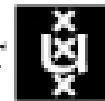


Development of an extraction method for roots of *Thuja occidentalis* and a bio-assay to test its attractiveness for *Heterorhabditis megidis*

Authors: Marieke Prins, Rob van Tol, Pim de Voogt and Maurice Sabelis



Pest problem

- The vine weevil is an important pest in the production of hardy ornamentals and small fruits worldwide
- Larvae cause most of the damage
- Biocontrol of larvae with entomopathogenic nematodes (EPNs)
- Treatment with EPNs is relatively expensive and variably effective in the field

Larva



Adult



Otiorhynchus sulcatus

Infochemicals in belowground tritrophic interactions

Maize

Western corn
rootworm

Entomopathogenic
nematode



Zea Mays

Diabrotica virgifera

Heterorhabditis megidis



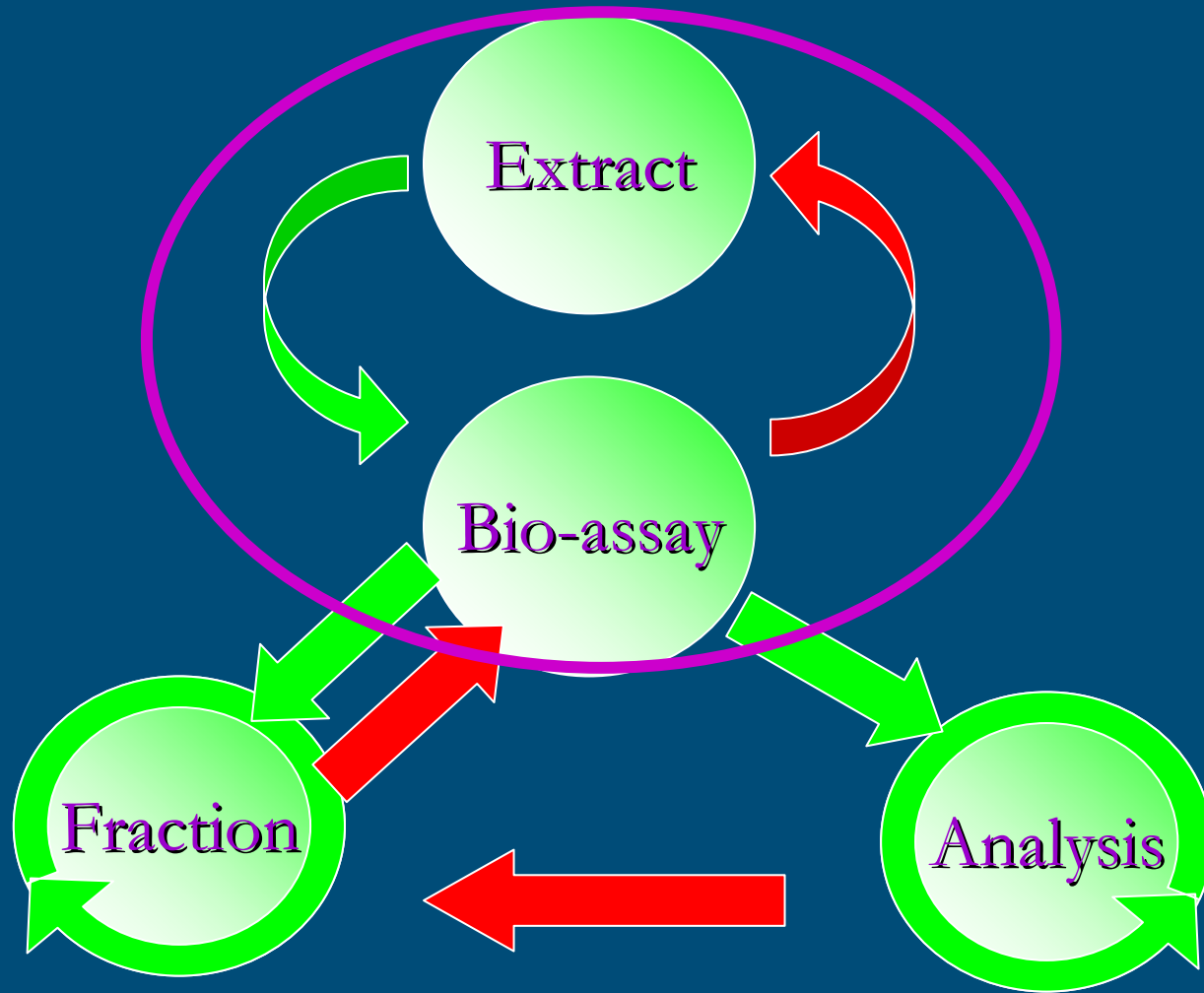
asmann, S., T. G. Kollner, J. Degenhardt, I. Hiltbold, S. Toepfer, U. Kuhlmann, J. Gershenson and T. C. J. Turlings (2005). Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434(7034): 732-737.

Objective PhD-research

- To identify the infochemicals released by weevil-damaged conifer roots that are attractive for *Heterorhabditis megidis*

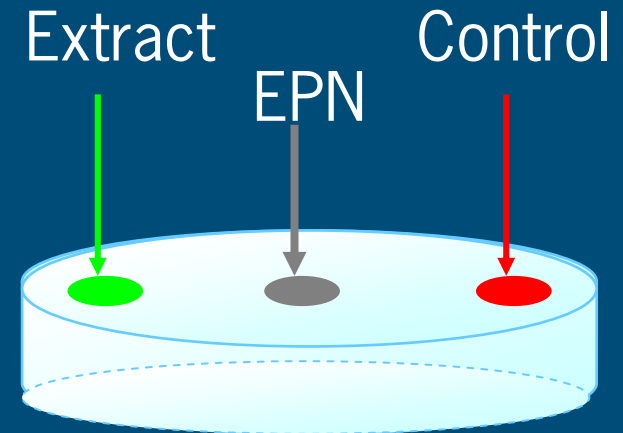


Bio-assay driven fractionation



Experiment

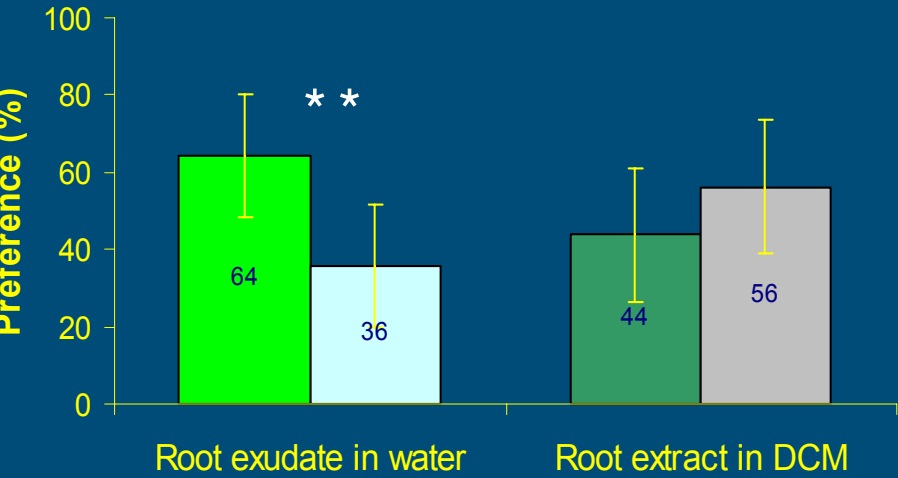
- Extracts
 1. Root exudate in water
 2. Root powder extract in dichloromethane (DCM)
- Petri dish bio-assays (Ø 90 mm)
 1. Water agar as a medium
 2. Silver sand as a medium



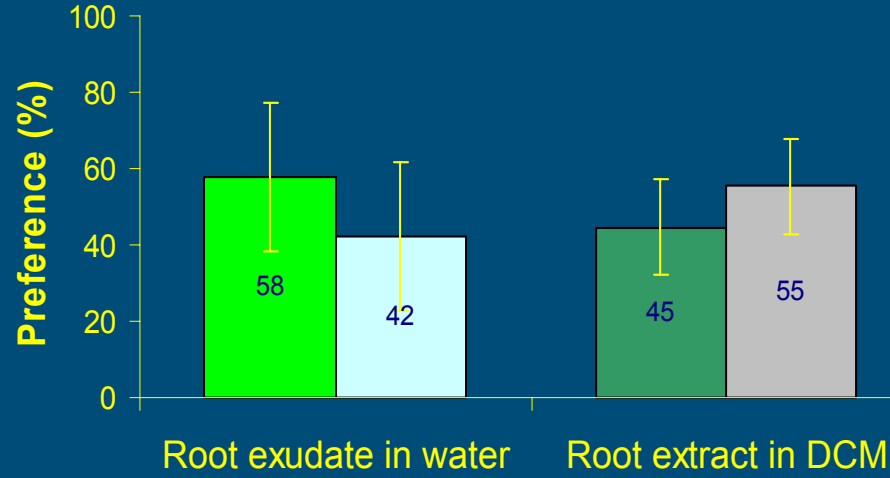
Results

Preference of *H. megidis*

Agar petri dish



Silver sand petri dish



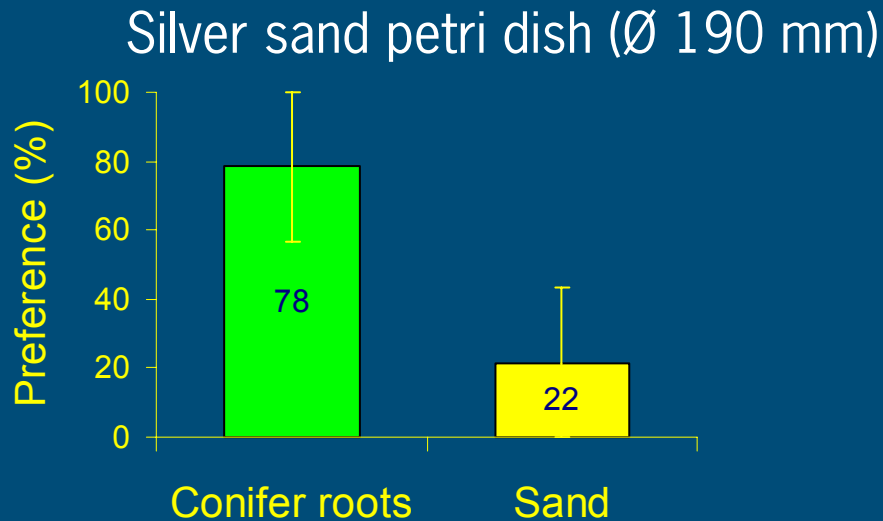
Legend

- Root exudate in water
- Control (water)
- Root powder extract in dichloromethane (DCM)
- Control (DCM)

** $P \leq 0.01$

Results

- Is silver sand as a medium in a petri dish bio-assay suitable?



- Attractiveness for conifer roots (78%) is stronger than for a conifer root exudate (58%) in a petri dish filled with silver sand

- Root exudate in water was significantly attractive with agar as a medium but not with silver sand
 - Water content (evaporation)
 - Pore size distribution (compactness of the sand)
 - Gas phase content
- Conifer roots were more attractive in a silver sand petri dish than the root exudate in water in a silver sand petri dish
 - Spot application versus continuous release
 - Dissimilarity in quantity/quality
 - Degradation/oxidation
 - CO₂ release
 - Dimension

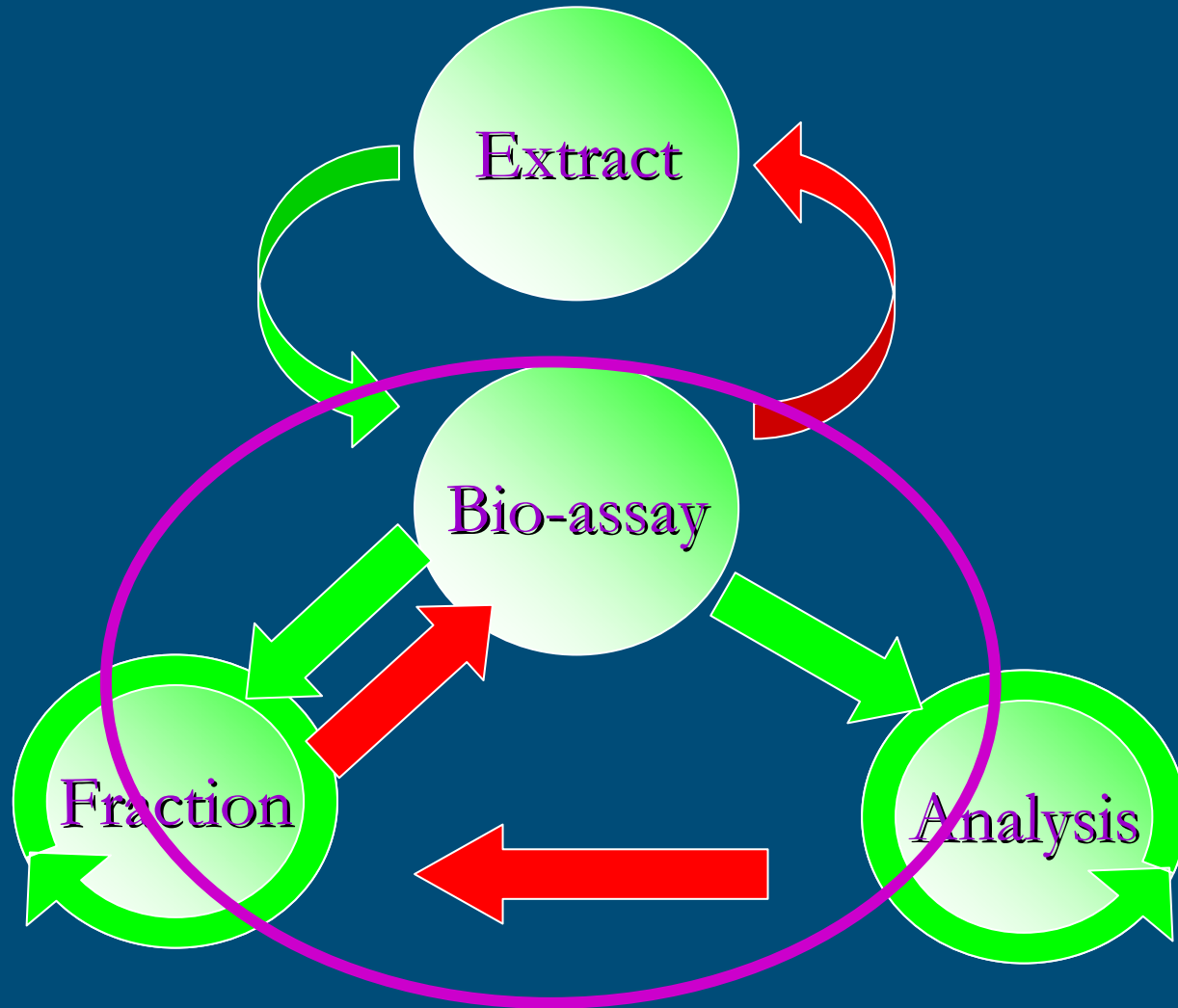
- Root powder extract in DCM is not attractive for *H. megidis*
 - Disturbing or repellent compounds extracted from the roots
 - Root powder is not representative for compounds released by the roots
 - Nematode attractants do not dissolve in DCM
 - Compounds are evaporated with the DCM after application in the petri dish bio-assay

Future work

- Composition of the infochemicals
volatile, water-soluble compounds or a combination?
 - Refinement of the bio-assay
 - Testing other extraction solvents – use of organic solvents
 - Testing other extracts – soil, water phase, gas phase
- Methodology of petri dish bio-assay
silver sand as medium as well as agar
 - Improving migration of nematodes
 - Improving gradient formation
 - Reducing external influences



Future work





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