

# PRELIMINARY EVALUATION OF PATHOGENS AFFECTING *Eleodes longicollis punctigerus* BLAISDELL (COLEOPTERA: TENEBRIONIDAE)

## EVALUACIÓN PRELIMINAR DE PATÓGENOS QUE AFECTAN A *Eleodes longicollis punctigerus* BLAISDELL (COLEOPTERA: TENEBRIONIDAE)

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### SUMMARY

The false wire-worm *Eleodes longicollis punctigerus*, despite of been registered more than 30 years ago, is until now considered as an emerging pest in some areas of the Sabana de Bogotá, in Colombia. Therefore, within an integrated management program, the use of biological control agents have to be considered. Under field conditions, larvae and adult wireworms were found to be affected by pathogenic microorganisms, such as *Paecilomyces* sp., *Beauveria bassiana* and *Metarhizium anisopliae* and nematodes. Preliminary studies to determine their pathogenicity in the laboratory were undertaken, showing that none of them were highly pathogenic. An alternative explanation may be that *E. longicollis* may be tolerant to the infection or the spore concentration used was too low, because the highest larval mortality observed was only 20% with the treatment pos. *Paecilomyces* sp., at  $1 \times 10^7$  spore concentrations. Adult mortality of 22.5% with *M. anisopliae* at  $1 \times 10^7$  was obtained. It was also found that the insect is susceptible to nematodes. Both nematodes and fungi should be studied under other conditions, with higher concentrations, in order to be incorporated as a complement into a strategy of preventive control.

Key words: False wire-worm, micro-biologic control, fungi, nematode.

### RESUMEN

El falso gusano alambre *Eleodes longicollis punctigerus*, a pesar de haberse detectado hace más de 30 años, apenas últimamente, se perfila como una plaga emergente en algunas

áreas de la Sabana de Bogotá, Colombia. Por ello, dentro de un plan de manejo integrado, se plantea el uso del control biológico y, luego de encontrar en campo larvas y adultos afectados por microorganismos entomopatógenos, tales como *Paecilomyces* sp., *Beauveria bassiana* y *Metarhizium anisopliae* y por nematodos, se decidió determinar la patogenicidad de éstos en el laboratorio. Los resultados mostraron que ninguno de ellos es altamente patogénico, existiendo la posibilidad que el insecto es tolerante a la infección o que la concentración de esporas empleada fue muy baja, ya que la mortalidad larval máxima observada fue del 20%, con el tratamiento de pos. *Paecilomyces* sp., concentración de esporas  $1 \times 10^7$ . Sobre el adulto, se observó una mortalidad del 22,5%, con el hongo *M. anisopliae*, a una concentración del  $1 \times 10^7$ ; también, se detectó que el insecto es susceptible a nematodos. Se concluye que, tanto estos últimos como los hongos mencionados, deben ser estudiados bajo otras condiciones y en concentraciones más altas, para poderlos incorporar como complemento de una estrategia de control preventivo, a su debido tiempo antes de la siembra, y con condiciones de adecuada humedad del suelo.

Palabras clave: Falso gusano alambre, control microbiológico, hongos, nematodo.

### INTRODUCTION

Biological control has been for years a tool for population management of insect crop pests, being defined by Nicholls (2008) as the use of beneficial organisms against pests that cause damage. This concept of biological control can be based on the principle that in nature all organisms possess

antagonists, which either compete or eliminate them, a process being essential for life equilibrium (Hanson, 1993).

Microorganisms are components of biological control, the so called entomopathogens, including viruses, bacteria, protozoa, rickettsia, fungi and nematodes, which can be found affecting insects, establishing symbiotic relationships such as parasitism and also causing disease and death, contributing therefore to the natural regulation of insect populations (Lacey & Kaya, 2007).

Currently, many organisms are studied and used in microbiological control programs. Successful alternatives, using entomopathogenic fungi to control arthropod pests, inhabiting water and soil environments have been developed, using primarily the genera *Metharizium*, *Beauveria*, *Sporothrix*, *Nomuraea*, *Paecilomyces* among others (Alves & Lopes, 2008). According to Posada & Pava-Ripoll (2010), *Bacillus* spp., *Beauveria bassiana* (Moniliales: Moniliaceae) and *Metharizium anisopliae* (Hypocreales: Clavicipitaceae) are the most studied fungi being mentioned in the literature, to control several insect pests. Vieira Tiago *et al.* (2014) also highlighted *M. anisopliae* as the fungus species most studied and used for control purposes, due, among other characteristics, to its persistence in the soil.

Nematodes have multiple relationships with insects; they can be parasites, affecting their reproductive ability (Kaya & Stock, 1997), but they can also enter insects and release bacteria that are responsible for the individual's death by septicemia (Rosales *et al.* 2009).

Both fungi and nematodes are recommended as biological control agents of insect pests in crops of economic importance; such is the case of *B. bassiana* for the management of the coffee berry borer (*Hypothenemus hampei*). This fungus is the most commonly used and widely distributed natural enemy of this pest (Posada & Pava-Ripoll, 2010).

*Beauveria bassiana* is also efficient against tenebrionids such as *Alphitobius diaperinus* in chicken houses (Steinkraus *et al.* 1991) and against *Tribolium castaneum* pest of stored grains (Pedrini *et al.* 2010). *Metharizium anisopliae* is used to control insects of several families of the Order Coleoptera, such as Curculionidae and Scarabidae, common pests of rice, citrus and sugarcane (Nicholls Estrada, 2008). It also affects wireworms of the genus *Agriotes* (Elateridae) (Kabaluk & Ericsson, 2007; Ericsson *et al.* 2007). Another species, *M. brunneum* has been inclusive, evaluated against ticks of the genus *Ixodes* (Acari: Ixodidae).

The nematode genera *Steinernema* and *Heterorhabditis* form part of the integrated management program of *Premnotrypes vorax* and *Tecia solanivora* in potato crops

(Maggiorani & Gudiño, 1996). *Steinernema feltiae* and *S. carocapsae* (Rhabditia Steinernematidae) are considered as promising tenebrionid control agents for *A. diaperinus* (Geden *et al.* 1987) and *Cyanaeus angustus* (Nansen *et al.* 2013), respectively.

The false wire-worm, recently identified as *Eleodes longicollis punctigera* Blaisdell, by Dr. Charles A. Triplehorn, Ohio State University, United States, based on specimens sent by Dr. Francisco Javier Posada Flórez, and previously referenced in Colombia as *Eleodes omissoides* Blaisdell (Coleoptera: Tenebrionidae), causes seed losses of various plant species, especially grasses and seedlings that reach germination. Larvae and adults of the pest are considered a limiting factor in the affected crops, because the attack requires replanting, and the use of insecticides that increase costs (Quiroga-Murcia & Posada-Flórez, 2013; Zenner de Polanía *et al.* 2014; Calkins & Kirk, 1973; Rogers *et al.* 1988). It is worthwhile mentioning, that *E. longicollis punctigerus* was recorded for the first time in Mexico and described by Blaisdell in 1935, but in this country apparently it never became a pest.

Information on the genus *Eleodes* is scarce and the literature refers primarily to the taxonomy and the description of new species from Mexico and the United States (Triplehorn, 2010; Triplehorn & Cifuentes, 2011; Triplehorn & Thomas, 2011); South-American data outside those from Colombia, to our knowledge, do not exist.

*Eleodes longicollis punctigera* receives the common name of false wireworm because of the larval resemblance to the larvae of the family Elateridae (Coleoptera) which are considered the true wire-worms. This generic name is given to the larvae of various insects of the genera *Epitragus*, *Anaedes*, *Blapstinus*, *Lobometopon* and *Ullus* spp. (Saunders *et al.* 1998), all of the family Tenebrionidae, known pests of rice, sorghum, corn, pineapple, cotton and pastures.

Previously identified as *E. omissoides* in Colombian publications and in the newsletter Entomological Notes and News (NNE), the insect was first recorded in the department of Boyacá in 1977 and then in Cundinamarca in 1980 attacking pea (*Pisum sativum*) seeds. From then on the bulletin sporadically mentions the insect in several municipalities of Cundinamarca such as Madrid, Mosquera, Bojacá and Facatativá and Tunja (Boyacá). In addition, the authors observed that the insect is susceptible to entomopathogenic fungi and nematodes. The susceptibility of *E. longicollis* was intended to be confirmed with this research, even though according to Pears (2009) false wire-worms do not have registered biological control agents.

This study, evaluated the potential of some naturally occurring entomopathogens, found at "El Remanso", research unit

of the University of Applied and Environmental Sciences, U.D.C.A Bogotá, Colombia. These biocontrol agents can be part of a sustainable control that could be integrated into a management program of *E. longicollis*.

## MATERIALS AND METHODS

Most studies, if not stated otherwise, were carried out under laboratory conditions,  $18 \pm 3^\circ\text{C}$ , 80% R.H. at the University.

**Collection, breeding and survey:** The collection of adults, larvae, pupae and eggs of the pest was done at "El Remanso", during three sampling periods. Two-hundred adults and some larvae were hand captured. Adults were separated by sex, resulting in 100 males and 100 females, then placed in sterile soil and fed corn and wheat seeds, and cooked rice. Their unaccounted F1 larval progeny was separated and fed with wheat. Eggs obtained from 31 adults of one sample date were counted, obtaining 70 eggs, from which 58 larvae emerged. Larvae were placed in sterilized moistened soil and fed with cooked rice.

Adults were in rectangular plastic containers (22 x 14 x 8cm high), fed with corn and wheat seeds. Most adults died, observing a survival of 15 after 10 months. Many of them were found dead with signs of cannibalism; while other showed signs of fungal infection, such as a rigid appearance, absence of odor and apparent absence of fungal infection signs. Dead adults were placed in vials with moist soil to observe sporulation.

During the first month larvae affected by *Metharhizum anisopliae* and *Beauveria bassiana* and adults affected by *M. anisopliae* were detected; two months later spores of the fungus identified as possibly belonging to the genus *Paecilomyces* were observed expanding on the soil surface, proceeding from one larva. No signs or symptoms of nematode infection were detected.

### Isolation and culture of entomopathogenic organisms:

After sporulation, the fungi obtained from the insects were placed in moist plastic growing chambers, and introduced to a PDA (potato, dextrose agar) medium inside polystyrene incubators, to obtain the fungal growth and development. A culture of the fungus pos. *Paecilomyces* was conducted in PDA obtaining a very good growth.

Once pure fungal cultures were obtained the effective SDLYM medium (Kaya & Stock, 1997), was prepared. SDLYM is composed of Agar-Sabouraud, milk, egg yolk, agar, dextrose and yeast extract.

Because no nematodes were found in false wireworm larvae, they were collected from the white grub *Clavipalpus*

*ursinus* at El Remanso. These larvae were placed in plastic containers with sterilized soil and moisture at field capacity. Approximately one month later, two larvae with possible symptoms of nematode infection (showing brown color) were observed. *Clavipalpus ursinus* larvae were introduced in modified White traps, which consist of plastic vials with a foam disc to prevent mites, from entering, that fed on the filter paper and the insect's cadavers. After obtaining nematodes, a solution of  $20 \text{ JI} / \text{cm}^3$  was prepared and within the same White traps, false wire-worm larvae of diverse instars were inoculated.

**Inoculation and evaluation of pathogenicity:** To determine the fungal pathogenicity on *E longicollis* larvae, 280 plastic vials, filled with 6g of previously sterilized soil were prepared, placing one larva per vial for each fungus species. As control 40 untreated larvae were used. Fungi produced in the above mentioned medium were applied to the larvae at two concentrations. Mortality was assessed every second day during 60 days for a total of 21 evaluations; during each observation distilled water, depending on the soil moisture, and two wheat grains as larval food were added to the vial.

Treatments corresponded to the inoculation with native strains of *Metarhizum*, *Beauveria* and pos. *Paecilomyces* at concentrations of  $1 \times 10^5$  and  $1 \times 10^7$ , and the control which consisted of distilled water. For this purpose, spore count was performed by dilution of a concentrated solution, using a hemocytometer (Kaya & Stock, 1997; Vélez *et al.* 1997).

Since only adults infected by *M. anisopliae* were detected, the experiment was performed with this fungus, extracted two months before inoculation and propagated in the medium SDLYM and with a control. A solution of  $1 \times 10^7$  spores was applied. Twenty females and 20 males per treatment, same number for the control were evaluated; for a total of 80 experimental units. During 20 days a total of eight observations were made, each every second day; and during this observation time food and water was provided as necessary.

To test the pathogenic nematode effect, those recovered in the white grubs, belonging to the family Sterneinematidae (identified using) (Kaya & Stock, 1997). Seventy two plastic vials, 36 for each treatment for each treatment, (nematode inoculation and control), with 6g of previously dried soil for 48 h received 3mL of distilled water. After 24 hours one *E. longicollis* larva and a corn grain was introduced in each vial. Larval instars were not determined at the start of the experiment and they varied from two-five instars. A nematode solution, containing  $20 \text{ JI} / \text{cm}^3$  was prepared (Saenz, 2003), which has been the most effective treatment invading larvae of *Clavipalpus ursinus* (Coleoptera). To each vial  $1 \text{cm}^3$  of the solution was applied; evaluations were performed every 72 hours for 15 days.

The soil was kept moist applying if necessary, every third day 1cm<sup>3</sup> of water. When noting dead larvae, the cadaver was extracted, washed with a solution of water and 0.5% hypochlorite, and placed in a modified White trap to confirm the presence of nematodes.

Data were analyzed with a qualitative approach and a descriptive-comprehensive scope, calculating and interpreting the larval and adult percent mortality.

## RESULTS AND DISCUSSION

Symptoms with the treatments with *Metharizium* were dots and brown spots on the cuticle of the larva; the spots were observed 8-10 days before the insect's death. Later a slight change in the color of the larva was noted and a rigid consistency was detected. After death, a white mycelium initiated at the ventral side between the junction of the externits and pleurits. Confirmation of the fungal infection was made when an olive green sporulation was detected and under the microscope spores in columns appearance were observed.

When infected with *Beauveria*, the larva revealed a dark coloration at its anal area; dead individuals had a rigid consistency and the translucent larvae showed a whitish color. The initial appearance of mycelium showed where the dark spot was originally perceived, then covered the entire body. *Beauveria* infection was confirmed by the observation of the spores which provided a powdery appearance to the larva and by small spherical sacks covering the body (Posada

& Pava-Ripoll, 2010). Under the microscope the globular spores, characteristics of this fungus, were detected.

Finally, in the case of pos. *Paecilomyces* the fungus did not produce spots, but a total color change of the whole dead body; the color ranged from intense yellow to salmon red. The larva was quite rigid and a few days after death, a rough texture of the cuticle was detected. The occurrence of white mycelium started between the abdominal segments; although no fungal sporulation was observed, the symptoms and signs were identical to those observed in the original specimens, from which the inoculum was taken.

Figure 1 shows the percentage of larval mortality after 15 days of inoculation. Before this all larvae were alive, except for those exposed to 1x10<sup>5</sup> of pos. *Paecilomyces*, all treatments produced the death of 5% of the 20 inoculated larvae. A maximum of 20% mortality throughout the trial was observed only with the high dose of this fungus at day 60. The concentration 1x10<sup>5</sup> achieved 15% mortality after two months. The higher dose, 1x10<sup>7</sup> of *M. anisopliae* caused 12.5% mortality, while the same high dose of *B. bassiana* at day 60 showed only 7.5% mortality.

Only *Paecilomyces* revealed a tendency to increase mortality over time. The other pathogens did not improve their mortality rate, *Metarhizium* from day 42 and *Beauveria* from day 36 on after inoculation.

Although, in general terms, all pathogens initiated their action and killed *E. longicollis* larvae, it was required a relatively

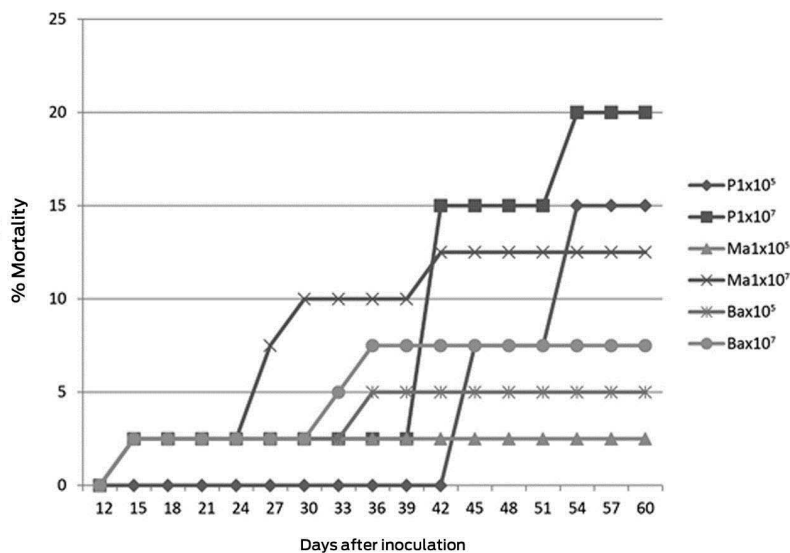


Figure 1. Percent mortality of the larvae of *Eleodes longicollis punctigera* subjected to different concentrations of entomopathogenic fungal spores. (P: *Paecilomyces*; Ma: *Metharhizium anisopliae*; Ba: *Beauveria bassiana*, followed by concentration employed).

long time for an increase in larval mortality. It is known that the three fungi are not fast acting. The low efficiency may be due to a natural larval tolerance to the infection process, i.e. the penetration of spores through the cuticle. For this action, entomopathogenic fungi produce extracellular enzymes, whose function in the pathogenicity corresponds to the suppression of the immune system and dissolution of the insect cuticle, among others (Gomes Fernandes *et al.* 2012; Tiemi Ito *et al.* 2007). It is possible, that the production of these enzymes from the strains used in this work was not appropriate to achieve a proper penetration through the chitin constituting the cuticle of *E. longicollis punctigerus*.

The same consideration may be true for the low pathogenic action of *M. anisopliae* against males and females at  $1 \times 10^7$ . Mortality of the first insects was detected after six days, with three dead males and one dead female. Three days later increased to 12% mortality with one more insect of each sex, for a total of five males (25%) and three females (15%) dead.

In figure 2 results using the nematode, *Steinernema* sp., at a concentration of 20 infective juveniles /  $\text{cm}^3$  is shown. At day three one dead individual was initially detected; at day ten 22.2% of dead larvae and at the end of the experiment 12 individuals representing 33.3%. The figure 2 shows a clear trend of increased larval mortality over time. Nansen *et al.* (2013) mention that the most important conditions for the development and the infection of another Tenebrionidae, *Cybaeus angustus*, by nematodes is soil moisture, which in this experiment was apparently adequate for the movement and action of the nematode. Maybe the relatively low efficiency has to do with the origin of the nematode, which was obtained from a beetle of another family, having a certain influence. Although nematodes are not considered specific, Geden *et al.* (1985) obtained satisfactory results controlling larvae of another tenebrionid, *A. diaperinus*, dispersing infective juveniles of *Steinernema feltiae* to the floor of poultry houses.

Comparing the maximum larval mortality produced by fungi and nematodes, it appears that the latter causes a faster and increased mortality of larvae of the false wire-worm, thus showing an advantage for the possible use within a management program of the insect.

The results of these preliminary experiments revealed a lower mortality rate as expected, based on previous field observations. Apparently, the insect is tolerant to infection by these fungi, at least under the conditions of the trial. In the case *M. anisopliae*, the low mortality rate can be attributed to adverse environmental conditions, since infection is influenced by temperature, moisture and particular conditions such as pH, organic matter content and texture, among other physical properties (Posada & Pava-Ripoll, 2010; Bidochka *et al.* 1998; Quesada-Moraga *et al.* 2007) not evaluated in this study. These and other experimental conditions also influence the efficiency of *B. bassiana*. Steinkraus *et al.* (1991) showed that the susceptibility to the fungus in the tenebrionid *Alphitobius diaperinus*, is influenced by the hosts instar, the substrate and the formulation of the inoculum.

In addition, it should be noted that the experiments were conducted with soil and although the presence of micro, meso and macro organisms were controlled, conditions such as the vials moisture, temperature and soil characteristics were difficult to control. They influence infection by microorganisms and the insects contact with the microorganism or its infective structures.

The contact of microorganisms with the host is a very important factor that should be taken into account when assessing its pathogenicity. In this context, low larval and adult susceptibility may be due to the low affinity of fungi with the rigid exoskeleton. In the case of nematodes, a certain soil humidity is required, sufficient to allow their movement through the soil and recognize the host to infect.

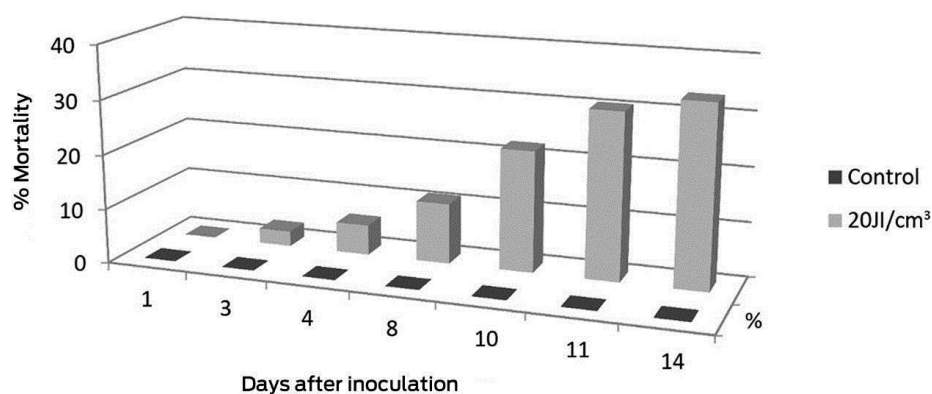


Figure 2. Percent mortality of larvae of *Eleodes longicollis punctigera* caused by a nematode of the family Steinernematidae at a concentration of 20 JI /  $\text{cm}^3$  in contrast to the control.

Another factor to consider, as mentioned above, is that the soil used was completely free of any organism, differing from natural conditions were mites, springtails and insects not susceptible to the infection of the microorganisms evaluated, could serve as means of transport so that larvae of the target species could come into contact with the pathogen employed.

From this preliminary and basic research, it can be concluded that both, the entomopathogenic fungi and the nematode could be part of an integrated management program of the false wire-worm, once a suitable dose of inoculation and the conditions of optimal field condition for adult and larval infection are established. Also it is considered important to study the molecular affinity of spores of entomopathogenic and the insects instar.

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