Nematodes in peat: old problems, new techniques.

Nematodes are microscopic small worm-like organisms. They are found in all kinds of habitats: woodlands, grassland, soils of arable land, gardens, sediments of rivers and seas, and also in peat soils. Some nematodes are harmful to plants, but the most are favourable to the soil quality and play an important role in the soil health. A few nematode species are harmful to animals and humans.

It is not desirable to have harmful nematodes in peat as raw material for substrate and substrate components for Horticulture. To avoid this, it is possible to analyse peat samples for nematodes.

Every year BLGG AgroXpertus analyses a few hundred peat samples. One of the problems in the analysis is the identifications of the nematodes. With the light microscope it is not always possible to identify nematodes to species or even genus level. It is also not possible to identify juvenile nematodes. New molecular techniques solve this problem. These new techniques make it possible to identify juvenile nematodes to species level and even to detect one single target nematode species among thousands of non target nematodes. This is important, especially for nematode species on the EPPO A1 and A2 list of pest recommended for regulation as a quarantine pest.
Nematodes in growing media – Old problems, new techniques.

Harm Keidel
Scientist Soil Quality
Introduction
Introduction
Annual percentages of cotton losses to nematodes (Dr. Don Blasingame).
Introduction

- What are nematodes?
- Plant Parasitic nematodes.
  - Quarantine nematodes.
- Nematodes, peat and other constituents.
- Conclusions.
What are nematodes?
Nematodes or eelworms

female

male
Nematodes, from egg to adult in four molts.

provided by Ayoub, 1980
Nematodes, their place in the Soil Food Web

**The Soil Food Web**

- **Nematodes**
  - Root-feeders

- **Arthropods**
  - Shredders
  - Predators

- **Fungi**
  - Mycorrhizal fungi
  - Saprophytic fungi

- **Protozoa**
  - Amoebae, flagellates, and ciliates

- **Bacteria**

**Organic Matter**
- Waste, residue and metabolites from plants, animals and microbes.

**Plants**
- Shoots and roots

**First trophic level**
- Photosynthesizers

**Second trophic level**
- Decomposers
- Mutualists
- Pathogens, parasites
- Root-feeders

**Third trophic level**
- Shredders
- Predators
- Grazers

**Fourth trophic level**
- Higher level predators

**Fifth and higher trophic levels**
- Higher level predators

Relationships between soil food web, plants, organic matter, and birds and mammals

Image courtesy of USDA Natural Resources Conservation Service


BLGG AGROXPERTUS
Nematodes, Headstructure and Food

Pictures: Hanny van Megen WUR
Nematodes, parasites of plants

Nematodes, parasites of nematodes
Nematodes, parasites of insects
Nematodes, parasite of humans (*elephantiasis*)
The maturity index: an ecological measure of environmental disturbance based on nematode species composition

Tom Bongers
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Received October 10, 1989 / Accepted December 12, 1989

Summary. Nematode assemblages constitute a potential instrument for assessing the quality of submersed, temporarily submersed, and terrestrial soils and for the development of an ecological typology and biomonitoring system. Interpretation of physical or pollution-induced disturbances has hitherto mainly been based on changes in diversity, dominance patterns or percentage of dorylaimids (Adenophorea). The maturity index, based on the nematode fauna, is proposed as a gauge of the condition of the soil ecosystem. Values on a coloniser/persister scale are given for nematodes that occur in The Netherlands. The possibilities of the use of this index are demonstrated by a retrospective interpretation of some literature data. The use of nematodes in environmental studies is discussed.

Key words: Nematodes – Maturity – Ecology – Colonization – Biomonitoring
Plant parasitic nematodes
Plant Parasitic Nematodes

- Important pest in Agriculture and Horticulture.
- Worldwide damage in 2003 estimated at $125 billion.
- Nematodes cause lower yields.
Plant Parasitic Nematodes

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- Nematodes cause lower yields.
- Nematodes cause problems in plant quality.
Plant Parasitic Nematodes

- Important pest in Agriculture and Horticulture.
- Worldwide damage in 2003 estimated at $125 billion.
- Nematodes cause lower yields
- Nematodes cause problems in plant quality
- Some nematodes cause problems with export
Plant Parasitic Nematodes

There are 2 main types of Plant Parasitic Nematodes:

1 Ectoparasitic: lives outside the plant, feeding on roots.
   - Trichodoridae
   - Longidoridae

2 Endoparasitic: penetrate the root, then enter and live inside it.
   - Pratylenchus
   - Meloidogyne
   - Heteroderidae (cyst nematodes)
A typical Plant Parasitic Nematode

Globodera rostochiensis

Picture: Hanny van Megen WUR
Pratylenchus penetrans
Cysts on a root
A single cyst with eggs and juvenile nematodes
Plant Parasitic Nematodes

- Not all Plant Parasitic Nematodes are important.

- Some Plant Parasitic Nematodes cause no damage, like most Tylenchidae.

- This nematodes have a stylet and can be mistaken for harmful species.

- Some nematodes with a stylet are not plant parasitic, like the most *Aphelenchoides* and *Ditylenchus* species.
● Problem is that some species of these genera are very dangerous.

● *Aphelenchoides*: over 70 known species, only 4 species are harmful.

● *Ditylenchus*: over 80 known species, only 3 species are harmful.

● Species within these genera are very difficult to identify.
Quarantine
Quarantine nematodes

- to prevent entry or spread of dangerous pests organisms (plant quarantine).

- In recent years, the identification of risk has been formalized.

- For example:
  
  **Council Directive 2000/29/EC of 8 May 2000** on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community.
### Quarantine organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>A1 list</th>
<th>A2 list</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>Fungi</td>
<td>39</td>
<td>23</td>
</tr>
<tr>
<td>Parasitic plants</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Insect and mites</td>
<td>93</td>
<td>54</td>
</tr>
<tr>
<td>Nematodes</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Viruses</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Invasive plants</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

[www.eppo.org/QUARANTINE/](http://www.eppo.org/QUARANTINE/)
### Quarantine nematodes

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><em>Bursaphelenchus xylophilus</em></td>
<td><em>Aphelenchoides besseyi</em></td>
</tr>
<tr>
<td><em>Nacobbus aberrans</em></td>
<td><em>Ditylenchus dipsaci</em></td>
</tr>
<tr>
<td><em>Radopholus similis</em></td>
<td><em>Globodera pallida</em></td>
</tr>
<tr>
<td><em>Xiphinema americanum</em></td>
<td><em>Globodera rostochiensis</em></td>
</tr>
<tr>
<td><em>Xiphinema bricolense</em></td>
<td><em>Heterodera glycines</em></td>
</tr>
<tr>
<td><em>Xiphinema californicum</em></td>
<td><em>Meloidogyne chitwoodi</em></td>
</tr>
<tr>
<td></td>
<td><em>Meloidogyne fallax</em></td>
</tr>
<tr>
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<td><em>Xiphinema rivesi</em></td>
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www.eppo.org/QUARANTINE/
Nematodes, peat and other constituents
Peat, habitat of nematodes? YES
# Nematodes in peat

<table>
<thead>
<tr>
<th>Nematode taxon</th>
<th>Feeding group</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acrobeloides</em></td>
<td>Bacterial feeders</td>
<td>272</td>
<td>1768</td>
</tr>
<tr>
<td><em>Cephalobidae</em></td>
<td>Bacterial feeders</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Rhabditidae</em></td>
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<td>39</td>
<td>239</td>
</tr>
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<tr>
<td><em>Diploscapter</em></td>
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</tr>
<tr>
<td><em>Plectus</em></td>
<td>Bacterial feeders</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Anaplectus</em></td>
<td>Bacterial feeders</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Dorylaimoidea</em></td>
<td>Predatory nematodes</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Qudsi anematidae</em></td>
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<td>0</td>
</tr>
<tr>
<td><em>Pseudhalenchus</em></td>
<td>Plant root feeders</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>390</td>
<td>2085</td>
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Nematodes in peat

- Can this peat used as growing medium?
- On the first sight: YES because:
  - No important Plant Parasitic Nematodes.
  - No Q-nematodes.
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**Dangerous for mushrooms?**
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Nematodes in peat

It is necessary to:

● Identify nematodes to species-level.

● To judge on genus- or family-level.

● To increase the knowledge about nematodes.
Nematodes in substrates

- In other substrates normally no nematodes.
  - Bark.
  - Lime.
  - Rice hulls.
  - Woodfibre.
  - ...

- Sometimes small numbers of non plant parasitic nematodes.

- In glasshouses when plants are growing on substrates, for example rock wool, nematodes can occupy these substrates.
Conclusions
Conclusions

- Nematodes are a serious problem in Agriculture and Horticulture.

- It is necessary to produce growing media without harmful nematodes.

- Peat is a natural habitat for nematodes and it is normal to find nematodes in it.

- Most nematodes are bactarial and fungal feeders.
Conclusions

- Sometimes you find harmless root feeders
  
  (but these nematodes have a stylet)

- For a good interpretation it is necessary to identify nematodes to species level.
Conclusions

For a few years ago identification only with a light microscope.

But this is:

- Specialistic and labor-intensive
- Hard to find good people.
- Some species are difficult to identify, for example *Meloidogyne*, *Aphelenchoides* and *Ditylenchus* species.
- It is not possible to identify juvenile nematodes to species-level

**Solution:**

New molecular techniques
Conclusions

● BLGG AgroXpertus has developed several molecular tests for identifying nematodes.

● In close cooperation with Wageningen University and Research (WUR).

● Based on the polymerase chain reaction (PCR).

● Use a small part of the genetic code of nematodes (18S rDNA).
Conclusions

● For these purposes the 18S ribosomal DNA gene of about 250 nematode-species has been sequenced.

● These tests enable simple identification of a broad range of nematode genera to species level.

● With Q-PCR it is also possible to give the numbers per nematode-species.

● Nematodes are easy to extract from the soil.
Extraction of nematodes
Conclusions

- Molecular laboratory
Test available

- *Ditylenchus dipsaci*

- *Meloidogyne*

- *Trichodoridae*

- *(Pratylenchus)*

- *(Aphelenchoides)*

- ...
Thank you

for your attention