

## **Isolation, identification and biocontrol activity of Colombian isolates of granulovirus from *Tecia solanivora* larvae**

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**Abstract:** Objective of the present work was to create a collection of native granulovirus for control of *Tecia solanivora*. Larvae were sampled from Colombian potato production areas. From 313 individuals analysed by dark field microscopy, 141 were preliminarily found positive for GV presence. Selected samples were used for viral propagation in order to reproduce the symptoms and signs of the disease and thus to demonstrate the presence of an infectious agent. Only 5 samples reproduced the symptoms and signs of granulovirus infection. These were purified on sucrose gradients and the identity confirmed by granulin identification using gel electrophoresis and by transmission electron microscopy. Biocontrol activity of formulated and unformulated native strains and a Peruvian reference granulovirus of *Phthorimaea operculella* were evaluated on *T. solanivora* larvae. Formulation enhanced biocontrol activity of the Peruvian reference strain and the native granulovirus isolates C0126 and C0404. Significant differences in mortality were found with unformulated strains ranging between 45% and 100% control.

**Key words:** *Tecia solanivora*, GV, potato tuber moth

### **Introduction**

In Colombia potato is grown on 180.000 ha and 90.000 families are linked to this production. The Guatemalan potato moth *Tecia solanivora* (Povolny) (Lepidoptera; Gelechiidae) is one of the most limiting potato pests in Venezuela, Colombia and Equador. Biological control using the granulovirus of *Phthorimaea operculella* (Zeller) has demonstrated to be one of the main tools for its management. CORPOICA has a commercial production plant for manufacturing using a Peruvian viral strain of *Phthorimaea operculella* granulovirus (PhopGV) for managing the Guatemalan moth under storage of potato seeds. This strain has demonstrated to be an efficient biocontrol agent for *T. solanivora*. However, a strain isolated from the target host might be more virulent than the strain from *P. operculella*. Actually, there are only few literature reports on a GV from *T. solanivora* (Zeddám et al., 1994; Niño and Notz, 2000). The present work pretended to isolate, identify and determine the biocontrol activity of a Colombian GV from *T. solanivora*.

### **Material and methods**

#### ***Collection of insects***

*T. solanivora* larvae collections were realized in the most important potato production areas of Colombia. Healthy larvae and those with signs of granulovirus disease were collected and placed individually in 1 ml saline solution (0,85%) in an Eppendorf tube. Thirty larvae were collected from each site. Larvae were transported to the laboratory and maintained at -70°C.

Each sample (larva) was homogenized in 1 ml saline solution and the suspension was divided in 5 sub-samples of 200  $\mu$ l which were maintained at -70°C.

#### ***Determination of granulovirus presence***

One sub-sample was utilized for checking granulovirus presence by using dark field microscopy. Samples with small white points with Brownian motion were classified as probably positive for granulovirus presence and used for infecting *T. solanivora* neonatal larvae in order to confirm the presence of virus. One sub sample of each larval suspension was diluted to 1 ml saline solution (0,85%) and dropped over a paper towel (10 cm<sup>2</sup>) with *T. solanivora* eggs (approximately 150 eggs per paper). Each inoculated paper was divided in smaller fragments of 1cm<sup>2</sup> and each fragment was placed over a clean potato. All potatoes inoculated with eggs from the same sample were placed inside a plastic cage. Cages were maintained at 22°C and 70% RH. Larvae were collected 25 days later and placed in Petri dishes. Larvae with signs of white coloration were classified as positive for viral presence.

#### ***Confirmation of granulovirus presence by SDS-PAGE***

Virus of each infected larvae was purified by using a sucrose gradient (45%, 65% and 80%) and purified virus was analyzed by SDS-PAGE according to Caballero et al. (2001).

#### ***Transmission electron microscopy***

Purified viruses were fixed on nets covered with foamvar. Samples were stained with phosphotungstic acid (2%, pH 6.3) and observed under an electron microscopy (Phillips 515).

#### ***Biocontrol activity of native granulovirus isolates***

The biocontrol activity of selected formulated and un-formulated Colombian isolates and the Peruvian reference strain was determined in bioassays. Purified virus OBs of each isolate were used to prepare a viral suspension adjusted to a concentration of 10<sup>5</sup> OBs/ml by using a previously standardized calibration curve (450 nm). Three clean potatoes were inoculated three times with each viral suspension by brushing the complete potato surface. All isolates were formulated with a commercial dry powder used for seed potato treatment. Formulated granulovirus isolates (10<sup>5</sup> OBs/g) application was carried out inside a plastic bag with three potatoes. Plastic bag was moved. Each potato was then placed in a plastic cage and 10 neonate *T. solanivora* larvae were placed over each tuber. Cages were cover with plastic lids and maintained at 22°C and 70% RH. Control treatment consisted in potatoes without viral inoculation. All larvae were collected 25 days later and mortality was determined. Mortality was corrected using the Schneider-Orelli equation (Zarr, 1999).

## **Results and discussion**

A total of 377 larvae were collected from 19 towns. Only in one town, larvae with typical granulosis infection symptoms were found. Results suggest that granulovirus infection symptoms are not frequently found under field conditions, as mentioned by Laarif et al. (2003) who studied the epidemiology of *Phthorimaea operculella* granulovirus under field conditions in Tunisia. Only with five samples, signs and symptoms of granulovirus infection were reproduced. Only five samples reproduced the granulovirus disease symptoms (Fig. 1) and were selected for further characterization. These isolates were codified as C0404, C0611, C0126 from Cundinamarca (centre of the country), Nr004 from Nariño (south, frontier with Equador) and N0108 from North of Santander (north-east, frontier with Venezuela).

SDS-PAGE showed only one band of approximately 35 kDa for all samples (Fig. 2) that coincide with granulin molecular weight, granulovirus main protein, which range from 25 to 38 kDa (Caballero et al., 2001). Other bands were not observed, possibly because of the very low concentration of other proteins, considering that granulin could be as much as 96% of granulovirus OBs total protein (Caballero et al., 2001). Structures observed with transmission electronic microscopy presented the typical morphological characteristics of granulovirus OBs (Fig. 3). For all samples oval particles of approximately 400  $\mu\text{m}$  length and 200  $\mu\text{m}$  wide were observed within the ranges described in the literature for granulovirus OBs (a length between 300 and 500  $\mu\text{m}$  and wide between 160 and 350  $\mu\text{m}$ ) (Caballero et al., 2001). Virions inside the protein matrix were visible in some pictures. Disease symptom reproduction, protein analysis and electron microscopy pictures confirmed the identity of larvae infectious agents as granulovirus.

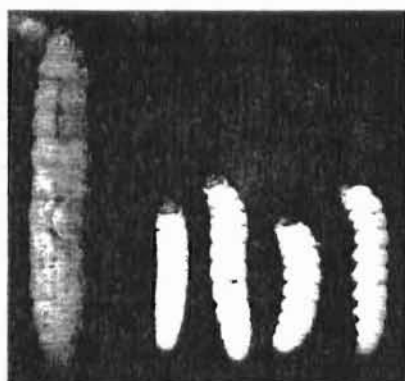


Figure 1. Healthy (A) and granulovirus infected (B) *T. solanivora* larvae

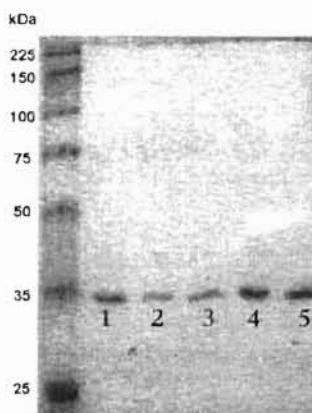


Figure 2. SDS-PAGE of native granulovirus isolates. 1. C0404, 2. N0108, 3. C0126, 4. C0611, 5. Nr004

Colombian granulovirus isolates N0108, Nr004 y C0611 produced a mortality of 100%, isolates C0126 and C0404 caused 45% and 50% mortality, respectively (Fig. 4). The Peruvian isolate produced 62% larval mortality. The statistical analysis using ANOVA and the Tukey test (95% confidence) revealed significant differences ( $P > 0.05$ ) between the three isolates. Results indicate that isolates N0108, Nr004 y C0611 are more virulent to *T. solanivora* than the Peruvian strain originally isolated from *P. operculella*. Harvey & Volkman (1983) reported that virus isolates obtained on the same host from different geographical zones could have the same genetic origin but could be more adapted to one particular host and to several other conditions in each ecosystem, elements that could generate some genetic variations and in consequence they might also have some differences in virulence. Significant differences were found when virus isolates were formulated ( $P > 0.05$ ). For isolation N0108, unformulated virus caused higher mortality than formulated virus (54%). Mortality of isolates C0126, C0404 and the reference strain from Peru increased significantly when formulated suggesting that formulation enhanced virus efficacy. Similar results were obtained by Ben Salah & Aalbu (1992), who evaluated a *P. operculella* granulovirus formulated using talcum under field conditions. The formulation enhanced viral activity, probably because small particle size of that powder caused spiracle blocking and larvae dehydration.

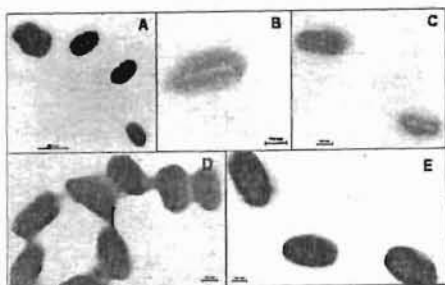


Figure 3. Electron microcopy of Colombian isolates A. N0108, B. C0611, C. C0404, D. C0126, E. Nr004

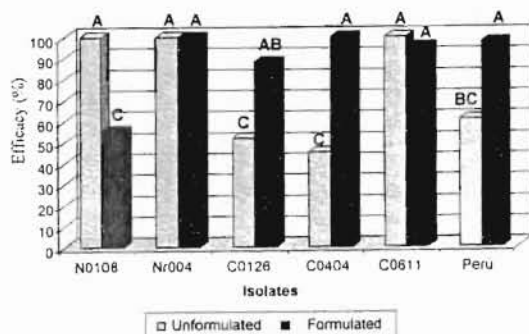


Figure 4. Efficacy of formulated and unformulated Colombian granulovirus isolates. Treatments with the same letter are not significantly different (Tukey 95%)

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