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Response to Reviewers: Estimado revisor: hemos agregado la palabra
"height" en la línea 15 del manuscrito.

Ética de la publicación

1. ¿Su trabajo ha comportado experimentación en animales?:

No

2. ¿En su trabajo intervienen pacientes o sujetos humanos?:

No

3. ¿Su trabajo incluye un ensayo clínico?:

No

4. ¿Todos los datos mostrados en las figuras y tablas incluidas en el manuscrito se recogen en el apartado de resultados y las conclusiones?:

No

1 **Infectivity by nematode-bacteria association on the potato weevil *Phyrdenus muriceus***

2 **Infectividad por asociación nematodo-bacteria en el gorgojo de la papa *Phyrdenus***

3 ***muriceus***

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Los autores declaran que no hay conflicto de intereses respecto al trabajo presentado.



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1 Bacteria of the genus *Photorhabdus* (Enterobacteriaceae) are motile, gram-negative,
2 symbiotically associated with juveniles of entomopathogenic nematodes from the genus
3 *Heterorhabditis* (Heterorhabditidae). Nematodes retain the *Photorhabdus* bacterial cells in
4 their intestine and transport them into an insect host⁶. Upon locating an insect host,
5 *Heterorhabditis* penetrates through natural openings (mouth, anus, spiracles)¹, or directly
6 into the hemocoel via the integument, subsequently releasing bacteria into the hemolymph⁴.
7 Once in the hemolymph, *Photorhabdus* begins multiplying simultaneously releasing toxins
8 virulent enough to kill the insect within 24 hrs². *Phyrdenus muriceus* (Coleoptera:
9 Curculionidae) is a pest of cultivated solanaceae affecting egg-plant, potato and tomato.
10 Considerable damages on these crops are produced by this pest affecting both the aerial and
11 underground parts of the plants^{3,5}. Infectivity of the entomopathogenic complex
12 *Heterorhabditis bacteriophora* and the symbiotic bacterium *Photorhabdus luminescens*
13 isolated from soil samples collected from an orchard of La Plata was tested against pupae
14 of *P. muriceus*. This stage was considered to be a suitable target to control at field due to its
15 permanence into the soil. Plastic containers (8.5 cm diameter x 5.5 cm height) were used
16 with sterile soil forming a 2 cm high layer. Pupae were added to the soil surface and
17 exposed to a concentration of 5.000 infective juveniles (IJs) / insect. Mortality of 70%
18 (n=10) occurred at 48 hrs after infection. In figure 1, we can appreciate nematodes inside
19 the body of the pupae (B and C). The cadavers showed the typical red wine coloration due
20 to the release of the endosymbiotic bacteria in the hemocoel (Fig. 1 B-D) unlike non-
21 parasitized pupae (Fig. 1A). The complex nematode-bacteria was effective to parasite and
22 kill *Phyrdenus muriceus* pupae. Future studies should be carried out to determine its use as
23 biological control for this horticultural pest.

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Figure 1 Pupae of *Phyrdenus muriceus* taken with an Olympus DP-71 camera. (A)

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Pupa with no infection (B) Dorsal view of infected pupa with nematodes inside the body

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(arrows). (C) Ventral view of infected pupa with nematodes inside the body (arrows). (D)

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Emerged nematodes from the lesion of the tegument (arrow).

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36 **References**

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