Final Report

Project title (Acronym)
Inventory of living collections of cyst and root knot nematodes in Europe and their maintenance techniques (Cyst and Melo Collect)

Project duration:

Start date:	2017-03-01
End date:	2019-02-01

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2. Short project report

2.1 Short executive summary

Various countries keep important nematode populations in (reference) collections for the purpose of research and identification. As funding has been scarce on projects concerning fundamental taxonomic research or the maintenance and accessibility of collections and archives, relevant collections cannot be managed optimally and maintained (i.e. indexing, updating) properly (see Euphresco Strategic Research Agenda). The main aim of this project was to gather together experts of nematode collections in different countries with the objective to make an inventory of live nematode collections in the participating countries: a list of the quarantine nematode populations hosted in the various countries was produced. Another objective was to exchange information on how nematodes are reared (maintenance and storage) in the different collections. Due to current legislation, in particular the Nagoya protocol and the new EU Plant Health Regulation, exchange of (reference) material is hampered severely: this has an impact on research collaboration and on the sustainability of reference collections. This point needs attention.

2.2 Project aims

The objectives of the project were:

- to inventory existing live collections of *Globodera* and *Meloidogyne* spp. present in the participating countries and extend it to countries not officially involved in the project, when possible. This inventory will be a useful document to facilitate the retrieval of information (live nematodes, DNA, slides) for diagnostic laboratories involved in test validation or proficiency test activities.
- to collect information on the different maintenance and storage techniques used in the participating countries, and to compare the protocols in order to support standardisation and the adoption of best practices: guidelines for the maintenance of cyst and root knot nematodes will be developed by the end of 2019.

2.3 Description of the main activities

A survey was organised during the Autumn 2017 with the aim to collect information on the live nematode collections in different countries. 32 (reference) collections in Austria, Belgium, Canada, Czech Republic, France, Germany, Italy, Latvia, Mexico, Netherlands, Portugal, Spain, United Kingdom, and United States of America participated in the survey.

A workshop was organised (Wageningen, 2017-09-05/06) jointly by the Dutch National Plant Protection Organization (NVWA) and the European and Mediterranean Plant Protection Organization (EPPO) to gather information on the nematode maintenance and storage techniques; the Workshop presentations can be found on the EPPO website.

The workshop also allowed discussion on other nematodes such as *Bursaphelenchus xylophilus* and look-alikes, *Pratylenchus* and *Ditylenchus* spp. and entomopathogenic nematodes. Collections based on DNA or slides were also discussed. During the Workshop it was proposed to develop a Standard on the maintenance of live nematode collections, using the protocols that were discussed and agreed during the workshop. EPPO will lead the work with the contribution of the scientists involved in the project.

2.4 Main results

Of the 32 mentioned collections the preferred nematode populations are clearly different between the USA and Europe: the tropical *Meloidogyne* species are the most represented in North America while *Globodera* species are in Europe (see Annex I).

The comparison of the protocols for live nematode storage for short and long periods of time showed high variability, although in general, *Globodera* spp. can be stored at 4 °C for longer time (>20 years) than *Meloidogyne* spp., whose optimal storage time is 8 months at 14 °C, when kept in soil.

The need to verify the population for its trueness, with other words, the frequency of identification differs; various possibilities are mentioned between never and almost monthly to frequently, each time the nematodes are extracted from the soil (90 days for PCN, 16 weeks for *Meloidogyne*) to yearly or only upon arrival. The frequency depends on the amount of time people can afford to put into this work and the risk assessment for getting cross contamination.

The conditions for rearing and maintaining were inventoried as well. For *Meloidogyne* spp., host differences for rearing and maintaining them are rarely observed (often *Solanum lycopersicum* -tomato- is used for both), although *Ficus carina* for long maintenance of *Meloidogyne* spp. and *Solanum dulcamara* for *M. fallax* have been used in France (pers. comm. Fabrice Ollivier, ANSES). For cyst nematode species their specific host is used for both rearing and maintenance purposes: *Solanum tuberosum* (potato), *Nicotiana tabacum* (tobacco), or *Glycine max* (soybean) are used respectively for the potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*), *G. tabacum* and *G. glycines*. Details on the hosts used for the rearing and maintaining are listed in Annex II.

During the Workshop, the issue of reference material exchange was discussed. The scientists clearly expressed their interest for collaboration and provided their availability for distributing material but barriers associated to legislation (such as the Nagoya protocol or the new EU Plant Health Regulation) and the administrative burden associated to institutional procedures (material trade agreement), make it difficult to establish international collaborations.

2.5 Conclusions and recommendations to policy makers

Based on the questionnaire and the workshop it can be concluded that the most frequent populations in collections are species of the root knot nematodes *Meloidogyne*, the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* and the pine wood nematode *Bursaphelenchus xylophilus,* often species with quarantine status. Laboratories keep these populations, as immediate access to this material is often needed in the diagnostic process and for research; exchange of populations is a challenge due to administrative (MTA, Nagoya) and plant health regulatory issues. As new regulations within the EU make proficiency testing mandatory for National Laboratories, it is important that reference material is easily accessible and readily available.

2.6 Benefits from trans-national cooperation

The project allowed nematologists from plant protection organisations and institutes in different European and North American countries to collaborate. Nematologists have learned from each other. Precious information on collections not readily available was collected that

will be made public for the benefit of the entire nematologist community and will allow the development of a Standard to support the work of collections.

3. Publications

3.1. Article(s) for publication in the EPPO Bulletin

None

3.2. Article for publication in the EPPO Reporting Service

None

3.3. Article(s) for publication in other journals

None

4. Open Euphresco data

The results of the questionnaire on nematode collections is available from Zenodo:
https://zenodo.org/record/1442874#.W7PSJGgzbIU

Annex I Collections of preferred nematode populations in the USA and Europe

Species	Country where populations are maintained*
Globodera ellingtonae	12,20
Globodera pallida	1,2,4,6,7,8,10,12,13,15,18
Globodera rostochiensis	1,2,3,4,5,6,7,8,10,12,15,18
Globodera tabacum	12,17,26,27,28
Heterodera glycines	16,22,28
Meloidogyne africana	
Meloidogyne ardenensis	12
Meloidogyne arenaria	3,11,12,16,17,21,22,26,27,28,31
Meloidogyne artiellia	12
Meloidogyne chitwoodi	2,11,12,24
Meloidogyne enterolobii	2,11,12,21
Meloidogyne ethiopica	12
Meloidogyne exigua	12
Meloidogyne fallax	2,12
Meloidogyne floridensis	21
Meloidogyne graminicola	
Meloidogyne hapla	2,3,5,6,11,12,16,17,20,23,24,25,26,27,30
Meloidogyne haplanaria	16
Meloidogyne hispanica	12
Meloidogyne incognita	2,3,6,9,11,12,14,16,17,19,21,22,23,25,26,27,28,29,31
Meloidogyne inornata	12
Meloidogyne javanica	6,12,16,17,21,23,26,28
Meloidogyne kralli	12
Meloidogyne luci	12
Meloidogyne mali	2,12
Meloidogyne minor	2,12
Meloidogyne naasi	12,30

*often various populations from 1 species present, for details on origin of populations see excel file

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Annex II Hosts on which nematode populations can be reared or maintained

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