



Nederlandse Voedsel- en
Warenautoriteit
Ministerie van Economische Zaken

Inventory of living collections of cyst and root knot nematodes and their maintenance techniques

an Euphresco project

Loes den Nijs, NRC
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Introduction:

Euphresco project:

EUPHRESCO= **E**uropean network for **phytosanitary research coordination and funding**



Network for phytosanitary research coordination and funding



Why this project:

- live collection important for reference material, demonstrations, instructions, proficiency test material
- shortage of means (people, time, money, knowledge)
- sharing knowlegde, materials

Contents of project: Cyst nematodes & Root knot nematodes



Objectives of project

- to have an overview of existing live collections of *Globodera* and *Meloidogyne* present in the participating countries
- to make an inventory of the different maintenance and storage techniques used in the participating countries
- write a maintenance manual for cyst and root knot nematodes.



Workplan:

- make up a questionnaire and send it to all participants for collecting information on their live collections of PCN and *Meloidogyne*, and on maintenance of populations
- Organise a workshop (to be held before the EPPO panel on diagnostics in nematology, sept 2017) in which maintenance issues will be discussed and information will be exchanged,
- To compose a manual, based on the information received from the participants



Euphresco project: status quo

Participants: Austria, Belgium, Canada, France, (Germany (2x)), Netherlands, UK, USA (17 participants) (additional: participants from EPPO Panel on Diagnostics in Nematology?)

List of populations in collection:

Parameters on how to rear/maintain/store these nematodes: host plants, inoculum density, growing conditions, time

Exchange of information: SOP's at workshop (today and tomorrow 😊)



Questionnaire:

The following questions are therefore raised:

1. Do you keep populations of the following nematodes genera *Globodera* or *Meloidogyne*?
If yes, go to question 2 . If no, please can you tell whether you have other genera or species live in collection?. (this might be of interest for participants but is not an issue in this project)

[Yes](#)

2. If you answered positive on above question, do you have different populations of one species, and in what aspect are they different?

Please list the species and the different populations within these species:

Globodera	G. pallida	G. pallida from
Globodera	G. pallida	Chavornay (Switzerland)
Globodera	G. rostochiensis	Scotland
Meloidogyne	M. incognita	INRA Avignon

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Meloidogyne	M. minor	
Meloidogyne	M. javanica	Seed company
Meloidogyne	M. hapla	Seed company



List of populations in the participating countries:

species	total frequency	frequency	# populations	frequency	# populations
		Euphresco -USA	Euphresco-USA	in USA	in USA
<i>Globodera ellingtonae</i>	2	1	1	1	1
<i>Globodera pallida</i>	8	7	> 42 *	1	1
<i>Globodera rostochiensis</i>	9	8	>57	1	2
<i>Globodera tabacum</i>	5	1	1	4	4
<i>Heterodera glycines</i>	2			2	2
<i>Meloidogyne africana</i>	1	1	3		
<i>Meloidogyne ardenensis</i>	1	1	1		
<i>Meloidogyne arenaria</i>	10	2	7	8	2
<i>Meloidogyne artiellia</i>	1	1	1		
<i>Meloidogyne chitwoodi</i>	3	2	10	1	1
<i>Meloidogyne enterolobii</i>	3	2	7	1	1
<i>Meloidogyne ethiopica</i>	1	1	1		
<i>Meloidogyne exigua</i>	1	1	1		
<i>Meloidogyne fallax</i>	2	2	9		
<i>Meloidogyne floridensis</i>	1			1	1
<i>Meloidogyne graminicola</i>	1	1	2		
<i>Meloidogyne hapla</i>	11	4	20	7	11
<i>Meloidogyne haplanaria</i>	1			1	1
<i>Meloidogyne hispanica</i>	1	1	1		
<i>Meloidogyne incognita</i>	17	4	9	13	26
<i>Meloidogyne inornata</i>	1	1	2		
<i>Meloidogyne javanica</i>	11	4	14	7	7
<i>Meloidogyne kralli</i>	1	1	1		
<i>Meloidogyne luci</i>	1	1	3		
<i>Meloidogyne mali</i>	2	2	2		
<i>Meloidogyne minor</i>	2	2	2		
<i>Meloidogyne naasi</i>	2	1	1	1	1
*populations; includes all pathotypes					



Hosts for collection use

species	host plants		maintaining*
	rearing		
<i>Globodera ellingtonae</i>	potato		
<i>Globodera pallida</i>	potato		
<i>Globodera rostochiensis</i>	potato		
<i>Globodera tabacum</i>	tabacco		
<i>Heterodera glycines</i>	soybean		
<i>Meloidogyne africana</i>	tomato		
<i>Meloidogyne ardenensis</i>	ligustrum vulgare		
<i>Meloidogyne arenaria</i>	tomato, peanut, tabacco		
<i>Meloidogyne artiellia</i>	kale		
<i>Meloidogyne chitwoodi</i>	tomato, wheat		
<i>Meloidogyne enterolobii</i>	tomato		
<i>Meloidogyne ethiopica</i>	tomato		
<i>Meloidogyne exigua</i>	tomato		
<i>Meloidogyne fallax</i>	tomato		
<i>Meloidogyne floridensis</i>	tomato		
<i>Meloidogyne graminicola</i>	echinochloa		
<i>Meloidogyne hapla</i>	tomato, pepper, tabacco		
<i>Meloidogyne haplanaria</i>	tomato		
<i>Meloidogyne hispanica</i>	tomato		
<i>Meloidogyne incognita</i>	tomato, corn, tabacco		
<i>Meloidogyne inornata</i>	tomato		
<i>Meloidogyne javanica</i>	tomato	papaya, pineapple	
<i>Meloidogyne kralli</i>	carex acuta		
<i>Meloidogyne luci</i>	tomato		
<i>Meloidogyne mali</i>	elm		
<i>Meloidogyne minor</i>	tomato		
<i>Meloidogyne naasi</i>	wheat, creeping bentgrass		
* when different from rearing			



Globodera rearing conditions

country	species	Host for rearing	Host for maintaining	remarks	Environment	Soil characteristic	Temperature, light, day/night	pot size, etc	water regime
Austria	<i>Globodera spp.</i>	Potato	Potato	desiree/hermes	Greenhouse	mixture of heat sterilized soil with 40% sand	Temp.: 17,5°C – 19,5°C Light: 16h (D), 8 h (N)	pots with 1500 ml soil, Inoculum density: 5 eggs+larve/ml soil	Before sprouts emerge water apply is done manually, after sprouting automatic water supply
Belgium	<i>Globodera spp.</i>	Potato	Potato	mostly Desiree	Greenhouse	artificial substrate Wageningen	max 25 (min 10 h)	2 liter pots	slow-release fertilizer watering weight based about weekly
Canada	<i>Globodera</i>	Potato	Potato	Desiree	climate chamber	sandy loam soil, pH 6-6.5	light: 16h (D), 8 h (N)	20 cm (8 inch) pots	watering once a week
Germany (B)	<i>Globodera spp.</i>	Potato	Potato	Variety Desirée	Greenhouse	Mixed soil, depending on availability	10 kLux, 18°C/16°C		Handwatering as needed
Germany (M)	<i>Globodera spp.</i>	Potato	Potato	Variety Desirée	Climate chamber	silica sand with 10% mole earth	12 hours light; 18/15°C	7cm clay pot	moderately moist, depot fertilizer
France	<i>Globodera spp.</i>	Potato	Potato	Variety Desirée	Greenhouse	potting soil with sand	20-28°C	1.5L pots,	watering regularly depending of need, no watering at the end of the cycle for female cyst maturation
Netherlands	<i>Globodera</i>	Potato	Potato	Bintje	Greenhouse	Fine sand/clay pellets/kaolin/N PK mixture	Day 20C, night 15C; 70% RV; 6-20 hours light	10 l clay pots	watering regularly depending of need, no watering at the end of the cycle for female cyst maturation
UK, Scotland	<i>Globodera</i>	Potato	Potato	G. pal on Maris Piper, G. ros on Desiree	outdoor enclosure- peat plunge	low organic content loam/sand mix	ambient to Scotland	1-4 litre pot	na



Meloidogyne rearing conditions

country	species	Host for rearing	remarks	Environment (greenhouse, lab, climate chamber etc.)	Soil characteristic (growing medium, pH, organic matter, etc)	Temperature , light, day/night	pot size, etc	water regime
Belgium	<i>Meloidogyne</i>	tomato		Greenhouse	Mix of potting soil / sterilized sandy soil: 1/3 or sterilised sandy soil (100%) + 1 tablespoon FLORANID/ 3L pot	> 22°C day (min 12h) >15 °C night	2-3 liter pots depending on space	watering when needed, liquid fertilizer weekly, , weekly cutting, removing flowers
Belgium	Glo + Melo	Potato		closed containers in climate chamber	pure autoclaved river sand	24h dark	30 ml (sterile) water / closed plastic pot of 0,5 liter filled with 200 g dry sand	
Canada	<i>Meloidogyne</i>	tomato	Rutgers	climate chamber	sandy loam soil, pH 6-6.5	light: 16h (D), 8 h (N)	20 cm (8 inch) pots	watering once a week
France	<i>Meloidogyne</i>	tomato	Nainesporom	Greenhouse	potting soil with sand	20-28°C	5L pots	watering regularly depending of need
Netherlands	<i>Meloidogyne</i> temperate	tomato	Domestica	Greenhouse	Fine/course sand mixture with Osmocote	Day 20C, night 15C; 70% RV 6-20 hours light	5 l clay pots	
Netherlands	<i>Meloidogyne</i> tropical			Greenhouse	Fine/course sand mixture with Osmocote	Day/night 22C, 70% RV; 6-20 hours light	5 l clay pots	



Rearing conditions, additional/different

	species	host	Environment (greenhouse, lab, climate chamber etc.)	Soil characteristic (growing medium, pH, organic matter, etc)	Temperature , light, day/night	Water regime, pot size, etc
Austria	PCN	potato	greenhouse	mixture of heat sterilized soil with 40% sand	Temp.: 17,5°C – 19,5°C Light: 16h (D), 8 h (N)	Before sprouts emerge water apply is done manually, after sprouting automatic water supply), 500 ml soil, Inoculum: Cyst inoculum which is available after extraction from field samples but not more than 8 cysts per pot
Germany	PCN	potato	greenhouse	Mixed soil, depending on availabillity	10 kLux, 18°C/16°C	Handwatering as needed
Germany	PCN	potato	nursery bed	sandy soil, open air	natural	10 square meters, fertilization and irrigation as required
UK, Scotland	PCN	potato	outdoor enclosure- peat plunge	low organic content loam/sand mix	ambient to Scotland	1-4 litre pot
USA	M. javanica	papaya or pineapple				



Storage conditions:

country	species	storage	period of time
Austria	Globodera	yes, at 4 degrees	1 year
Belgium	Globodera + Meloidogyne	Belgian Globodera populations and references (Chavornay, Harmerz, Ecosse) stored at 4°C. Cysts are kept in petri-dishes or staining glasses, as picked out cysts or still in the float (if not too many). Several hundreds of cysts. The populations with few cysts from EU and outside were stored at room temperature for months/years and are now stored at 10°C, a few at 4°C. 2 populations (G. rostochiensis and G. pallida) in 2 microplots outside, not multiplied anymore since 2014.	Stored at 4°C: We do not know, have not tested. We assume for 10 years at least.
		Reference material of M. chitwoodi and M. fallax in plant material (potato) and in soil for the diagnostic lab at 4°C. Stored at 10°C-14°C: some tropical RKN populations. At -20°C: juveniles of RKN in Eppendorf tubes, or extracted DNA.	Stored at 4°C: M. chitwoodi and M. fallax in plant material (potato tubers) and in soil 1 year. Soil survival was tested with nematodes stored in soil at 14 ±4 °C for 8 months: between 20 and 44% survived. DNA was still OK for identification with PCR. Stored at 10°C-14°C: tropical nematodes. We assume about 6 months, not tested. Stored at -20°C, we take 5 year as storage time (not tested).
Canada	Globodera	yes, Store at 4°C	> 10 years
	Meloidogyne	yes, Store at 4°C	30 days
Germany (B)	Globodera	yes, in big concrete boxes or plastic tubs mixed with soil	has not been tested yet, expectation is several years
Germany (MV)	Globodera	yes, 4-8 degrees	6 month-6 years
France	Globodera	yes	1 year
	Meloidogyne	no	
Netherlands	Globodera+other:Heterodera/ Punctodera/ Cactodera	yes, airdried cysts 4 C	few month to several years
	Meloidogyne	no	yes
UK, Scotland	Globodera	yes, both extracted from and within dried float material in dry containers in a domestic fridge.	over 20 years
USA	Globodera, Heterodera	yes, at 4 degrees for Globodera, or 10 or 12 degrees for Heterodera in soil in plastic bags	1-3 years



Identification frequency:

country		frequency of confirmation of the identity
Austria	Globodera	at the beginning of the maintaining, rearing or multiplication action
Belgium	Globodera + Meloidogyne	once per year if possible
Canada	Globodera + Meloidogyne	once a year
Germany	Globodera	never
Germany	Globodera	Each year at the beginning of the season for the resistance testing
France	Globodera + Meloidogyne	each year or each test
Netherlands	Globodera/Heterodera/ Punctodera/ Cactodera + Meloidogyne	once a year and every time a population is delivered
UK, Scotland	Globodera	Resistance testing populations are confirmed every 2 years by comparing performance on differential potato clones
USA	Globodera, Heterodera & Meloidogyne	various possibilities between never, yearly, upon arrival and almost montly



Summary (1):

Populations:

- Cysts: 5 species, 1-59 pop. PCN abundant, at 9 places
- *Meloidogyne*: 22 species, 1-35 pop. *M.incognita* & *M.javanica* & *M.hapla* abundant, at 17 (13) places

Storage maximum:

- Cysts: > 20 y. cysts in dry float or without in dry containers at 4 degrees
- *Meloidogyne*: 8 months. in soil at 14 degrees

Identification frequency:

- Cysts: once per year
- *Meloidogyne*: at the beginning of any action



Summary (2):

Growing conditions: in general no big differences in rearing and maintenance for hosts plants, water, light, temperature (5x greenhouse, 2x climate chamber, 1 outdoors)

exception:

- Pot sizes vary (<1-10 liter)
- For *Globodera* use of nursery bed or outdoor-enclosure peat plunge
- For *Globodera* and *Meloidogyne* use of small closed containers in climate rooms for maintenance
- For *Meloidogyne* maintenance on pineapple or papaya

To do: compose a manual on basis of colated information



Aim versus Future:

Will we change our collections and safe some means?

Extend this project between countries to gain information on other nematode genera and species?

questions?

