Fungi–Nematode interactions between *Esteya* spp. and the pinewood nematode, *Bursaphelenchus xylophilus*

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INTRODUCTION

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a **quarantine organism** in several countries and the **causal agent of pine wilt disease** (PWD). Controlling it is difficult, but **fungi of 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.

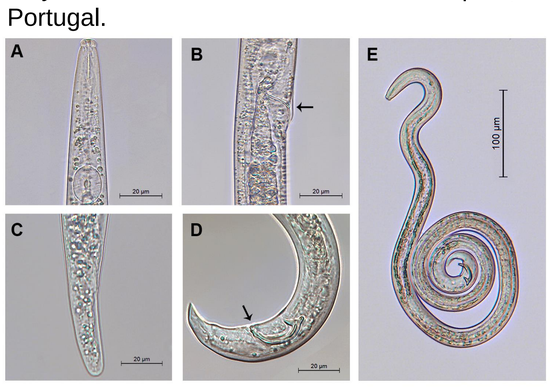


Figure 1. Morphological characters of *Bursaphelenchus xylophilus*. **A** – head; **B** – vulva and vulval flap (arrow); **C** – tail of a female; **D** – tail and spicule of a male (arrow); **E** – full body of a male.

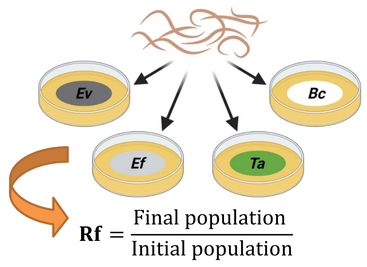
Figure 2. *Esteya vermicola* observed under the microscope (1600X). **A** – conidiophores with cylindrical conidia; **B** – conidiophores on the main hypha with lunate conidia.

METHODS

Fungus-nematode

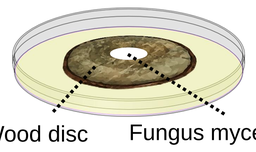
Determine the PWN **reproduction factor** (Rf) on *E. vermicola* G810 (*Ev*), *E. floridanum* V14639 (*Ef*), *Botrytis cinerea* (*Bc*) and *Trichoderma alni* (*Ta*)

ca. 500 nematodes



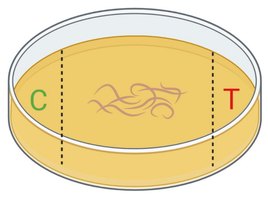
$$\mathbf{Rf} = \frac{\text{Final population}}{\text{Initial population}}$$

Fungus-plant

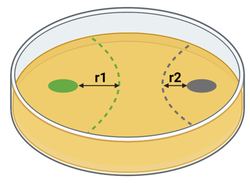


Fungus-fungus

ca. 100 nematodes



Calculate the **chemotaxis index** (CI): <0 → preference for control; 0 → no preference; >0 → preference for tested fungus



Determine the **percentage inhibition of radial growth** – I (%), between the potential antagonist (r1) and test fungus (r2)

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

$$\mathbf{I}\ (\%) = 100 \times \frac{\text{Potential antagonist (r1)} - \text{Test fungus (r2)}}{\text{Potential antagonist (r1)}}$$

OBJECTIVES

Study plant-nematode-fungus interactions → **Determine the attraction effect exerted by *Esteya* on the PWN** → **Infer the most promising *Esteya* spp. for biocontrol strategies**

RESULTS

Table 1. Reproductive ability of the PWN on fungal mats of *Esteya vermicola*, *E. floridanum*, *Trichoderma alni* and *Botrytis cinerea*. Values represent the mean ± SE of 4 technical replicates. Values followed by the same letter are not statistically different (p<0.001).

Fungus	Initial population	Final population	Rf
E. vermicola	500	0	0[a]
E. floridanum	500	1	0,0015[a] ± 0,002
T. alni	500	100	0,1995[a] ± 0,054
B. cinerea	500	2240	4,4795[b] ± 0,323

Table 2. Percent inhibition of potential antagonists against *Esteya vermicola*. Values represent the mean ± SE of 3 technical replicates (p<0,001).

Potential antagonist	Inhibition (%)
Esteya floridanum	7 ± 0,017
Ophiostoma ips	67*** ± 0,276
Trichoderma alni	91*** ± 1,138

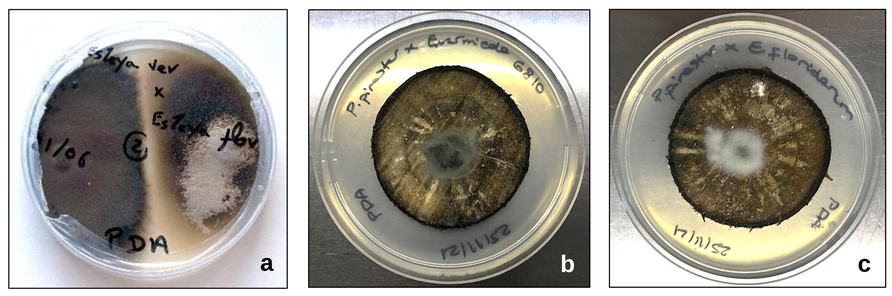


Figure 3. Biotic interactions between: a) *Esteya vermicola* and *E. floridanum*, with a clear inhibition zone in the middle; b) *E. vermicola* and wood disc of *Pinus pinaster*.; c) *E. floridanum* and wood disc of *P. pinaster*.

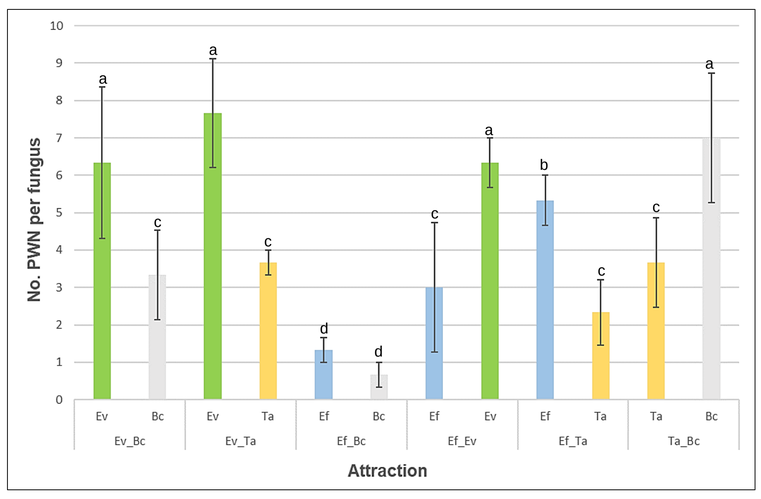


Figure 4. Attraction of PWN to the living mycelia of *Esteya vermicola* (*Ev*), *E. floridanum* (*Ef*), *Trichoderma alni* (*Ta*) and *Botrytis cinerea* (*Bc*), after 1 h. Bars represent the mean ± SE of 3 technical replicates. Values followed by the same letter are not statistically different (p<0.05).

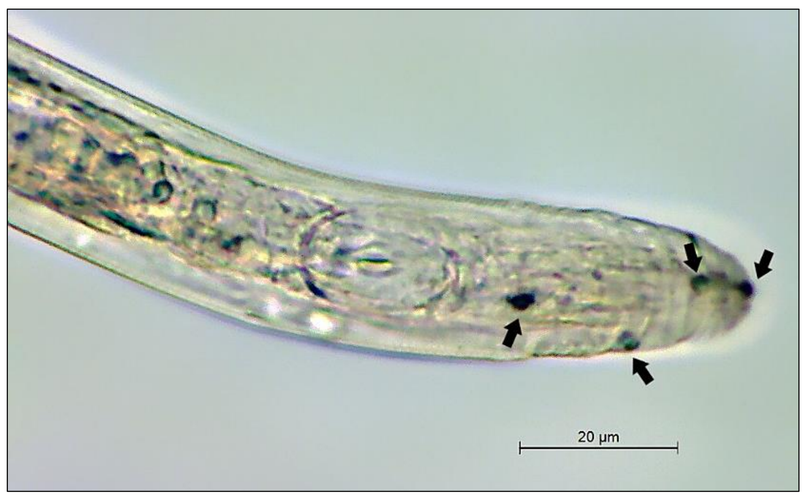


Figure 5. Cephalic region of a decaying specimen of *Bursaphelenchus xylophilus*, infected by *Esteya vermicola*, with visible spores (arrows) attached to the cuticle.

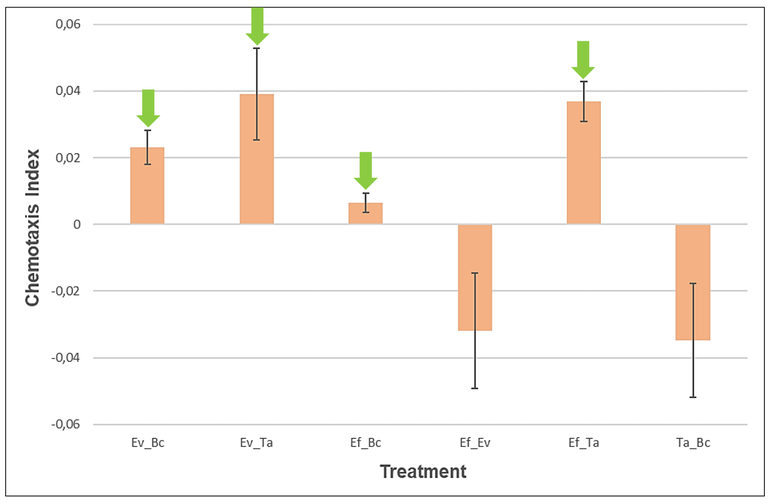


Figure 6. Chemotaxis index for all treatments after 1 h. Bars represent the mean ± SE of 3 technical replicates. Arrows indicate a stronger attraction to *Esteya* spp.

CONCLUSIONS

- **Strong attraction exerted by *Esteya* spp. on the PWN**, especially *E. vermicola*, compared to a naturally-occurring fungus in *P. pinaster*, *T. alni*, and the common PWN food source *B. cinerea*;
- No living PWNs were recovered from the mycelium of *E. vermicola* and *E. floridanum* dramatically decreased the initial population 7 days after inoculation, indicating that **both fungi successfully killed the nematodes** *in vitro*;
- Both ***E. vermicola* and *E. floridanum* can grow on and colonize *P. pinaster* discs**;
- These results suggest a **promising potential of *Esteya* spp. for biocontrol of the PWN in maritime pine**, but more isolates need to be considered and tested.

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