolate from Costa Rica (PhopGV-CR1) against the insect pests *P. operculella* and *Tecia solanivora* under field conditions was evaluated. The virus reduced damage between 50 and 80% in fields compared with the untreated controls. These data favor the inclusion of PhopGV-CR1 formulations in IPM programs.

Key words: granulovirus, microbial control, field control, *Tecia solanivora*, *Phthorimaea operculella*

Introduction

The tuber moths, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) (Zeller) and *Tecia solanivora,* (Lepidoptera: Gelechiidae) (Povolny), are the most devastating potato pests in Latin American field crops (Arthurs et al., 2008). *P. operculella* attacks the aerial parts of the tomato plant not causing major damages to tuber quality or yield. Infestation of tubers occurs only in storage conditions. *T. solanivora* only infests the tubers, both in the field, before harvest, and in storage. Protection of tubers entering in warehouses is thus not sufficient to avoid *T. solanivora* derived losses. Pre-harvest intervals limit the use of broad spectrum pesticides, when the most economically significant damage typically occurs (Arthurs et al., 2008). Therefore, alternative options for late-season and postharvest control that also provide tools for insecticide-resistance management and organic production are needed. Field applications of biopesticide formulations have demonstrated not only to protect the pest natural enemies but also maintain the ecological balance in the environment. Several baculovirus-based biopesticides have been successfully used against several pests (Berling et al., 2009; Moscardi et al., 1999; Lasa et al., 2007), most which can be included in Integrated Pest Management (IPM) programs.

Successful IPM programs against the tuber moths have included granulovirus-based formulations for field use (Chaparro et al., 2010). However, in Costa Rica, IPM programs for these pests have not yet included them given the lack of indigenous strains, which have not been isolated and characterized until only very recently (Gómez-Bonilla et al., submitted). The novel Costa Rican *P. operculella* GV (PhopGV) strains have proved their efficiency under laboratory conditions against the two pests. Their inclusion in IPM programs in this country only depends on their field efficiency. This paper describes the performance of a highly pathogenic strain from Costa Rica (PhopGV-CR1) under field conditions.

Material and methods

Insect rearing

The insect populations of *P. operculella* and *T. solanivora* originally came from Costa Rica and were maintained at 25ºC and a 16:8 light:dark photoperiod at the Research Center Carlos Duran (Cartago, Costa Rica). Larvae were fed on potato tubers previously treated with 0.5% chlorine solution. Adults were fed honey or 30% (w/v) sugar.

Viral formulations

Viral formulations were based on the Costa Rican PhopGV-CR1 strain, previously characterized (Gómez-Bonilla et al., submitted). A solid formulation was manufactured with 20 *P. operculella* infected larvae (2.13 x 10^{10} OBs) which were homogenized and added: 0.2% Tween 20, 1l water and 1kg talc. The resulting mixture was air dried at room temperature for at least 15 days and then grinded. Finally, it was packed in 2 plastic bags containing 500g each. The liquid formulation was prepared with 20 *P. operculella* infected larvae which were homogenized and added, 0.2% Tween 20 and 1l water (Zeddam et al. 2003).

Efficiency assays

Field assays were carried out in Alvarado (Cartago, Costa Rica) throughout the two seasons in 2009: summer assays from February to May and winter assays from April to July. Both fields were divided into 25m^2 plots, each consisting of five 5m long rows with 20 potatoes seeded 25cm apart in each row. A row was kept empty between plots to avoid treatment drift. Plots were subjected with one of the following three treatments: i) baculovirus formulations alone (Bac); ii) baculovirus formulations together with insecticide and fungicide applications (Bac+I+F); and iii) insecticide and fungicide applications (I+F). Three additional plots were kept untreated as controls. The treatments were applied as scheduled in Fig. 1 and arranged in a complete randomize block design. The whole assay was replicated six times. The weight of the potatoes produced by the four plants placed along 1m of two central rows in each plot was registered at the end of the season and damage (those potatoes with at least one tunnel) calculated as a percentage between the weight of damaged potatoes and the total weight. Pheromone traps for both moths were placed in the four field corners from sowing to harvest and monitored each week.

A modified Shapiro-Wilks test was applied to check for the normality of the data and the analysis of the variance (ANOVA), and the Duncan test (SAS software package, SAS Institute Inc., USA) was applied to determine statistical differences.

Results and discussion

Field applications of a PhopGV-CR1-based powder and liquid formulations, alone or together with an insecticide, protected field potatoes as effectively as chemical insecticides (Table 1). Damage in the treated plots was significantly reduced: between 56.1 and 78.6% in the summer field and between 61.1 and 82.5% in the winter field compared with untreated control plots, where damage reached 19.1 and 14.4% in summer and winter, respectively. No significant differences were obtained between treatments in any of the two experimental fields, indicating that PhopGV-CR1 can be used against these pests alone, as an alternative to chemicals or, rather, included in IPM programs to diminish the chances of chemical resistance development.

Table 1. Percent damage by *P. operculella* and *T. solanivora* in summer and winter assays.

Treatment	Damage (%) ± S.E.	
	Summer	Winter
Control untreated	19.05 ± 1.62 a	14.38 ± 2.23 a
Bac	8.37 ± 2.27 b	5.59 ± 0.90 b
Bac+I+F	4.07 ± 1.43 b	2.64 ± 0.95 b
I+F	4.27 ± 0.97 b	2.52 ± 0.71 b

Damage inflicted in the untreated plots was moderately low because of the temperate climate of this region of Costa Rica, with frequent rains. Although rain leads to low moth densities, this pest has gradually adapted to higher moisture conditions. As occurred in the warehouses, incidence of *P. operculella* was lower than *T. solanivora* during both seasons (Fig. 1). Both populations suffered drastic population decreases upon chemical insecticide applications against moths (Fig. 1).

Frequency of virus application may seem too numerous in comparison with other baculovirus-host systems. However, weekly PhopGV applications are a common practice against potato tuber moths in Southern America given the overlapping host generations commonly found in tropical regions. Up to 10 *T. solanivora* generations per year can occur under optimal conditions (Notz, 1996), with a length cycle of 43 days at 25°C (Niño, 2004). In Venezuela, Niño captured adults from January to October (Niño, 2004).

In conclusion, the results of this study indicate that PhopGV-CR1 based formulations can be incorporated into IPM practices for field use against *P. operculella* and *T. solanivora* to protect potatoes in Costa Rica and that they may be as efficient as chemical insecticides.

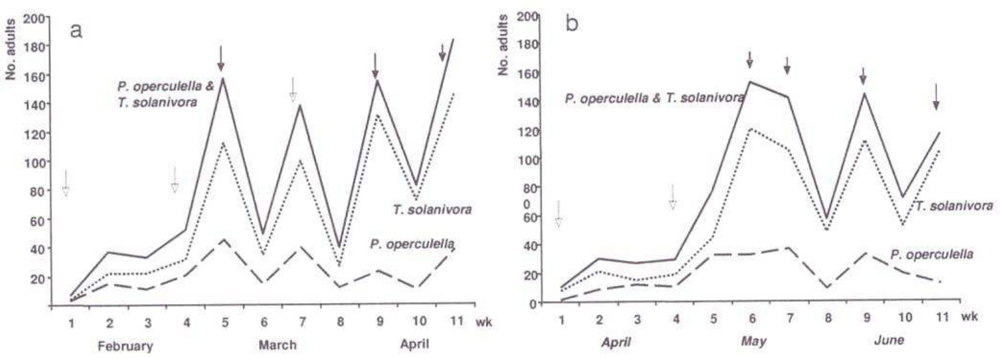

Figure 1. Population fluctuations of *T. solanivora* and *P. operculella* along the potato crop cycle. Black arrows indicate chemical insecticide applications against moths in Bac+I+F treated plots as well as in I+F treated plots, whereas grey arrows indicate chemical applications only in I+F treated plots.

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