Available online at www.sciencedirect.com
ScienceDirect
Journal of Invertebrate Pathology 97 (2008) 237–244
Journal of INVERTEBRATE PATHOLOGY
www.elsevier.com/locate/yjipa

# Evaluation of *Metarhizium anisopliae* (Metsch) Sorok. to target larvae and adults of *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae) in soil and fiber band applications

Pierdomenico Marannino [a,b], Cándido Santiago-Álvarez [a], Enrico de Lillo [b], Enrique Quesada-Moraga [a,*]

[a] *Departamento de Ciencias y Recursos Agrícolas y Forestales, ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edificio C4 Celestino Mutis, Cordoba 14071, Spain*
[b] *Dipartimento di Biologia e Chimica Agro-forestale e Ambientale, Università degli studi di Bari, Via Amendola 165/a, Bari 70126, Italy*

Received 29 March 2007; accepted 10 September 2007
Available online 15 September 2007

**Abstract**

The aim of this work has been to evaluate in the laboratory the potential of entomopathogenic fungi against adults and larvae of *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae) through fiber band application and a potted plant bioassay with soil application, respectively. Our previous findings revealed that *Metarhizium anisopliae* EAMa 01/58-Su isolate was the most virulent against neonate larvae of the buprestid. In the present work, *M. anisopliae* EAMa 01/58-Su isolate has been also shown to be highly virulent against adult beetles by immersion in a conidial suspension; thus it was selected to accomplish our objectives. When adult beetles were stimulated to climb $100 \times 200$ mm non-woven commercial fiber bands impregnated with conidia of *M. anisopliae* EAMa 01/58-Su isolate, total mortality rates varied from 85.7% to 100.0%; whereas no significant correlation was detected between the time needed to cross the band (mean value $648.7 \pm 22.4$ s) and the time of death, with mean average survival time ranging between 10.3 and 16.0 days, compared to 28 days of the controls. Potted seedlings (5–6 months old) of cherry plum (*Prunus myrobalana* Lois.), a commonly used apricot rootstock, were used to study the efficacy of soil treatment with *M. anisopliae* EAMa 01/58-Su isolate against neonate *C. tenebrionis* larvae. The soil inoculation with *M. anisopliae* EAMa 01/58-Su isolate had a significant effect on the mean number of dead larvae recovered from the roots, with mean mortality ranging from 83.3% to 91.6%; whereas no significant differences were detected between the three fungal doses. In all cases, dead larvae found within roots exhibited external signs of fungal growth. Hence, it may be possible to use *M. anisopliae* EAMa 01/58-Su isolate in a biocontrol strategy targeting both adults and larvae of *C. tenebrionis*.


*Keywords:* *Beauveria*; *Metarhizium*; Soil treatment; Virulence; Neonate larvae; Endophitic; Biological control; *Capnodis tenebrionis*; Buprestidae; Fiber bands; Tree borers; Soil dwelling pest; Entomopathogenic fungi; Coleoptera

## 1. Introduction

The Mediterranean flatheaded peachborer, *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae), poses an enormous threat to stone-fruit crops of the family Rosaceae in the Mediterranean basin due to the feeding behavior of both adults and larvae (Alfaro-Moreno, 2005). Adults cause defoliation by feeding on twigs and young branches throughout the warm season (Garrido, 1984). Neonate boring larvae, which hatch from eggs deposited on the ground close to the base of the tree stem, reach the host by crawling through the soil (Marannino and de Lillo, 2007a) and may cause tree death or severe plant weakening in consequence of root/collar tunnelling under the bark. In consideration of the high injury it produces, *C. tenebrionis*

* Corresponding author. Fax: +34 957218440.
*E-mail address:* cr2qumoe@uco.es (E. Quesada-Moraga).

0022-2011/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2007.09.006

has been included on the European list of harmful organisms impairing quality of stone-fruit propagating material (Comm. Dir. 93/48/EEC).

To date, no effective methods for controlling this jewel beetle are available in organic fruit production, and the only *C. tenebrionis* management tactic in conventional fruit production is nonselective chemical insecticide application by repeated foliar sprays against adults and by soil dusting against neonate larvae. This approach is generally unsatisfactory due to its adverse effects on humans, non-target fauna and environment ecological balance (Ben-Yehuda et al., 2000); thus biological control might represent a viable option. Neonate larvae of *C. tenebrionis* are susceptible to steinernematid and heterorhabditid nematodes (Marannino et al., 2004; García del Pino and Morton, 2005); whereas the use of these nematodes against the adults in the aerial part of the plant is highly limited, not only by the lack of efficacy, but mainly by desiccation (Begley, 1990). Despite the possible use of nematodes, the most important natural enemies of *C. tenebrionis* are the entomopathogenic fungi (Marannino and de Lillo, 2007b), which are attractive potential biocontrol agents for *C. tenebrionis* because they may be economically produced in large quantities and they can be formulated in a variety of ways (Wraight et al., 2001). Moreover, they could provide an additional advantage as they could be used in a biocontrol strategy targeting both adults and larvae. Such a strategy will require the identification of a fungal isolate active against both insect stages.

In a previous study, we found five isolates of the fungal pathogens *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* to be very effective for the control of *C. tenebrionis* neonate larvae using a bioassay method simulating their natural endophytic growing conditions (Marannino et al., 2006). However, the pathogenicity of these candidate isolates against *C. tenebrionis* adults has not yet been investigated.

In order to develop a reliable *C. tenebrionis* microbial control strategy, not only is the lethality of the candidate fungal isolate to larvae and adults important, as revealed by laboratory bioassays, but also critical is its ability to operate satisfactorily when applied in the soil, to target larvae, or to the aerial part of the plant, to target the adults. When emerging from soil, *C. tenebrionis* adults must feed intensively before mating and ovipositing; due to their weakness and to the lack of optimal temperature, especially in springtime after over-wintering, they are not able to fly and are thus forced to reach the host-tree canopy by climbing the trunk (Caponero et al., 2006). On the basis of this behavioral feature, the efficacy of entomopathogenic fungi could be tested through application on the stem. To this end, as shown by several authors (Nobuchi, 1993; Higuchi et al., 1997; Dubois et al., 2004), fiber bands impregnated with the mycopathogen conidial powder and wrapped around the trunk represent the most effective microbial control measure available to date to combat tree-boring Cerambycidae and Buprestidae.

Consequently, the first objective of this work was to select from among our best isolates for *C. tenebrionis* larvae, those that are also pathogenic against adults. Among them, we selected a candidate fungal isolate for further evaluation against both stages, administering it by soil application and by fungal bands, respectively.

## 2. Materials and methods

### 2.1. Entomopathogenic fungi

The isolates used in the experiment belong to the fungal collection of the Agricultural and Forestry Sciences and Resources (AFSR), Department of the University of Córdoba (Spain). *Beauveria bassiana* isolate EABb 04/01-Tip was obtained from *Timaspis papaveris* (Hymenoptera; Cynipidae) at Sevilla (Spain) and *M. anisopliae* EAMa 01/58-Su isolate from the soil of a wheat crop at Córdoba (Spain). Both isolates were chosen due to their high level of virulence against peach borer neonate larvae (Marannino et al., 2006). Fungal cultures were grown on Malt Agar (12.75 g/l malt extract, 2.75 g/l dextrine, 2.35 g/l glycerol, 0.78 g/l gelatine peptone and 15.0 g/l agar) at 25 °C in the dark. Conidial suspensions for experiments were prepared by scraping conidia from 15-day-old well sporulated cultures into an aqueous solution of 0.2% Tween-80. Suspensions were then filtered through several layers of cheesecloth to remove mycelium and the concentrations of viable conidia were estimated as colony forming units, using a dilution plate count method.

### 2.2. Insects

About 500 recently emerged adults (both males and females) of *C. tenebrionis* were collected from heavily infested stone-fruit orchards in the provinces of Córdoba and Murcia in Southern Spain during September and October 2006. The beetles were fed on fresh apricot twigs under laboratory conditions (28–30 °C, 40–50% RH, 16:8 L/D photoperiod). This allowed them to reach sexual maturity, mate and lay eggs in Petri dishes filled with sifted sand, according to the method described by Garrido et al. (1987) to separate the soil from the eggs. Incubation took place under the same conditions. Adults of approximately the same size were selected for the experiments. In those experiments performed with larvae, we selected neonates from the rearing boxes within 24 h after hatching.

### 2.3. Susceptibility of C. tenebrionis adults to B. bassiana and M. anisopliae

The beetles were immersed individually for 10 s in a conidial suspension ($1.0 \times 10^8$ conidia/ml) or in 0.2% Tween 80 aqueous solution (for controls). They were then moved to clean rearing boxes (240 × 80 mm) kept at 25 °C, fed every day on fresh apricot twigs and monitored daily for mortality. There were three replicates (boxes) per