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Fig. 6. *Enoplolaimus australis* n. sp. Fig. 7. *Enoplus Michaelsoni* v. Linst.



Nederlandse Voedsel- en  
Warenautoriteit  
*Ministerie van Economische Zaken*

# Nematode collections at the NPPO (NL)

Gerrit Karssen



## •Introduction

- Why do we have biological collections?

## •Collections at the NPPO (NL)

- What collections do we have?
- What is in it?
- How do we maintain them?

## •Spin-off

- NCE
- Delivery on demand
- Courses.





# Introduction

*Why do we have biological collections at the NPPO (NL)?*

\*Serve as a reference standard for taxa or symptoms:

→ identification & comparison.

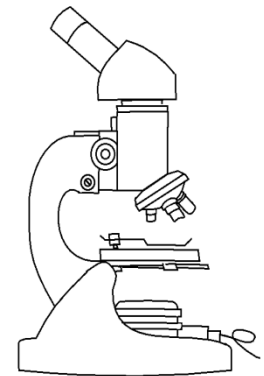
\*Needed for research studies:

→ taxa description & revisions

→ host-plant relation

→ molecular test development.

\*Training & education.





# Collections

- *What kind of collections do we have?*
  - Symptoms: pictures (slides) & plant (parts) in jars
  - Permanent slides: regular & types
  - Green house cultures
  - in vitro cultures
  - Micro-plots.





## Collections: symptoms

- *What is in it? How do we maintain them?*

→Photo's of plant disease symptoms caused by nematodes:

→± 1600 color slides

→stored in a closed cabinet at room temp

→± 400 have been digitalized so far.

→Plant or parts with symptoms in jars:

→185 sealed jars

→6% formaline

→stored in a closed cabinet in (aerated) lab

→maintained according to protocol.





## Collection: permanent slides

- *What is in it? How do we maintain them?*

- Regular slides:

- + 50.000 Cobb slides

- stored in fireproof cabinets at room temperature

- Excel database

- maintained by technician.

- Type slides:

- nearly 3800 slides (Cobb & glass)

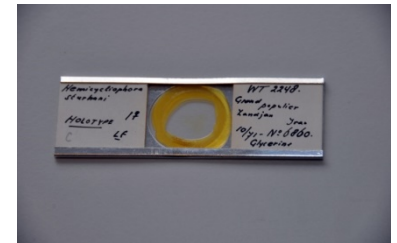
- stored in fireproof cabinets at room temperature

- Excel database

- on-line available at [www.nce.nu](http://www.nce.nu)

- slides maintained by researcher & technician

- website maintained by researcher & 'Wiesweb'.







## Collection: green house cultures (1)

- *What is in it? How do we maintain it?*
  - Root-knot nematodes:
    - ± 100 populations
    - ± 22 species (see next slide)
    - temp. (20-15C) & "tropical" compartments (21C)
    - main host: tomato (cv Dometica)
    - sandy soil mixture (melo soil)
    - all year round
    - renewed & ID check 1x year
    - some on other hosts or in micro-plot
    - maintained according to protocols.





## Collection: green house cultures (2)

- **Melo species in culture:**

Temperate species:

- *M. chitwoodi*
- *M. fallax*
- *M. minor*
- *M. hapla*
- *M. artiellia* (on cabbage)
- *M. naasi* (on wheat)
- *M. kralli* (on *Carex acuta*)
- *M. ardenensis* (micro-plot on *Ligustrum vulgare*)
- *M. mali* (micro-plot on *Ulmus hollandica* "Wredei").

"Tropical" species:

- *M. javanica*
- *M. incognita*
- *M. arenaria*
- *M. hispanica*
- *M. enterolobii*
- *M. ethiopica*
- *M. inornata*
- *M. luci*
- *M. exigua*
- *M. ichinohei* (on *Iris levigata*)
- *M. graminicola* (on *Echinochloa crus-galli*)
- *M. africana* (on *Coffea arabica*)
- *M. camelliae* (on *Camellia japonica*).







## Collection: green house cultures (3)

### →Cyst nematodes:

- $\pm$  30 populations (incl. pathotypes)
- 12 species (see next slide)
- temperate compartment
- "tropical": *H. glycines*, *H. ciceri* & *C. cacti*
- host: several
- soil: sandy/clay mixture (cyst soil)
- not all year round (stored at 4C)
- renewed & ID check 1x year
- maintained according to protocols.

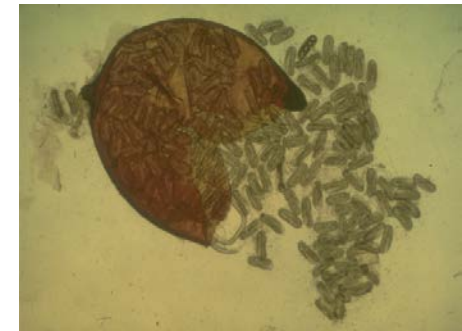




## Collection: green house cultures (4)

- Cyst species in culture:

- <i>Globodera</i>	<i>pallida</i> (Pa2 & 3)	potato cv Bintje
- <i>G.</i>	<i>rostochiensis</i> (Ro 1, 2/3, 4 & 5)	„
- <i>G.</i>	<i>tabacum</i>	tobacco
- <i>Heterodera</i>	<i>glycines</i>	sojabean
- <i>H.</i>	<i>schachtii</i>	cabbage
- <i>H.</i>	<i>betae</i>	cabbage
- <i>H.</i>	<i>trifolii</i>	clover
- <i>H.</i>	<i>daverti</i>	clover
- <i>H.</i>	<i>cruciferae</i>	cabbage
- <i>H.</i>	<i>ciceri</i>	lentil
- <i>H.</i>	<i>carotae</i>	carrot
- <i>Cactodera</i>	<i>cacti</i>	cactus



-Testing: *H. koreana*, *H. bifenestra*, *H. elachista*, *Globodera ellingtonae* & *G. mexicana*.

- Micro-plot: *H. humuli*, *H. riparia*, *H. urticae*, *H. ustynovi* & *Punctodera punctata*.



# Collection: green house cultures (5)

→ Other nematodes

→ 16 populations

→ 12 species

→ o.a.:

- <i>Nacobbus abberans</i>	tomato
- <i>Radopholus similis</i>	banana
- <i>Pratylenchus goodeyi</i>	banana
- <i>P. zae</i>	grasses
- <i>P. bolivianus</i>	tomato
- <i>Rotylenchulus reniformis</i>	jasmine
- <i>Tylenchulus semipenetrans</i>	citrus

→ all maintained as for melo's

→ some are highly experimental!



## Collection: in vitro cultures (1)

- *What is in it? How do we maintain them?*
  - In petri dish on fungi
    - 28 populations
    - 14 species (next slide)
    - *Botrytis cinerea* on malt extract agar
    - incubator at room temperature
    - renewed & ID check  $\pm$  12 weeks
    - maintained according to protocols.



## Collection: in vitro cultures (2)

- Species in vitro culture:

- *Bursaphelenchus xylophilus*

- *B. mucronatus* (EU-type & East-Asian type)

- *B. fungivorus*

- *Cryptaphelenchus* sp.

- *Laimaphelenchus pannocaudatus*

- *Aphelenchoides besseyi*

- *A. subtenuis*

- *A. saprophilus*

- *A. fragariae*

- *A. blastophthorus*

- *A. composticola*

- *A. sinodendroni*

- *Ditylenchus arachis*

- *D. myceliophagus*.





## Collection: in vitro cultures (3)

→ In petri dish on carrot

→ 7 populations

→ 3 species

→ on *Daucus carota* disc

→ incubator at room temperature

→ renewed & ID check  $\pm$  8 weeks

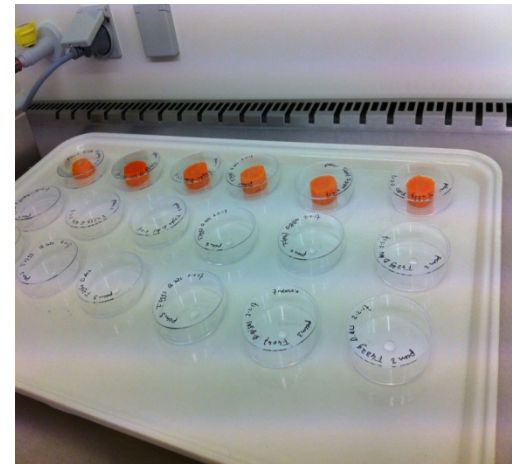
→ maintained according to protocols.

→ in culture:

→ *Aphelenchoides ritzemabosi*

→ *Ditylenchus dipsaci*

→ *D. destructor*.







## Collection: micro plots

- *What is in it?*
  - 40 micro plots
  - restricted area
  - different soil types
  - clover/grass main hosts
  - all PPN checked 6 years ago
  - PPN: 14 genera & 46 species
  - includes some q's
  - PPN mainly used for courses
  - maintained by greenhouse technicians
  - o.a.
    - M. ardenensis* on *Ligustrum vulgare*
    - M. chitwoodi* on grasses/clover
    - M. mali* on *Ulmus hollandica* "Wredei"
    - M. minor* on grasses.





# Spin-off

- Nematode Collection Europe (NCE)
  - on-line database for type collections from D, UK, B & NL
  - nomenclatorial database for Nematoda (excl. animal parasites)
  - [www.nce.nu](http://www.nce.nu)
  - maintained by Wiesweb and researcher.
- Delivery on demand
  - about 75 populations delivered/per year
  - mostly used for DNA tests (reference or ID test development)
  - free for NPPO's.
- Courses
  - slides & living nematodes used for teaching
  - UGent & WUR-NPPO ID course once a year
  - several courses on request each year (1-2 persons).



## The team

- **Meloidogyne:** Evelyn van Heese & Patricia Bor
- **In vitro & others:** Anne Sophie van Bruggen, Evelyn van Heese & Patricia Bor
- **Cysts & micro-plots:** Anne-Marie Heij & Patricia Bor
- **Slides:** Evelyn van Heese & Rens van de Biggelaar
- **WaNeCo:** Evert Wiesenekker (Wiesweb) & G. Karssen.



THANKS!