

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* and *E. floridanum*, 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

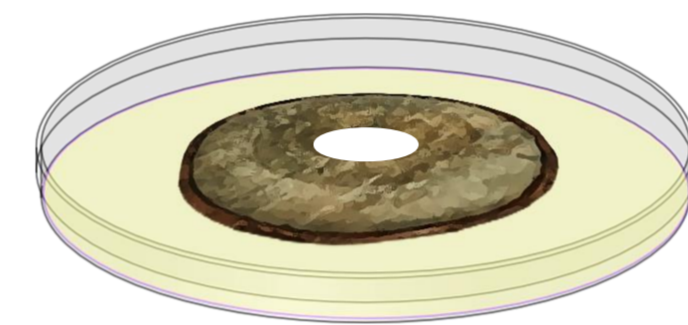
Feeding trials

Determine the reproduction factor (Rf) of the PWN on *E. vermicola* G810 (Ev), *E. floridanum* V14639 (Ef), *Botrytis cinerea* (Bc) and *Trichoderma alni* (Ta)

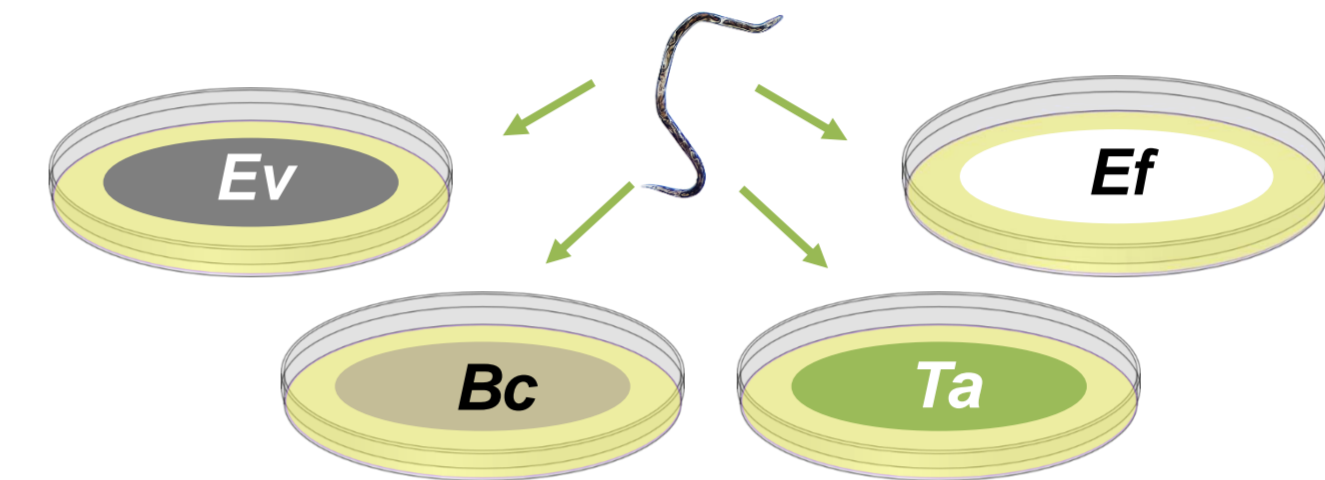
Chemotaxis assays

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

Biological interactions

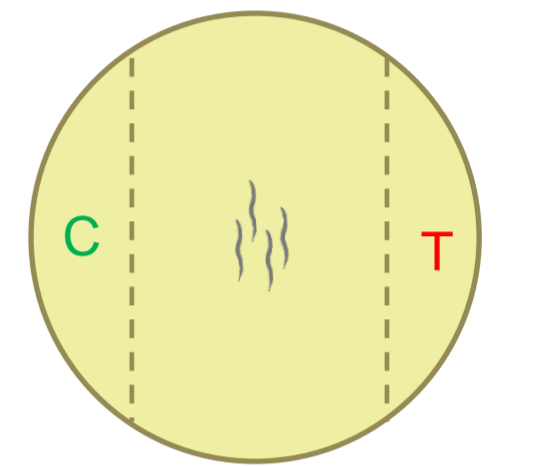


Feeding trials



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

Chemotaxis assays



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

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

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 7 DAI.

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

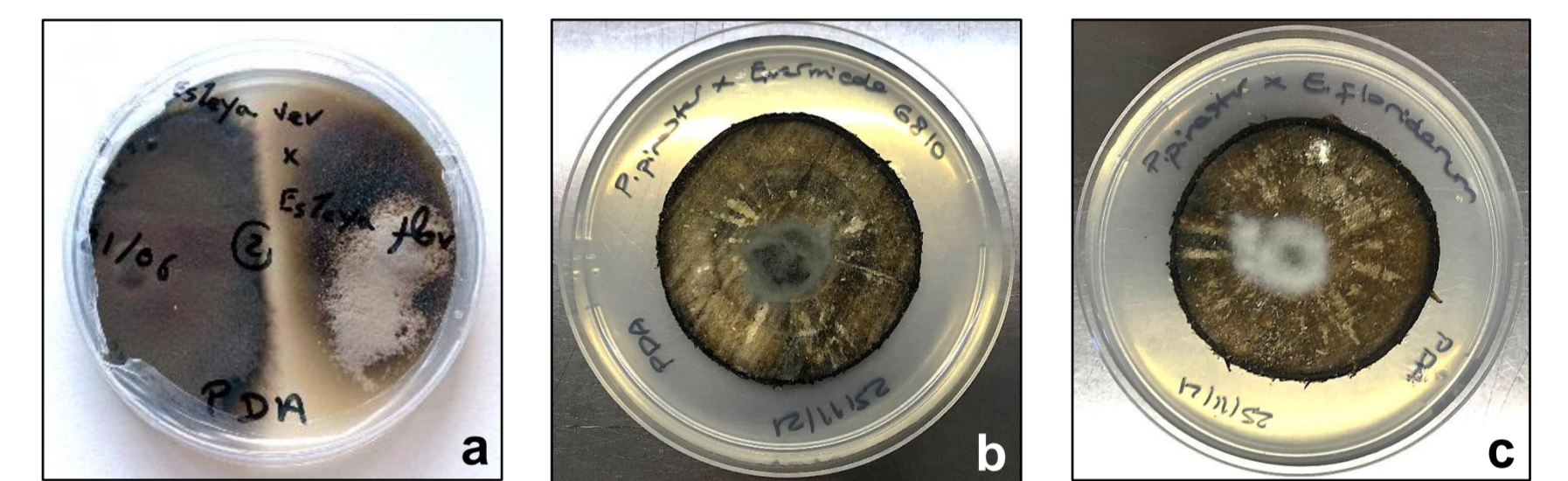


Figure 1. Biological interactions between: a) *E. vermicola* and *E. floridanum*, with a clear inhibition zone; b) *E. vermicola* and wood disc of *P. pinaster*; c) *E. floridanum* and wood disc of *P. pinaster*.



Figure 2. Cephalic region of *B. xylophilus*, with visible spores of *E. vermicola* (arrows) attached to the cuticle.

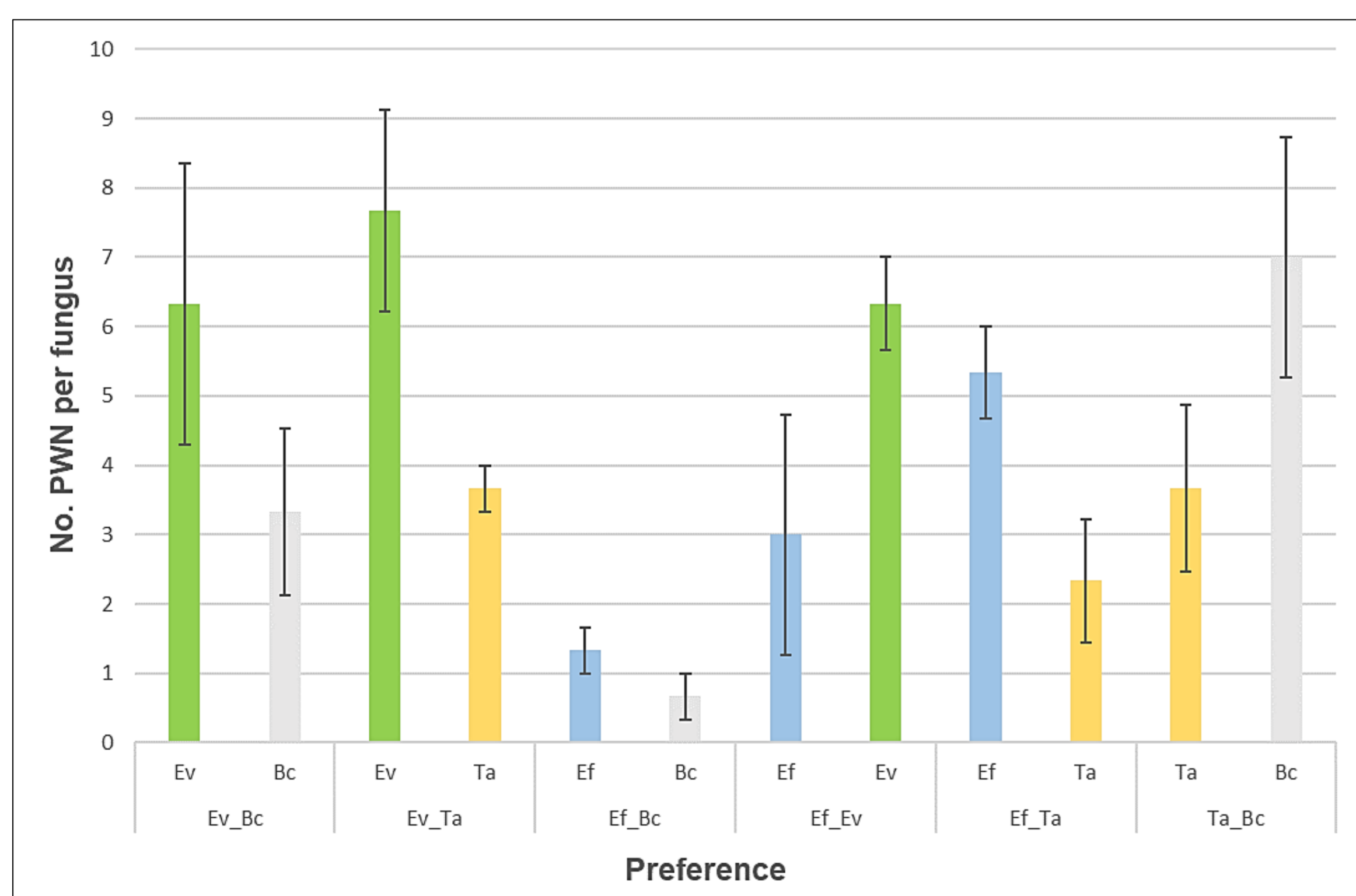


Figure 3. Attraction of PWN to the living mycelia of *E. vermicola* (Ev), *E. floridanum* (Ef), a naturally-occurring fungus of maritime pine, *T. alni* (Ta), and a non-sporulating strain of *B. cinerea* (Bc) after 1 h. Bars represent the mean ± SE of 3 replicates.

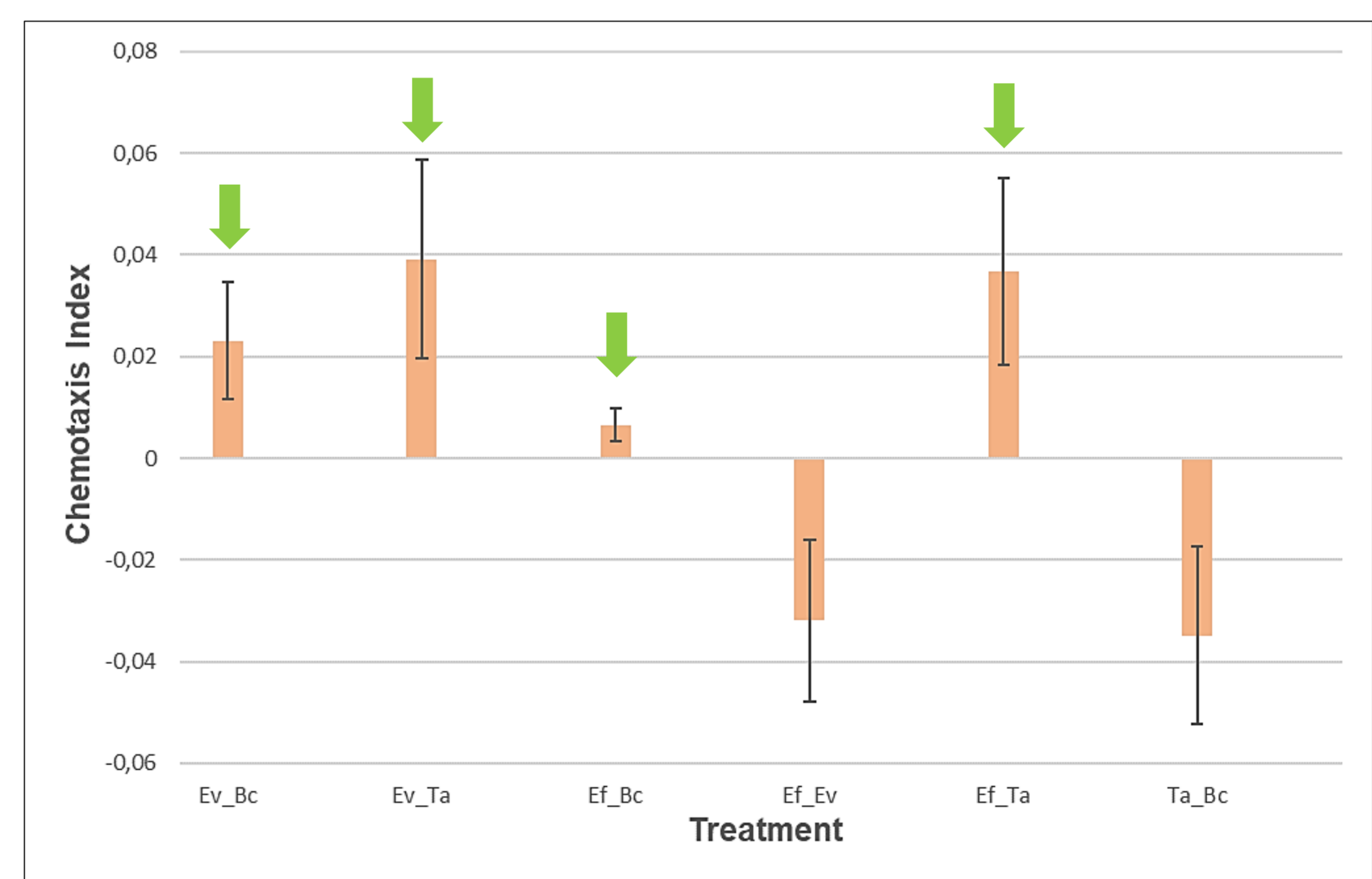


Figure 4. Chemotaxis index for all treatments after 1 h. Bars represent the mean ± SE of 3 replicates. Arrows indicate PWN preference for *Esteya* spp.

- Our preliminary results reveal a **clear attraction of *B. xylophilus* to *Esteya* spp.**, 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* dramatically decreased the initial population 7 DAI, indicating that **both fungi successfully 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.