Microbiology –WP4.2

Aerosolisation of fungal fragments, microconidia and macroconidia of *Botrytis cinerea* as affected by growth material, air jets and changing air humidity

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Release/aerosolisation of fungal spores

Spore release is the process during which spores become set free from the parent tissue.

The term spore release is also often used as a term for aerosolisation or emission of fungal spores.

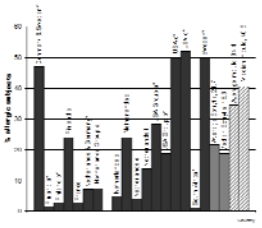
Fungal spores are described to be released by different mechanisms:

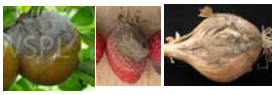
- Rain splash (indoor –water splash?)
- Dew (indoor ?)
- Increasing or decreasing vapour pressure/rh
- Wind (indoor ?)
- Animals (indoor ?- transport)

Rh
Decreasing Rh
Rh+Wind

Particle aerosolisation from *Botrytis* cultures- why *Botrytis*

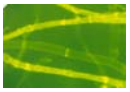
- Relatively many people are allergic to the grey mould *Botrytis cinerea* compared to its low airborne prevalence
- Slight tendency for a higher *Botrytis* level in complaint homes than in non-complaint environments
- *Botrytis cinerea* has many potential indoor sources – it is not known whether it can grow on indoor building materials

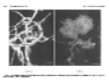




Botrytis cinerea morphology

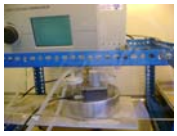
- Micro-conidia – to become airborne : Globose 2.5-3.0 µm, function unknown, probably spermatia, not able to grow agar.
- Macro-conidia – to become airborne: obovoid 8.0 x 6.0 µm, function make a new colony.
- Sporangia
- Hyphae
- Sclerotia, visible with the naked eye, function- survival.
- Fragments ? - to what? – different sizes, we measure particles smaller than micro-conidia size





Chitin in the cell wall is labelled, it has also glucan in its cell wall
Old macro-conidia are strongly pigmented
Glucan

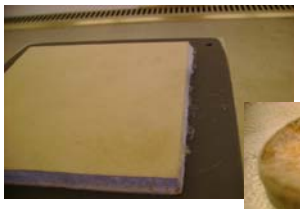
Aerosolisation of particles



- PM1 triplexcyclon : Fungal fragments
- PM2.5 triplexcyclon : Fungal fragments + some Micro-conidia
- Inhalable (GSP): do + Macro-conidia
- APS: Particle size and number
- P-FLEC 1.5 m s^{-1}
- Glucan
- NAGase
- TIP

Can *Botrytis* grow on indoor building materials?

Test different building materials
Model vegetable/waste material



Aerosolisation of particles of *B. cinerea* during decreasing rh: Gypsum board

- The aerosolisation from a *B. cinerea* culture on gypsum board not exposed to air jets was studied for 5 hours.
- The number of aerosolised particles was measured every sec.
- No particles were aerosolised during this period.
- In the period of measure the air humidity decreased continuously from 83% to 62%.
- In water damaged buildings viable *B. cinerea* spores have been found during demolition activity, but neither before nor after demolition.

Aerosolisation of particles of *B. cinerea* during decreasing rh: Floor paper

- The aerosolisation of particles from *B. cinerea* on floor paper was studied for a 14 hour period where the humidity decreased from 78% to 20%.
- During the whole period almost no particles were aerosolised.
- The fastest decrease in rh was a decrease from 76% to 56% during 38 minutes.
- In that period no particles smaller than microconidia size were aerosolised; a fraction of 0.034% of both microconidia and macroconidia sizes were aerosolised per minute relative to the number aerosolised during exposure to air jets (1.5m s^{-1}) at a rh of 62%.

Gypsum-boards – exposure to an airflow

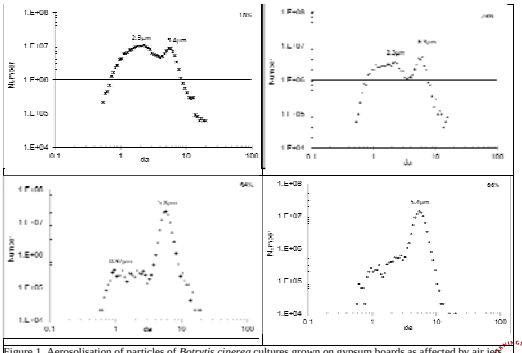


Figure 1. Aerosolisation of particles of *Botrytis cinerea* cultures grown on gypsum boards as affected by air jets of 1.5m s^{-1} and as influenced by air humidity. Numbers over the curves are the sizes of the in terms of numbers dominating size fraction.

Floor paper – exposure to an airflow

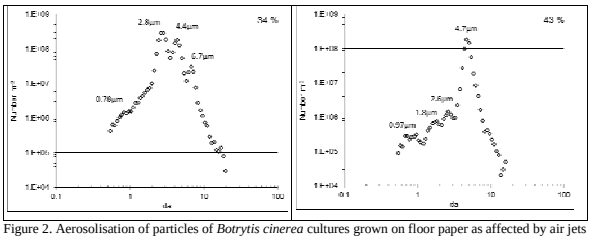


Figure 2. Aerosolisation of particles of *Botrytis cinerea* cultures grown on floor paper as affected by air jets of 1.5 m s^{-1} and as influenced by air humidity. Numbers over the curves are the sizes of the in terms of numbers dominating size fraction.

Aubergines – exposure to an airjet

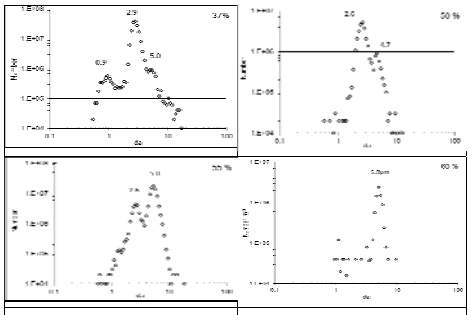


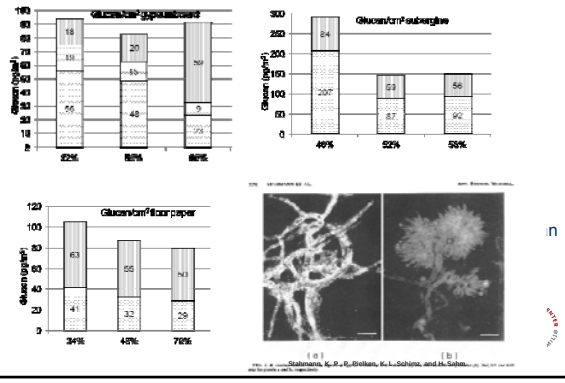
Figure 3. Aerosolisation of *Botrytis* particles with different da (aerodynamic diameters in µm) from cultures on aubergines as affected by air jets (1.5m s^{-1}) at different rh. Numbers over the curves are the sizes of the in terms of numbers dominating size fraction.

Influence of relative humidity on the number of particles (x1000) aerosolised per cm^2 gypsum board, floor paper or aubergine when exposed to air jets of 1.5m s^{-1} during 5 minutes (n=3).

		Number of particles of				
	RH %	Fragment size* 0.54-1.6µm	Microconidia size 1.8-3.3µm	Macroconidia size 3.5-10.4µm	Sum 0.54-19.8µm	Respirable fraction
Gypsum boards	19	460[a]	675[a]	534[b]	1669[a]	74%
	23	321[a]	362[a]	891[ab]	1574[a]	70%
	65	19[b]	27[b]	736[ab]	780[b]	31%
	72	38[b]	49[b]	1202[a]	1378[ab]	22%
Floor paper	34	31870[a]	1141090[a]	761480[a]	1947440[a]	68%
	43	4360[b]	9210[b]	550010[a]	567270[a]	36%
	79	30[c]	90[c]	6250[b]	6390[b]	29%
Aubergines	37	211[a]	6312[a]	952[b]	8348[a]	79%
	50	16[b]	1182[b]	237[c]	1441[b]	79%
	55	30[b]	926[b]	4020[a]	4987[a]	39%
	60	15[b]	5.8[c]	226[c]	318[c]	33%
	65	12[b]	9.0[c]	432[bc]	446[bc]	29%

Comparison (within a material, within a certain size fraction) of number of aerosolised particles at different rh, numbers followed by the same letter are not significantly different.* The sizes are the aerodynamic diameter measured by an APS.

Glucan in PM1 and inhalable fraction



In conclusion

- *Botrytis cinerea* is able to grow on building materials
- It produces micro-conidia which seem to be spermatia which are not able to grow on agar media – and will not be detected by cultivation methods.
- A decrease in rh does not cause an aerosolisation of *B. cinerea* fragments, micro-conidia or macro-conidia
- When exposure to an airflow cultures at low rh aerosolise much more particles of respirable size than cultures at higher rh.
- This may be because more micro-conidia in addition to fragments are released during dry conditions and thus can be aerosolised during exposure to an airflow.
- Drying out of building material cause an exposure to more particles of respirable size if exposed to an airflow.
- A very high exposure to glucan is found from the PM1 fraction – this may be fragments of the fungus or extracellular produced glucan (cinerean).

Future:

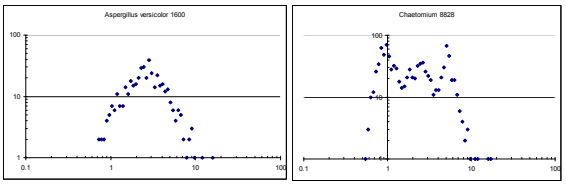
Spore aerosolisation:

- Many different fungi exist, with different structures, different cell walls, different biology etc.
- From the 5 homes bioaerosols have been sampled, with a mixture of different microorganisms
- Mika is going imitate a water damage and expose the water damaged building material to the bioaerosol components
- Study the growth and aerosolisation as a function of air humidity

Botrytis cinerea:

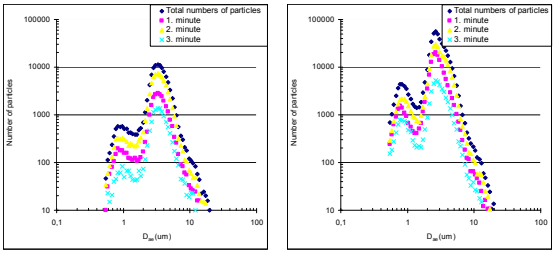
Presence in the 5 homes?

8 år gamle kulturer!



This is because the spores (= ascospores) are produced within flask-shaped bodies (= perithecia) and not exposed to air like those of moulds such as *Penicillium* and *Aspergillus*. When the spores mature, they are released inside the perithecium and then squeezed out in a column like toothpaste through an opening at the top of the perithecium. The coiled hairs trap the spores such that they are not easily dispersed into the air by wind

Example of particle profile from sedimented dust and growing microorganisms on a surface



'Wet' (max 3.05 um, 0.965 um) (43% in material)

1.5 m/s

'Dry' (max 2.64 um, 0.835 um) (13% in material)