Assessment of the combined effect of temperature and relative humidity on fungal growth

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Biological background and motivation

Fungi are resilient organisms that are able to grow in almost any environment. They generally form a **mycelium** (Fig. 1 a-b), a wide-spread network of narrow, thread-like structures called **hyphae** (Fig. 1 c). Growth occurs at the hyphal tips, known as **apices**. New biomass is generated either by the extension of existing hyphae or by the creation of new apices, a process called **branching** (Schmidt, 2006).

Fungal growth is determined by the **environmental conditions** when nutrients are not limiting. Each fungal species grows under a certain range of environmental conditions and most species achieve their maximum growth rate under very specific circumstances only, referred to as the **optimal growth conditions**. Of those environmental conditions, **temperature** and **relative humidity** are the most influential (Vereecken & Roels, 2012).

Defining the optimal growth conditions has been frequently done, since they allow to optimize **industrial processes** and provide information about fungal species that cause **damage to crops** (e.g. *Rhizoctonia solani*) and in **construction** (e.g. *Coniophora puteana*) (Fig. 1 d-e).

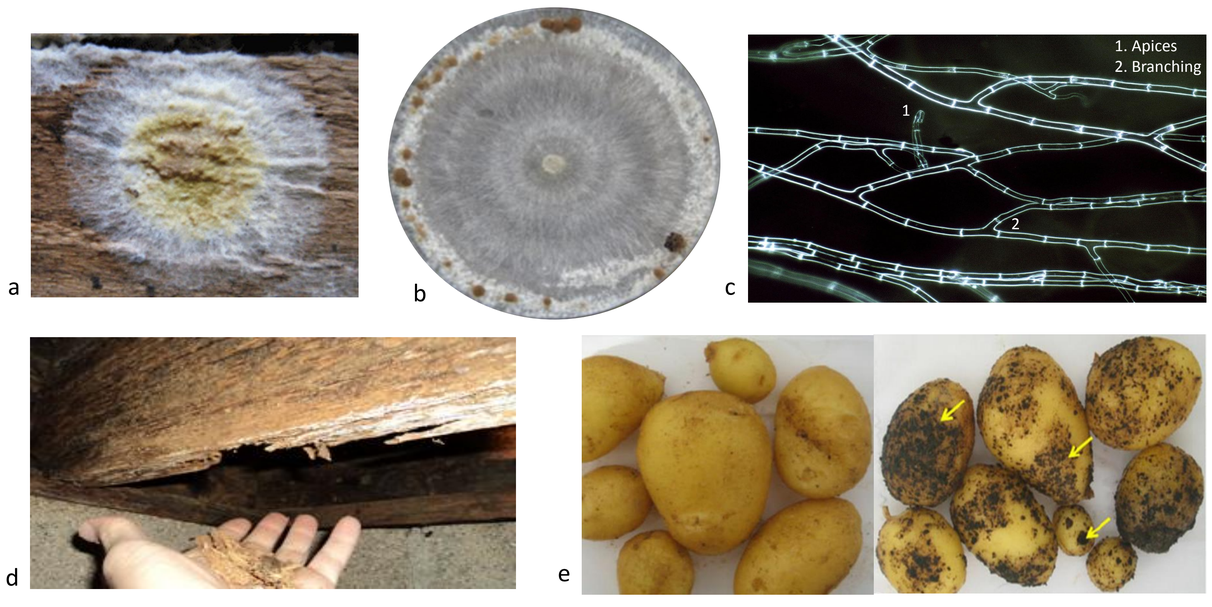

Fig. 1 *Coniophora puteana* mycelium (a) and wood damage (d); *Rhizoctonia solani* mycelium (b) and potato crop damage (e); Fungal hyphae (c)
Sources: (a) and (d) Sachverständigenbüro für Holzschutz, 2016; (b) Lu et al., 2016; (c) Barron, 2013; (e) Djebali et al., 2014

Material and methods

The optimal growth conditions of two frequently studied fungal species are examined, the brown rot fungus ***Coniophora puteana*** and the plant pathogen ***Rhizoctonia solani.***

Mother cultures of these fungi were used to extract a **disk-shaped inoculum** of about 1 cm diameter. Then, the inoculum was placed at the centre of the bottom lid of a Petri dish, surrounded by 12 small substrate disks (Fig. 2). The top lid of the Petri dish was placed on top of the bottom lid, as such **restricting the height** between the lids to 0.6 mm and enforcing **growth in two dimensions** only.

Images of the growing fungi were captured using a **flatbed scanner** on top of which the Petri dishes were mounted. **Growth was tracked for 72 hours.** In order to assess the effects of the environmental conditions on fungal growth, we placed the flatbed scanner in a **climate chamber** where temperature and relative humidity could be adjusted. **Temperature** was varied from **15 to 30 °C** in steps of 5 °C, while **relative humidity** was varied from **65% to 80%**, in steps of 5%.

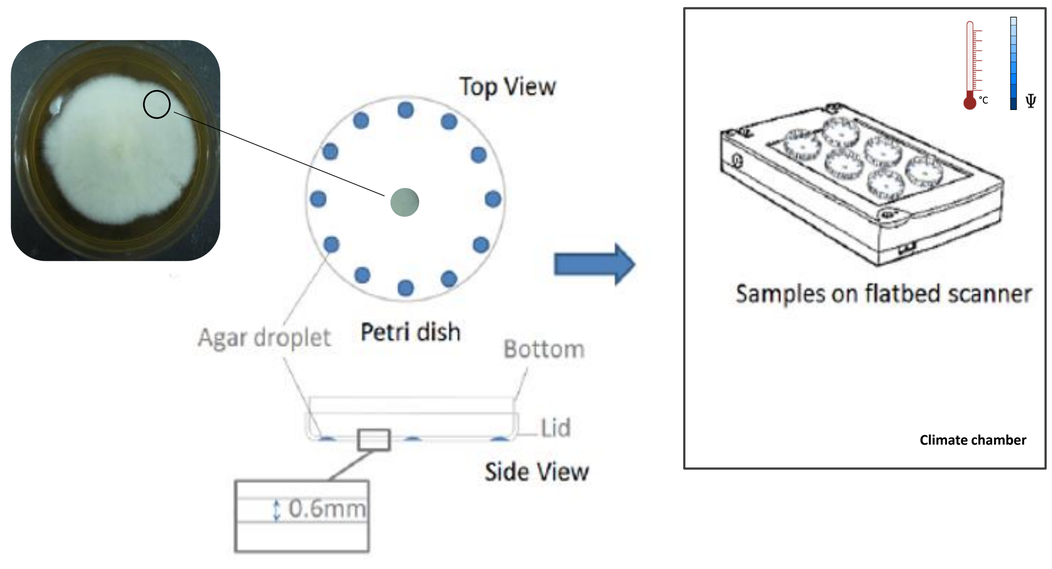

Fig. 2 Scheme of the experimental set-up.

Image Analysis

In order to extract fungal growth features from the initial image (Fig. 3 a), four steps need to be taken.

Step 1: Removing noise in the images, such as droplets of agar and the initial inoculum (Fig. 3 b)

Step 2: Extracting the fungal network. A **line detection algorithm** (Lopez-Molina *et al*., 2015) is used to extract a thin **binary ridge map** from each image, which represents the fungal network (Fig. 3 c). Binary ridge maps for *Rhizoctonia solani* growing in vitro are represented in Fig. 4.

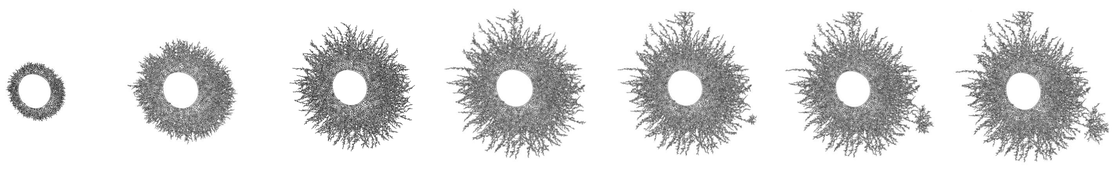

Fig. 4 Evolution of the fungal network of *Rhizoctonia solani,* extracted using a line detection algorithm (Lopez-Molina et al., 2015). The pictures represent the growth from 10 to 72 hours at intervals of 10 hours.

Step 3: Converting the ridge map into a mathematical graph. The MorphologicalGraph function of Mathematica converts images into **mathematical graphs** (Fig. 3.d), where the intersections represent junctions of hyphae and apices of the mycelium and the line segments represent the hyphal segments connecting them.

Step 4: Extracting fungal features. Using the information contained in the graphs we can compute some of the most important fungal kinetic parameters: **total length of the mycelium, total number of tips, area of the mycelium, etc.**

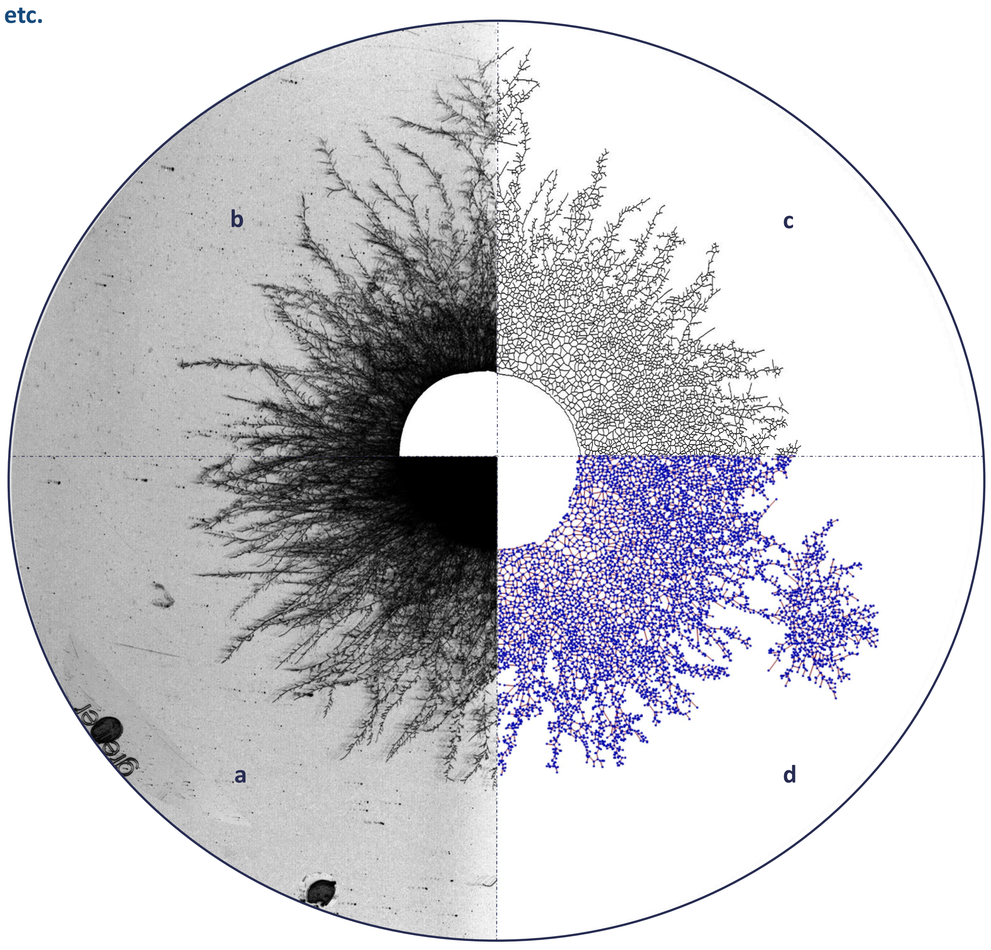

Fig. 3 Summary of the complete process of fungal growth feature extraction from an image of *Rhizoctonia solani*.
a) Initial image; b) Cleaned image; c) Binary ridge map; d) Mathematical graph.

Results

The evolution of the total length of the mycelium and the number of tips, as a function of temperature and relative humidity, can be found in Fig. 5 for *Rhizoctonia solani*.

Some of the main findings:

- Maximal mycelial length and number of tips at 20°C and 65% relative humidity
- At the highest temperature (30°C) there is rapid growth only at the beginning, after which a plateau is reached and growth stabilizes

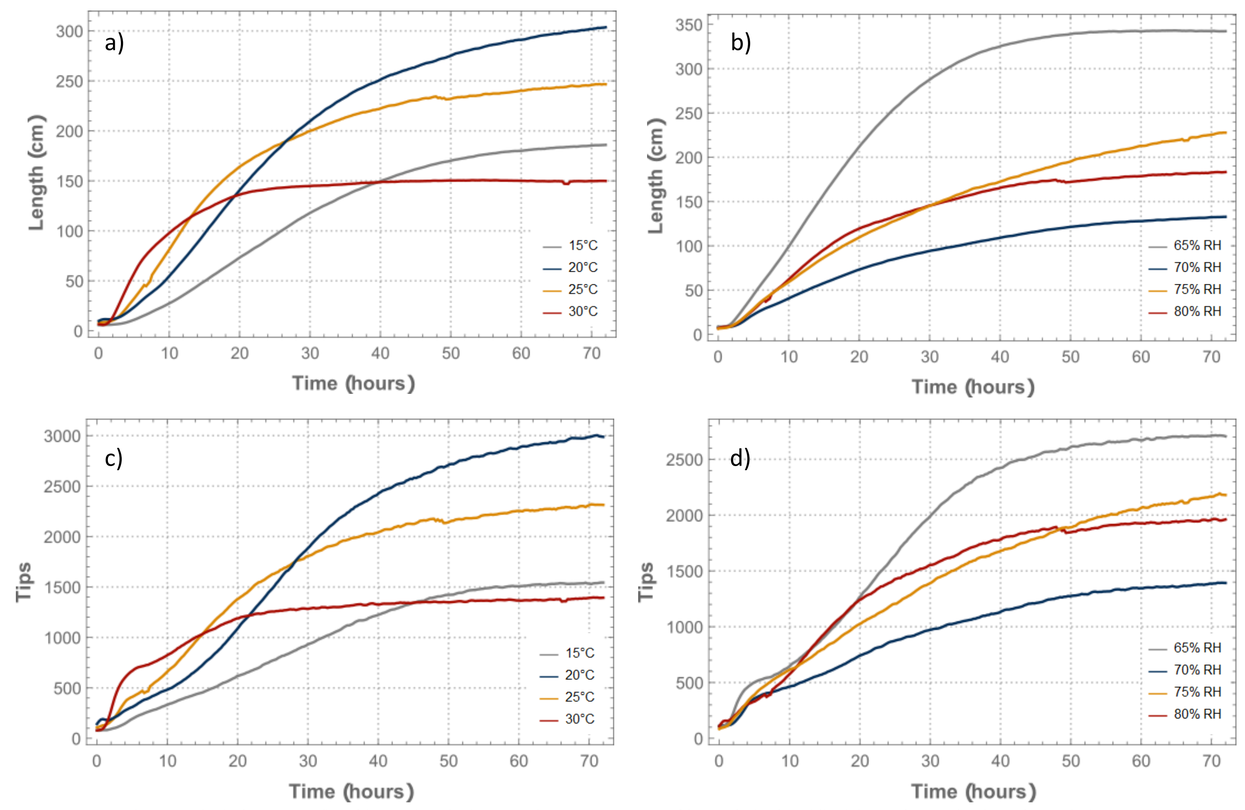

Fig. 5 Topological measures for *Rhizoctonia solani* averaged over temperature (°C) or relative humidity (%RH). The graphs show the evolution over time of the total length of the mycelium (a-b) and the number of tips (c-d) grouped by temperature and relative humidity, respectively.

References

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Acknowledgements

FWO Research Foundation Flanders (Fonds Wetenschappelijk Onderzoek – Vlaanderen).

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