

Research proposal: Can heavy metals protect *Brassica napus* from pathogens and pests?

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Background

Some plants show unusually high levels of heavy metals accumulation in their tissues (Brooks et al. 1977). Over 500 plant species and 0.2 per cent of all angiosperms have been described as metal hyperaccumulators (Sarma, 2011). Many hypotheses have been raised to explain such phenomenon. One of these suggests that heavy metal accumulation can protect plants from pathogens and pests (Boyd and Martens, 1992).

Several studies have focused on determining whether metals can defend plants from biotic stress (e.g., Fones et al. 2010; Poschenrieder et al. 2011). They reached the conclusion that, sometimes, it is actually possible.

Plant defense by metals is relevant on many fields such as phytoremediation, phytomining or metallomics (Boyd, 2007), which may benefit from further research. Also, new insights on specificity and cross talk in plant responses to environmental stresses can be provided (Poschenrieder et al. 2006).

Objectives and hypotheses

The **objectives** of the research project are to:

- 1 Obtain new information about the biology and ecology of *Brassica napus*
- 2 Provide additional insights on the role of heavy metals in plant-pathogen and plant-herbivore interactions
- 3 Become a data source for further *Brassica napus* studies or applied uses

The **initial hypotheses** are the following:

- 1 Heavy metals accumulation can protect *Brassica napus* from pathogens and pests
- 2 Different metals offer different grades of protection

<h3>Why <i>Brassica napus</i>?</h3> <p>It is cultivated worldwide Many uses</p> <ul style="list-style-type: none"> Vegetal oil production Phytoremediation Biofuel production Animal fodder High biomass production Well known pathogens and pests Defense by metals not studied yet 	<h3>Which metals will be tested?</h3> <p>Cd and Zn</p> <p>Its accumulation in <i>B. napus</i> leaves has been proven and quantified</p> <p>Using two metals allows to test whether they offer different protection</p> <p>They are mobile elements that can concentrate along the food chain</p>	<h3>Which pathogens and herbivores will be used?</h3> <p><i>Alternaria brassicicola</i> <i>Peronospora parasitica</i> <i>Pieris rapae</i> <i>Helix aspersa</i></p>
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Materials and methods

Experiment 1: Metal concentration in plant

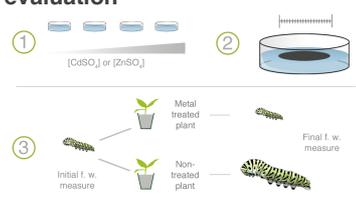
Objective: Determine which concentration of Cd and Zn should plants be exposed to for an optimum accumulation and quantify metal incorporation into leaves.



Procedure: Different hydroponic solutions with a variable metal concentration will be prepared. Plants will be visually examined. The higher metal concentration showing no apparent symptoms of toxicity in plants will be used in the following experiments. Metal concentration in tissue will be quantified by inductively coupled plasma spectrometry.

Experiment 2: Biotic factors toxicity evaluation

Objective: Determine whether pathogens and herbivores are susceptible to Cd and Zn.

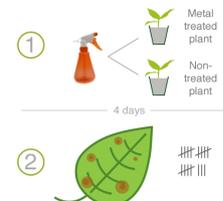


Procedure: Pathogens tolerance will be estimated by inoculating the fungi on to MEA plates and mesuring resultant mycelium diameter.

Herbivores will be placed on metal treated and non treated plants and their fresh weight gain will be measured.

Experiment 3: Pathogen infection

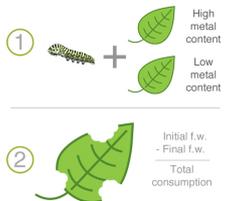
Objective: Verify whether accumulation of Cd or Zn in *B. napus* protects it from *Alternaria brassicicola* and *Peronospora parasitica* infection.



Procedure: Inoculation of pathogens will be carried out by spraying plants with a spore suspension. 4 days after inoculations, the number of lesions per leaf will be counted as a measure of plant infection degree

Experiment 4: Herbivore choice feeding

Objective: Verify whether accumulation of Cd or Zn in *B. napus* protects it from *Pieris rapae* and *Helix aspersa* herbivory.



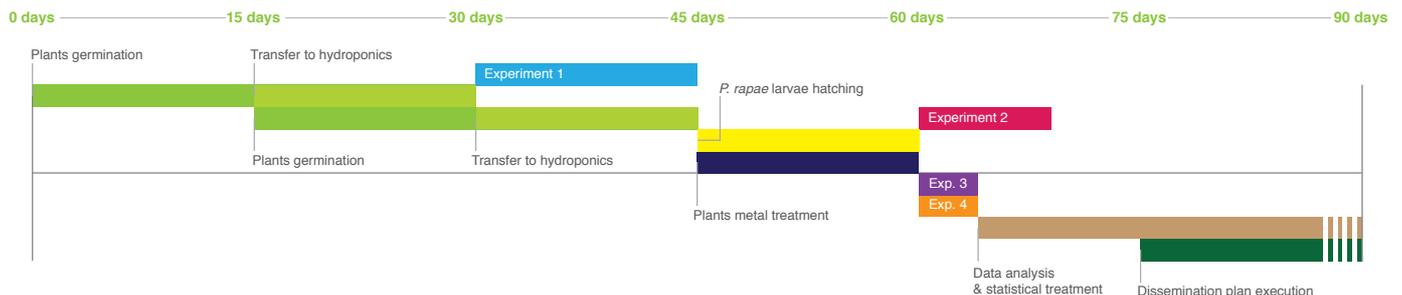
Procedure: One herbivore and two pieces of leaf will be placed on a recipient, one containing high metal concentration and one containing low metal concentration. Leaves fresh weight will be measured before and after the experiment. Feeding will be quantified by measuring fresh weight loss.

Expected results

How expected results would look like:



Working plan



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