GartneriRådgivningen A/S
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Herewith we sent you the results of the measurements of spores sedimentation and collection efficiency for *Botrytis cinerea* and *Rhizopus stolonifer* conducted in Gartneri Knud Jepsen A/S.

In addition to various gases and condensed vapors, air also includes various biological elements such as mould spores, yeast, bacteria and viruses. The longest lifespan of microbes in air is found among bacterial and fungal spores. Mould fungi from *Penicillium* sp. and *Rhizopus* sp. genera together with several other genera, constitute more that 90% of all microbial forms in the air. *Rhizopus* spp. is a typical "indoor mould" that can be found in high concentrations in many buildings and places where plants are grown or where fruits and vegetables are kept (packing houses, canteens etc). Plant pathogenic fungus, *Rhizopus stolonifer* has a cosmopolitan distribution. It is a common plant pathogen but it is also capable of causing opportunistic infections of humans (zygomycosis). Its spores are also commonly found in many greenhouses were plants are grown and many plants can be infected by this pathogen. Spores of *Rhizopus stolonifer* becomes airborne to make a new colony. Spores of this fungus are big, 4-11 µm and by being "very sticky" they easily settle on different surfaces.

Another airborne plant pathogenic fungus, *Botrytis cinerea* is a typical "outdoor mould" and can become airborne/released due to different mechanisms, but increasing or decreasing vapor pressure and rain splash plays very important role in this process. Spores of *Botrytis cinerea* are ovoid 8.0x6.0 µm. They are often released in groups (known as aggregation). The presence of this fungus in the buildings and greenhouses is an evidence of continuous input of microorganisms from outside *via* visiting/working people and *via* windows and doors. Research shows that *Botrytis cinerea* survives better in humid environment. In dry environment this fungus will mostly produce much smaller microspores that aerolise much better when exposure to an airflow.

Fungi can move laterally and vertically in air. They may drift attached to dust particles and immersed in water. Most airborne spores will bypass an immobile object, but a proportion impact on the object when the spore is too heavy to be carried in the air flow. Efficiency of impaction increases with wind speed, increasing mass of spores, small diameter of the object, and by the spore being sticky. Most spores would not impact on a tree trunk, but many plant pathogens have heavy spores that enable impaction on narrower objects such as stems and leaves.

Sedimentation takes place when the air current is too slow to carry the spores. Gravity causes the spores to drop. Usually sedimentation only takes place in the boundary layer, which is normally up to 1mm above a surface. This process will take place between plants in the greenhouse.

The Agam unit operates closed to the principle of the Andersen air sampler: an electric motor sucks air causing spore-laden air to enter at a perforated lid (arrowhead in Picture 1) and to pass down through the instrument with filters fitted inside it. The measurements were done according to that principle.

As air is aspirated through, it impacts onto the surface of growing media in a standard 90 mm Petri dish arranged at an angle of 45° (based on the pilot experiment, where this placement and orientation displayed the smallest level of result ambiguity). Microorganisms impact the culture media and the colonies were counted after proper incubation period (10 days for *Botrytis cinerea* and 3-5 days for *Rhizopus stolonifer*). The system measures the flow of air and regulates the aspirated volume to constant value of 9000 m^3/hour. As this process continues down the stack, the same volume of air is forced to travel through successively smaller perforations, and so the air speed is progressively increased. The path taken by this air is shown in Picture 2.

Picture 1. Air in (left) and air out (right). Petri dishes of 90 mm Petri dish arranged at 45° angle on the lid.

Measurements were performed for two wind direction and at constant speed with inside temperature and humidity.

Spore concentration was measured as "inlet" (C_{ind}) by using Petri dishes with selective medium as spore traps placed on the entry lid, disposed at about 20cm from the surface. Spore concentration "exit" (C_{out}) was measured by using Petri dishes with a selective medium as spore traps placed on the exit lid, disposed at about 20cm from the surface (Picture 1).

The total spore deposited in the greenhouse (A) was estimated based on sedimentation by means of 16 Petri dishes with Botrytis selective medium arranged slanted at an angle of 45° (above the crop canopy in regular pattern) to measures the spore sedimentation over the plant canopy. Linear placement of Petri dishes in relation to spore trap was chosen. Petri dishes were exposed in the glasshouse for 24 h (8.30 – 8.30) period.

In the glasshouse used for this experiment no forced ventilation was used and the Agam unit was switched off 48 h before measurements started for the first time measurements. For the second measurements of *Botrytis cinerea* and *Rhizopus stolonifer* spores, the measurements were performed in the different greenhouse with more severe human activity close to the Agam unit (transport wagons, packing unit, computer etc.). The Agam unit was not switched off in that greenhouse unit prior to the measurements.

The Petri dishes with different selective medium were removed and taken for incubation after the Agam unit had run for 5-15 minutes in the first measurement and 1 hour for the second measurement. Mean of the number of colonies per spore trap was calculated.

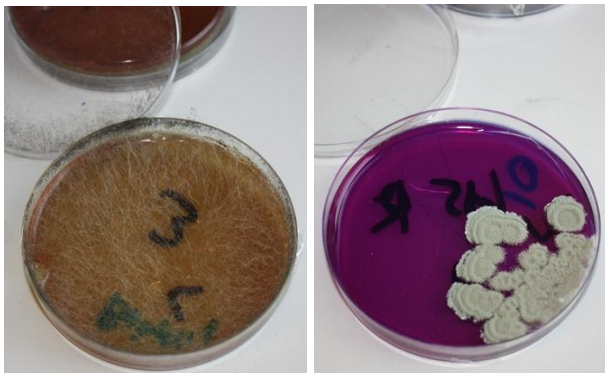
The spore traps were custom-made. Each trap was built by attaching a wire support to the bottom surface of the Petri dish containing the selective medium.
After incubation on selective medium, colonies of *Botrytis cinerea* were counted as colony forming units. There was not possible to present results for *Rhizopus stolonifer* as a colony forming units due to fungus growth, therefore number 1 (as a 1 colony) was used to indicate that the fungus growth covered the whole plate.

Picture 2. The path taken by air, in spore-trap ventilation unit.

Results

The colony forming unit (CFU) of each organism was quantified in terms of their number per unit volume of air collected by Agam unit.

As for *Rhizopus stolonifer,* a total removal of spores was observed and no spores were detected on "outside" placed Petri dishes with selective medium (Picture 3). This can be also due to a good ability/morphology of spores of *Rhizopus stolonifer* to settle down on the different surfaces.



Picture 3. *Rhizopus stolonifer* captured in the "in" air (left) and no *Rhizopus stolonifer* in air "out" (right). The grey colonies on the right picture are *Penicillium* sp. fungus.

The results shows that penetration of *Botrytis cinerea* spores through the filters in Agam unit is difficult to prevent. A high number of spores were collected by Agar flow in both measurements but spores were capable to penetrate the filters. This penetration could be related to:

- aggregation of botrytis spores (only 1-2 spore from aggregate are captures on Petri dishes, while the other passes through the unit);
- filters too coarse, thus allowing passage of the smaller fungal spores;
- The airspeed velocity on the exit measurement points was considerably higher than at the inlet points, thus forcing more spores into the calm boundary layer;
- Moisture condensation and subsequent spore aggregation on the top of the equipment should be taken into account as well.

It was not possible to calculate E_c or N_L for the second measurement, due to negative results.

The penetration factors can be highly dependent on the pressure difference between inside flow and outside flow. This difference disables make any comparison between the measurements for *Botrytis cinerea* spores for in- and out -flow.

The results in Table 1 shows, that Agam unit is collecting a great number of fungi and bacteria spores. Removal efficiency is very satisfactory for *Rhizopus stolonifer* spores, but removal efficiency for *Botrytis cinerea* spores indicated a need for better filters system.

The collection efficiency (Ec) and the number of loaded particles (N_L) were calculated from the following equations:

$A=a*100*100/pi*r2*t*1/5$

$Ec=1-C_{out}/C_{ind}$

$N_L=Q_L*E_C*C_{ind*}t_{L.}$

$A_f=A*\ Q_L$

Type of spores	Spores size	Sedimentation of spores on the plants A (CFU m^{-3})	Concentration of collected spores in flow, A_f (CFU m^{-3})	Load N_{LS} (CFU cm^{-2})
Rhizopus stolonifer	4x11 µm	Not possible to measure*	16	$5,4 \times 10^5$
Botrytis cinerea	8.0x6.0 µm	39	$1,06 \times 10^6$	$7,7 \times 10^5$

*medium used for this measurement was highly selective over for other fungi and only *Botrytis* sp. could grow on it.

The results from the two measurements for to plant pathogenic fungi *Botrytis cinerea* and *Rhizopus stolonifer* suggest that Agam unit can play an important role in reducing fungal spores in greenhouses. Unit presents high loading ability for fungal spores.

Reduction in air humidity will have big impact on fungal spores dispersion and sedimentation in the greenhouse.

References

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