

Fungicide Simulation (Growth Experiment and Resistance Test)

- First, determine the appropriate amount of fungicides to be used in laboratory scales according to the provided safety data sheet (here: Proline® and Folicur® from Bayer AG, Leverkusen, Germany) by converting field scales to laboratory scales. For example:

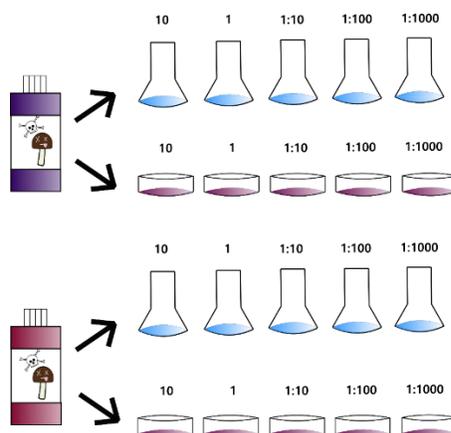
$$1 \text{ ha} = 10,000 \text{ m}^2$$

$$1 \text{ agar plate} = 58 \text{ cm}^2$$

- Example of the calculation table:

Fungicide	Usage Dose		Volume for every plate (58 cm ²)	For every 10x fungicide solution (in 21 ml water)
	Field	Plate		
Proline	0.8 l/ha in 200-400 l water/ha	4.64 µl in 2.32 ml water	2.32 ml	420 µl
Folicur	1.5 l/ha in 200-400 l water/ha	8.7 µl in 2.32 ml water	2.32 ml	787.5 µl

- Dissolve the fungicide stock solution into the highest concentration needed (e.g. 10x) and perform a serial dilution up to the lowest concentration needed.
- Mix the appropriate volume of fungicide with the appropriate amount of growth media (suspension and agar) and inoculate an overnight culture of the desired model organism.
- Perform growth experiments with the desired fungicide concentrations on suspension growth media and agar media like in the following illustration:



- Plan a time span for the growth experiment (e.g. 8 hours), measure the culture's OD regularly (e.g. every hour), and document the time and the measured OD values.
- Plate a sufficient amount of the overnight culture on the agar plates containing the desired concentrations of fungicide. Incubate at the appropriate temperature for several days.
- **Important:** take a negative control by performing the growth experiments on growth media without fungicide.
- Plot the documented OD-values along with the time where the OD-measurement was performed. Determine the concentration where the organism couldn't survive anymore (commonly starting from the recommended usage dose).
- Observe the growth on agar plates as soon as growth spurs are visually identifiable.
- Note: for growth experiments with filamentous fungi such as *A. niger*, it is advised to additionally measure the bio dry mass along with the OD-measurements.

Resistance Test:

- Determine the highest fungicide concentration in which the desired model organism still survive.
- From the organism's overnight culture, inoculate a start OD from 0.1 to the respective growth media with the determined fungicide concentration.
- Measure OD value at the following points of time: 0 (right after inoculation), 12 h, 24 h.
- After 24 hours, inoculate the grown culture into a fresh growth media with a softly increased amount of fungicide concentration (e.g. 1:10 to 1:8). Save a negative control by inoculating the culture to the same fungicide concentration.
- Proceed this experiment until the recommended usage dose is reached (or any concentrations where the original organism wouldn't survive) and observe for growth (resistance).

From: iGEM Bielefeld-CeBiTec