

# Biological interactions between nematophagous fungi, *Esteya* spp., and the pinewood nematode, *Bursaphelenchus xylophilus*

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## OBJECTIVES

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a **quarantine organism** in several countries and the **causal agent of pine wilt disease (PWD)**. Controlling the PWN is difficult, but **nematophagous fungi belonging to the *Esteya* genus**, *E. vermicola* (*Ev*) and *E. floridanum* (*Ef*), are **promising candidates for biocontrol**. However, they were never tested in the maritime pine, *Pinus pinaster*, the main and most affected species in Portugal.

Study host-nematode-fungus interactions

Determine the attraction effect of *Esteya* spp. on the PWN

Infer the most promising *Esteya* spp. for biocontrol strategies

## MATERIAL & METHODS

### Biological interactions

Fungus-nematode (feeding trials & chemotaxis assays)  
 Fungus-fungus (percent inhibition - I)  
 Fungus-plant

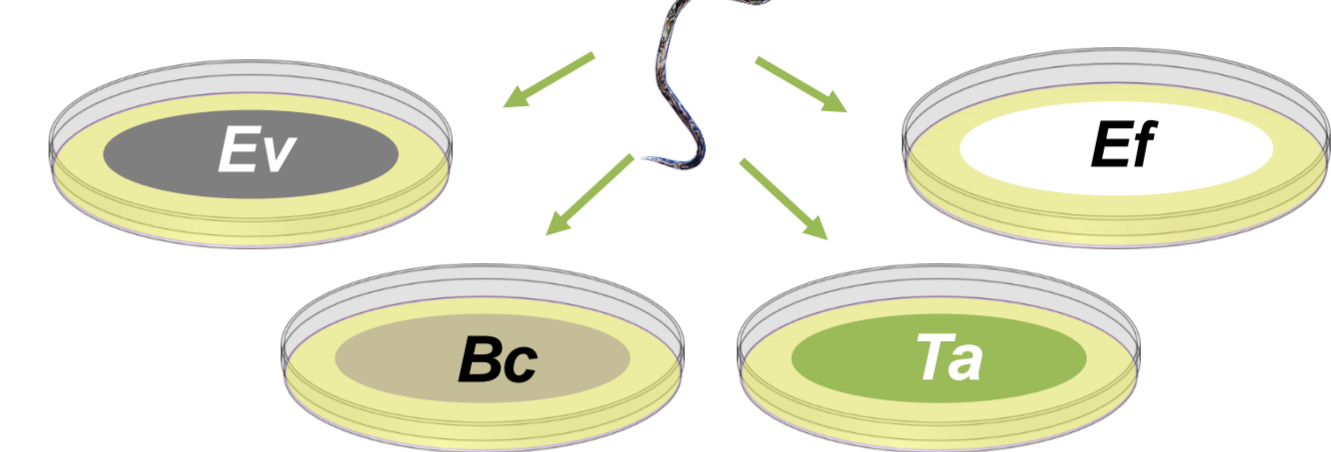
### Feeding trials

Determine the reproduction factor (Rf) of the PWN on fungal mats of *Esteya* spp., *Botrytis cinerea* (*Bc*) and *Trichoderma alni* (*Ta*)

### Chemotaxis assays

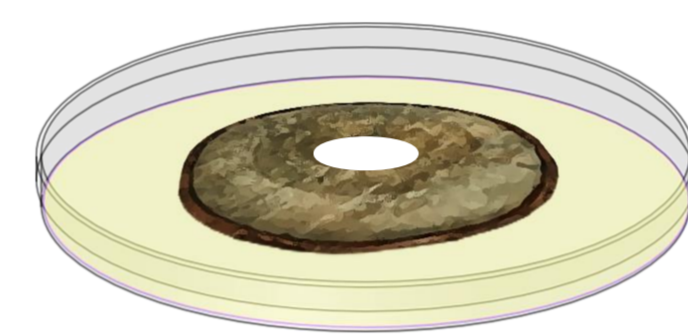
Chemotaxis index (CI) after 1 hour  
 CI: <0 → preference for control; 0 → no preference; >0 → preference for tested fungus

### Feeding trials



$$Rf = \frac{\text{Final population}}{\text{Initial population}}$$

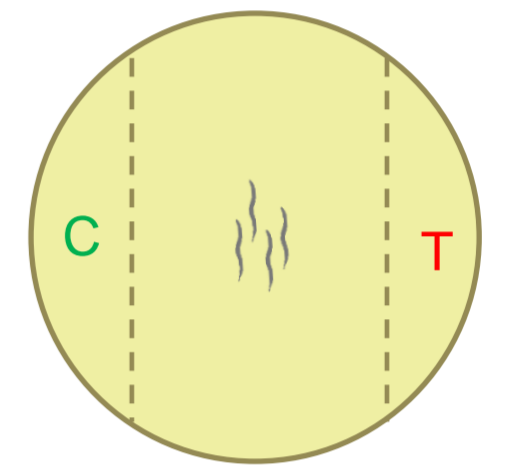
### Biological interactions



$$I (\%) = 100 \times \frac{\text{Potential antagonist} - \text{Tested fungus}}{\text{Potential antagonist}}$$

$$CI = \frac{\text{No. PWNs in tested fungus (T)} - \text{No. PWNs in control (C)}}{\text{Total no. PWNs}}$$

### Chemotaxis assays



## RESULTS

### Fungus-nematode

- No living PWNs were recovered from the mycelia of *E. vermicola* 7 days after inoculation (DAI);
- E. floridanum* dramatically decreased the initial population 7DAI.

### Fungus-fungus

- Antagonism between *Ev* and *Ef*;
- Growth inhibition of *E. vermicola* by *Ophiostoma ips* and *T. alni*.

### Fungus-plant

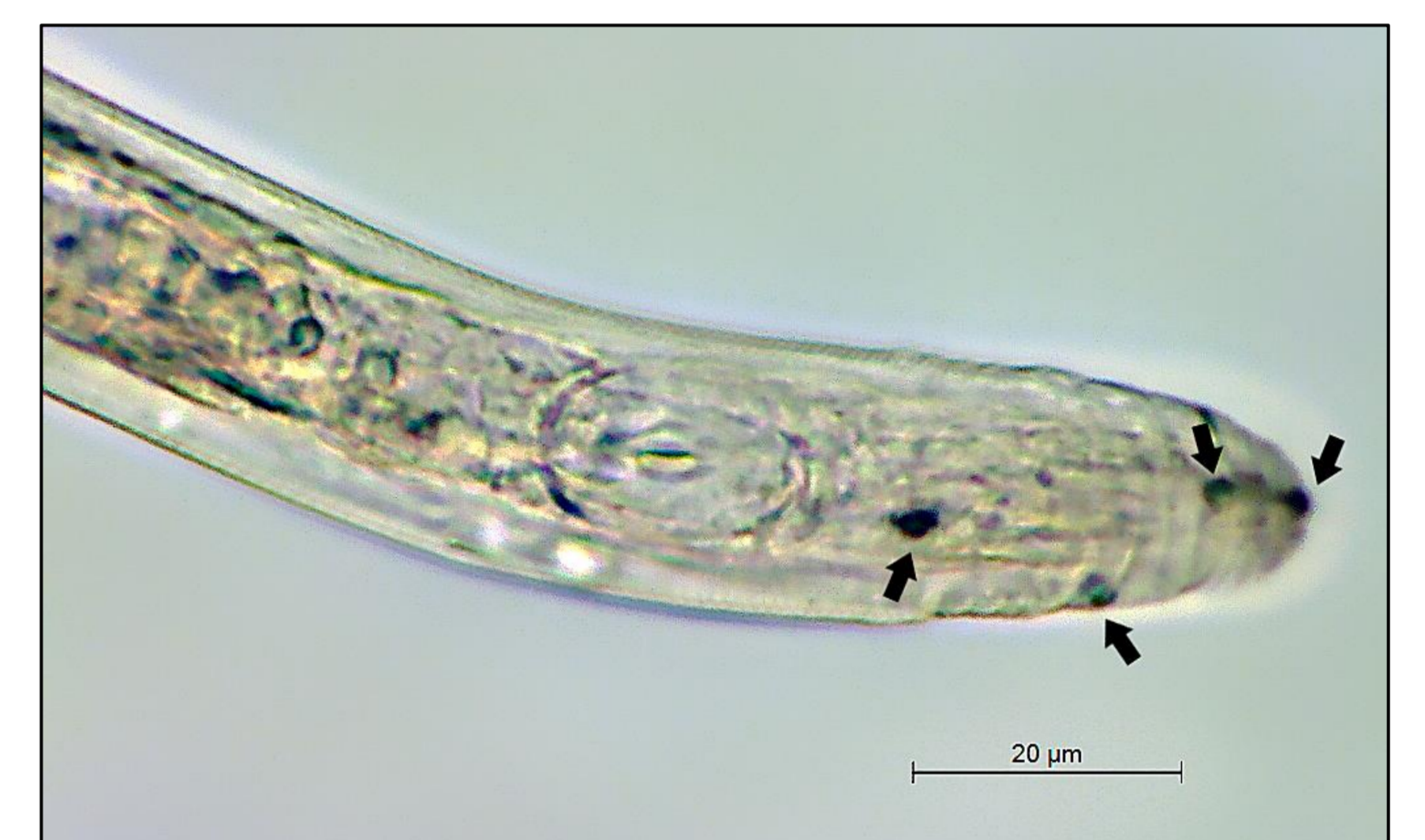
- Esteya* spp. grew on and colonized *P. pinaster* discs.

**Table 1.** Percent inhibition of potential antagonists against *E. vermicola*. Values represent the mean ± SE of 3 replicates.

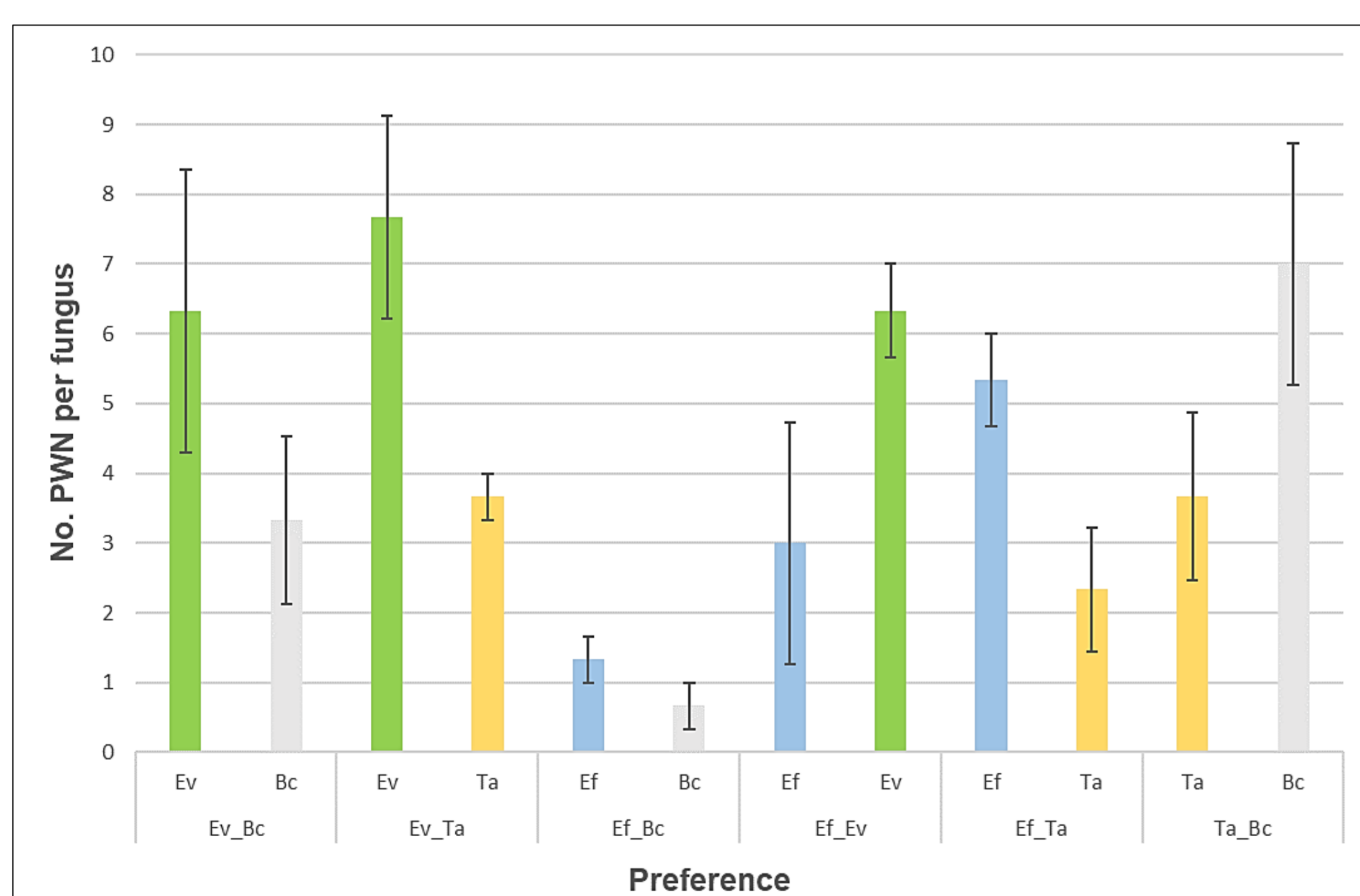
| Potential antagonist     | Inhibition (%) |
|--------------------------|----------------|
| <i>Esteya floridanum</i> | 7 ± 0,017      |
| <i>Ophiostoma ips</i>    | 67 ± 0,276     |
| <i>Trichoderma alni</i>  | 91 ± 1,138     |

**Table 2.** Reproductive ability of PWNs on fungal mats of *E. vermicola*, *E. floridanum*, *T. alni* and *B. cinerea*. Values represent the mean ± SE of 4 replicates.

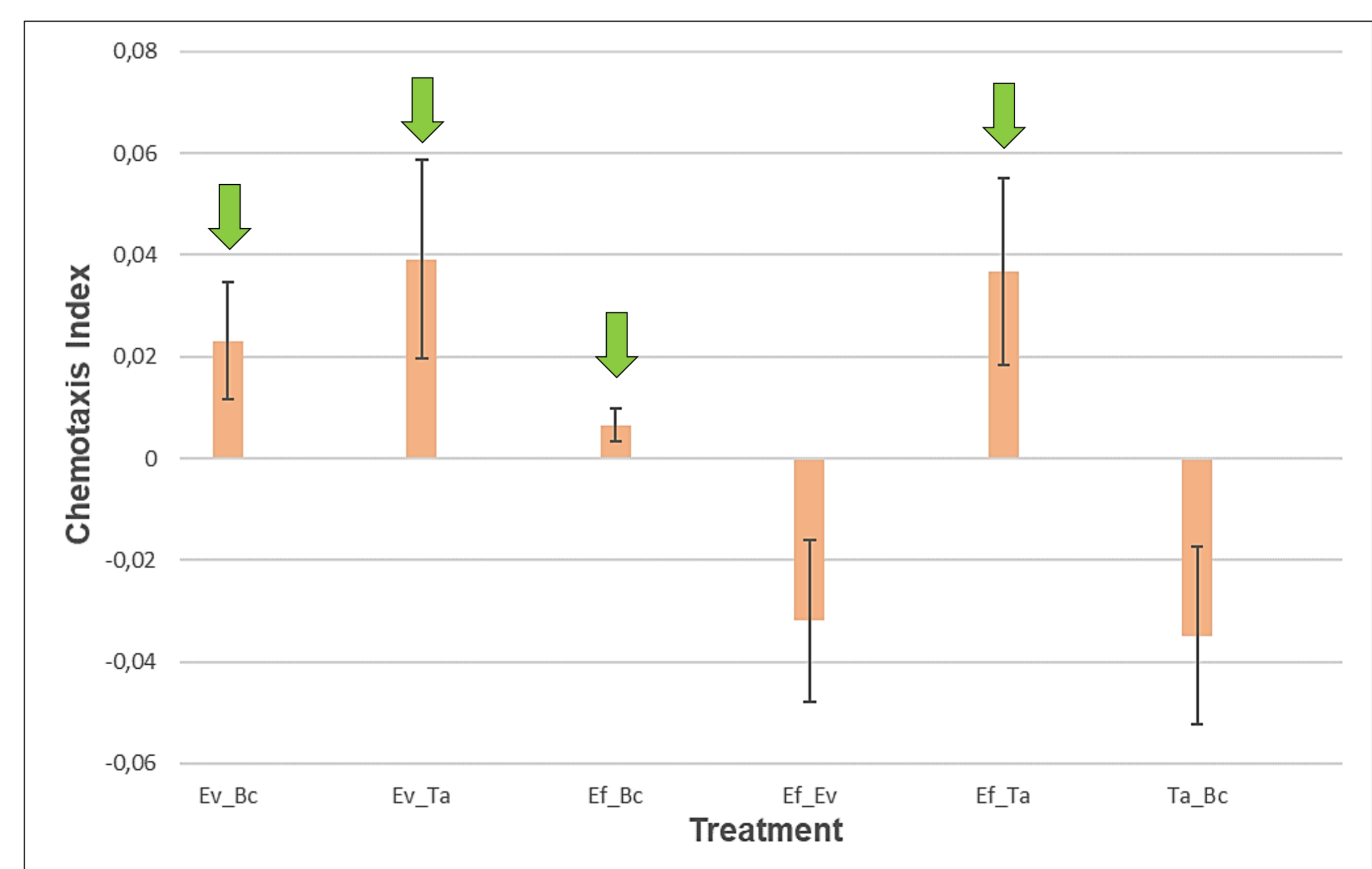
| Fungus               | Initial population | Final population | Rf             |
|----------------------|--------------------|------------------|----------------|
| <i>E. vermicola</i>  | 500                | 0                | 0              |
| <i>E. floridanum</i> | 500                | 0,75             | 0,0015 ± 0,002 |
| <i>T. alni</i>       | 500                | 99,75            | 0,1995 ± 0,054 |
| <i>B. cinerea</i>    | 500                | 2239,75          | 4,4795 ± 0,323 |



**Figure 1.** Cephalic region of *B. xylophilus*, with visible spores of *E. vermicola* (arrows) attached to the cuticle.



**Figure 2.** Attraction of PWN to the living mycelia of *Esteya vermicola* G810 (*Ev*), *E. floridanum* V14639 (*Ef*), a naturally-occurring fungus of maritime pine, *Trichoderma alni* (*Ta*), and a non-sporulating strain of *Botrytis cinerea* (*Bc*) after 1 h. Each bar represents mean ± SE of 3 replicates.



**Figure 3.** Chemotaxis index for all treatments after 1 h. Each bar represents mean ± SE of 3 replicates. Arrows indicate PWN preference for *Esteya* spp.

- Our preliminary results reveal a **clear preference for *Esteya* spp. by *B. xylophilus***, especially *E. vermicola*, compared to naturally-occurring fungi in *P. pinaster*, like *T. alni*, and common PWN food source *B. cinerea*;
- No living PWNs were recovered from the mycelia of *E. vermicola* and *E. floridanum* 7 DAI, indicating that **both fungi killed the nematodes in vitro**;
- Both *E. vermicola* and *E. floridanum* can **grow on and colonize *P. pinaster* discs**, but they are antagonistic to one another;
- These results suggest a **promising potential of *Esteya* spp. for biocontrol of the PWN in maritime pine**, but more isolates need to be tested.