



# Topographically triggered mycelial bundles in *Fusarium* species

## or “Unusual growth conditions reveal new morphologies”

Anne D. van Diepeningen<sup>1,\*</sup>, Colin Ingham<sup>2</sup>, Wim van Egmond<sup>3</sup>, Jan Dijksterhuis<sup>4</sup>

<sup>1</sup>BU Biointeractions and Plant Health, Wageningen University and Research, The Netherlands; <sup>2</sup>Hoekmine BV, Utrecht, The Netherlands;

<sup>3</sup>www.wimvanegmond.com; <sup>4</sup>Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; \* anne.vandiepeningen@wur.nl

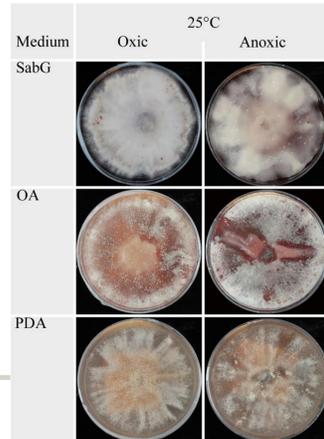
### Background/Objective

Growing fungi in the laboratory or for production purposes, we use standard media and growth for fungal growth. The objective of this research is to illustrate the profound changes in fungal morphology caused by altered organization of the availability of nutrients.

#### 1. Oxygen levels

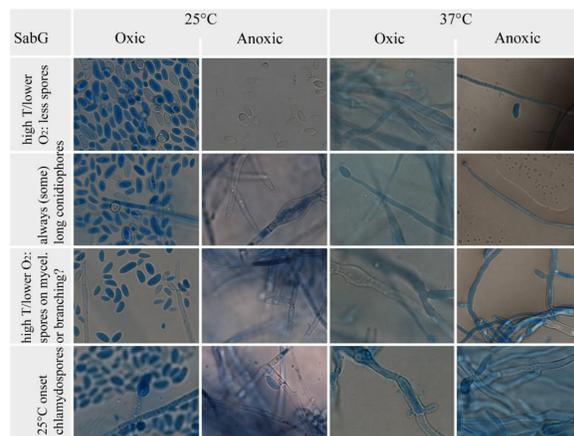
*Fusarium solani* s.s grown under standard growth conditions; Sabouraud dextrose agar (SabG), oatmeal agar (OA) and potato dextrose agar (PDA) and environmental (oxic) O<sub>2</sub>/CO<sub>2</sub> levels and intraorganismal (more anoxic) reduced O<sub>2</sub> rates shows growth rate, density, sporulation and pigmentation differences (Fig. 1).

**Figure 1.** Effect of oxygen levels on *Fusarium solani* s.s. growth on three different standard media.



#### 2. Oxygen & temperature effects

Microscopically, reducing O<sub>2</sub> leads to reduced sporulation (conidia and chlamydospores), while increasing temperatures to 37°C reduces the production of the tall conidiophores typical of *Fusarium solani*. Spores directly produced on hyphae may explain why in e.g. human infections *Fusarium* can easily be transported via blood (Fig.2 ).



**Figure 2.** Effect of oxygen levels and elevated temperatures on *Fusarium solani* s.s. growth on Sabouraud dextrose agar (SabG).

#### 3. Micro-cultivation chips (MCC)

Micro-cultivation chips (MCC) are a novel method for growth and screening of fungi as microcolonies. MCC comprise an array of microwells (from 20 to 300 microns diameter) with a porous ceramic base, which can be placed on standard growth media. *F. oxysporum* produces mycelial bundles when cultured on MCC (Fig. 3).



**Figure 3.** Topographically triggered mycelial bundles in *F. oxysporum*

#### 4. *F. oxysporum* ‘mushrooms’

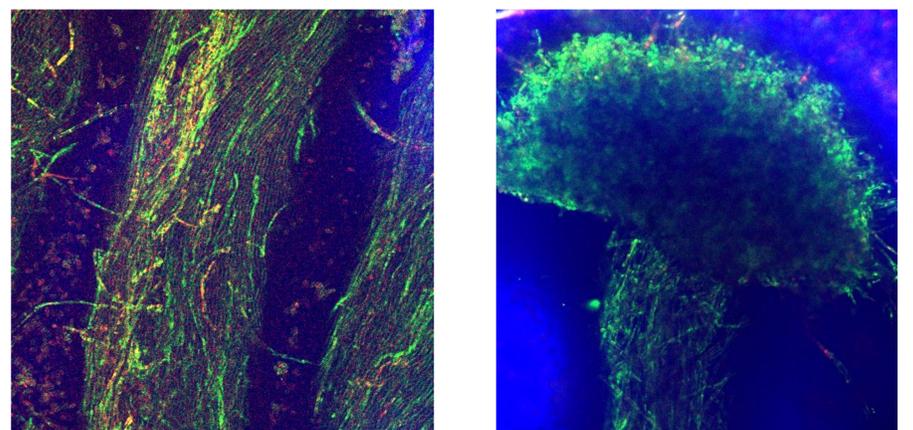


**Figure 4.** Topographically triggered bundle and cap formation in *F. oxysporum*.

Spores of *F. oxysporum* on micro-cultivation chips on PDA, produce bundles of mycelium (diameter ca 180 μm) sprouting from the microwells, headed with a small mushroom cap in as little as 3 days.

#### 5. Mycelial bundle and cap composition

Using an inverted confocal laser scanning microscope and green fluorescent transformants of *F. oxysporum* we can study the stalk of the formed mini mushroom in more detail: the stalk proves formed by parallel hyphae occasionally linked by anastomoses (Fig.5A).



**Figure 5A.** Green fluorescent *Fusarium oxysporum* bundle of hyphae grown on micro-cultivation chips on potato dextrose agar (PDA). **B.** The cap of the *gfp-F. oxysporum* culture on MCC/PDA is formed by spreading mycelium bundles and numerous microconidia

The cap of the green fluorescent *F. oxysporum* ‘mushroom’ is formed when the parallel bundles of hyphae expand and are interspersed with formed microconidia (Fig. 5B).

#### Conclusion

By growing in *Fusarium* in new ways, new developmental processes have been revealed. These require an explanation as to how these processes are controlled and triggered, and whether there is a direct relationship with the survival and pathogenicity of these strains in the environment.

