MSc Computational Science
joint programme UvA/VU

FROM CONIDIAL GERMINATION TO HYPHAL GROWTH: A SPATIAL MODEL OF *ASPERGILLUS* SPP.

THESIS TOPIC PROPOSAL

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1 Introduction
Research Relevance
Research Context and Data
2 Model
Model Goals
Preliminary Ideas
3 Outlook
Tentative Schedule

ASPERGILLI

Food Spoilers and Cell Factories

- Filamentous moulds from the genus *Aspergilli*, phyllum Ascomycota
- Notable species:
  - *Aspergillus fumingatus* - pathogen causing aspergillosis;
  - *Aspergillus niger* - food contaminant, possible pathogen, use in chemical industry;
  - *Aspergillus nidulans* - research organism in cell biology;
  - *Aspergillus oryzae* - used in the fermentation of soy products;
  - *Aspergillus terreus* - producer of organic acids, can cause opportunistic infection in humans.

A. NIGER AS MODEL SPECIES

- Extensively researched species;
- Production of citric acid recognised as far back as 1917;
- Production of enzymes (e.g. glycoamylase).

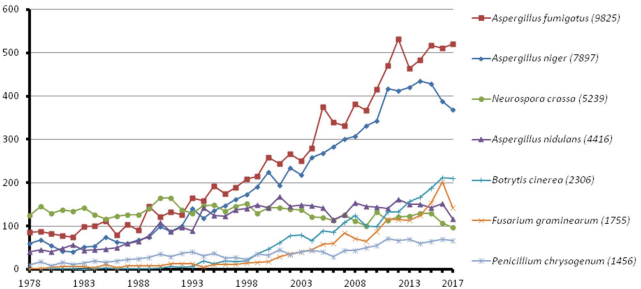
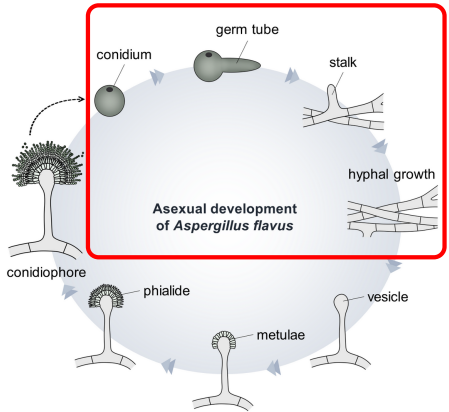


Figure: Number of published papers on PubMed about specific filamentous fungi[2].

ASPERGILLUS LIFE CYCLE

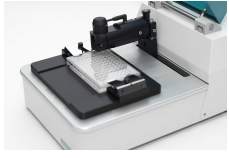
- Stages of interest - inoculation to hyphal outgrowth (excluding reproduction).

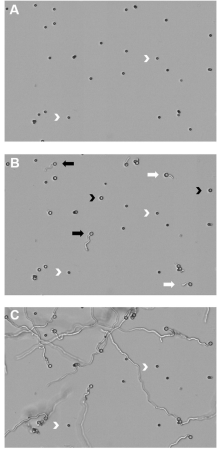


STUDIES BY IJADPANAHSARAVI ET AL.

Microscope imaging with oCelloscope

- Frames of conidia samples in well plates, taken at regular intervals for 24 hours[7];
- Computer vision algorithm detects properties of observed spores: **Area**, **Circularity**, **Elongation**, **Granularity**, **Thinned Length**, **X and Y Position**, **Branch Points**, **Bounding Box**;
- Overlapping objects yield ambiguous measurements and are therefore removed from analysis.
  - Therefore: data on germination up to early hyphal extension.





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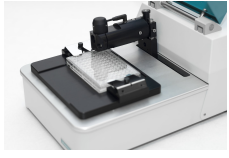

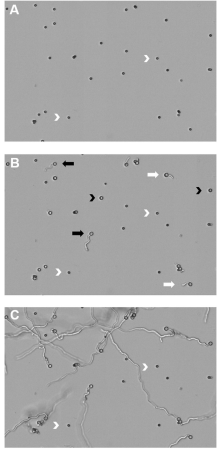

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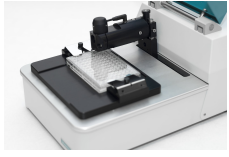

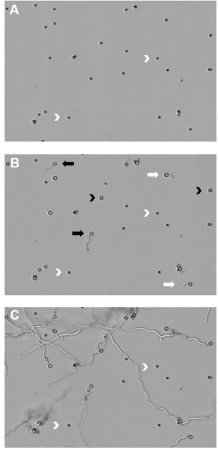

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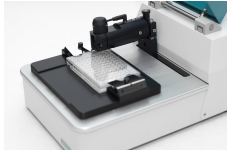

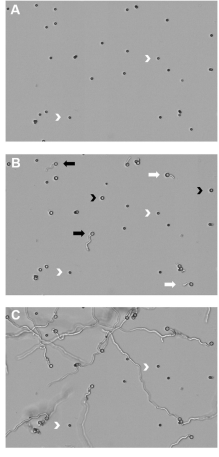

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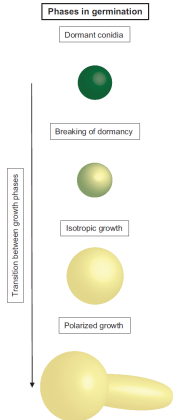
Germination phases[1]

- Dormancy
- Swelling
- Polarisation
- Germ tube formation
- Hyphal extension



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Minimal nutrient requirements for germination

- Glucose + either inorganic phosphate, inorganic nitrogen or magnesium sulphate: sufficient for swelling and germination;
- These conditions are not sufficient for outgrowth;
- Therefore, a fraction of the spores "takes a chance" to germinate.

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Effect of highly inducing amino acids on *A. niger*

- Alanine and proline - highly inducing amino acids (stronger effect on germination than glucose);
- They are sources of carbon and nitrogen, usually forming in plants under stress.

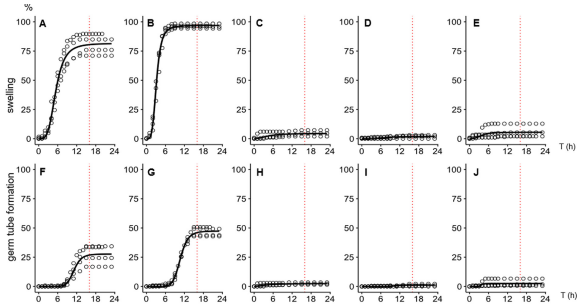

Figure: Fitted asymmetrical model[4] on *A. niger* data measured in different media: alanine (A, F), proline (B, G), glutamate (C, H), histidine (D, I), phenylalanine (E, J)[7].

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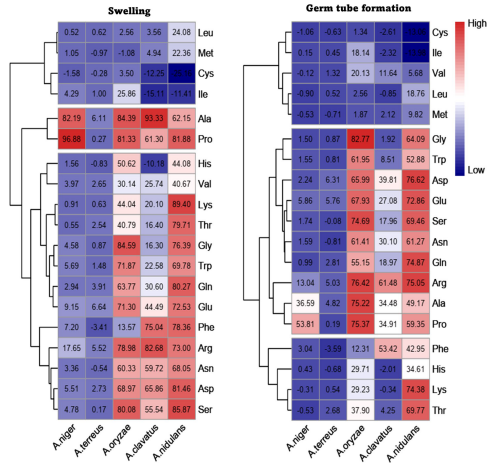
Amino acid effects on other *Aspergilli*

- Germination in different species is induced by different amino acids.[6]



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Carbon sensing

- G and Ras proteins play a role in environmental sensing.

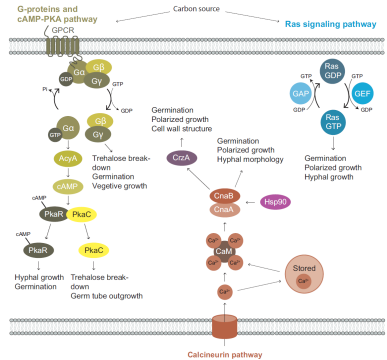

Figure: Membrane-related regulatory pathways involved in conidial germination[1].

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Effect of spore size, density and co-cultures

- *Aspergilli* form conidia of different sizes to accommodate different germination responses.
- Large spores more sensitive to germination inducers (surface area proportional to number of carbon receptors).
- High density of same-species conidia **inhibits germination incidence** (but not germination time).
- Presence of other-species conidia may **inhibit germination incidence** (but not germination time) depending on medium.

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RESEARCH ON HYPHAL OUTGROWTH

Free filaments vs. pellets

- Single conidia form free filaments.
- Agglomerated conidia form pellets.

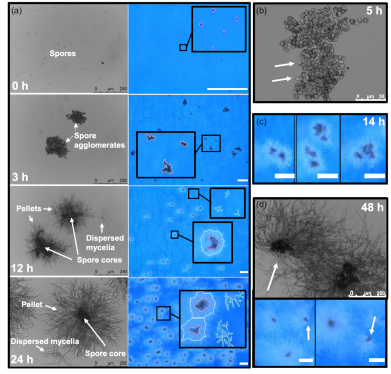

Figure: Pellets at different cultivation stages: differential interference contrast (DIC) images (grayscale) and stereomicroscopic images (RGB).[8]

RESEARCH ON HYPHAL OUTGROWTH

Synchrotron radiation-based microcomputed tomography

- Available data on 3D pellet morphologies of a hyper-branching *A. niger* mutant.
- Can serve as an extreme case verification.

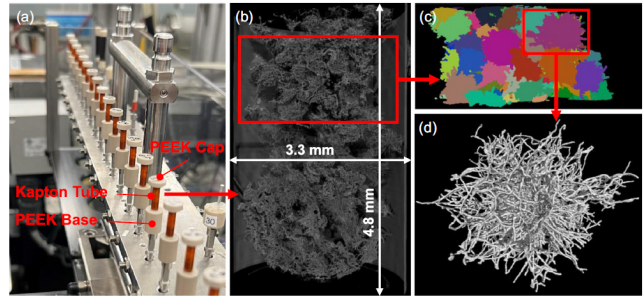

Figure: Process of obtaining 3D imagery of an *A. niger* pellet through microtomography[9].

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THE ASPECT OF SPACE

- The spatial colonisation of substrate is important for tracing pathogenicity or productivity of an *Aspergillus* fungus.
- Spatial heterogeneities may be indicative e.g. of competition effects or irregular distribution of resources.
- Results of spatial results can be qualitatively and quantitatively compared to microscope imagery.
- The spatial spread and morphology should be traceable back to the inoculation and germination conditions.
- Current models predominantly focus either on germination or on outgrowth, but not on both.

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BRIDGING THE GAP

Considering space in germination

- Asymmetrical model by Dantigny[4] is used to fit germination parameters P_{max}, τ and d:

$$P = P_{max}\left[1 - \frac{1}{1 + \left(\frac{t}{\tau}\right)^{d}}\right] \quad (1)$$

- It results in a good fit but is not directly based on biological principles (derived from non-competitive inhibition model).

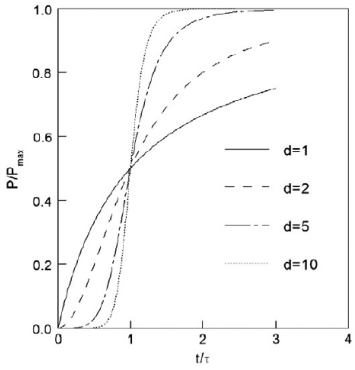

Figure: Effect of different values for the design parameter d.[4]

BRIDGING THE GAP

Considering space in germination

- Can spatially distributed interactions based on actual mechanisms (carbon sensing, mutual inhibition) result in the same average germination curves?
- Parameters can be used to construct distributions for randomised germination times of spatially distributed spores.

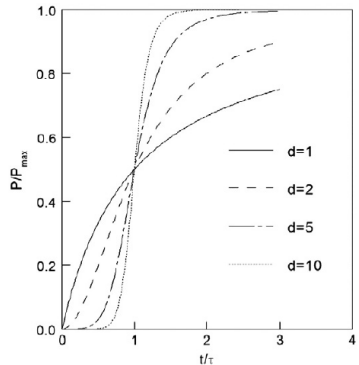

Figure: Effect of different values for the design parameter d.[4]

BRIDGING THE GAP

Connecting germination to outgrowth

- Germination time is correlated with mycelium outgrowth lag time[5];
- Inoculum consistency (concentration, agglomeration) affects pellet morphology[8];
- Culture mixes and medium may impact not only germination but also pellet formation.

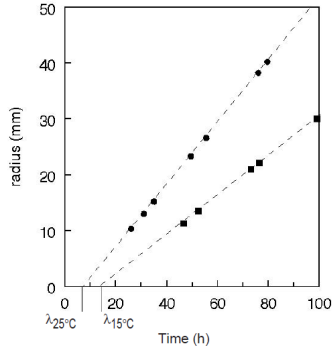

Figure: Linear growth of the radius of two *Mucor racemosus* colonies over time, grown at different temperatures[3]

PRELIMINARY IDEAS

- The heterogeneous properties and interactions of spores can be captured through agent-based modelling.
- Hyphal growth can be represented through successive (branching) vector additions to each conidium source.
- If evidence shows a high importance of diffusive transport, lattice-based PDE techniques can be considered.

MODEL SUMMARY

- Encompassing stages from inoculation to pellet/filament formation.
- Simulated growth in three-dimensional space.
- Initial behaviour informed by germination data.
- Final morphologies verified by tomographic data (comparison of volume, fractal dimension, branching ratio etc.)

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TENTATIVE SCHEDULE

1. Submission of Thesis Proposal - by 21.10.2024;
2. Literature review (carbon signalling during germination, spore agglomeration, mechanics of hyphal growth) - by 01.12.2024;
3. Minimal model completion and data access setup - by 17.01.2025;
4. Verification and correction of model - by 28.02.2025;
5. Analysis of model results - by 28.03.2025;
6. Expansion of model (application to other species, incorporation of other mechanisms) - by 25.04.2025;
7. Documentation (writing of Thesis) - by 30.05.2025.

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