Using insects as vectors for inoculation of beneficial resistance-inducing microbes in plants

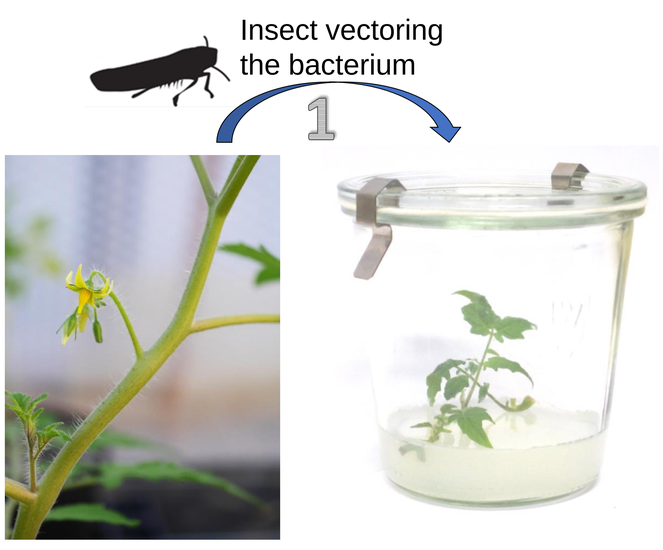
Mohamed Matared[1,2], Imke Hutter[1], Philipp Rödel[1] & Philipp Franken[2,3]

1 INOQ GmbH, 29465 Schnega, Germany, matared@inoq.de
2 Department of Plant Physiology, Institute of Biology, Humboldt University of Berlin, 10115 Berlin, Germany
3 Department for Plant Nutrition, Institute of Vegetable and Ornamental Crops, Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany

INTRODUCTION
EU-ITN project MiRA aims to use beneficial microorganisms that induce plant resistance against agricultural pests. We are working with the chosen model system that comprises tomato - *Solanum lycopersicum* - as a host plant, beet armyworm - *Spodoptera exigua* - as a pest and bacterial and fungal species as beneficial microorganisms.

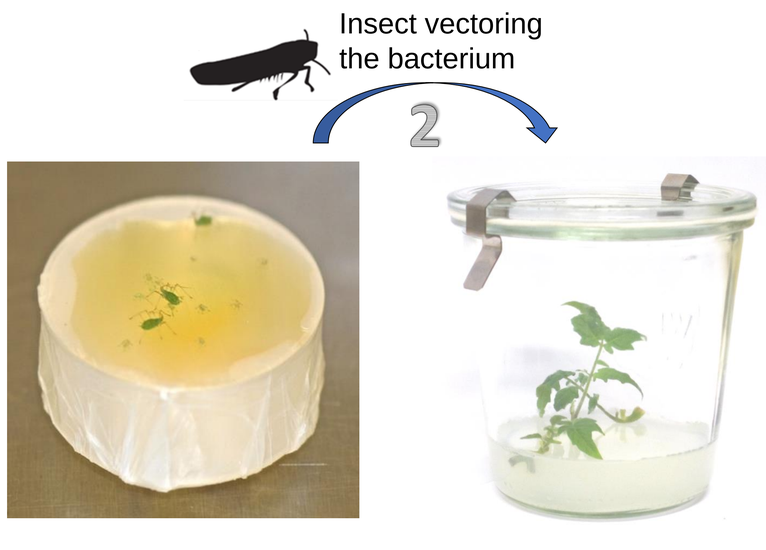
OBJECTIVE
Transmission of beneficial bacterial endophytes into plants by sap feeders and pollinators as insect vectors.

EXPERIMENTS
1. Vectoring a bacterium from an infected plant into an axenic *in-vitro* plant with a sap feeding insect
2. Vectoring a bacterium from an artificial diet into an axenic *in-vitro* plant with a sap feeding insect
3. Vectoring of a bacterium by inoculated bumble bees with hive dispenser



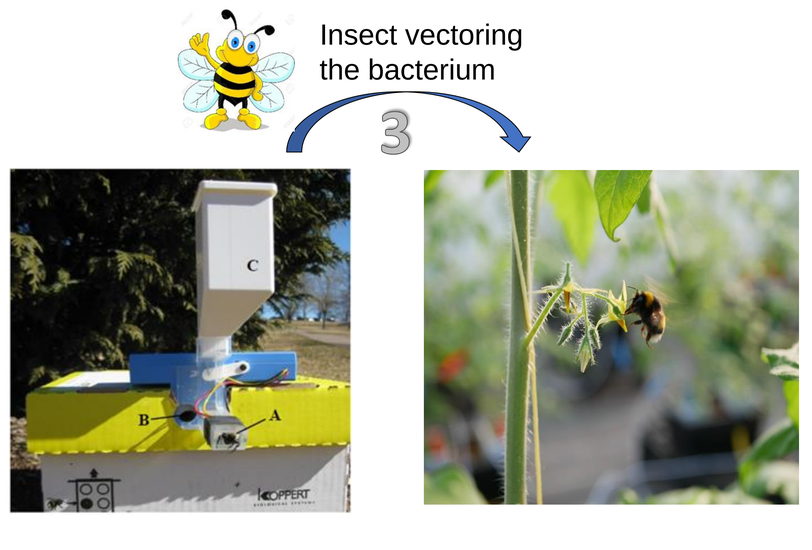
Source plant: colonized by bacterium

Sink plant: free of bacteria



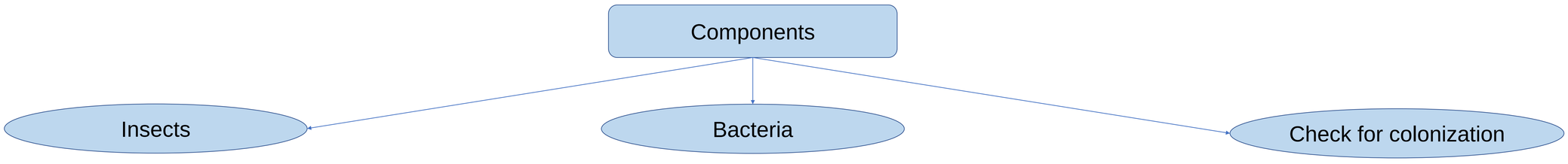
Source: feeding chamber with artificial diet colonized by bacterium

Sink plant: free of bacteria



Source: hive dispenser with bacteria powder

Sink plant & bumble bee inoculated with the bacterium



Criteria for a suitable insect as a vector for the inoculation:
→The insect should be attracted by tomato host-plants
→The insect should be vector for bacteria
→The insect should not be affected by the bacteria

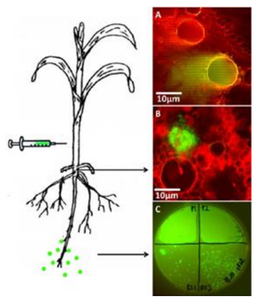
The potential insect vector models:
→Green peach aphid *Myzus persicae*
→Whitefly *Bemisia tabaci*
→Potato leafhopper *Empoasca fabae*
→Green stink bug *Nezara viridula*

→Bumblebee *Bombus terrestris*

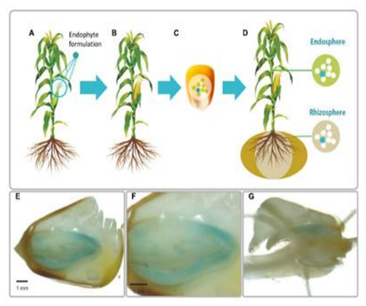
What is the criteria the bacterium needs to fulfill?
→Should have ability to be transferred by insects (sap feeders or pollinator)
→should have systemic colonization behaviour inside plants
→Should have induce systemic resistance (ISR) effect
→Could be beneficial endophytes

The potential bacterial endophyte models:
→*Bacillus subtilis* B26
→*Kosakonia radicincitans* (DSM 16656)
→*Bacillus amyloliquefaciens* Blu-v2; CM-2 & T-5

After inoculation of *in-vitro* tomato plant tissues with bacteria, we will check for colonization inside the plant by appropiate methods:
→ Qualitative / Visual: GFP; GUS
→ Quantitative PCR



(Johnston-Monje and Raizada 2011)



(Mitter et al., 2017)

PCR

OUTLOOK
After identifying a successful transfer of bacteria by insect vector and achieving plant colonization by a bacterial endophyte, we can develop the experiment further and answer questions like:
1.How many bacteria will be necessary to inoculate the insect vector to achieve an appropriate transmission rate?
2.How many insect vectors are needed to cover a target plant?
3.How long is the longevity of the insect vector during, and after transmission?
4.How much do bacteria influence viability of insect vectors? (Mortality)
5.How many insect vectors need to be released into a large greenhouse in commercial production?

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