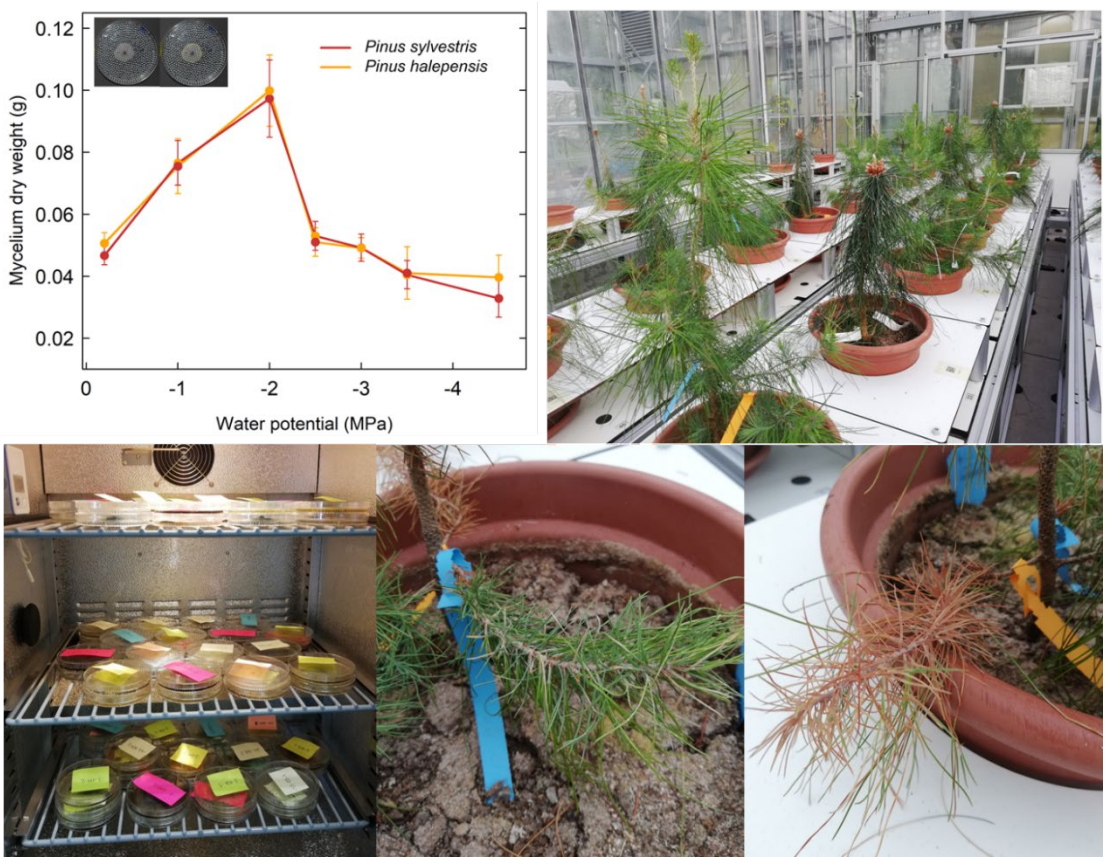
ADDITion



Adaptation of the pathogen DIplodia sapinea to pine hosts with contrasting Tolerance to drought

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Thematic action(s) concerned: : *WP1*

Context —
European forests are exposed to increasingly frequent and severe drought episodes due to climate change. Forest diseases associated with drought and heat waves have thus emerged during last years in Europe. Those diseases are caused by cryptic pathogens, which are present asymptomatically on their hosts and develop after abiotic stress. However, the mechanism that triggers the pathogenic behaviour of latent infections is poorly understood. One example of drought-induced forest pathogens is Diplodia tip blight of conifers. The causal agent, the ascomycete *Diplodia sapinea*, is one of the most important pathogens of pine species worldwide.

Objectives — The general objective was to test the link between the host's drought resistance and its tolerance to the disease. Particularly, we tested how host's resistance to cavitation and proline accumulation as a response to drought is linked to disease development.

The two objectives were then to 1) test whether *D. sapinea* isolates obtained from two different pine hosts with contrasting vulnerability to cavitation present different growth rates in agar medium under different water potentials; and to (2) evaluate if inoculations of pine with those *D. sapinea* isolates lead to the same in vitro growth pattern and it is linked to water potential in pine shoots.

Approaches —
We chose two pine species with contrasting drought tolerance and vulnerability to the *Diplodia* tip blight disease: *Pinus sylvestris* and *Pinus halepensis*, with the latest being relatively less affected by the disease and more drought tolerant. The first approach was a laboratory experiment by which we compared the growth of 5 strains isolated from *P. halepensis* and 8 strains isolated from *P. sylvestris* in culture media modified to obtain a gradient of 7 water potentials levels: -0.2, - 1, -2, -2.5, -3, -3.5, -4.5 MPa. As no difference was found between those strains, the transcriptomic analysis was not appropriate. Instead, we tested the hypothesis of different proline accumulation in the two hosts resulting in different vulnerability to the disease. A test is being performed this spring in the context of an M2 internship, where the strains will be grown in proline-enriched culture media. The second approach was a greenhouse experiment where a total of 104 plants were exposed to the following combination of treatments: *P. halepensis* and *P. sylvestris* plants, inoculated or non-inoculated (control) under a well-watering regime, a moderate or a severe drought.

Key results —
There was no significative difference in the growth pattern under the gradient of water potentials between the *P. halepensis*- and *P. sylvestris*-isolated *D. sapinea* strains, in terms of growth in surface and biomass.

- An optimal growth is found at -2 MPa, which confirms the capacity of *D. sapinea* to grown under negative water potentials, close to the water potential that pine species can experience in the field.
- However, we did find a marginal difference in the growth speed of *P. halepensis* strains, that perform better than those from *P. sylvestris* at an extremely low water potential (-4.5 MPa).

Main conclusions including key points of discussion —
The rather uniform *D. sapinea* population across hosts does not exclude our second hypothesis which implies that cavitation at less negative values of water potential results in disease development, that explains the different susceptibility of the two hosts. This cannot be confirmed yet, as an internship (part of the ADDITion project) is now performing the final measurements and analyses of the samples from the greenhouse experiment.

Perspectives —
The role of proline in disease development is also being analyzed in the samples from the greenhouse experiment. An additional experiment will be performed by the M2 student to monitor the growth in proline-enriched culture media. Those complementary trials and the work already done on water potentials, will give a clearer idea of the link between drought resistance and susceptibility to *D. sapinea* and new research questions will be set up.

Valorization —
The work of the project ADDITion was presented at the Symposium on Emerging Needle and Shoot Diseases of Conifers in Europe held in the University of Freiburg July 3-5, 2023. The experiment on growth in a gradient of water potentials and the hypotheses tested were very welcome by the research community. The experiment in the greenhouse is not finalized yet. The publication will be started when we have all the results. I also plan to present the results in the next IUFRO conference on foliar and shoot diseases.

Leveraging effect of the project—
This project has allowed me to establish a collaboration network around the work on *D. sapinea* in Europe. New research questions have arisen and they will be developed in new projects, particularly the ANR JCJC call. It has also contributed to a solid collaboration with the DSF (French Forest Health Department).