Mortality of *Phyllophaga vetula* larvae by the separate and combined application of *Metarhizium anisopliae*, *Steinernema carpocapsae* and *Steinernema glaseri*

Jaime Ruiz-Vega1,*, Carlos I. Cortés-Martínez2, Teodulfo Aquino-Bolaños1, Pastor T. Matadamas-Ortíz1, Cipriano García-Gutiérrez2 and José Navarro-Antonio1

1Instituto Politécnico Nacional, CIIDIR U. Oaxaca, Protección y Producción Vegetal, Santa Cruz Xoxocotlán, Oaxaca 71230, México.
2Cuerpo Académico de Biotecnología Agroalimentaria, Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Tulancingo de Bravo, Hidalgo 43600, México.
3Instituto Politécnico Nacional, CIIDIR U. Sinaloa, Bulevard Juan de Dios Bátiz Paredes 250, Colonia San Joachin, Guasave, Sinaloa, CP 81101, México.

*E-mail: jruizv@yahoo.com*

This paper was edited by Raquel Campos-Herrera.

Received for publication December 4, 2019.

Abstract

*Phyllophaga* spp. are a complex of edaphic insect pests that are present in the corn crops (*Zea mays*) in México, which are usually controlled with increasing dosages of broad-spectrum chemical insecticides. Several entomopathogenic nematode species can produce acceptable control levels of these larvae. However, the synergistic interaction between fungi and entomopathogenic nematodes (EPN) could improve the control of this insect. This study investigates the mortality of larvae of *Phyllophaga vetula* by the effect of the separate or combined application of the fungus *Metarhizium anisopliae* M1cog strain (Ma) and the nematodes *Steinernema carpocapsae* All strain (Sc) or *Steinernema glaseri* NJ-43 strain (Sg).

In laboratory, dosages of $1 \times 10^6$ or $1 \times 10^8$ spores/larva and 250 infective juveniles were applied on medium or large size *P. vetula* larvae contained in vials with sterilized agricultural soil as the assay arena. The separate application of Ma did not kill any larvae, but Sg killed 40 and 80% of the larvae, respectively. However, the Ma and Sc combination had an important antagonistic interaction that decreased the mortality to 40%, but the combination Ma and Sg had a slight additive interaction that increased the mortality to 47%. The most determining factor in larvae mortality was the nematode used, with Sg as the species with best performance in 6 of the 12 treatments evaluated and with a maximum effectivity of 80% on medium-size larvae if combined with a low dosage of Ma.

The combined application of an entomopathogenic fungus and EPN showed no consistent effects on the mortality percentage of *P. vetula*, mostly because the fungus was not isolated from *Phyllophaga* larvae.

Keywords

Entomopathogenic fungi, Entomopathogenic nematodes, Mixed infection, White grub, *Zea mays*.

White grubs of *Phyllophaga* spp. (Coleoptera: Scarabaeidae) are the principal soil-living pests in corn production (*Zea mays*), affecting seed quality and yield (Jackson and Klein, 2006). In corn fields of the central valleys of Oaxaca, Mexico, one of the most abundant species is *P. vetula* (Horn), which causes yield losses up to 50% depending on plant stand losses (Ruiz-Vega et al., 2003). In Central Mexico, a yield loss of 500 to 1,400 kg ha$^{-1}$ is expected (Villalobos, 1992). However, the use of chemical pesticides for the control of white grubs is causing increased resistance of this insect pest and has resulted in negative effects on the environment and human health, which make necessary the development of effective biological
control products with low impact on the environment and human health (Cory and Franklin, 2012; Chandel et al., 2019; Karabörklü et al., 2018).

The combined application of entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) through a formulated product is an alternative solution that is being tested to improve the effectiveness of the microbial pesticides used in integrated pest management programs (Wakil et al., 2017). It is believed that by the action mode of EPF, their previous application on the insect hosts increases their susceptibility to EPN infection (Ansari et al., 2008; Shaurub et al., 2016). However, in combination with nematophagous fungi, a reduction in the EPN and EPF activity has been reported (Bueno-Pallero et al., 2018).

The first challenge in the development of these products is to get a combination of entomopathogenic agents (EAs) that favor a synergist or additive interaction in order to achieve an effective control because biological, environmental, and management factors influence the performance of EAs.

The separate application of the entomopathogens *Metarhizium* spp., *Heterorhabditis* spp., and *Steinernema* spp. usually shows a good percentage of control over *Phyllophaga* spp., an edaphic pest that feeds on roots, decreases the yield and quality of the corn-crop *Zea mays* (Jackson and Klein, 2006; Gassmann and Clifton, 2017), but in Mexico *Phyllophaga* spp. is usually controlled with broad-spectrum chemical insecticides such as carburofan and heptachlor (Ruiz-Vega et al., 2003).

*S. glaseri* (NJ-43 strain) is a nematode of greater effectiveness than *S. carpocapsae* (All strain) against some species of *Phyllophaga* spp. (Ruiz-Vega et al., 2003; Girón-Pablo et al., 2015; Cortés-Martínez et al., 2017), while *S. carpocapsae* had an intermediate performance because it required four days to control 50% of the larvae when applied at doses of 200 IJs (Ruiz-Vega et al., 2003). The fungus *M. anisopliae* (HI-019) is more effective than *Bauveria bassiana*, killing up to 86% of *P. vetula* larvae treated with $1 \times 10^6$ conidia.mL$^{-1}$ after 12 days post-inoculation (Pérez et al., 2016).

In laboratory, additive interactions have been observed between *H. megidis* and *B. bassiana*, and between *H. bacteriophora* and *B. bassiana* or *M. anisopliae* in most of the combinations applied on *Cyclocephala lurida* (Coleoptera: Scarabaeidae). However, the combined effect was not significantly different for simultaneous applications of nematodes or fungi or carried out at different times (Wu et al., 2014). In a study in laboratory and greenhouse conditions, where the interaction between four EPN and two EPFs was evaluated, synergies were found between nematode species applied after the fungi at different times, while the separate application of *B. bassiana* and *M. anisopliae* did not reduce the population of third-stage larvae of *Cyclocephala lurida* Bland (Martínez-Hernández et al., 2015).

Except for a few studies, the effect of the combined application of certain species such as *Metarhizium* spp. and *Steinernema* spp. on *Phyllophaga* spp. is still little explored and could result in a synergistic or additive effect with better effectiveness than applied separately. Therefore, the aim of this work was to evaluate the effect of separate and combined application of the fungus *M. anisopliae* and the nematode *S. glaseri* or *S. carpocapsae* on the mortality of *P. vetula* and to determine its type of interaction in a four-day deferred application between EAs.

**Materials and methods**

**P. vetula larvae**

Larvae of *P. vetula* were collected from two different corn-crop fields in the village of Cuilapam de Guerrero, Oaxaca, Mexico (17° 00´ N, 96° 46´ W, 1596 masl) in July, 2019. The presence of larvae in the earlier stages of growth of corn plants (*Zea mays*) and the appearance of visible damage in the plants were the criteria for selecting the sampling site. Each larva was collected manually from a 0 to 20 soil depth and confined individually in a 50 mL cylindrical plastic container that was filled with sterilized agricultural soil from the collection site and a slice of carrot as feed. In laboratory, the *P. vetula* larvae were classified by their size as medium (1.8 cm < Me < 2.5 cm) or as large (2.5 cm < La ≥ 3.2 cm) and kept at room temperature (23 ± 6°C). During a week, insects were observed in order to separate the dead, injured, or infected larvae.

**Entomopathogenic agents**

The EPF used was *M. anisopliae* M1cog strain (Ma) Access GenBank KF998522 (Metchnikoff) Sorokin (1883), which was isolated from *Spodoptera frugiperda*, was provided by the “Laboratorio de Bioinsecticidas de CIIDIR IPN Unidad Sinaloa.” The nematodes *Steinernema carpocapsae* All strain (Sc) Access GenBank CM016762.1 (Weiser, 1955) and *Steinernema glaseri* NJ-43 strain (Sg) Access GenBank AF122015.1 (Steiner, 1929) are from the collection of the “Laboratorio de Nematodos Entomopatógenos of CIIDIR IPN Unidad Oaxaca.”
which was initiated with nematode species donated by the EPN Laboratory of the University of California, Davis. The nematodes were reproduced using third-instar larvae of the wax moth Galleria mellonella (Kaya and Stock, 1997) and stored at 12°C for 15 days before use.

**Infectivity assay**

Infectivity, defined as the proportion of dead insects after being exposed to entomopathogens (Ricci et al., 1996), was tested every seven days from the moment of application of Ma. Eight observation periods were completed, i.e., for a total of 56 days. The assay arena was a 100 mL cylindrical container filled with loamy-sand soil and a larva of size Me or La. P. vetula larvae were inoculated first with Ma in high (H) or low (L) doses, 1 × 10^6 and 1 × 10^5 spores/insect, respectively. Four days later, the nematodes Sg or Sc were applied at a dose of 250 IJs/insect (20 IJs/cm^2) on the surface of the arena. This apparently large amount was applied to ensure infection because the larvae were already buried into the soil. The dilution procedure proposed by Stock and Goodrich-Blair (2012) was followed to estimate the IJ’s concentration.

Insect mortality was determined when the larvae did not move when stimulated with a dissecting needle. To confirm the cause of death, each cadaver presenting signs of infection was transferred to a petri dish lined with a filter paper disc (90 mm in diameter, Whatman No. 1) and incubated at 23 ± 2°C until the appearance of spores of Ma (indicative of fungal infection) or the emergence of IJs (indicative of infection by nematodes) happened.

**Experimental design and treatments**

To evaluate the effect of the application of fungus Ma and the nematodes Sc and Sg on the mortality of P. vetula, two sizes of larvae were inoculated in a multifactorial experiment 2^3, where the factors were: (i) size of the larva (Me or La), (ii) dose of Ma (H or L), and (iii) species of EPN (Sc or Sg). The eight resulting treatments were: LaHSc, LaHSc, LaLSc, LaLSc, MeHSc, MeHSc, MeLSc, and MeLSg. One larva of size Me or La was placed in each of 15 containers filled with field-capacity moist agricultural soil per treatment to determine insect mortality, including the control treatment. The experiment was conducted twice. The treatments LaH, LaSg, MeL, and MeSc were included to evaluate the type of interaction on the LaHSc and MeLSc treatments and the effect of increasing the dose of Ma in larvae of size Me or La.

**Determination of the interaction between M. anisopliae and nematodes on P. vetula**

The type of interaction (synergistic or antagonistic) between EPF and EPN was determined in the combined treatment LaHSc considering LaH and LaSg effects, whereas for the treatment MeLSc, the effects of MeL and MeSc were considered. The expected mortality was estimated using the formula $E_m = M_H + M_l (1-M_e)$, where $M_H$ and $M_l$ are the mortality percentage observed for nematodes and fungi inoculated separately, respectively. Then, a Pearson test was carried out with the estimated value of observed mortality $M_{n+f}$ in combined applications and compared with the value in tables of $X^2$ for 1 degree of freedom, with $p < 0.05$. According to the criterion established, if it is fulfilled that $X^2 > X^2_t$, the interaction is additive, or it is synergistic/additive if that criterion is not met. Then, if the difference between $M_{n+f}$ and $E_m$ has a positive or negative value, it is considered that there is a synergistic or antagonistic interaction, respectively (Mcvay et al., 1977).

**Statistical analysis of data**

The accumulated mortality per treatment after 56 days was used to estimate the mortality percentage, i.e. the number of dead larvae in proportion to the number of larvae that was used. The data were transformed into ranges to satisfy the normality criteria. The effect of the factors of insect size, dose of Ma and EPN species on mortality percentage was determined by using a factorial analysis of variance (ANOVA) and applying the Tukey test ($p < 0.05$). Also, a Pearson correlation analysis was performed to determine the relationship between increasing doses of Ma before the application of Sg or Sc and the mortality percentage of larvae of size Me or La ($p < 0.05$). All analyzes were performed on SigmaPlot® 12 (Systat Software, Inc., San José, CA, USA).

**Results**

**Interaction between fungi and EPN on P. vetula larvae**

The average mortality percentage of larvae of two sizes (Me and La) of P. vetula by the application of the fungus Ma in two doses (H or L) and the nematodes Sc and Sg during a 56 days period is presented in Figure 1. In treatments with only one entomopathogen, the highest
Mortality of Phyllophaga vetula larvae: Ruiz-Vega et al.

Larvae mortality was observed with MeSc (80±11.5%) and no mortality was recorded in the LaH or MeL treatments. In combination treatments, the highest mortality was achieved in MeLSg (80±11.5%) and the lower mortality was observed when the MeHSc (27±17%) and LaHSc (27±13%) treatments were applied. No larval mortality was observed in the untreated control (data not shown).

The statistical comparison of all treatments with respect to the control shows that only six treatments (Table 1) were statistically different (p<0.05). In the combined treatments, the larger difference was observed with the MeLSg treatment (p=0.002), followed by the MeHSc treatment (p=0.047) and the LaLSg and LaHSc ones. In the uncombined treatments, MeSc was the one with the greatest control difference (p=0.002), while LaSg showed the minimum mortality difference. Five of the six better treatments applied included Sg.

The interaction analysis shows that the combined treatments LaHSc (47±13.3%) and LaSc (40±11.5%) presented a synergistic interaction $\chi^2 < \chi^2$ between the fungus Ma and the nematode Sg, while an antagonistic one ($D = -33.33$) was observed between Ma and Sc.

**Effect of EPN on the mortality of Phyllophaga vetula larvae**

The ANOVA analysis between factors showed that the only significant difference was in the mean values

| Treatment  | Mortality of Phyllophaga vetula (%) ± SD | p-value (ANOVA) |
|-----------|----------------------------------------|-----------------|
| LaHSc     | 47±13.3                                | 0.031           |
| LaLSg     | 60±11.5                                | 0.009           |
| MeHSc     | 60±20                                  | 0.047           |
| MeLSg     | 80±11.5                                | 0.002           |
| LaSg      | 40±11.5                                | 0.034           |
| LaHSc     | 27±13.3                                | 0.116           |
| LaLSc     | 33.3±13.3                              | 0.086           |
| MeHSc     | 27±17.6                                | 0.219           |
| MeLSc     | 47±29                                  | 0.185           |
| MeSc      | 80±11.5                                | 0.002           |
| LaH       | 0                                      | 0               |
| MeL       | 0                                      | 0               |

Notes: H, high dose of *Metarhizium anisopliae*; L, low dose of *Metarhizium anisopliae*; Sg, *Steinernema glaseri*; Sc, *Steinernema carpocapsae*; La, large-sized larvae; Me, medium-sized larvae.
Effect of *Metarhizium anisopliae* dose on the mortality of *Phyllophaga vetula*

The mortality trend of two *P. vetula* larvae sizes due to the increase in the dose of Ma (H, L, and a control without fungus) before the treatment with Sg or Sc is shown separately. It can be seen that in the treatments with Sg (Fig. 3A), there was no significant relationship between dose and mortality among the different levels of the EPN factor and the comparison between Sg and Sc levels; Tukey tests showed that the difference of the two groups that were compared was statistically significant (N=24, q=3.438, p=0.027). This difference can be seen in the graph of mean mortality for EPN by levels of Ma (Fig. 2), where the mortality percentage follows the same increasing trend in both levels of EPN with increasing doses of Ma, but without a significant influence of Ma on larvae mortality.

By analyzing the tested factors separately, it was found that the species of EPN applied was the most important component in the mortality percentage of *P. vetula*, with Sg being the nematode of best performance (N=24, q=3.438, p=0.027). The factors that did not have a significant influence on the mortality percentage were insect size or dose of Ma. When the entomopathogens were combined, larvae mortality of size Me increased when the dose of Ma decreased, particularly in the combination Ma with Sc (Pearson r = −1.0, p = 0.0175).

The mortality trend of two *P. vetula* larvae sizes due to the increase in the dose of Ma (H, L, and a control without fungus) before the treatment with *S. glaseri* or *S. carpocapsae* is shown separately. It can be seen that in the treatments with Sg (Fig. 3A), there was no significant relationship between dose and mortality.
of La larvae (Pearson $r = -0.162, p = 0.896$), whereas in the treatments with Sc the mortality of Me larvae tended to increased significantly (Pearson $r = -0.856, p = 0.0345$) when the dose of Ma decreased (Fig. 3B).

**Discussion**

This is the first study to evaluate the mortality of *P. vetula* larvae of two sizes due to the separate or combined application of the fungus *M. anisopliae* and the nematodes *S. glaseri* or *S. carpocapsae*. In respect to separate applications, the results show that the mortality percentage by EPN went from regular to high, while no larvae died by the application of *M. anisopliae* alone. However, when the EPN and the fungus *M. anisopliae* were applied in combination, the interactions were of the additive and antagonistic types. The additive interaction happened between Ma and Sg and the insect mortality increased, whereas with the combined application of Ma and Sc, the interaction was antagonistic and insect mortality diminished.

The deferred application between EPF and EPN has been evaluated on third-instar larvae of *Phyllophaga polyphylla* by Martínez-Hernández et al. (2015) who reported an antagonistic effect and a low percentage of insect mortality. In that study, they first applied *Metarhizium pingshaense* ($1 \times 10^8$ conidia.mL$^{-1}$) and six days later *H. bacteriophora* (50 IJs.mL$^{-1}$), obtaining a mortality of 9.1 to 11.5% after 39 days. However, combining a *B. pseudobassiana* isolate ($1 \times 10^5$ conidia.mL$^{-1}$) and one isolate of *H. bacteriophora* (100 IJs.mL$^{-1}$), where the fungal pathogen was inoculated first, an additive interaction was identified.

The nematode Sc is usually not effective against larvae of *Phyllophaga* spp. This species has been tested at a dose of $5 \times 10^8$ IJs.ha$^{-1}$ against *P. anxia* larvae (LeConte) in Abeto Fraser tree production fields and no effective control was observed in relation to the control treatment over two years of the experiment, because it only killed 1 insect/m$^2$ in the first year and less than 1 insect/m$^2$ in the second year ( Liesch and Williamson, 2010). When evaluated against third-instar larvae of *P. bicolor*, the nematode Sc (All strain) was slightly infective (22.92%), but it did not cause the death of any insect (Melo et al., 2010).

Third-instar larvae of *P. hirtula* were confronted to 24-h-old Sc at a concentration of 1,000 IJs/larva, but even though there were IJs on the body of the insect, the entry route was through the digestive tract (anus and mouth) and not through the spiracles or perforations in the cuticle (Forschler and Gardner, 1991), resulting in a lower percentage of penetration, and a low percentage of larvae mortality.

Sg is a nematode of greater effectiveness than Sc against some local species of *Phyllophaga* spp. In the laboratory, the dose of 1000±50 IJs on third-instar larvae of *P. vetula* has achieved a mortality rate of 40 to 97.5% (Girón-Pablo et al., 2015; Cortés-Martínez et al., 2017), while Sc (All strain) had an intermediate performance because it required four days to control 50% of the larva when applied at doses of 200 IJs (Ruíz-Vega et al., 2003). In contrast, third-instar larvae of *P. crinita*, *P. congrua*, and *P. georgiana* were not very susceptible to Sg (NC strain) in doses of 400 IJs per insect, since the mortality percentage was 6 to 23% (Koppenhöfer et al., 2004). Only depending on application rate, Sc (All strain) combined with *B. bassiana* resulted in additive effect on *Curculio caryae* (Coleoptera: Curculionidae) (Shapiro-Ilan et al., 2004).

*M. anisopliae* is more effective than *B. bassiana* against *P. vetula*; the pathogenicity of HI-019 strain native of Morelos, Mexico, applied at a dose of $1 \times 10^6$conidia.mL$^{-1}$ was 86% after 12 post-inoculation (Pérez et al., 2016). However, our study reports different results as *M. anisopliae* M1cog strain at doses of $1 \times 10^6$ and $1 \times 10^8$ spores/insect did not kill any larvae.

This result suggests that *M. anisopliae* M1cog strain tends to be a specialist rather than a generalist fungus, mainly because it is an isolate from *S. frugiperda*, which limits their ability to cause disease in multiple insects as Zimmermann (2007) and Wang et al. (2016) argued. Santi et al. (2010) pointed out that the insect cuticles induce the secretion of specific proteases to respond in a precise and specialized way to specific hosts.

On the basis of the results presented in this study and the hypothesis that suggests that the synergistic interactions strongly depend on the target host, pathogens that are combined and the application timing (simultaneous vs sequential) (Wakil et al., 2017), the combined application of an EPF and EPN showed no consistent effects on the mortality percentage of *P. vetula*, but certainly, additional exploratory studies in relation to these factors must be carried out to confirm this proposition.

**Conclusions**

This study conducted under laboratory conditions showed that the separate application of EPN on *P. vetula* larvae caused mortalities of 40 and 80% using the nematodes *S. glaseri* (NJ-43 strain) and *S. carpocapsae* (All strain), respectively. However, the application of *M. anisopliae* (M1cog) did not cause any deaths because the fungus was isolated from *S. frugiperda*. 
As for the combined application, an additive interaction between the time deferred application of the fungus *M. anisopliae* and *S. glaseri* caused a slight increase in mortality from 40 to 47% on *P. vetula* large-sized larvae, while the antagonistic effect by the deferred application of *M. anisopliae* and *S. carpocapsae* significantly diminished its mortality percentage from 80 to 27% on medium-sized larvae.

**Acknowledgments**

This work was supported by the SIP Project 20196287. The authors thank the Instituto Politécnico Nacional (IPN) of México and the IPN Comisión de Operación y Fomento de Actividades Académicas (COFAA-IPN). The authors have no financial interest and gain no benefit from the direct applications of the research described in this manuscript.

**References**

Ansari, M. A., Shah, F. A. and Butt, T. M. 2008. Combined use of entomopathogenic nematodes and *Metarhizium anisopliae* as a new approach for black vine weevil, *Otiorynchus sulcatus* (Coleoptera: Curculionidae) control. Entomol Experimentalis et Applicata 129:340–7.

Bueno-Pallero, F. A., Blanco-Pérez, R., Dionisio, L. and Campos-Herrera, R. 2018. Simultaneous exposure of nematophagous fungi, entomopathogenic nematodes and entomopathogenic fungi can modulate belowground insect pest control. Journal of Invertebrate Pathology 154:85–94.

Chandel, R. S., Soni, S., Vashisth, S., Pathania, M., Mehta, P. K., Rana, A., Bhatnagar, A. and Agrawal, V. K. 2019. The potential of entomopathogens in biological control of white grubs. International Journal of Pest Management 65:348–62.

Cortés-Martínez, C. I., Lewis, E. E., Ruiz-Vega, J. and Martínez-Gutiérrez, G. A. 2017. Mechanical production of pellets for the application of entomopathogenic nematodes: effect of pre-acclimation of *Steinernema glaseri* on its survival time and infectivity against *Phyllophaga vetula*. Biocontrol Science and Technology 27:940–51.

Cory, J. S. and Franklin, M. T. 2012. Evolution and the microbial control of insects. Evolutionary Applications 5:455–69.

Forschler, B. T. and Gardner, W. A. 1991. Parasitism of *Phyllophaga hirticula* (Coleoptera: Scarabaeidae) by *Heterorhabditis heliothidis* and *Steinernema carpocapsae*. Journal of Invertebrate Pathology 58:396–407.

Gassmann, A. J. and Clifton, E. H. 2017. “Current and potential applications of biopesticides to manage insect pests of maize”, In Leland, J. and Gore, J. (Eds), Microbial Control of Insect and Mite Pests: From Theory to Practice Elsevier, London, 173–84.

Girón-Pablo, S., Ruiz-Vega, J., Pérez-Pacheco, R., Ortiz-Hernández, Y. D. and Aquino-Bolaños, T. 2015. Biological control of *Phyllophaga vetula* (Horn), and lethal concentrations and times of entomopathogenic nematodes. Southwestern Entomologist 40:291–6.

Jackson, T. A. and Klein, M. G. 2006. Scarabs as pests: a continuing problem. Coleopterists Bulletin 60:102–19.

Karabörkül, S., Azizoglu, U. and Azizoglu, Z. B. 2018. Recombinant entomopathogenic agents: a review of biotechnological approaches to pest insect control. World Journal of Microbiology & Biotechnology 34:14.

Kaya, H. K. and Stock, S. P. 1997. “Techniques in insect nematology”, In Lacey, L. A. (Ed), Manual of Techniques in Insect Pathology Elsevier, London, 281–324.

Koppenhöfer, A. M., Fuzy, E. M., Crocker, R. L., Gelernter, W. D. and Polavarapu, S. 2004. Pathogenicity of *Heterorhabditis bacteriophora*, *Steinernema glaseri*, and *S. scarabaei* (Rhabditida: Heterorhabditidae, Steinernematidae) against 12 white grub species (Coleoptera: Scarabaeidae). Biocontrol Science and Technology 14:87–92.

Liesch, P. J. and Williamson, R. C. 2010. Evaluation of chemical controls and entomopathogenic nematodes for control of *Phyllophaga* white grubs in a Fraser fir production field. Journal of Economic Entomology 103:1979–87.

Mqvay, J. R., Gudauskas, R. T. and Harper, J. D. 1977. Effects of *Bacillus thuringiensis* nuclear polyhedrosis virus on Trichoplusia Mixtures nor larvae. Journal of Invertebrate Pathology 29:367–72.

Martínez-Hernández, A., Alatorre-Rosas, R., Guzmán-Franco, A. W. and Rodríguez-Leyva, E. 2015. Effect of dual inoculation with nematodes and fungal pathogens on the survival of *Phyllophaga polyphylla* larvae (Coleoptera: Scarabaeidae). Biocontrol Science and Technology 25:1221–32.

Melo, M. E. L., Ortega, O. C. A., Gaigl, A. and Bellotti, A. 2010. Evaluation of entomopathogenic nematodes for the management of *Phyllophaga bicolor* (Coleoptera: Melolonthidae). Revista Colombiana de Entomología 36:207–12.

Pérez, O. S., Gutiérrez, A. C., Chora, G. P., Andrés, A. G., Morales, M. M. S., Ramón, S. R. and Velázquez, V. M. H. 2016. Pathogenicity, virulence and the interaction of *Metarhizium anisopliae* and *Beauveria bassiana* against *Phyllophaga vetula* (Coleoptera: Melolonthidae). Journal of Pure and Applied Microbiology 10:2607–13.

Ricci, M., Glazer, I., Campbell, J. F. and Gaugler, R. 1996. Comparison of bioassays to measure virulence of different entomopathogenic nematodes. Biocontrol Science and Technology 6:235–46.
Ruiz-Vega, J., Aquino-Bolaños, T., Kaya, H. K. and Stock, P. 2003. Colecta y evaluación de nematodos entomopatógenos para el control de gallinas ciegas *Phyllophaga vetula* (Horn) en Oaxaca, México. Folia Entomológica Mexicana 42:169–75.

Santi, L., Silva, W. O. B., Pinto, A. F. M., Schrank, A. and Vainstein, M. H. 2010. *Metarhizium anisopliae* host–pathogen interaction: differential immunoproteomics reveals proteins involved in the infection process of arthropods. Fungal Biology 114:312–9.

Shapiro-Ilan, D. I., Jackson, M., Reilly, C. C. and Hotchkiss, M. W. 2004. Effects of combining an entomopathogenic fungi or bacterium with entomopathogenic nematodes on mortality of *Curculio caryae* (Coleoptera: Curculionidae). Biological Control 30:119–26.

Shaurub, E. -S. H., Reyad, N. F., Abdel-Wahab, H. A. and Ahmed, S. H. 2016. Mortality and nematode production in *Spodoptera littoralis* larvae in relation to dual infection with *Steinernema riobrave*, *Heterorhabditis bacteriophora*, and *Beauveria bassiana*, and the host plant. Biological Control 103:86–94.

Steiner, G. 1929. Neaaplectana glaseri, ng. n. sp. (Oxyuridae), a new nemic parasite of the Japanese beetle (*Popillia japonica* Newm). Journal of Washington Academy of the Science 19:436–40.

Stock, S. P. and Goodrich-Blair, H. 2012. Nematode parasites, pathogens and associates of insects and invertebrates of economic importance. In Lacey, L. A. (Ed.), Manual of Techniques in Invertebrate Pathology, London: Academic Press, pp. 373–426.

Villalobos, J. F. 1992. “The potential of entomopathogens for the control of white grub pests of corn in México”, In Jackson, T. A. Y. and Giare, T. R. (Eds), Use of Pathogens in Scarab Pest Management Intercept Ltd, Andover, 253–60.

Wakil, W., Yasin, M. and Shapiro-Ilan, D. I. 2017. Effects of single and combined applications of entomopathogenic fungi and nematodes against *Rhynchophorus ferrugineus* (Olivier). Scientific Reports 7:5971.

Wang, J. B., St. Leger, R. J. and Wang, C. 2016. “Advances in genomics of entomopathogenic fungi”, in Lovett, B. and St. Leger, R. J. (Eds), Advances in Genetics Elsevier, London, 67–105.

Weiser, J. 1955. Neaaplectana carpocapsae n. sp. (Anguillulata, Steinernematinae). novy cizopasnik housenik obalece jablecného, Carpocapsa pomonella L. Vestnik Cesk Zool Spolecnosti 19:44–52.

Wu, S., Youngman, R. R., Kok, L. T., Laub, C. A. and Pfeiffer, D. G. 2014. Interaction between entomopathogenic nematodes and entomopathogenic fungi applied to third instar southern masked chafer white grubs, *Cyclocephala lurida* (Coleoptera: Scarabaeidae), under laboratory and greenhouse conditions. Biological Control 76:65–73.

Zimmermann, G. 2007. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Science and Technology 17:879–920.