Aims and tools for aerobiological monitoring

focused on sampling systems for airborne pathogens
in sentinel experiments

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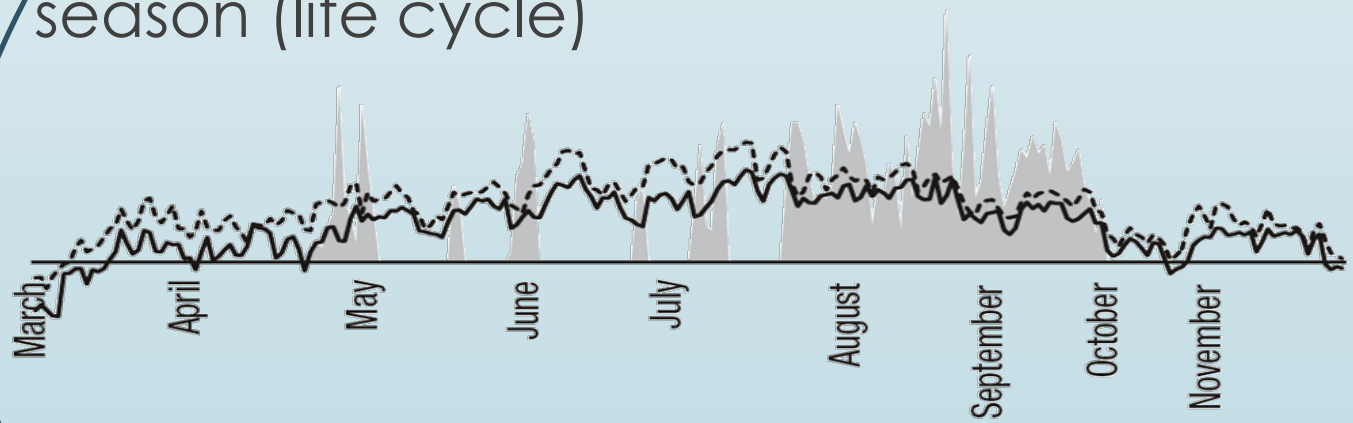
Aerobiology is a branch of biology that studies the passive transport of airborne organic particles, such as bacteria, **fungal spores**, very small insects, pollen grains and viruses (= bioaerosol) and their impact on human and plant health.

Why to detect fungal spores?

- Pathogenic spores can be detected in the very early stage of introduction, even before the symptoms occurr.

- To discover the biology of the pathogen ≠ spread in the environment and during a season (life cycle)



How it helps in Sentinel experiments' monitoring?

It monitors the space between planted and native trees

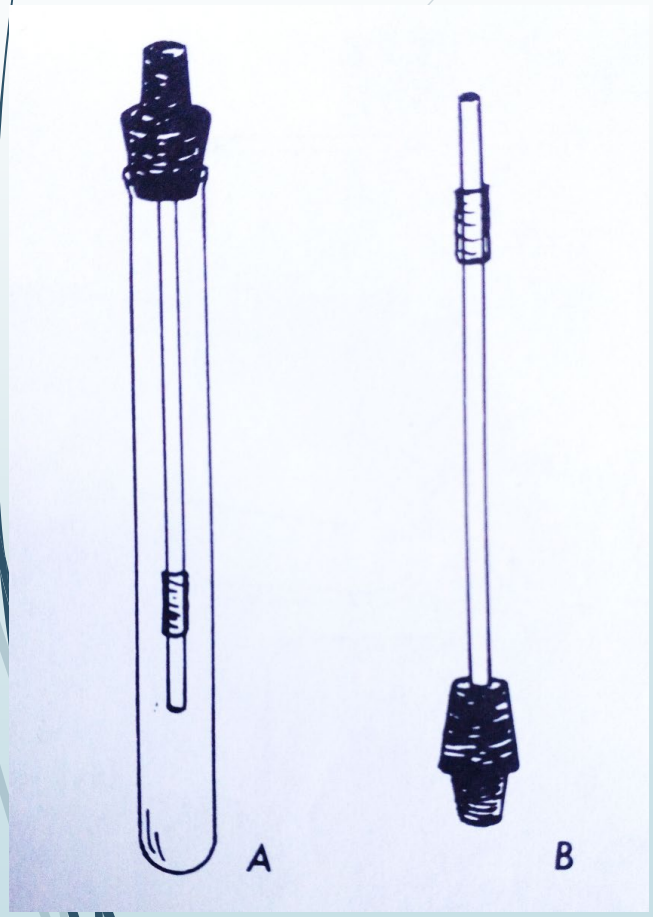
= between source of infection and symptoms.

- Sentinel plantations:
  - Are the **symptoms** a consequence of present airborne pathogens?
  - Are the airborne pathogens present, but the **syptomless** plants are resistant?
- Sentinel nurseries:
  - Monitoring of airborne pathogens spreading from the seedlings

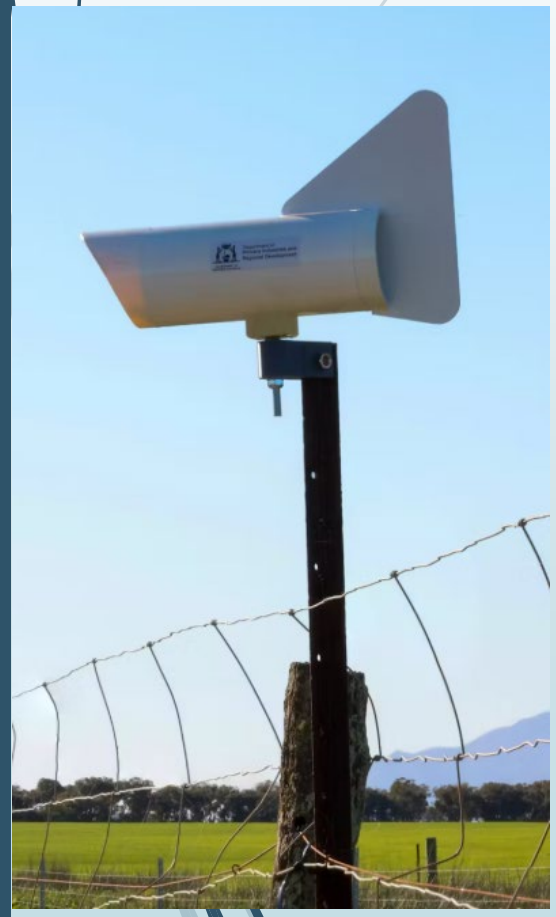
How to do it? With spore traps!

- Passive: particles are naturally landing on an adhesive surface (microscopic slides, glass rod, filter paper, tape)
- Active: active mechanism to increase the capture (rotorods, columetric, cyclon and others)

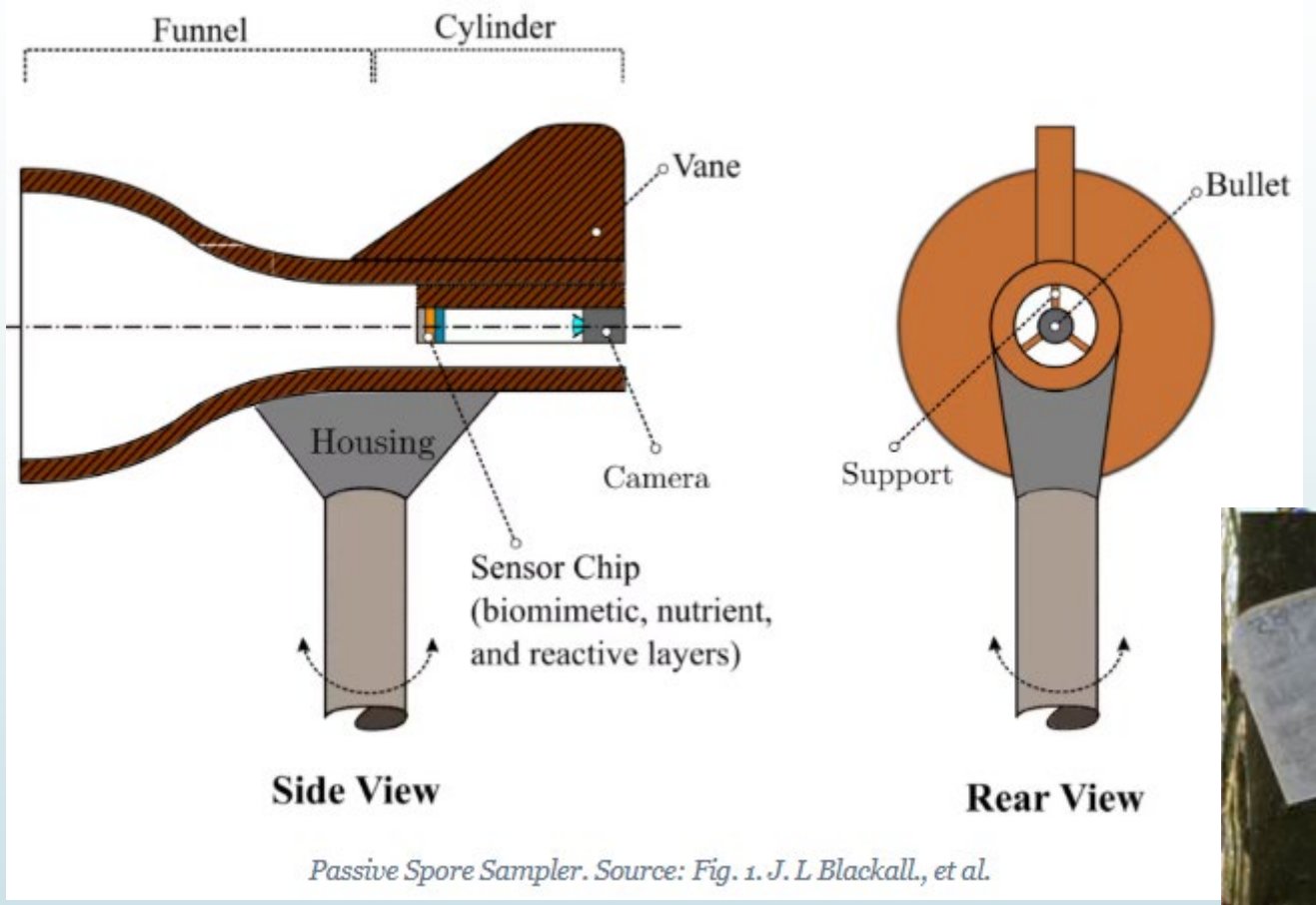
Passive spore traps



Passive spore traps



BioScout



Passive Spore Sampler. Source: Fig. 1. J. L Blackall., et al.

Burgdorf et al. (2022)

Passive spore traps

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Cheap
Light
Independent on electricity
All sizes of spores
Easily homemade
Exhibit natural mechanism of spore deposition
Rather easily microscoped
DNA extraction easy
Low risk of overloading = long sampling periods

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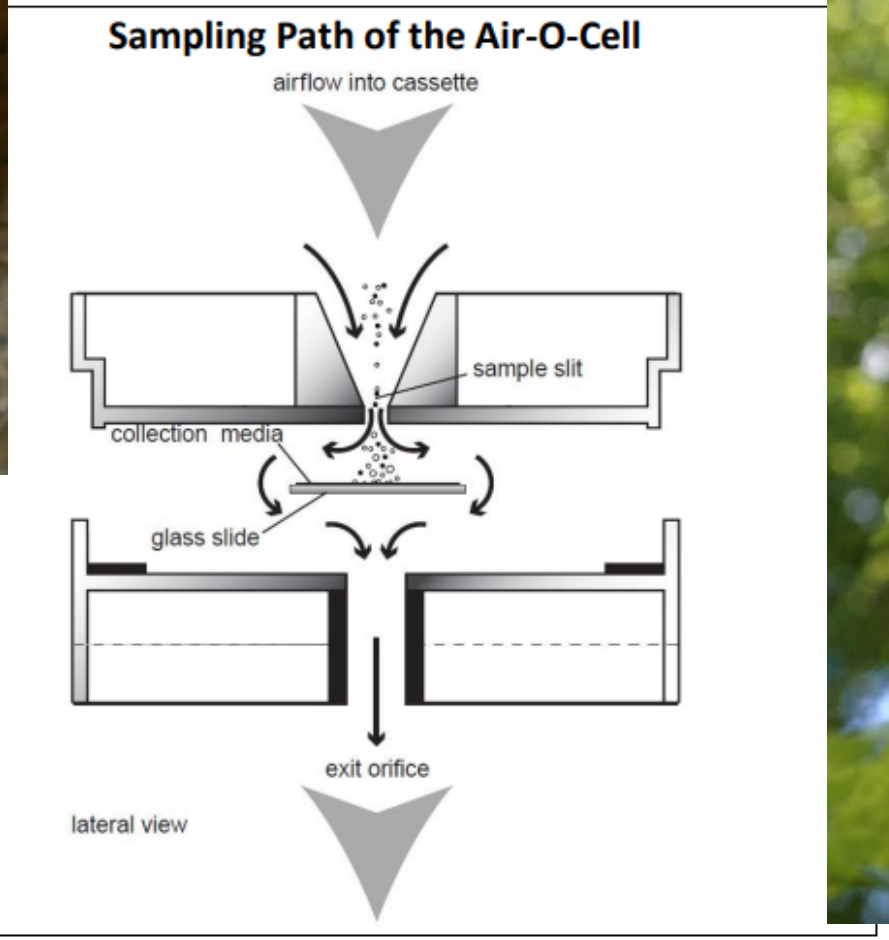
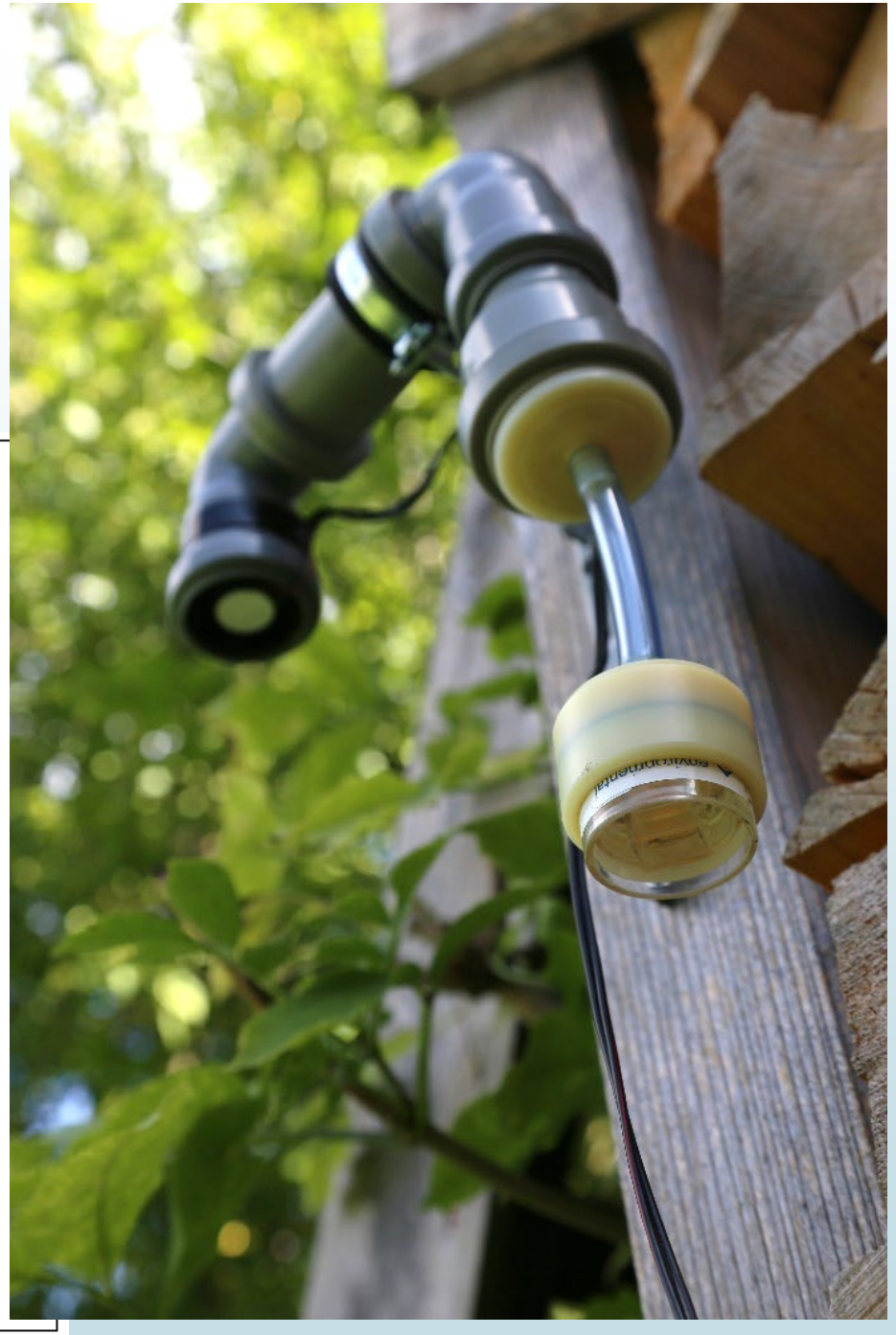
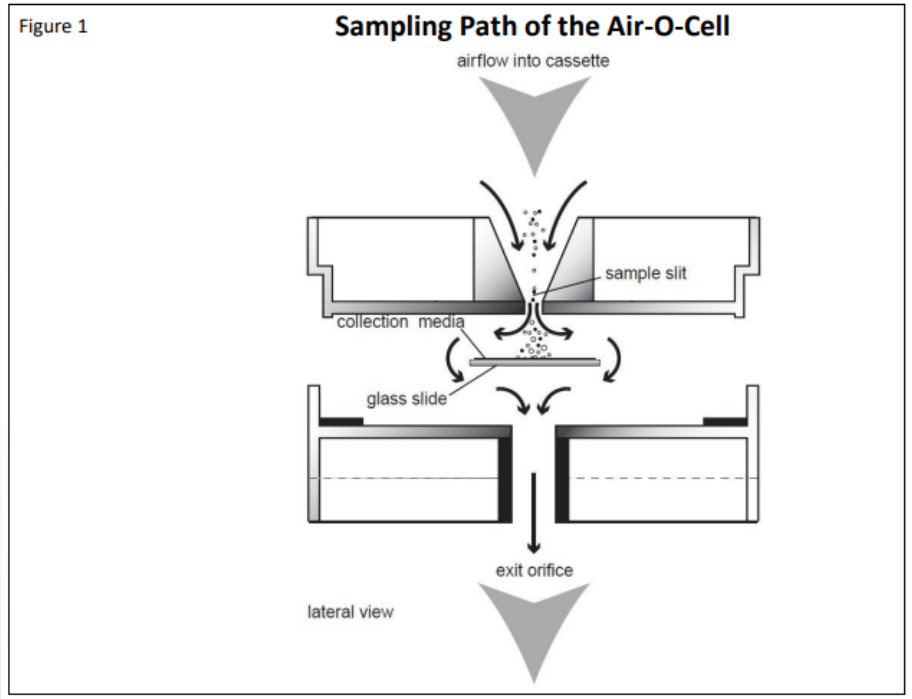
Lack of the dimension of time
Low sensitivity
Unknown spore concentrations

Active: slit sampler





Slit sampler



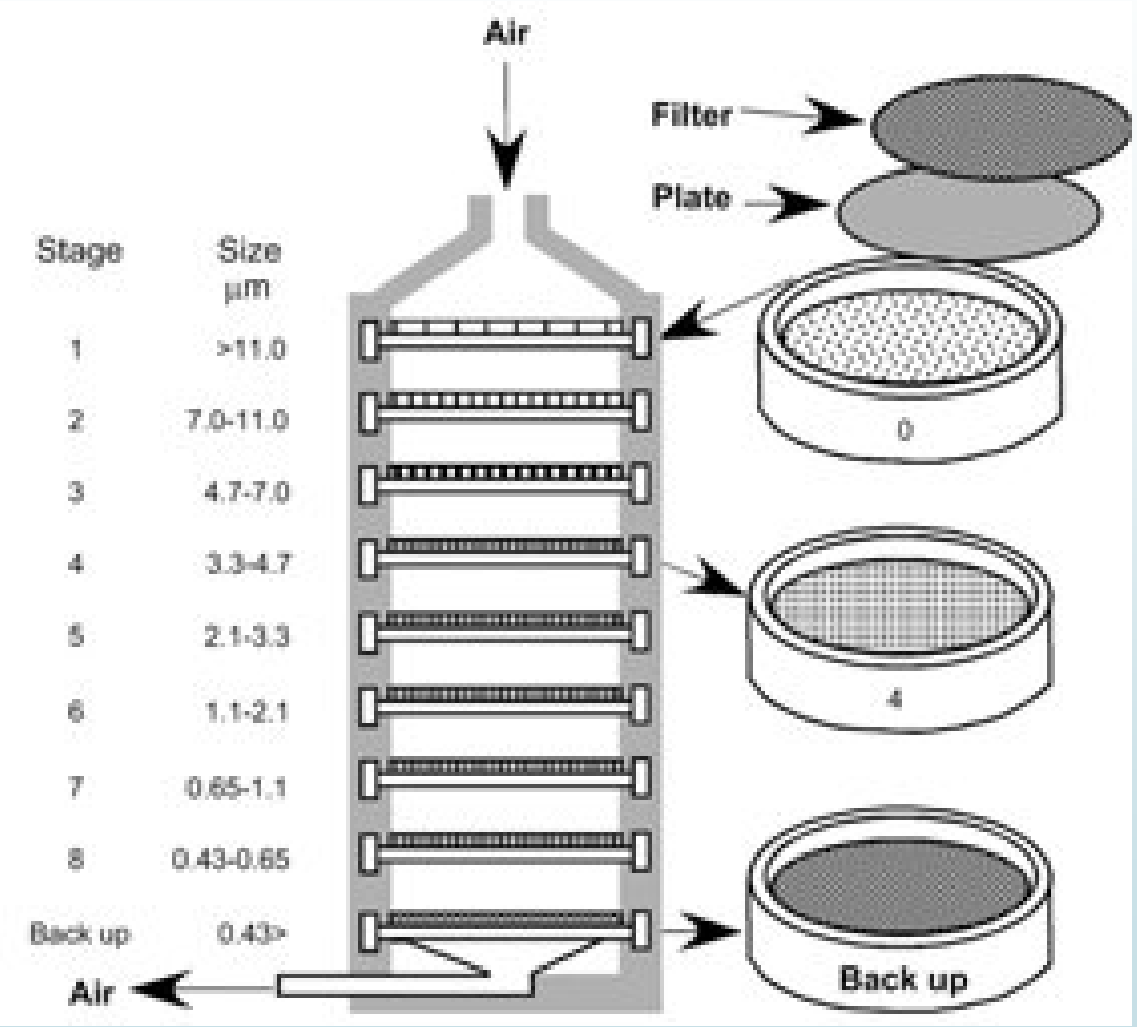
+

Cheap
Light
Fair sampling rate
All sizes of spores
Easily microscoped
DNA extraction easy
Low consumption

−

Lack of the dimension of time
Sensitive to wind (in-doors)
High risk of overload = short sampling periods
Electricity dependent

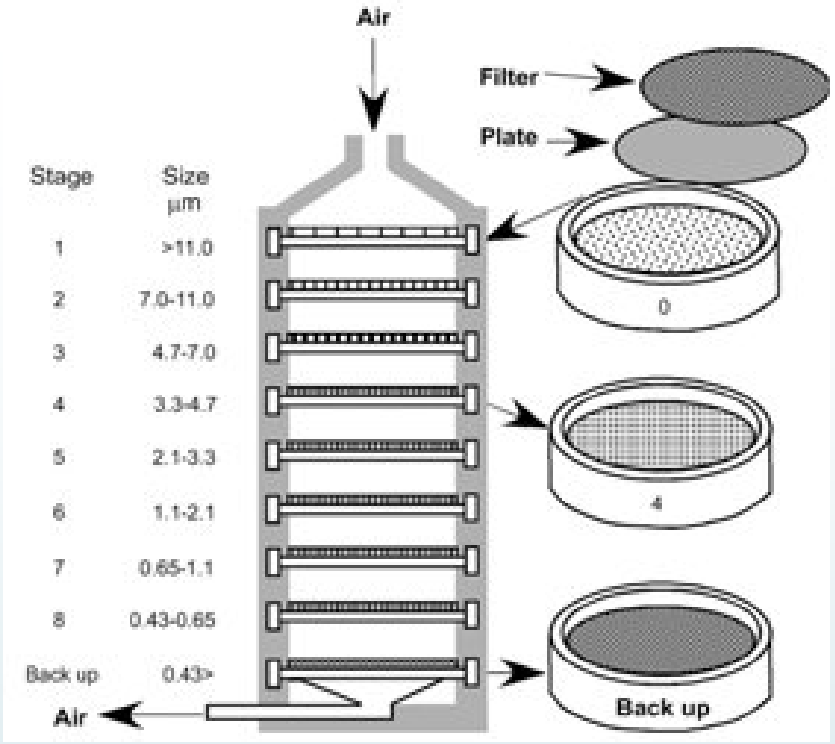
Active: Andersen sampler



Andersen sampler

+

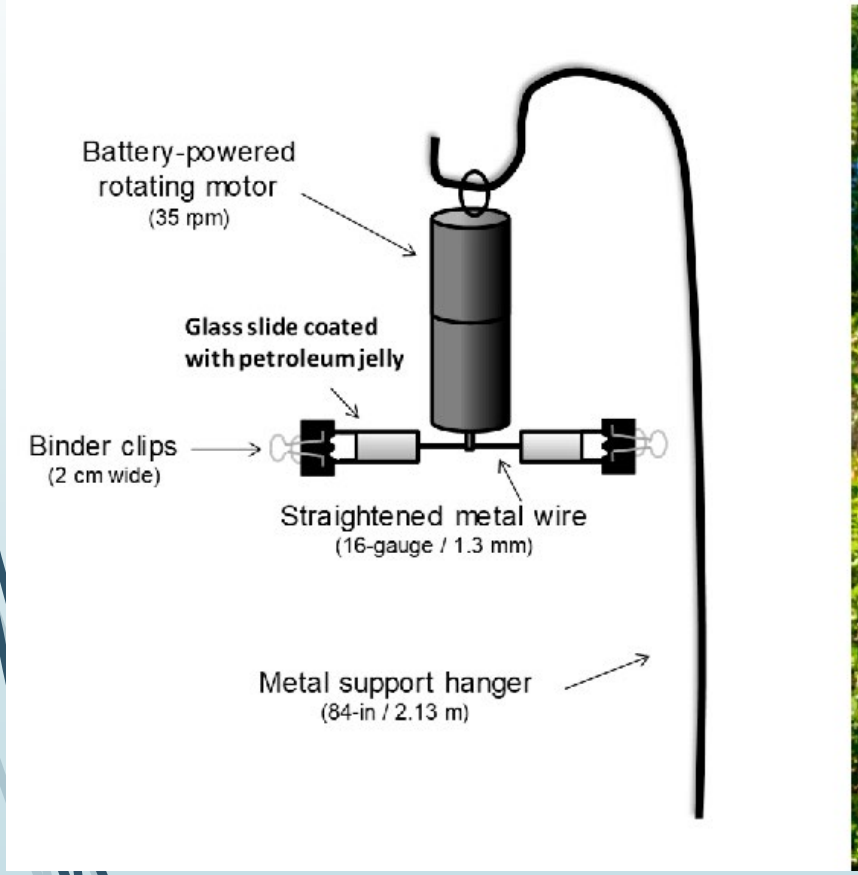
Fair sampling rate
All sizes of spores
Spores asorted = no overload



−

Rather expensive
Lack of the dimension of time
Sensitive to wind
High el. Consumption
Designed for in-doors

Active: rotorods/rotoslides



Active: rotorods

Rotorods

+

Cheap
Light
High sampling rate (over 50l/min)
High sensitivity
Rather easily homemade
No wind sensitivity

−

Lack of the dimension of time
Not suitable for small spores (bellow 8 µm)
Higher el. consumption
Fragile
Risk of overload
Maintenance needs practice

Active: rotoneedles

Rotoneedles

\+

Cheap
Light
High sampling rate (over 50 l/min)
High sensitivity
Rather easily homemade
No wind sensitivity
Easy to maintain

\-

Lack of the dimension of time
Not suitable for small spores (bellow 8 µm)
Higher el. consumption
Risk of overload
Not exact spore concentrations

$$rpm = \propto \times T \times 0.1667$$

where:

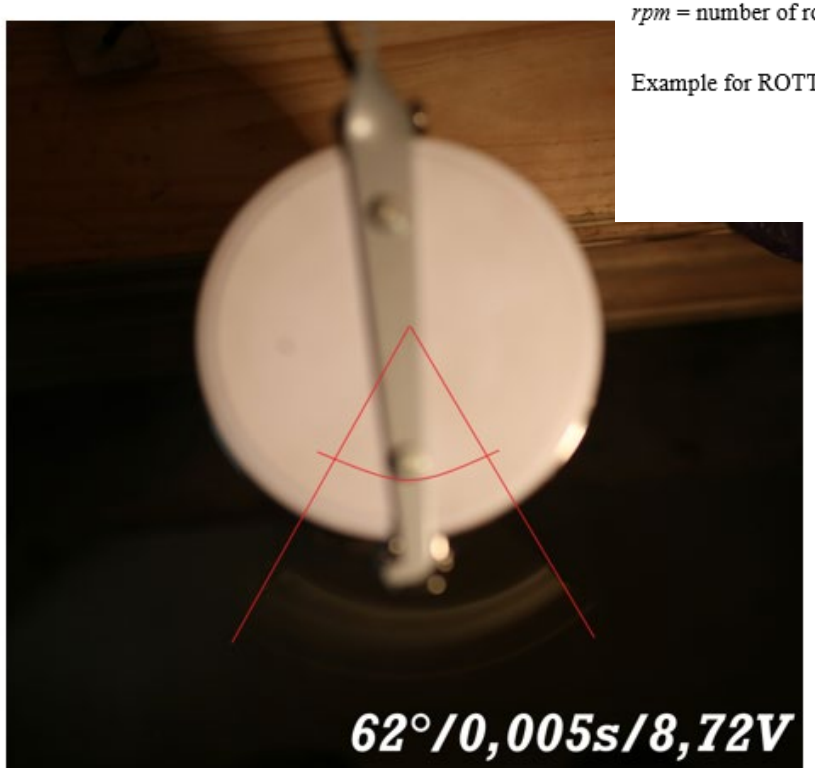
rpm = number of rotations per minute

α = angel drawn by the arm (rod) in the photograph (°)

T = shutter time selected on the camera (1/T s; for 1/200 s: T = 200)

The example in figure 8 would be calculated:

$$rpm = 62 \times 200 \times 0.1667 = 2067$$



$$v = \pi \times D \times \frac{rpm}{60}$$

where:

v = impaction rod velocity (cm/s)

π = constant (= 3.1416)

D = rotating diameter; length of the horizontal rotating arm

rpm = number of rotorod rotations per minute

Example for ROTTRAP 104 (9V DC):

$$v = 3.1416 \times 20 \times \frac{2067}{60} = 2164\ cm/s$$

$$d = \sqrt{H \times \frac{W}{v}}$$

where:

d = particle diameter (cm)

H = constant (= 0.0162)

W = width of impaction rod (cm)

v = impaction rod velocity (cm/s)

$$d = \sqrt{0.0162 \times \frac{0.08}{2164}} = 7.739 \times 10^{-4} = 7.739\ \mu m$$

$$R = 0.12 \times v \times W \times L$$

where:

R = Sampling rate (l/min)

v = impaction rod velocity (cm/s)

W = width of impaction rod (cm)

L = length of impaction rod (cm)

Example for ROTTRAP 104:

$$R = 0.12 \times 2164 \times 0.08 \times 5 = 103.872$$

Active: Seven-day automatic volumetric sampler

Burkard Manufacturing, Ltd. (UK)

Lanzoni VPPS 2000 (I)

AMET (CZ)

7-day automatic volumetric sampler

\+

Dimension of time
Spores of all sizes
Fair sampling rate (10 l/min)
High sensitivity
No risk of overload
No wind sensitivity

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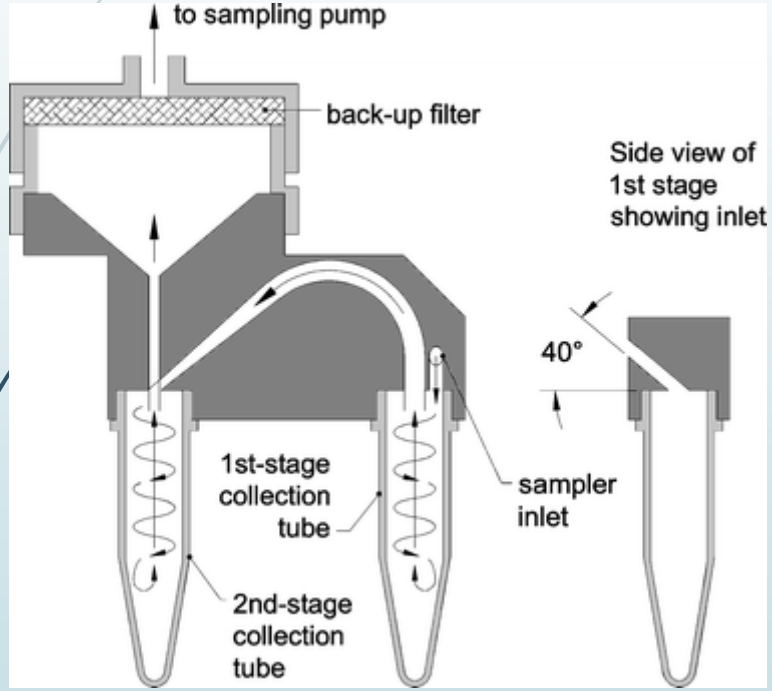
Expensive
Heavy
Higher el. Consumption
DNA extraction - intercontamination

Active: cyclon sampler



Cyclon sampler

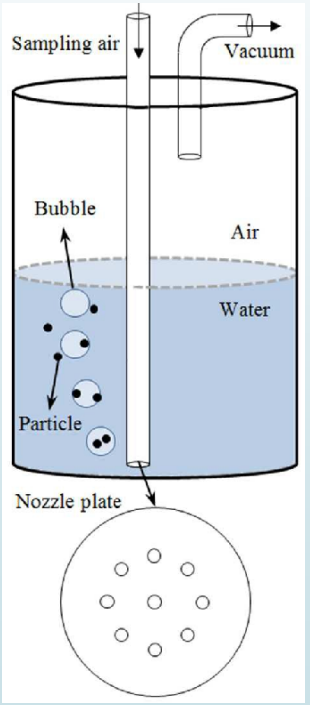
\+

DNA methods friendly – eppendorf tube mounted
Spores of all sizes
Fair sampling rate (10 l/min)
High sensitivity
No wind sensitivity

\-

Expensive
Heavy
Higher el. consumption
No dimension of time

Active: impinger



Impinger

\+

Cheap
Small
Better for waterborne spores?
DNA methods friendly
No overload
Special buffers

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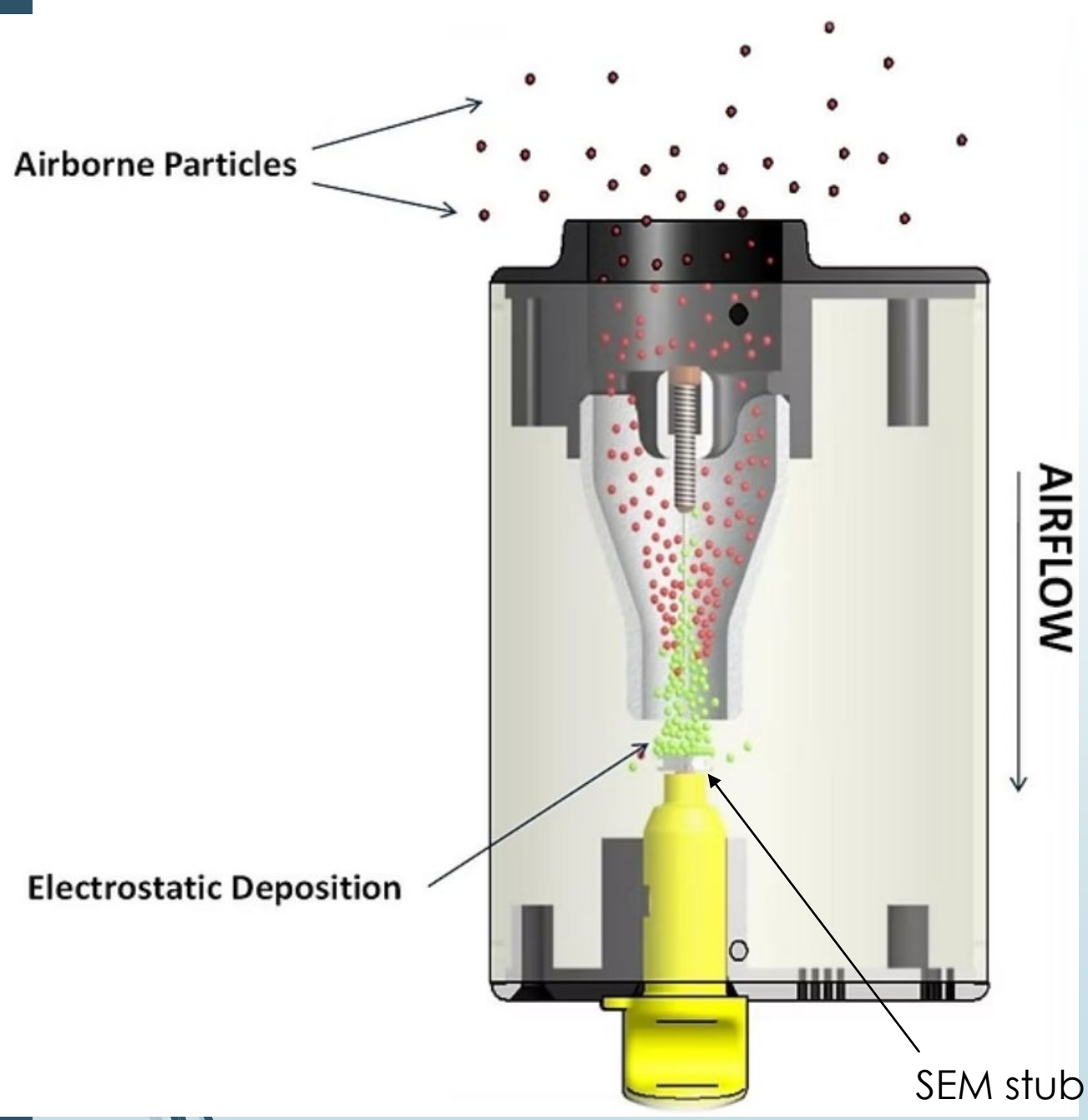
Higher el. consumption
No dimension of time
Spores diluted in bigger volume
Risk of evaporation
Wind sensitivity

Active: Ionic Spore Trap



Maverick PDM

Ionic spore trap

\+

DNA methods friendly
SEM friendly (stub inserted)
Spores of all sizes + very small particles

Ionic Spore Trap in use | Fungal spores under SEM

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Expensive
Higher el. consumption
No dimension of time
Selection of spores?
Wind sensitive
Risk of overload

Virtual impactor

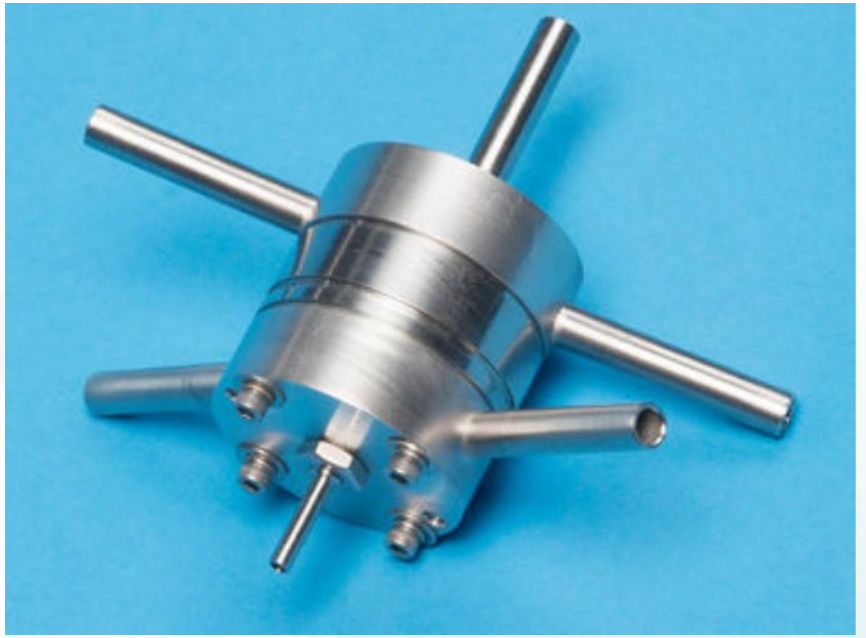
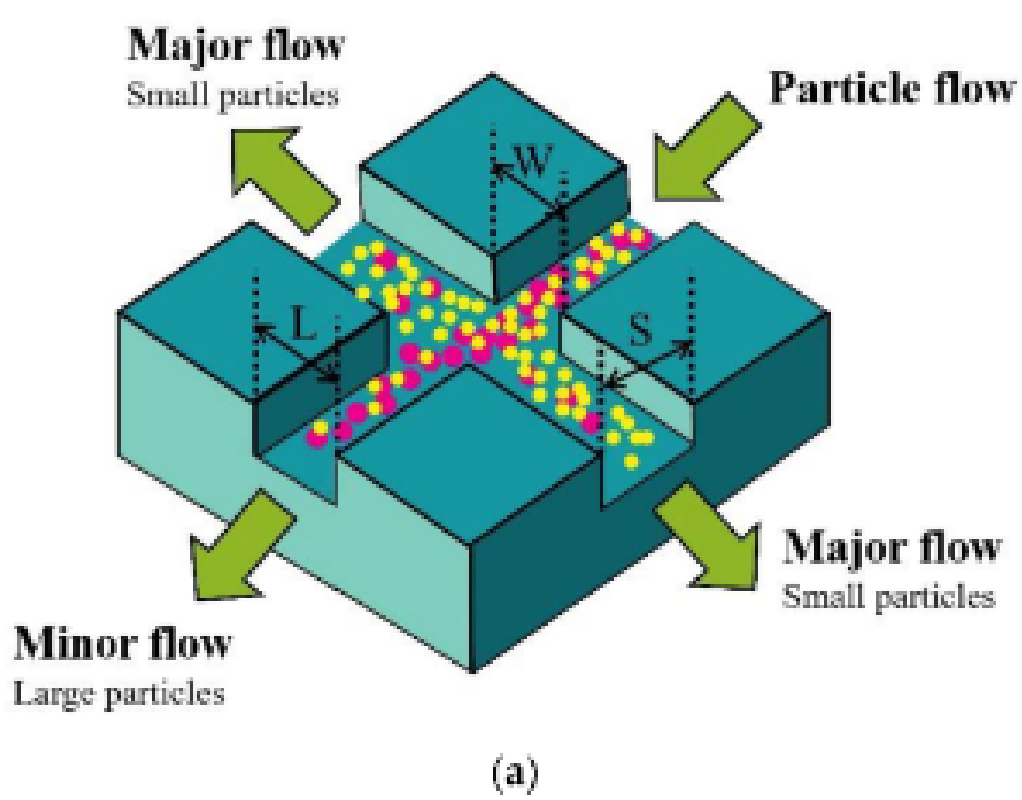
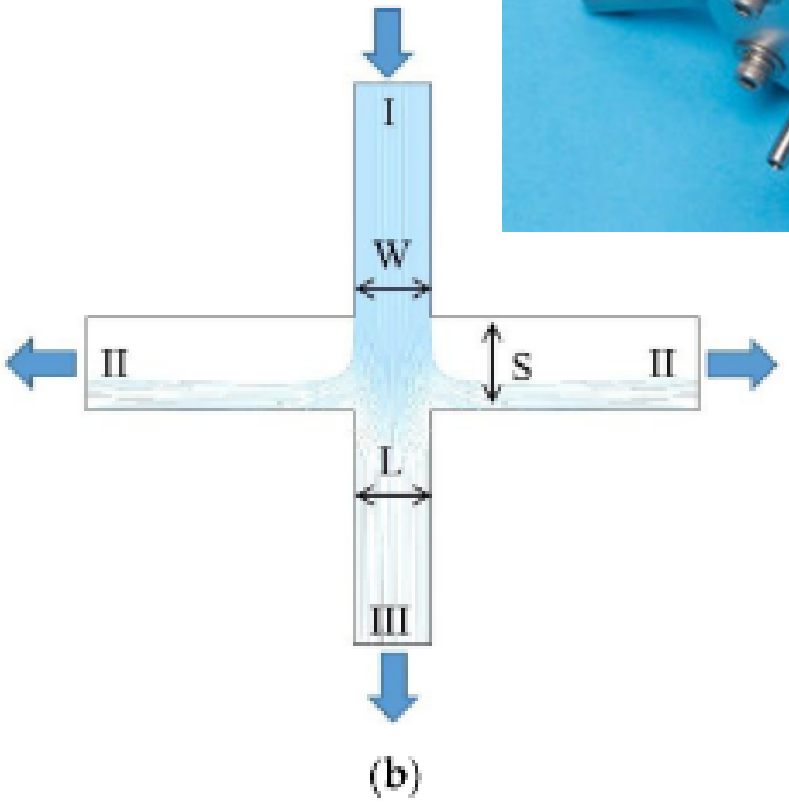
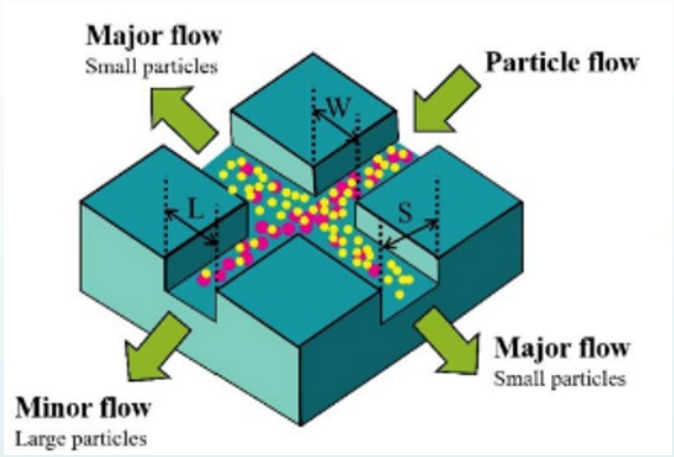


Figure 1. (a) 3D model of virtual impactor; (b) 2D model of virtual impactor.





Virtual impactor



\+

Spores asorted = two groups of sizes with different sampling rate
DNA methods friendly
Low risk of overload

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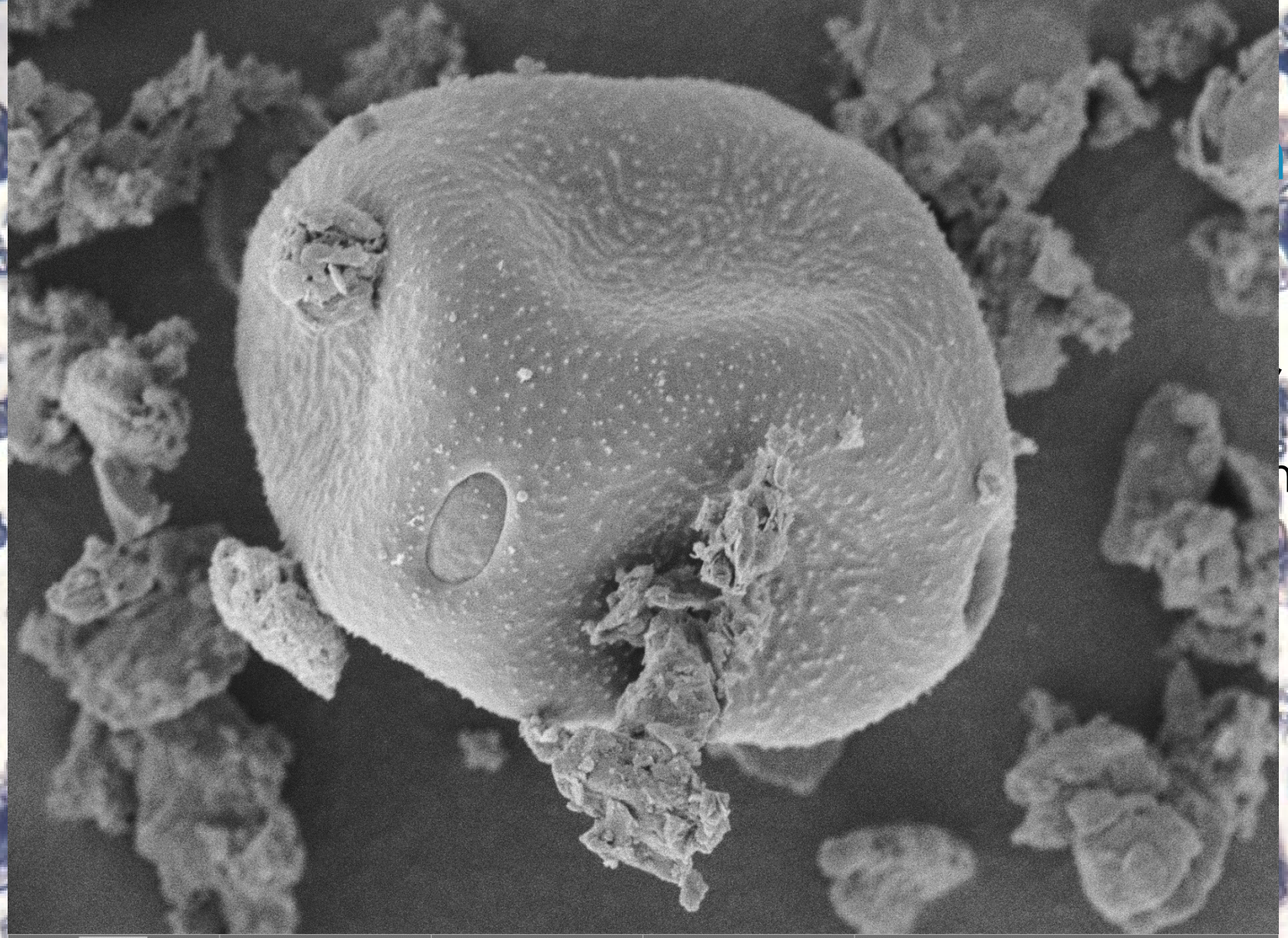
Expensive
Higher el. consumption
No dimension of time
Selection of spores
Wind sensitive

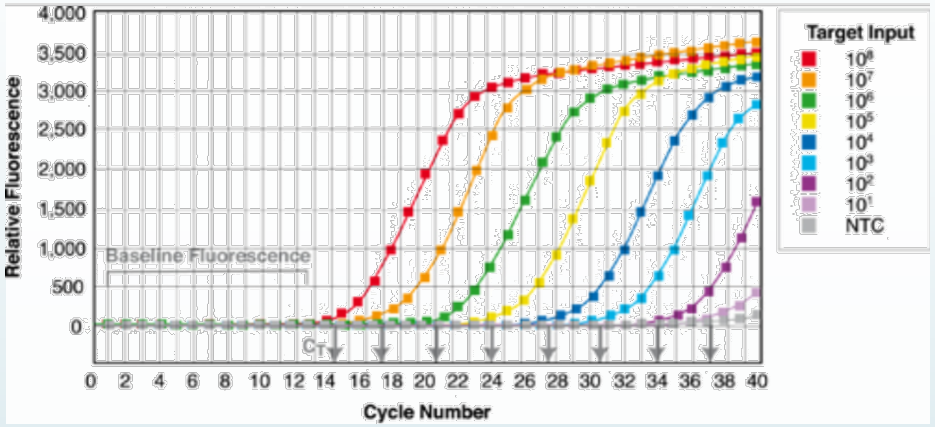
How to identify and quantify the samples?

- Microscopically
- DNA based methods
- Image analysis
- HPLC (High Performance Liquid Chromatography – chemical substances)
- FCM (Flow CytoMetry)


Mag
14.0 kx
WD
4.05 mm
BC
38 pA
10 µm
Det
SE
Energy
5 keV
Date
2025-03-14
Pressure
HighVac

DNA based methods for target pathogens

First step = DNA extraction with proper spore disruption (mechanical, chemical). Then:



- PCR (lower sensitivity)
- Quantitative real-time PCR with absolute quantification (highest sensitivity)
- Isothermal amplification (high sensitivity, no need of thermocycler):
  a) LAMP (Loop mediated isothermal amplification)
  b) RPA (Recombinase polymerase amplification)
  c) HDA (Helicase-dependent amplification)
  d) SDA (Strand displacement amplification)

DNA based methods to detect „everything“

First step = DNA extraction with proper spore disruption (mechanical, chemical). Then:

METABARCODING:

- Amplicon-based metagenomics = targeted sequencing (Illumina, Oxford Nanopore) to e.g. ITS1/ITS2 region
- Long read metagenomics (PacBio, Oxford Nanopore) = targeted sequencing of whole gene regions – more specific

DATA ANALYSIS:

Identification of sequences in nucleotide databases (GenBank, UNITE)

Image analysis

BioScout

Pathology | 20 Feb 2023

How BioScout Works

What's in the box!? Find out more about BioScout technology and how we are improving disease management.

Edward Gubbins
6 Min Read

What's in the box!?

Well, if we told you that, we'd have to kill you...

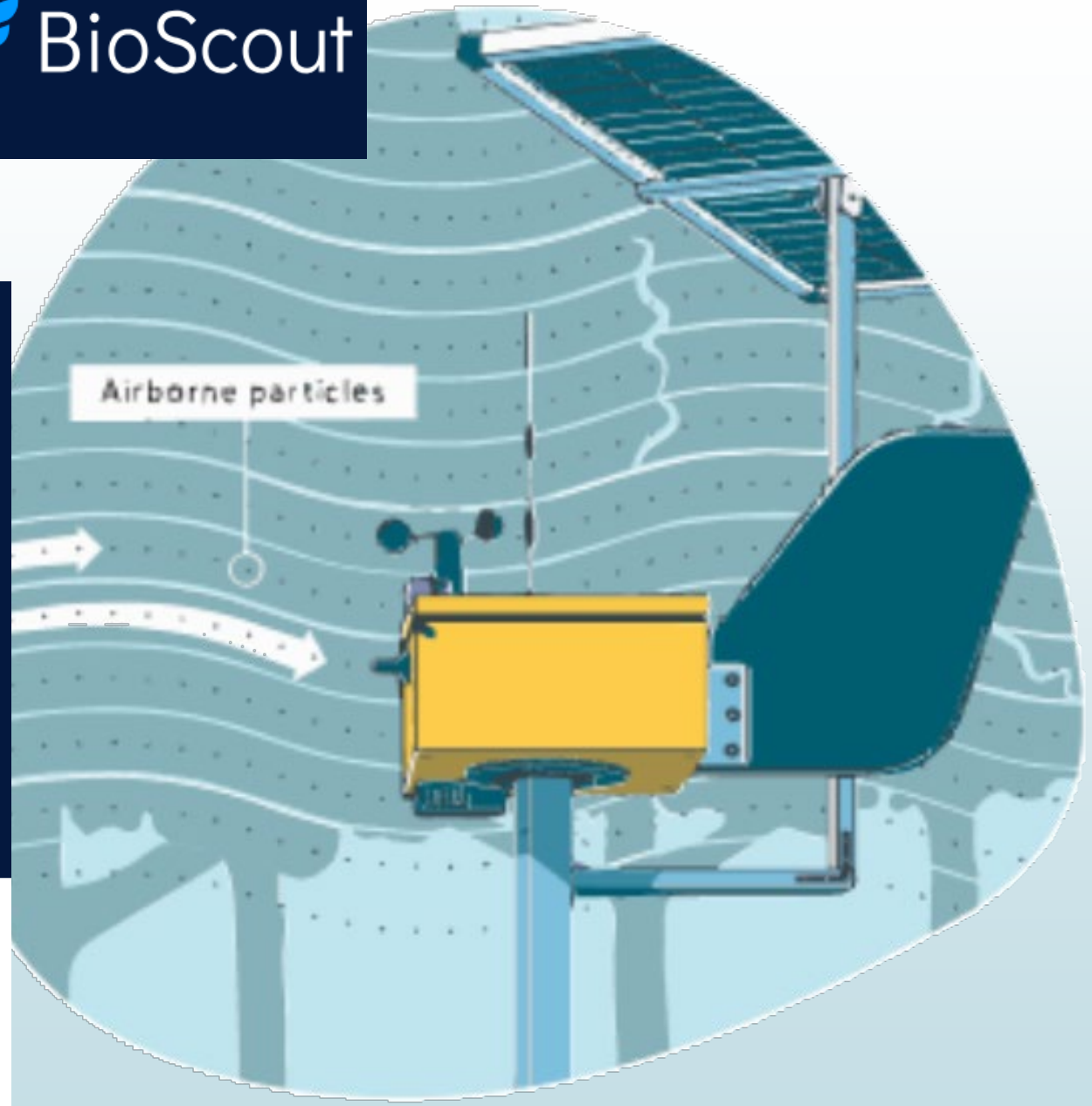


Image analysis

1. Volumetric spore trap sampling on a sticky tape
2. Exposed tape shotted by a camera via automatic microscope
3. Hundreds of images sent via 4G cellular network technology to a data centre
4. In the data centre, images are automatically compared with a database of determined microscopic images of spores via machine learning and counted.
5. Spore numbers together with weather data are available to customers via app.
6. Next step: disease and weather data integration into a next-generation disease model and forecast.

Dashboard

Your Site

Choose a device
North

Weather at 07/02/2023 08:37 AM

14.06 °C
Temperature

73.32 %
Humidity

18.09 km/h
Wind speed

2 mm
Rainfall

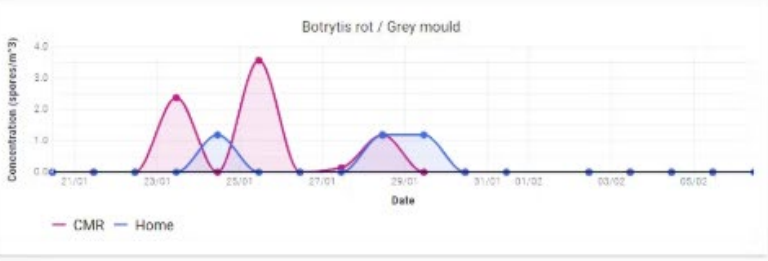
PATHOLOGY NOTES | SPRAY LOGS | FIELD REPORTS

Date	Site	Note
30/01/2023	Example Site	All target spores have been observed throughout January. We have also observed cereal and stone fruit rust, Gramineanum pollen, Cladosporium, Alternaria and other Pleosporaceae conidia
05/01/2023	Example Site	An increase in spore load was observed from the middle of December, particularly for Botrytis, Powdery Mildew and Alternaria. We observed background levels of grass pollen and spores of cereal rust, Cladosporium spp., Ustilago spp. and Epicoccum spp.

Rows # 25 1–2 of 2

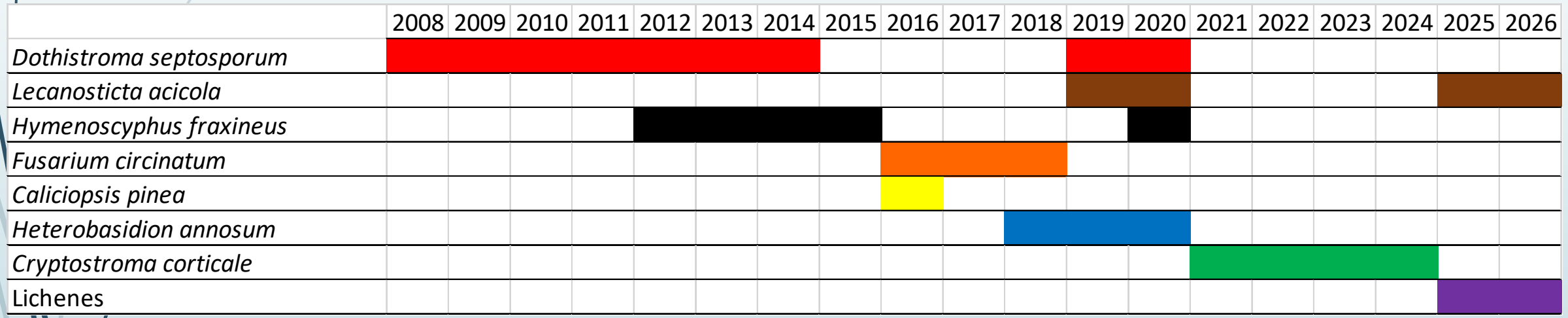
Filters

Devices | Diseases | From 21/01/2023 | To 07/02/2023 | DOWNLOAD CSV



Application

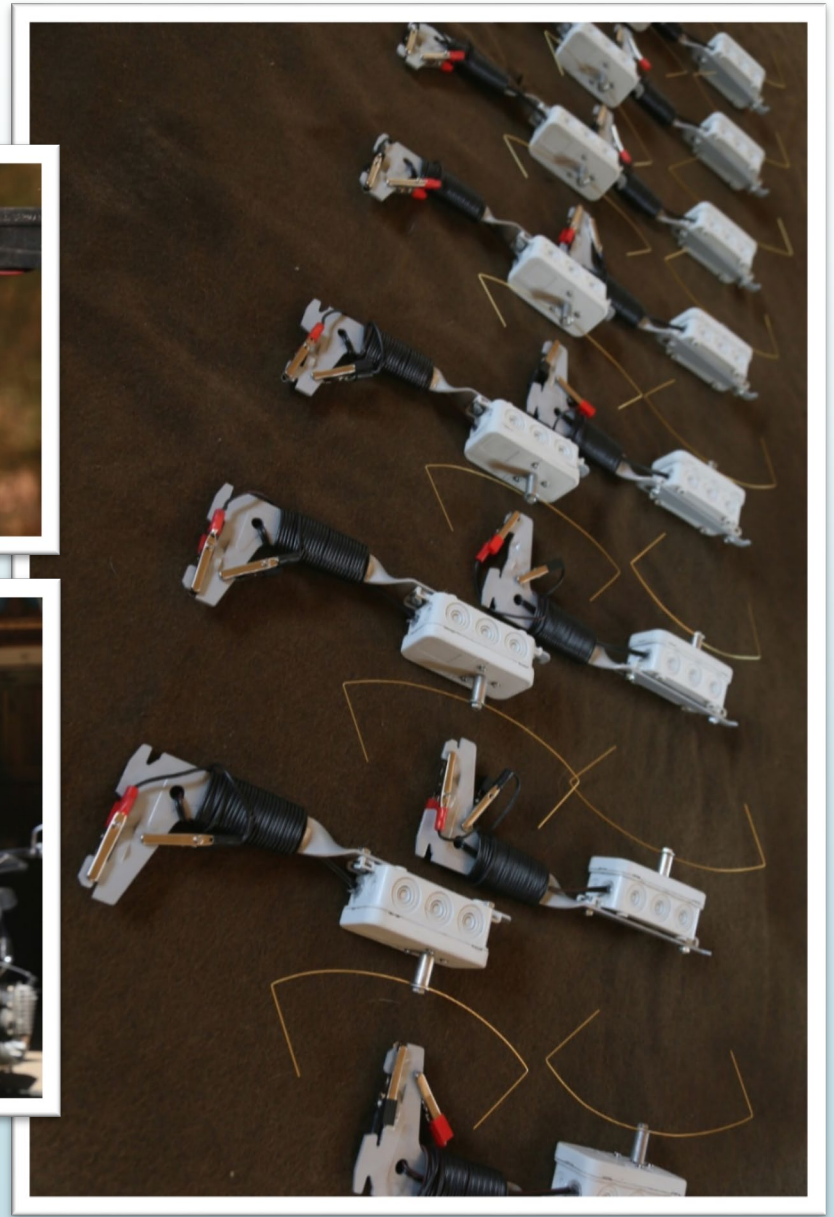
Pathogens air-sampled by Mendelu team



7-day automatic volumetric spore trap

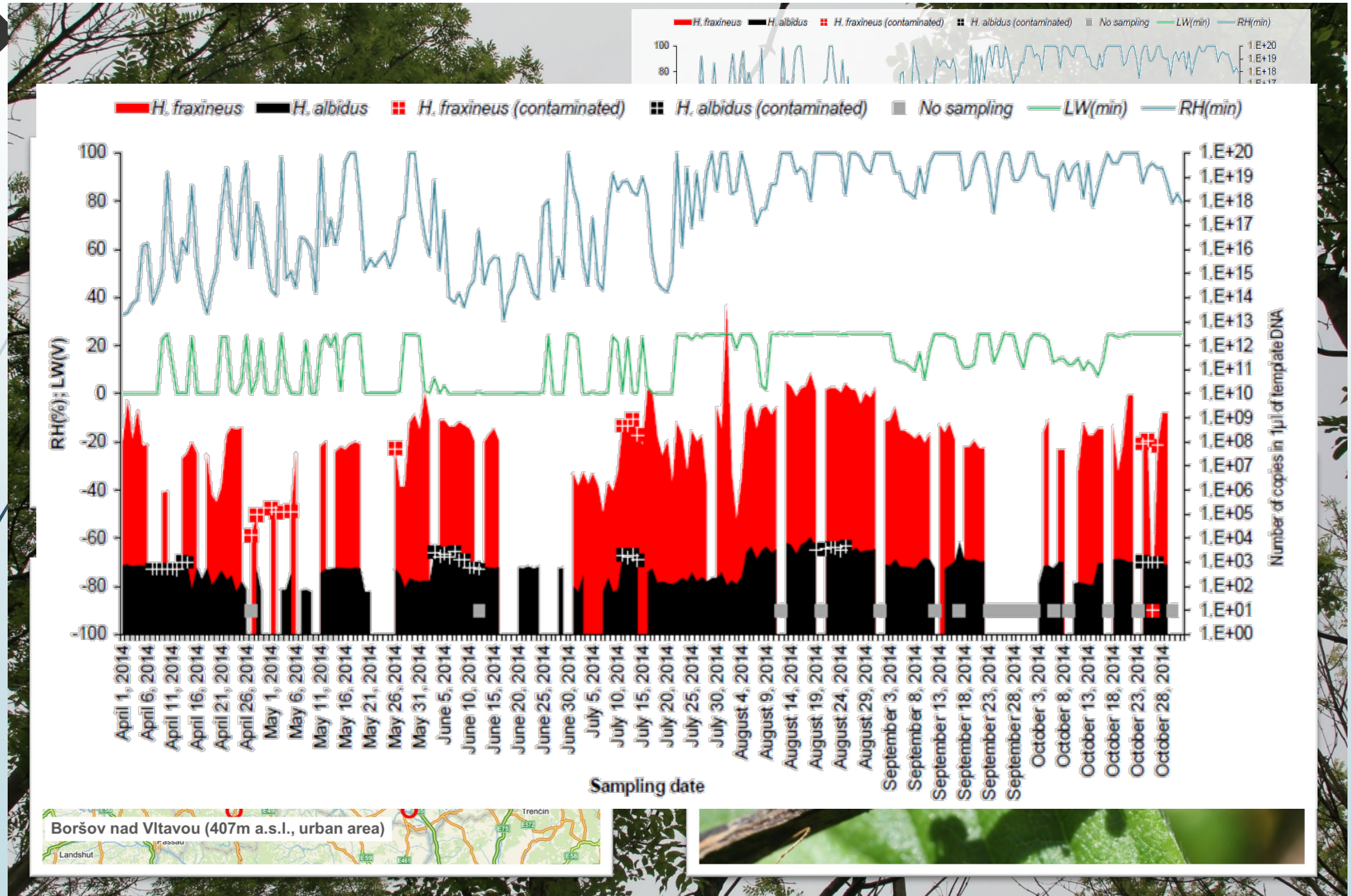
- AMET (Velké Bílovice)

Rotorods (ROTTRAP 120, 52)



- Miloň Dvořák (Boršov nad Vltavou)

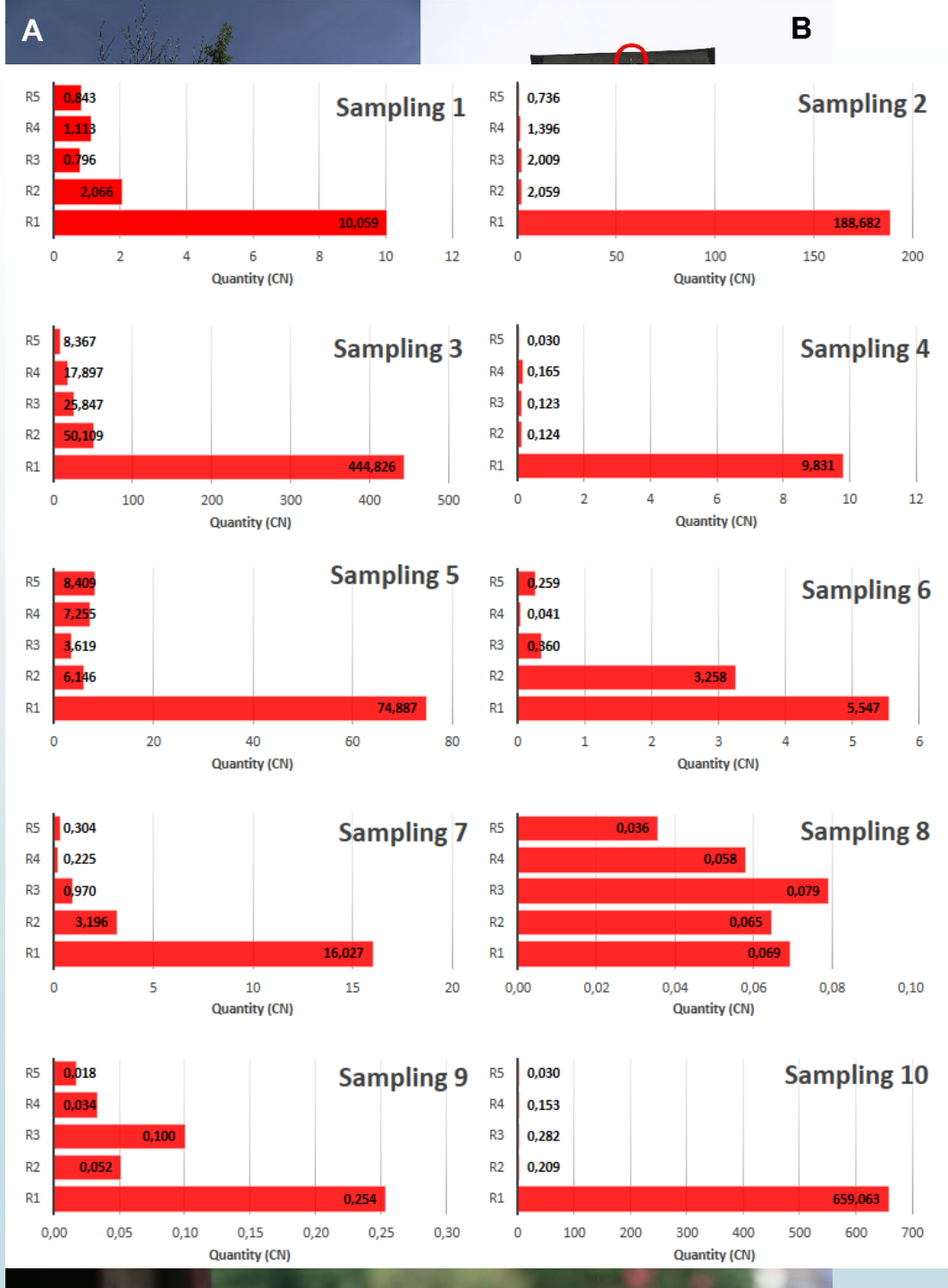
Hymenoscyphus fraxineus – Ash dieback



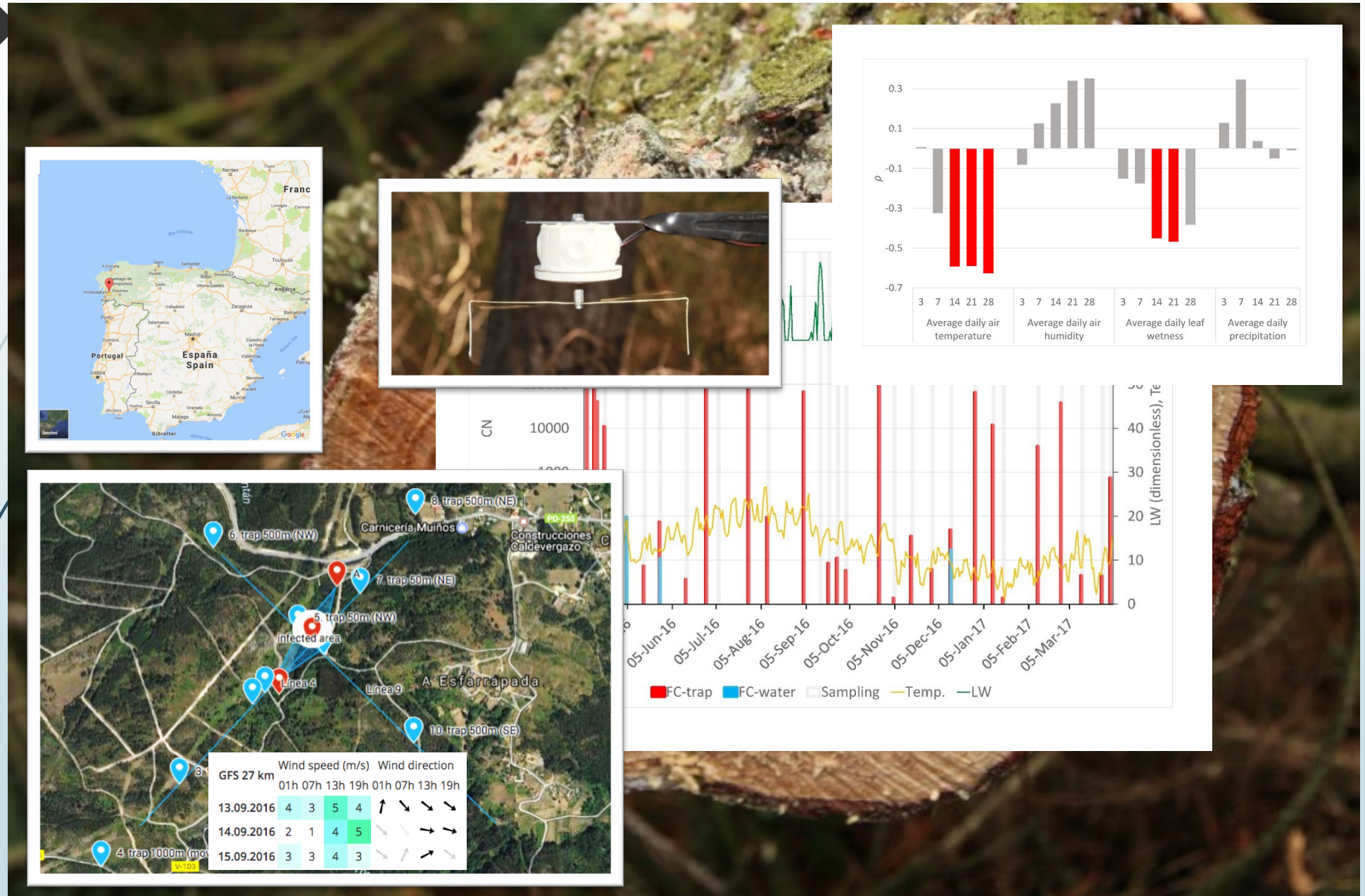
Hymenoscyphus fraxineus – Ash dieback

Vertical pattern of spread

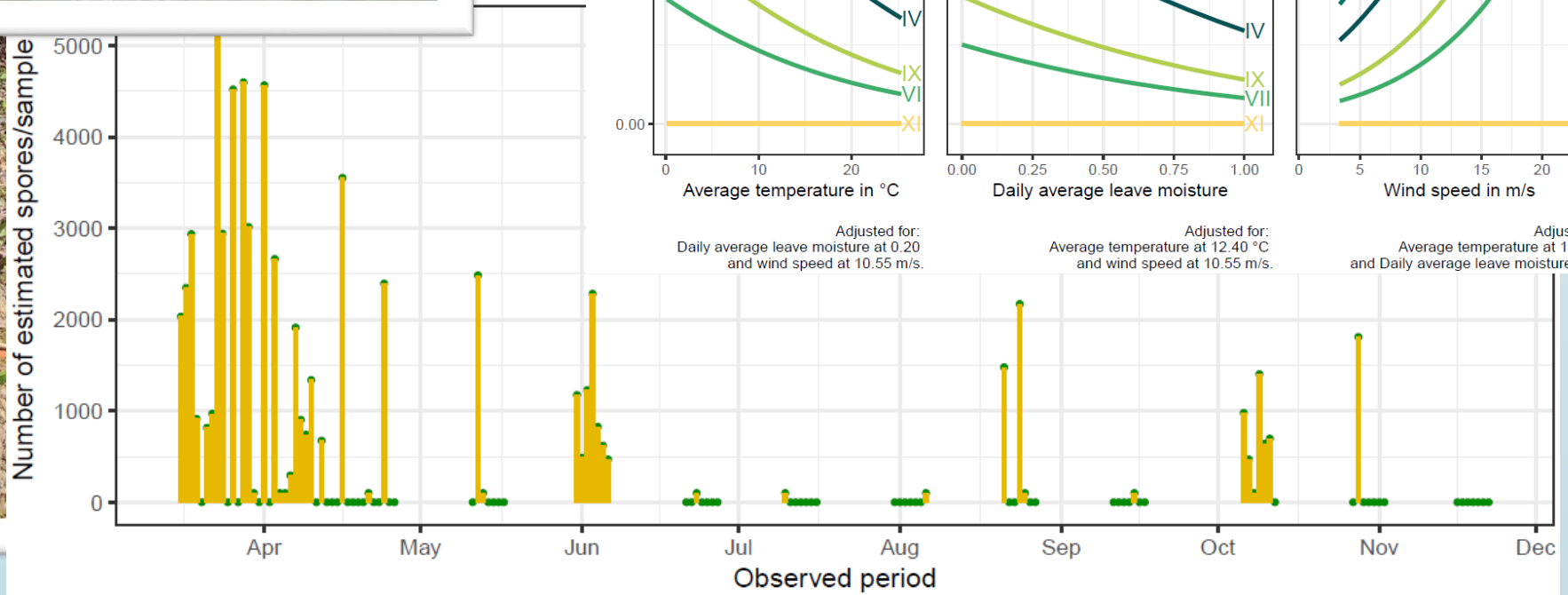
- 11 samplings per 48h during 2020 season (July and August),
- Sampling at 48, 37, 26, 14 m above and on the ground
- Rotating arm spore trap ROTTRAP 52,
- qPCR detection (Chandelier et al., 2010)

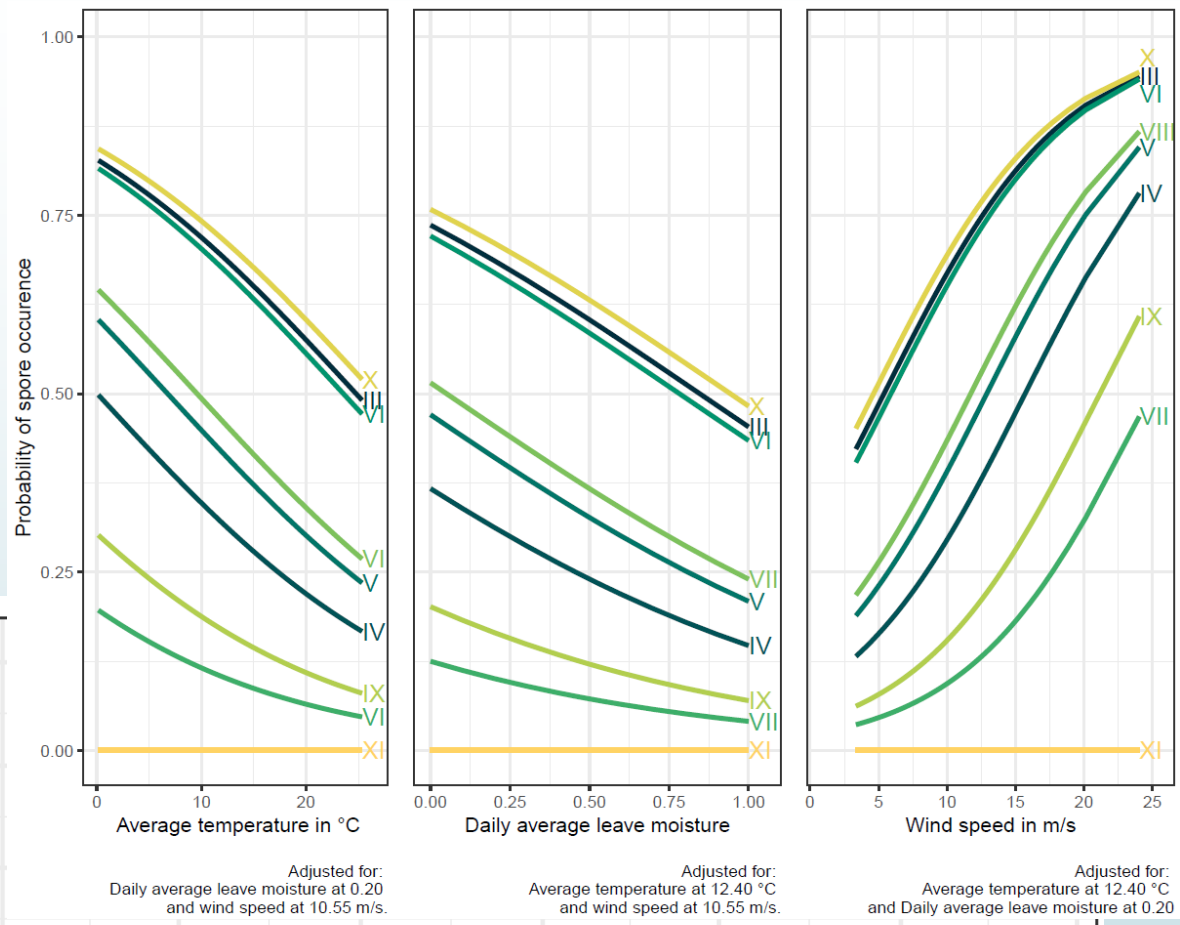


Fusarium circinatum – Pine pitch canker



Cryptostroma corticale – Sooty bark disease of sycamore





Thanks for attention!

Miloň Dvořák (milon.dvorak@mendelu.cz)