Which biotic and abiotic soil factors affect the establishment of *Metarhizium*-based fungal biocontrol agents?

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Introduction

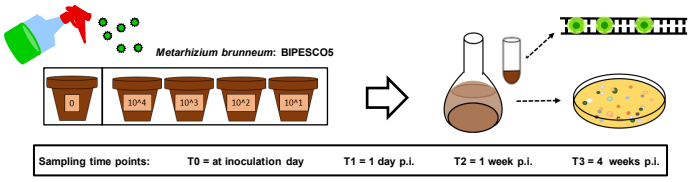
- ***Popillia japonica*** is an invasive pest insect in Europe.
- Invested areas in Northern Italy and Ticino, Switzerland.
- 300 host plants including many high-value crops.
- Entomopathogenic fungi of the genus *Metarhizium* are tested as biological control agent (BCA) against the soil dwelling larvae.

Goal: What are the biotic and abiotic soil factors that affect fungal BCA establishment?

Establishment of biological control agent (BCA) quantification methods

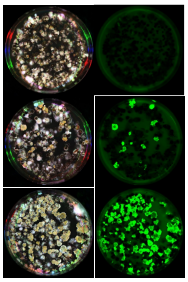
Quantification of green fluorescent protein (**GFP**) **labelled BCA strain** (***Metarhizium brunneum***) with two approaches:

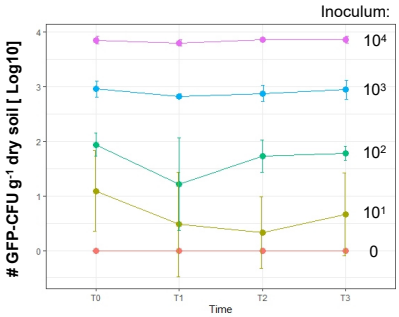
1. Cultivation-dependent: colony forming unit (**CFU**);
2. Cultivation-independent: **qPCR**.



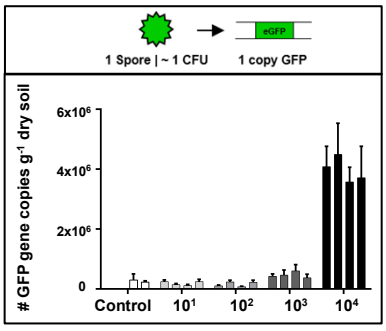
Results:

1. **CFU:** Plated soil samples on a semi-selective medium. Reliable detection possible down to one hundred spores per gram dry soil.





1. **qPCR :** BCA detection by GFP gene amplification. Theoretical detection limit for qPCR: 10^4 GFP gene copies per gram dry soil (one gene copy per PCR reaction).



Conclusions:

- CFU method is 100 times more sensitive than qPCR.
- Further optimization of GFP-qPCR required.

Abiotic and biotic soil factors that influence native *Metarhizium* abundance

Hypothesis: Screening of native *Metarhizium* abundance in grasslands across Switzerland and correlation to abiotic and biotic factors will reveal factors with potential relevance for BCA establishment.

Methods:

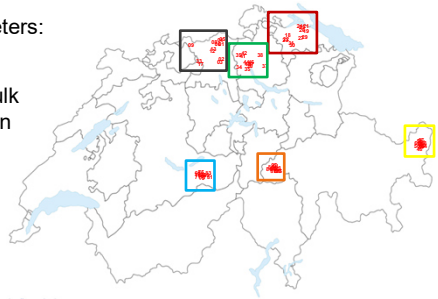
- 72 grassland sites defined and investigated in the frame of the EU project BIOINVENT
- Determined soil parameters:

  Abiotic factors:

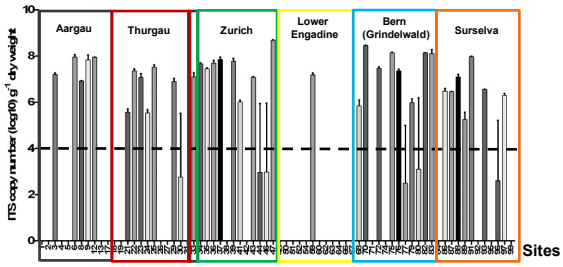
  pH, soil composition, bulk density, nitrogen/ carbon content, wetter, treatments

  Biotic factors:

  Bacterial-, fungal- and plant-communities



- Quantification of native *Metarhizium* abundance using genus specific qPCR: ITS1 gene amplification



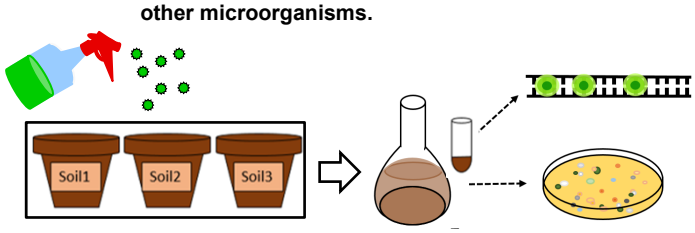
Conclusion: 30 sites with low or no *Metarhizium* presence, 42 sites with middle to high *Metarhizium* abundance.

Outlook

Pot Experiment:

- Soil from sites with characteristic factor combinations defined based on the results of the survey study above.
- Monitoring establishment of applied GFP-labelled BCA strain using the GFP-quantification methods developed (CFU, qPCR).

Influence of different soils on BCA establishment: BCA development and competitiveness towards other microorganisms.



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