



**PROJECT DELIVERABLE REPORT**  
**D.4.3 Technical documentation for application of  
entomopathogenic fungi FF OFF-Season-IPM tool made available  
for WP6**



**Fruit Flies In-silico  
Prevention & Management**  
**FF • IPM**

**Project Title:**

In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies  
(‘OFF-Season’ FF-IPM)

SFS-2018-2

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## 1. Summary

The control of fruit flies (Diptera: Tephritidae) is mainly based on insecticide treatments targeting epigeal adults. Targeting the soil-dwelling stages by soil treatment with a strain of entomopathogenic fungus could be a strategy to be considered. The main objective of the present deliverable was to examine the effectiveness of a commercially available mycoinsecticide product based on strains of the *Beauveria* entomopathogenic fungus as a tool for soil application in orchards against the Mediterranean fruit fly (medfly), *Ceratitidis capitata*, in spring and/or in autumn (Off-Season).

This deliverable presents the results of screening process starting with five commercial products (Naturalis®, Botanigard® WP22, Ostrinil®, Serenisim® and Betel®) against medfly, *C. capitata*, in laboratory studies with the selection of the most effective product to be used in the field experiments, especially for an Off-Season application.

GHA strain, corresponding to Botanigard® WP22 was selected because: i/it was the most virulent in the pathogenicity screening test carried out by dipping larvae in an inoculum of  $10^6$  and  $10^8$  conidia/ml of five registered *Beauveria* strains corresponding to five commercial products on *C. capitata* and two other fruit flies, *Bactrocera dorsalis* and *Zenogodacus cucurbitae*, on their late third instar larvae; and ii/ because this product is the one with the highest spore density, which makes it possible to treat soils with the highest spore quantity. The proof of concept that the use of this product in soil treatment was able to control the soil-dwelling stages was achieved using treated sand with Botanigard® WP22 at the dose of  $10^7$  conidia/g before depositing a larva of *C. capitata* or *B. dorsalis*. This treatment allowed, whatever the fly species, to reduce the rate of emergence. It induced a high rate of mortality and mycosis on the cadavers of late third instar larvae, pupae but also adults contaminated during their emergence. The growth of the GHA strain of Botanigard® WP22, modelled as a function of temperature, allowed to validate that the soil temperatures in Italy, where the field trials were planned, were compatible with those allowing the growth and survival of the fungus. Finally, the laboratory test of the temperature and dose effects on the pathogenicity and virulence induced by sands treated with Botanigard® WP22 showed that it induced a mortality of *C. capitata* significantly different from the control whatever the dose ( $10^5$  to  $10^7$  conidia/g) and at all the temperatures tested (10, 15, 20 and 25°C). *C. capitata* mortality was positively correlated with the dose of Botanigard® WP22, whereas it was negatively correlated with the temperature. This was probably due to the fact that the insect did not develop or very slowly at low temperature while the fungus remained active. This suggests that the impact of the treatment may be greater off-season, in spring, and/or in autumn, when temperatures are lower and insect development is slow while the fungus remains active.

For the in-situ field testing in apple and peach orchards in Italy, off-Season (in spring and autumn) and on season (in summer) was carried out. For practical reasons of implementation, it was applied by drenching Botanigard® WP22 to the soil surface at the rate of 29 g/2 m<sup>2</sup>, corresponding to the equivalent of  $10^7$  conidia/g of soil in the first 5 cm. Tests performed after one-year revealed that the fungus was able to maintain itself in the soil, being pathogenic and able to reduce emergence of *C. capitata* for at least one year.

Our results showed that the soil treatment with Botanigard® WP22: i/ may have the potential to reduce emergence and kill *C. capitata* OFF-season and ii/ the soil temperatures of the apple and peach orchards in Italy where we will perform the field tests are compatible with those that allow the growth and survival of the fungus. Botanigard® WP22 applied off-season or on early season before medfly population build-up could provide a useful tool for a sustainable environmental-friendly way to control the medfly. Implementing this strategy in WP6, is an opportunity to evaluate this strategy in large scale. Application of Botanigard® WP22 can be performed with simple drenching with standard sprayer equipment beneath the canopy in the orchards but we recommend if possible to incorporate the product in spring at  $10^7$  conidia/g in the first 5cm horizon of soil for a better repartition and protection of the inoculum. However, costs at the dose tested in the field are very high and not sustainable in IPM practice, even if the product is applied

only under the tree canopy. Using lower dose might be more economically sustainable. The use of Botanigard® WP22 against medflies might be constrained by efficacy and by costs.