ECOLETTER

Periodic publication of trials that test the efficacy and properties of Futureco Bioscience products

Issue 29, September 1st 2020

Soil persistence of the fungus *Metarhizium brunneum* EAMb 09/01-Su: a product under development for the biological control of the olive fruit fly

C. Sant , I. Hernández y C. Fernández

Introduction

The olive fly (*Bractocera oleae*) is the most important pest of the olive tree. This fly deposits its eggs in the olives and the larvae feed on the pulp causing serious losses in terms of quantity (the larvae consume part of the pulp and can cause the fruit to fall) and quality (the presence of the larvae and their effects on the olive increase the acidity and diminish the oil's conservation capacity). In addition, the fly's bite can be the entrance door to other pathogens, mainly fungi, with the consequent secondary damages. In the INNOLIVAR* project, Futureco Bioscience is developing a bioinsecticide product based on the entomopathogenic fungus *Metarhizium brunneum* strain EAMb 09/01-Su to control this pest.

Efficacy is the main factor to be taken into account when marketing a plant protection product, but not the only one. The persistence in the different environmental compartments (soil, plant, surface and subway water, etc.) is also an important factor since it largely determines the environmental impact of the product and, sometimes, its effectiveness. Determining the persistence of products in the soil is especially important in the case of those that are directed at pathogens that develop part of their life cycle underground, as is the case of the olive fly, whose pupae develop entirely in the soil.

The aim of this phase of the project INNOLIVAR is to develop a method for detecting the strain EAMb 09/01-Su in soil samples and apply it in field trials.

Materials and Methods

To monitor the persistence of the EAMb 09/01-Su strain at Futureco Bioscience we have developed a real-time quantitative PCR method in which 3 strain-specific DNA sequences are simultaneously quantified. To develop this method we sequenced the genome of the fungus and applied the approach described by our researchers for bacteria (Hernández et al 2020; https://doi.org/10.3389/fmicb.2020.00208).

The method was applied in two parallel field trials, one in Mont-roig del Camp (Tarragona) and another in Avinyonet del Penedès (Barcelona). In both trials, prototypes of the EAMb 09/01-Su strain were applied in different formulation formats [dispersible oil (OD), wettable powder (WP) and microsclerotia (MS)]. The prototypes were applied directly to the soil at two points in the crop: one in late November, when the flies are pupating in the soil, and another in early February, in anticipation of the adult emergence.

Results and Discussion

Although some of the values presented (Figure 1) are below the limits of quantification (LOQ)s (and therefore not quantifiable with this method), the values obtained were mostly consistent by comparing the 3 markers with each other, confirming the robustness of the methodology despite the problems in absolute quantification, especially in the plots treated with MS (Figure 1).

The populations of the fungus went from being below the LOQ before the first application, to detectable levels after this one (Figure 1), in both trials. This result is expected since *M. anisopliae* EAMb 09/01-Su is an allochthonous strain and the method is specific to this strain. After the second application, we observed an increase in the levels of the fungus, which was more rapid in the substrate of Barcelona (Figure 1). On the other hand, the oscillations observed were not

Para más información contactar a
info@futurecobioscience.com
Futureco Bioscience SA,
Avenida del Cadí 19-23
Sant Pere Molanta 08799

corresponded with other additional treatments applied by the farmer - nutritional deficiency correctors (N and B), systemic herbicides and fungicides.

The OD formulation maintained higher levels of the fungus throughout the approximately 200 days of both trials. The WP formula showed similar behavior to OD, although differences were noticeable in the Barcelona trial after the second application. Finally, the MS formula showed very variable results in the Tarragona trial, possibly due to the methodological difficulties involved in the quantification of DNA in MS. However, an increase of up to 7 orders of magnitude is observed with the arrival of spring, and there is a positive correlation between the levels of the fungus and the minimum daily temperature ($r2 = 0.726$; $P < 0.05$), which suggests that the levels of the fungus in the plots treated with MS depend largely on climatic conditions. This behavior would be consistent with the biological function of MS, which are forms of resistance composed of condensed hyphae that develop and sporulate when environmental conditions are appropriate.

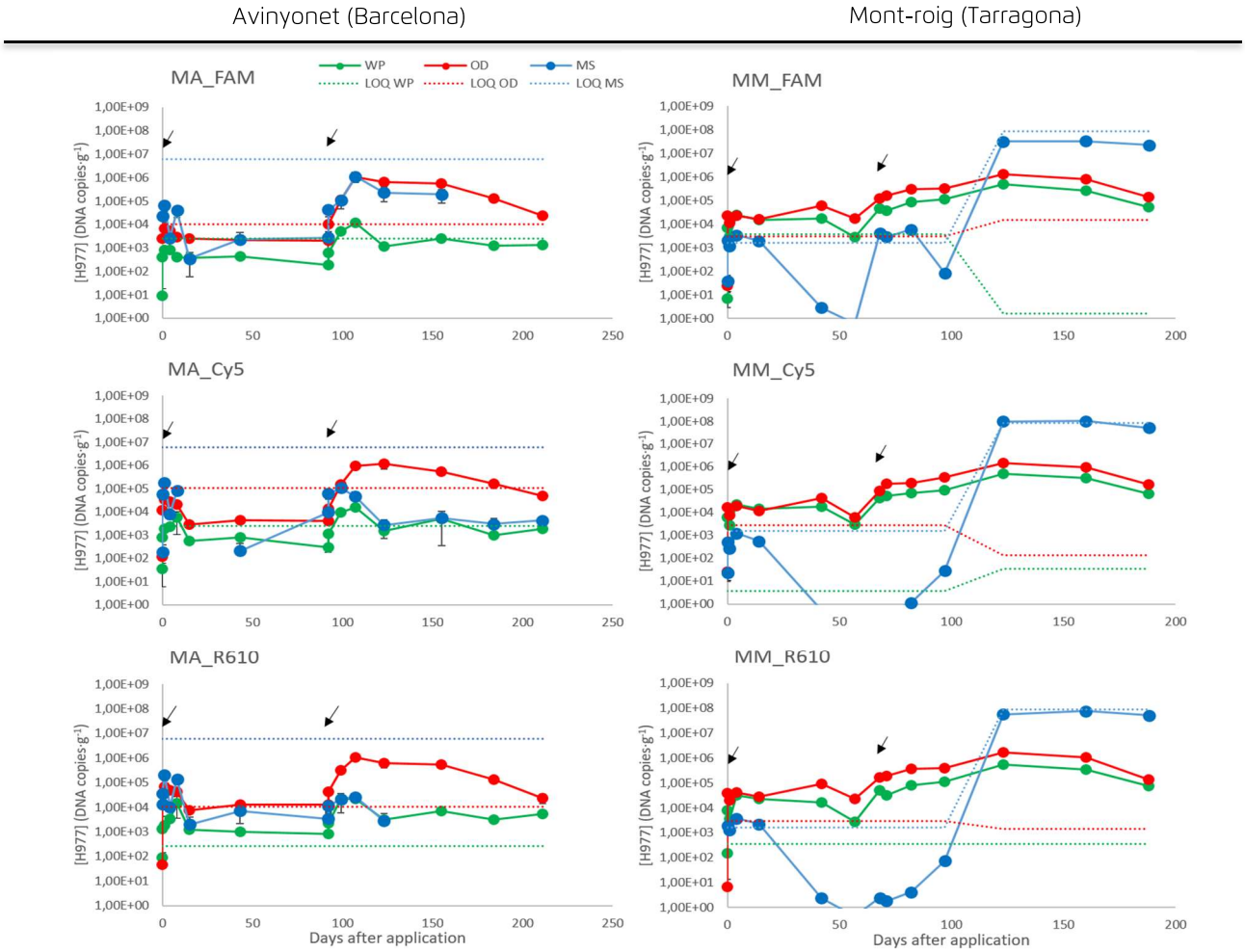

Figure 1. Quantification of the levels of *Metarhizium brunneum* strain EAMb 09/01-Su in soil samples from commercial olive plots treated with prototype formulations OD (dispersible oil), WP (wettable powder) and MS (microsclerotia) in experimental fields of Avinyonet del Penedès (Barcelona; MA) and Mont-roig del Camp (Tarragona; MM). Each panel shows the values for one of the three markers analyzed (FAM, CY5 and R610). The arrows indicate the moments of application of the products. LOQ, limit of quantification estimated from the calibration lines for each molecular marker and treatment.

Conclusions

The described method allows determining the persistence detecting the EAMb 09/01-Su strain of *Metarhizium brunneum* in agricultural soils.
- The fungus in WP and OD formulations can persist for more than 200 days.
- OD and WP formulations show similar persistence dynamics, although OD formulation offers consistently higher persistencies, especially in the Barcelona trial.
- Fungal levels in plots treated with MS formula are very dynamic. This phenomenon may be partly due to methodological limitations, but shows a significant correlation with environmental conditions (temperature).

* Innolivar Project: In 2019 a pre-commercial innovation public purchase contract was formalized between the company Futureco Bioscience SA and the University of Cordoba (UCO), according to the agreement between the Ministry of Economy, Industry and Competitiveness and the UCO, co-financed with FEDER funds.