Assessing the effect of soil treatments with the entomopathogenic fungus *Metarhizium anisopliae* (Metcchnikoff) Sorokin against puparia of *Bactrocera oleae* (Diptera: Tephritidae) on soil dwelling non target arthropods

Inmaculada Garrido-Jurado1,2, Cándido Santiago-Álvarez1, Mercedes Campos2, Enrique Quesada-Moraga1

1Department of Agricultural and Forestry Sciences, ETSLAM, University of Cordoba. Campus de Rabanales. Building C4 “Celestino Mutis”. Cordoba 14071 (Spain)

e-mail: g72gajui@uco.es

2Department of Environmental Protection. Zaidín Experimental Station (CSIC). Profesor Alhareda no 1. 18008 Granada (Spain)

Abstract: The objective of this study was to determine the persistence of the autochthonous *Metarhizium anisopliae* EAMa 01/58-Su isolate in the soil when applied beneath olive trees for controlling olive fly puparia and to elucidate its possible effect on non-target soil dwelling arthropod communities. For that, we selected 200 olives trees in an organic olive orchard at the province of Málaga (Spain) to be sprayed either with a 2.5 x 10^7 conidia m^-3 suspension of the fungus on the ground beneath the tree canopy (100 trees) or with the blank formulation as controls (100 trees). Before fungal treatments, we selected 10 trees from the treated ones for evaluating both the possible presence of indigenous entomopathogenic fungi in the soil by using the Galleria Bait Method and the evolution of the conidial densities in the soil after spraying. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuill. was the most common species, being found in all the samples, while *M. anisopliae* was found only in one sample. After spraying the 100 treated and 100 control trees, soil samples beneath the 10 selected trees from the top 10 cm were taken to calculate the number of conidial forming units per gram of soil at 1, 7, 14, 28, 35, 42, 49, 56 and 63 days after treatment respectively. Our preliminary data indicate that the soil ecosystem favours the persistence of this autochthonous isolate, which could allow long term protection of the crop against olive fly puparia. In order to assess the possible effect of the fungal treatment on soil arthropod populations, 40 pitfall traps (7.5 cm diameter by 10 cm deep) placed beneath the tree canopy of randomly selected 20 treated and 20 control trees, were sampled every two weeks. Our preliminary data indicate that formicidae species are the most abundant arthropods trapped, but no infected insects have been found in field as a result of the treatment to now.

Key words: Bactrocera oleae, Beauveria bassiana, Metarhizium anisopliae, fungal persistence, biological control, nontarget organisms

Introduction

Olive is a major crop in the Mediterranean basin, the cultivation of olive trees goes back to ancient times. A quarter of the olive world cultivated area is found in Spain, in particular, olive orchards are concentrated in the southern (Civantos, 1999).

The olive fruit fly *Bactrocera oleae* Gmelin (Diptera: Tephritidae) is the most harmful pest of olive orchards in Mediterranean region. The predominant method of this insect pest control has been through the use of chemical insecticides. Continued use of these products has been questioned in recent years, because the insecticidal treatments have ecological and
toxicological effects such as environmental contamination or destruction of non-target organisms. Recent studies show the entomopathogenic fungi may be a potential source of control of tephritid adults and pupae (Kostantinopolou and Mazomenos, 2005, Quesada-Moraga et al, 2006). Since success in controlling B. oleae pupae with entomopathogenic fungi in field assays depends on the presence of inoculum in olive orchard soils we carried out a study to determine the persistence of Metarhizium anisopliae (Metchnikoff) Sorokin conidia in olive orchard soils and to elucidate the impact of the treatment with this entomopathogenic fungus on the arthropods fauna of the olive orchards.

Materials and methods

The study site was located at Antequera, Málaga (37° 01'N, 4° 41'W) on an organic olive orchard. The investigated area was divided into two square sub-fields, each one having 100 trees.

Before fungal treatment, 10 soil samples were taken completely randomized for determining the presence of entomopathogenic fungi in the soil. All soil samples were taken to a depth of 10 cm using a hand trowel. In each point, 5 samples were collected within an area underneath the canopy, these ones were mixed up together in a polyethylene bag. In the laboratory, each soil sample was sifted with 1 mm sieve.

The Galleria Bait Method was applied to each soil sample to isolate entomopathogenic fungi (Zimmermann, 1986). Slightly moistened soil was placed in a petri dish (9.0 mm Ø, 1.5 cm high), and then 10 late instar larvae of Galleria mellonella were added to each dish. Baiting was conducted in darkness at 25°C, and for 7 days the plates were turned daily to keep the larvae moving in the soil. After this, the larvae were placed in humid chamber to be checked for fungal infection.

For this field trial, it was used a semi-commercial product based on the entomopathogenic fungus Metarhizium anisopliae. The treatment was applied at 2.5 X 10³ conidia m⁻². After spraying the soil underneath the canopy from 100 trees, soil samples were taken at 1, 7, 14, 28, 35, 42, 49, 56 and 63 days after treatment as described before. To assess the conidial density in each sample, the number of colony forming units (CFU) per gram of dry soil was determined onto Sabouraud glucose agar chloramphenicol in petri dishes (Goettel and Inglis, 1996). One gram of the homogenized sample of soil was added to 9 ml of sterile distilled water, and shaken for 20-60 min. After homogenization, aliquots of 100 µl were spread onto the medium. In some cases, it was necessary to dilute the soil solution before spreading. The density of M. anisopliae was analyzed using ANOVA, and Tukey's multiple range test (α=0.05) was used to separate means.

Arthropods populations were sampled using pitfall traps (7.5 cm diameter by 10 cm deep) every two weeks, from 4 July until 15 August 2007 with a total of four replacements. Traps were buried with the lip of the container flush with the soil surface. Each trap contained 125 ml of soaped water to keep the insects inside. Twenty traps were randomly located on each sub-field, using two positions, each one repeated twice. Traps were removed after 2 days and carried to the laboratory for counting and identification of insects. Determination of possible fungal infection was performed with humid chamber. Insects were washed off with sterilized water to remove soil particles, then were deep in 1% sodium hypochlorite for 3 min and rinsed twice with sterilized water. After that, insects were placed on a humid sterilized filter paper contained in a petri dish which was sealed with parafilm®. Data were compared by non-parametric analysis t-test.
Results and discussion

The Galleria Bait Method is a very sensitive method for determination of entomopathogenic fungi in soil samples (Keller et al., 2003). By using this method, we isolated two fungal species, B. bassiana that was obtained from 100.0% of the analysed soil samples, and M. anisopliae that was found only in one soil sample, coexisting with the former species (Figure 1). This result agrees with our previous work (Quesada-Moraga et al., 2007; Eudesouki, 2007), in that B. bassiana is the most abundant entomopathogenic fungal species in soils from Spain, and particularly form olive orchards, followed by M. anisopliae.

![Mortality percentage of Galleria mellonella larvae infected with autochthonous B. bassiana and M. anisopliae from assessed field.](image)

Figure 1: Mortality percentage of Galleria mellonella larvae infected with autochthonous B. bassiana and M. anisopliae from assessed field.

The changes in the density of M. anisopliae were assessed weekly by counting CFUs on a selective medium. The time had a significant effect on the evolution of conidial counts ($F_{8,39} = 24.87, p < 0.0001$), with a significant decrease from $3.9 \times 10^5$ to $1.4 \times 10^3$ conidial g$^{-1}$ soil in the first week, which remained almost constant until day 42 after treatment, after which a slightly decrease until $9.9 \times 10^2$ conidial g$^{-1}$ soil was noted (Figure 2). Such a decline in fungal density of entomopathogenic fungi has been previously reported by Hu and St. Leger (2002), who found a decrease of the population of M. anisopliae in soil from $10^5$ to $10^3$ conidia g$^{-1}$ soil after several months. Our preliminary studies corroborate this fact, since in only two months there was a slow dwindling. However, the fungal inoculum tended to establish reaching a constant level, by extending our sampling period for 3 years, we probably could address whether or not this establishment occurs. From our results it could be concluded that a long term protection of the crop against olive fly puparia could be possible with spaced soil treatments.

Finally, the incidence of the fungal treatment on soil dwelling insect was weigh up with pitfall traps. There was no significant differences between treatments in catches per traps, except for the second day ($t$-test, $P = 0.024$) (Figure 3).

Arthropods from pitfall traps were identified to orders, but due to the highest number of captures of the order formicidae, ants were identified to genera. Cataglyphis was the ant genera more abundant, it appeared in 52.6% of the catches, followed by Pheidole and Messor, 23.3 and 22.9% respectively. The traps sampled 2457 specimens from 16 arthropods orders. Apart from the formicidae, Isopoda was the most abundant group followed too far by Araneae and Diptera (Figure 4). No significant differences were observed between control and treated plots in the number an type of arthropod species.
Figure 2: Temporal evolution of the EAMa 01/58-Su conidial per gram of olive orchard soils, for 56 days. Data shows means ± standard error.

Figure 3: Arthropods caught per trap recorded for EAMa 01/58-Su treatment and control, throughout the complete sampling period and SD

Figure 4: Relative importance of orders captured in pitfall traps
After that, the possible infection of the sampled specimens with entomopathogenic fungi was checked by humid chambers. We did not observe any sign of fungal infection in the sampled specimens neither from treated plots nor from the control ones. Consequently, the application of entomopathogenic fungi for olive fly puparia control in the soil may offer some advantages as it reduces the amount of inoculum and applied area treated with the fungus, thereby minimizing the potential adverse effects of the fungi on non-target organisms. In addition, from our work, it could be concluded that there is no negative effect of the treatment on the soil non-target fauna.

References


