

ABOUT THE FCT **NEMATransFER PROJECT** (PTDC/AGR-FOR/4391/2014)

The Pine Wilt Disease (PWD) is a complex interaction established between three very distinct organisms: the Pine Wood Nematode (PWN), *Bursaphelenchus xylophilus*, an insect-vector (gen. *Monochamus*) and a conifer tree host (insect species and tree species varies throughout the world). The nematode is native from North America, where it doesn't cause disease to the native coniferous trees, but when accidentally introduced elsewhere causes the wilt of millions of pine, like in Asia (Japan, China and Korea) and in Portugal, since its detection in Portugal in 1999, where it only wilts *Pinus pinaster* (until 2012, when it was found in *P. nigra*) and is carried only by *M. galloprovincialis* (unique native species of the genus).

Dead pine trees infected with PWN are colonized by many wood and boring insects that spend months inside the wood to complete their life cycles. However, PWN is only detected in *Monochamus galloprovincialis* adults, never in pupae or larvae and never in other insect species, as morphologically close cerambycids found colonizing the same trees. The *Monochamus* beetle is a secondary insect unable to lay the eggs on healthy pine (they would be covered and destroyed by the resin exudation from the wound the beetles make to lay the eggs), and depended on finding weakened trees by abiotic (i.e. forest fires) or biotic agents (i.e. scolytid beetles). Now it carries the fast decline agent and their populations increased without control, spreading the nematode and the PWD.

The insect-vector overwinters as larvae inside the host tree until the adult is formed and emerges, by the end of May. If the tree is infected, the nematode migrates only to the newly formed callow-adult beetles, still inside the pupal chamber, dig by the larvae 5cm deep into the xylem. After emerging the adult beetle flies carrying nematodes and needs to feed on the thin bark of young healthy pine shoots for maturation, providing the entry for the nematodes into healthy pines, which within few weeks wilt and die. Few studies were made worldwide on the PWN transmission and its unknown why only few insect species from genus *Monochamus*, transport the PWN.

The proposed research plan aims to fill in the main knowledge gaps about the interaction between the Pinewood Nematode (PWN), the pine host tree and the *Monochamus galloprovincialis* insect-vector, with potential use to break the disease infection cycle. So, how does the PWN recognize the newly formed insect-vector adult, still inside the pupal chamber in the wood and what makes the nematode leave the wood to enter into the *Monochamus* breathing apparatus?

The second question concerns the second phase of the disease transmission, and is, what triggers the PWN transfers from inside the free flying insect-vector to the healthy pines, during insect feeding, to continue the infection cycle?

For the first question two different approaches will be developed, where the first (Task 1) will involve detailed anatomic study of the insect-vector and the other similar cerambycids, in an attempt to find some physiological barrier that could prevent the nematode entry. The second study (Task 2) will be based on discovering chemical volatiles emitted by the *Monochamus* callow adult, which gives the clue for the nematode to find the right insect species in the precise stage for the transmission to occur.

The second question must be related to the nematode recognition of chemical volatiles (Task 3), emitted by the healthy host tree, either the same that the insect-

vector is sensitive to find the adequate weakened host, or some more specific compounds, probably herbivore-induced plant volatiles release as a consequence of the insect feeding.

These are question still unanswered that will be tackle with innovative approaches, and it's our conviction that the knowledge of the mechanisms behind the PWN transmission would certainly provide new control methods against the disease spread.

Control methods available worldwide are based on the cut and destroy of the symptomatic and dead trees before insect-vector emergence, and the use of traps with chemical lures to capture flying insects

However, these lures are only effective against insects in reproductive stage. Immature adults, less than two weeks old, don't respond to these lures and that's the period when nematode transmission from the insect to the host mainly occurs, and this is a major gap in the PWD control strategy that will be address in the last, Task 4, of this project.