# Tritrophic interactions in soil

Rob van Tol & Willem Jan de Kogel

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## 2 examples

- Vine weevil – plant – parasitic nematode
- Grubs – plants – entomopathogenic fungi

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Chemical information in soil: tritrophic interactions between plants, insects and parasitic nematodes

Rob van Tol
Marleen Riemens
Frans Zoon
Willem Jan de Kogel

*University of Amsterdam*
Pim de Voogt
André van Roon
Maurice Sabelis

*Thuja* and *Taxus*; host plants

*Otiorhynchus sulcatus;* larvae

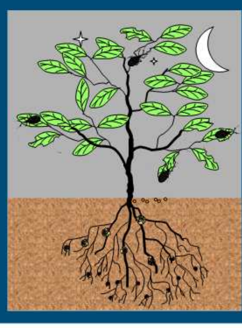
*Otiorhynchus sulcatus;* infected larva

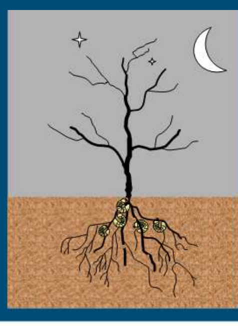
*Heterorhabditis megidis*

an Tol

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Field situation summer and winter





PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Control efficacy EPN

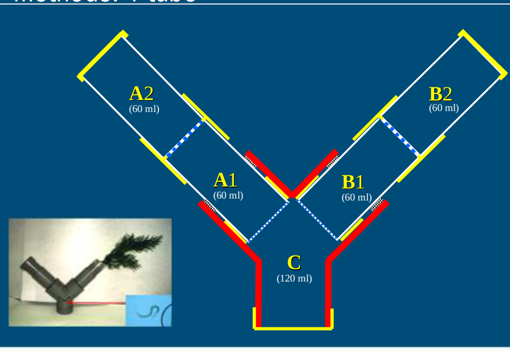
Efficacy in practice (autumn application)

- Control results in pots: high and predictable (80-100%)
- Control results in the open field: variable and unpredictable
- Drench with 1 million EPN/$m^2$ gives 60-70% control in field tests in the Netherlands

- How to improve efficacy?
- >>Understand host finding behaviour

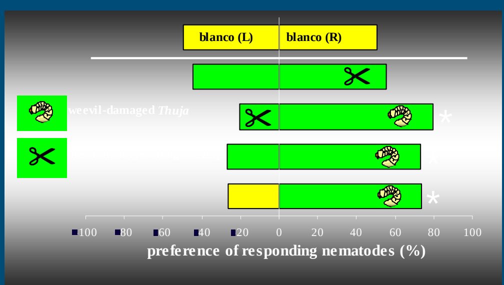


## Methods: Y-tube





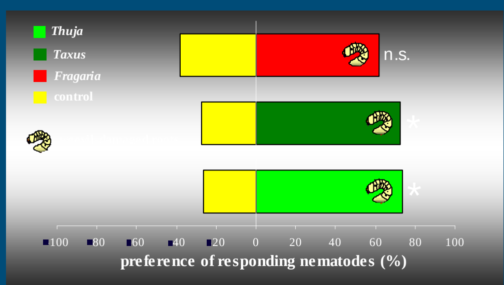

## Preference of *H. megidis* for herbivory-damaged roots



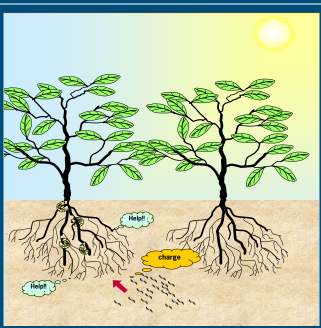

(*Ecology Letters (2001) 4: 292-294*)



## Preference of *H. megidis* for herbivory-damaged roots



## S.O.S. signalling of roots






## Exploitation of signals in soil

<u>EPNs</u>

- Quality control of product prior to application
- Selection for strains: searching and genetics

<u>General</u>

- Preventive attraction of natural enemies towards roots for plant protection
- More effective use of introduced antagonists

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

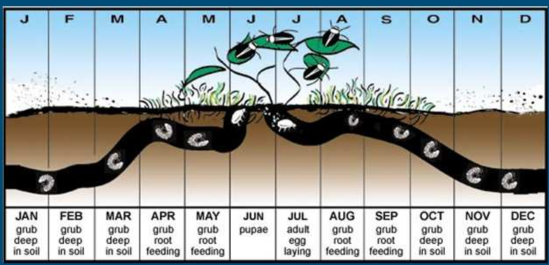
## Attraction and repellence of grubs in soil systems as part of pest control strategies

### The cockchafer *Melolontha melolontha* example

Rob van Tol
Gerrie Wiegers
Willem Jan de Kogel

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Life cycle




*M. melolontha* lives up to 3 years in soil as a grub

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Pest problem

- White grubs
  - Where?: Pasture, Nurseries, Fruit, Forest, Vineyards
  - Damage?: Grubs live several years in soil eating roots
  - Problem?: Chemical means ▼
    : Biological means ▲ but relative expensive, large areas, application difficult, limited efficacy, legal registration in EU difficult

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Control strategies

- Adults
  - Pheromones
  - Light traps
  - soil cover
- Grubs
  - insecticides
  - entomopathogenic nematodes
  - entomopathogenic fungi
  - bacteria (milky spore disease: *Bacillus* sp.)
  - rickettsia (*Rickettsiella popilliae*)
  - parasitoids (*Dexia rustica*)


PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Research strategies

- Lure and kill
  - attractive plants luring grubs to effective control means
- Push and pull
  - repelling plants protecting crop
  - attractive plants as alternative food
  - control of grubs on lure plants (removal, insecticide, biocontrol)
- above and belowground
  - Repelling adults from oviposition sites (repelling plants) and grubs from plant roots

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Olfactometer + plant selection

- Pasture grasses and herbs
  - 14 species
- Cover crops
  - *Tagetes*
  - Brassicaceae
- Crops to be protected
  - *Taxus*, *Quercus*, *Fagus*, *Carpinus*,.....

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Efficacy testing biological means

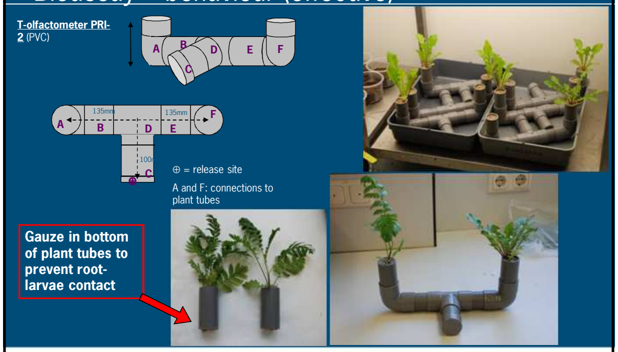
- Nematodes
  - EPN strain selected for *M. melolontha* (coded)
- Fungi
  - *Beauveria brongniartii* – commercial strain
  - *Beauveria bassiana* – commercial and other strains
  - *Metarhizium* species – `brunneum`, `guizhouense`, `robertsii`, `flavoviride`, `anisopliae`, ...........
- Others
  - *Bacillus sphaericus*, *B. popilliae*, *B. thuringiensis*, *Paenibacillus* sp.

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Bioassays – behaviour (not effective)

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Bioassay – behaviour (effective)

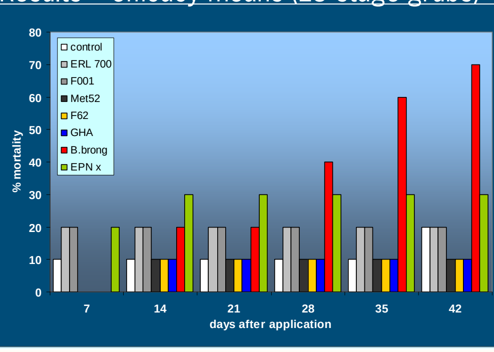



PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Bioassays – biological means

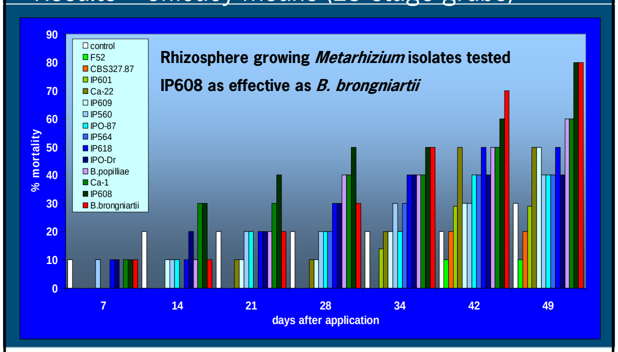


## Results – efficacy means (L3 stage grubs)





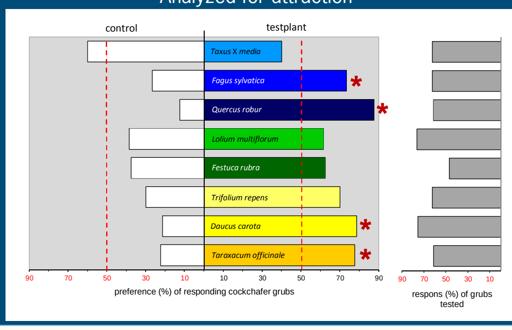

## Results – efficacy means (L3 stage grubs)





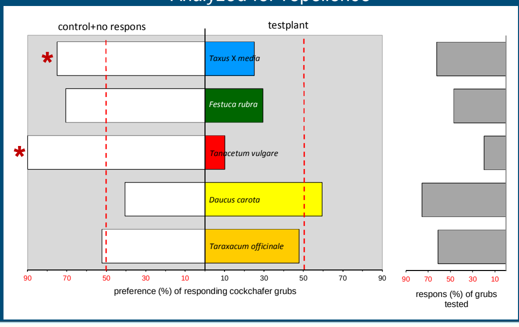

## Results – plant preference grubs (2007-2011)

Analyzed for attraction



## Results – plant preference grubs (2007-2011)

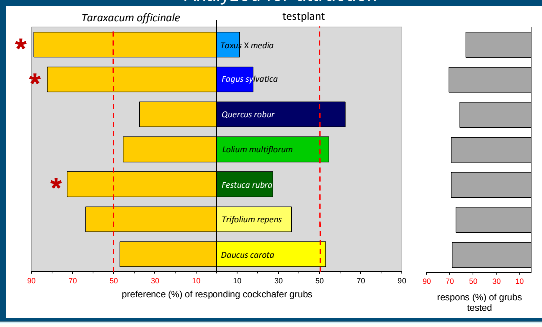
### Analyzed for repellence




PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Results – plant preference grubs (2007-2011)

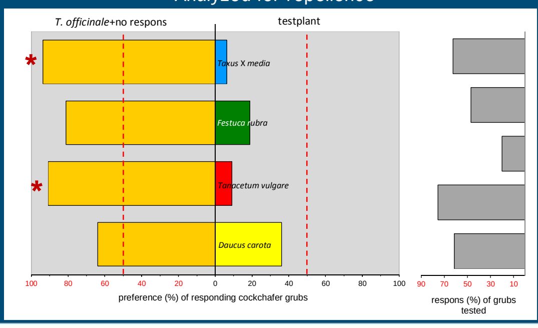
### Analyzed for attraction




PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Results – plant preference grubs (2007-2011)

### Analyzed for repellence




PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Results: beech without repellent plants

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Results: beech with repellent plants

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Continuation repellent strategy

- Large field tests in reforestation areas in Poland
  - Effect on grubs present
  - Prevention of migration grubs into fields
  - Push-pull with attractive alternative (carrot, dandelion, grass, other…..(tests in NL)
- Effect of cover crop on egg-laying beetles

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Rhizosphere Competence of *M. anisopliae*

Most previous work with entomopathogenic fungi has ignored the habitat preferences and survival of the fungus outside of the host. It is possible that factors associated with fungal biology outside of the host are more important when selecting an isolate than how pathogenic it is against a particular host in a laboratory bioassay.

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

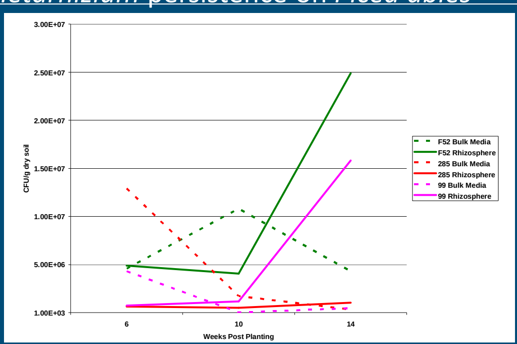
USDA ARS

## Efficacy of Rhizosphere Colonized Roots

- 76% of black vine weevil larvae feeding on fungal treated roots were dead after 2 weeks

USDA ARS

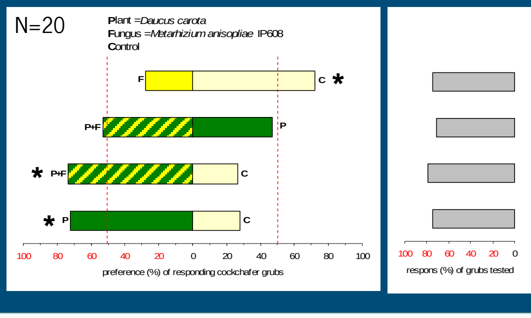
## *Metarhizium* persistence on *Picea abies*




USDA ARS

## Results – plant preference grubs (2010)

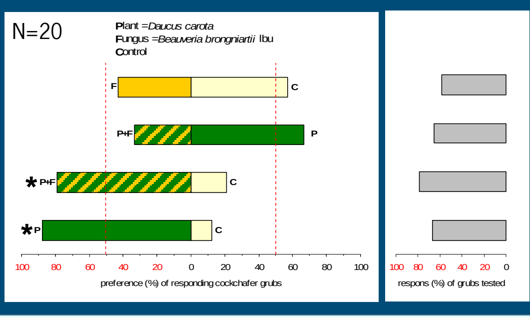
**Tests performed with plants only 1 week before inoculated with spores**




PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Results – plant preference grubs (2010)

**Tests performed with plants only 1 week before inoculated with spores**



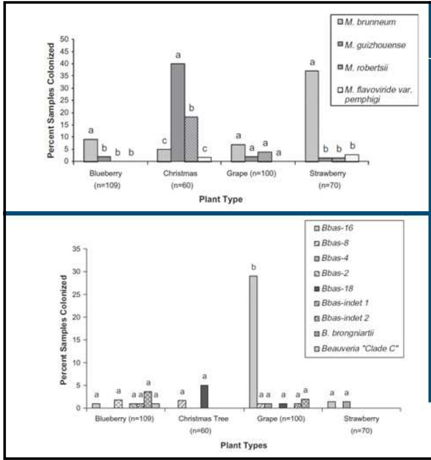

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

## Prevalence and Isolate Identification

- Strawberries, blueberries, wine grapes, Christmas trees
- Which fungi are present and how prevalent are they?
- Isolates more inherently suitable for use as a rhizosphere colonizer?

Fisher, Rehner and Bruck – Journal of Invertebrate Pathology *In Press*

USDA ARS




The percent of blueberry, Christmas trees, grape and strawberries samples collected from fields throughout the Willamette Valley, OR colonized by four *Metarhizium* taxa and nine *Beauveria* taxa.

USDA ARS

## Rhizosphere Competence: A New Approach???

- Plant host range (Underway)
- Persistence (5 yr study underway)
- Colonization of elongating roots (Underway)
- Efficacy (Underway)
- Compatibility with other biologicals

USDA ARS

## Main questions

- What is the role of rhizosphere competent fungal entomopathogens in regulating pest populations?
- How can we use and implement this for more effective microbial control programs?

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR — USDA ARS

## Main scientific questions

- Do plants benefit from the association?
- Is the `bodyguard` concept relevant in soil? What mechanism?
- Have different phylogenetic groups different strategies in association with plants?

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR — USDA ARS

## Main applied questions

- Most effective approach for inoculation roots?
- Persistence on root systems of different plant species?
- Will it provide consistent and acceptable levels of pest control?
- Are there fungal species with wide range of host-plants and target pest insects?

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Continuation options

- Screening/selection entomopathogenic fungi NL/USA (Biocontrol companies, PRI, USDA)
  - Rhizosphere competence/persistence
  - Efficacy against target pests
  - Host-plant range
- Olfactory preference/acceptance fungus-plant by grubs (PRI, USDA)
- Fungus-induced plant attraction (RU-Nijmegen, PRI)
  - Yes/no present for selected fungus-plant combinations
  - Chemical identification
  - Detection infested plants (grubs/fungi/.... via top soil odour profile)

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

## Existing/planned cooperation

- Research
  - University of Berlin - Germany (Prof. Dr. M. Hilker and E. Eilers)
  - Max Planck Institut for Chemical Ecology Jena - Germany (Dr. A. Reinecke)
  - Radboud University Nijmegen (Prof. Dr. N. van Dam, Dr. S. Cristescu)
  - Forest Research Institute Poland (Dr. Sukovata)
  - USDA - USA (Dr. D. Bruck)
  - Research Stations – Netherlands
  - **Biocontrol companies......**

The project `Interactions between soil pests, crop and biological means` is part of the BO program Plant Health financed by the Dutch ministry of Agriculture

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR