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Organisation of Inter-Laboratory Proficiency Tests: feedback from the Plant Health Laboratory's Nematology Unit after almost 10 years

Elsa Rulliat (elsa.rulliat@anses.fr) (1), Renaud Iooos (renaud.ioos@anses.fr) (2), Laurent Folcher (laurent.folcher@anses.fr) (1)

(1) ANSES, Plant Health Laboratory, National Reference Laboratory, Nematology Unit, Rennes Le Rheu, France.

(2) ANSES, Plant Health Laboratory, National Reference Laboratory, Mycology Unit, Malzéville, France.

This article describes the organisation of Inter-Laboratory Proficiency Tests (ILPTs) in the area of plant health through the example of ILPTs for the detection and identification of the potato cyst nematodes *Globodera pallida* (Stones) Behrens and *G. rostochiensis* (Wollenweber) Behrens. It first presents how participation in the network in France and Europe has changed, then describes the procedures for organising such tests. The main lessons learned by the organising unit are then given. Lastly, the issue of Inter-Laboratory Proficiency Tests in the area of plant health is broadened through other disciplines.

Introduction

In France, plant pests and particularly quarantine pests are monitored by a network of accredited laboratories in charge of undertaking official analyses for the French Ministry of Agriculture. One of the main missions of the National Reference Laboratory (NRL) is to ensure the reliability of such analyses by (i) overseeing this network, (ii) developing reliable and appropriate analytical methods in terms of performance for each intended use and (iii) organising Inter-Laboratory Proficiency Tests (ILPTs) requiring the participation of accredited laboratories and laboratories applying for accreditation. The primary aim of an ILPT is therefore to assess the participating laboratories by ensuring that they have the required capacities (proficiency) to conduct the analyses under their responsibility. As such, ILPTs rely on identical comparative media (test 'samples' or portions) for all of the participating laboratories and the results

are compared with satisfaction criteria (success) that are established before the samples are sent out.

Furthermore, a two-pronged quality approach is used for the organisation of ILPTs and participation in these tests.

- Participation in ILPTs has historically been voluntary with the aim of helping French and European laboratories implement quality assurance. In France, participation is recommended by the guidance document of the French Accreditation Committee, LAB REF 02 (COFRAC, 2012), which aims to provide clients with high-quality, verifiable analytical activities.
- In addition, the quality approach implemented by the Plant Health Laboratory (LSV) is currently being strengthened so as to ultimately offer ILPTs developed in accordance with the ISO 17043 (ISO, 2010) Standard by 2015. This international standard defines general requirements for the competence of providers of proficiency testing schemes and for the

Globodera pallida and *G. rostochiensis*: two quarantine nematodes

These two nematodes are obligate parasites specific to solanaceous crops, especially potatoes. Native to the Andes and particularly Peru (Picard *et al.*, 2004), they have proliferated widely through the spread of potato crops and can now be found on all continents. Protected inside a cyst (Photo 1), a form of preservation for these parasites, the larvae (Photo 2) can survive in the soil for around ten years in the absence of a host plant (Wright and Perry, 2006). This form of preservation gives them a dispersive advantage particularly through trade, agricultural tools and other physical means of transport.

Severe reductions in yield (Greco *et al.*, 1982) linked to the damage caused to potato crops by these parasites justify their classification as quarantine pests under Directive 2000/29/EC of 8 May 2000 (Anonymous, 2000) and therefore the adoption of mandatory control measures. Specifically, this European regulation stipulates (i) that potato tubers (*Solanum tuberosum* L.) intended for planting must come from fields free from these two nematodes and (ii) that potato plants must be produced on uncontaminated land.

Therefore, compliance with the regulations in force necessarily implies the use of nematode extraction techniques based on samples of soil and underground plant parts, and species identification techniques. In France, the National Reference Laboratory is responsible for developing such methods, which are officially published and applied by accredited French laboratories.

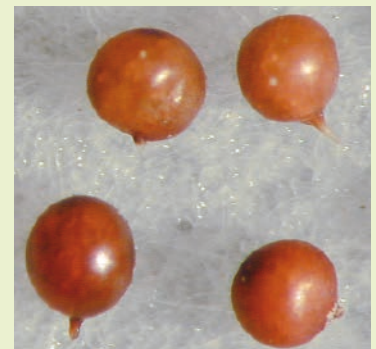


Photo 1. Nematode cysts of the *Globodera* genus (Source, LNPV).



Photo 2. Anterior end of *Globodera* larva (Source, LNPV).



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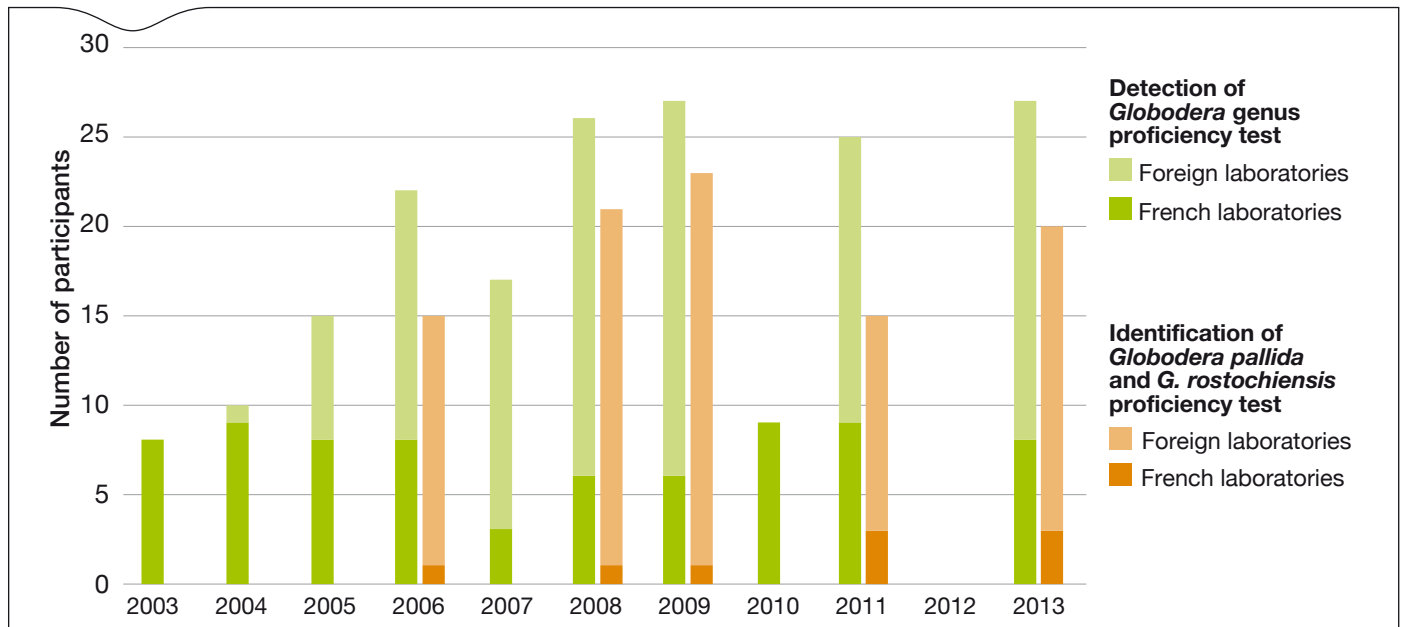


Figure 1. Changes in the number of French and foreign laboratories participating in ILPTs for the “Detection of *Globodera* genus nematodes” and the “Identification of the *G. pallida* and *G. rostochiensis* species”.

development and operation of such schemes. This process helps improve the reliability of the official analyses undertaken by the network of accredited and reference laboratories.

The Nematology Unit, which is the NRL for plant-parasitic nematodes, has been organising ILPTs for the potato cyst nematodes *Globodera pallida* (Stones) Behrens and *G. rostochiensis* (Wollenweber) Behrens for almost ten years (Box 1). After an initial section describing changes in participation, the second part of the text will present the methodology used in 2013. The last part of the article will be devoted to feedback from the NRL regarding ILPTs.

Changes in participation in ILPTs dedicated to *Globodera* genus nematodes

The Nematology Unit has been organising ILPTs for the detection of the *Globodera* genus for ten years and for the identification of the *G. pallida* and *G. rostochiensis* species for five; there have been significant changes over this period.

In 2003, the first ILPT devoted to the detection of the *Globodera* genus was organised and was initially open exclusively to French laboratories. Then in 2004, certain European laboratories were invited to participate on a voluntary basis. The number of ILPT participants has since increased over the years.

From 2003 to 2006, the French participants were mainly French Regional Plant Protection Laboratories (LRPVs) and professional laboratories. After 2006, when the network of laboratories in France was restructured, giving rise to the gradual disappearance of these LRPVs, the number of participating French laboratories decreased while the proportion of foreign laboratories rapidly increased (Figure 1). Also in 2006, the first ILPT for the identification of the *G. pallida* and *G. rostochiensis* species was held and was almost exclusively intended for

participants from other European countries (Figure 1). The panel of participating European laboratories is made up of National Reference Laboratories, regional laboratories and professional laboratories. As such, over the past ten years, a total of 54 laboratories⁽¹⁾ from 22 countries have participated in an ILPT session for the detection of *Globodera* genus nematodes (Figure 2), whereas 36 laboratories⁽¹⁾ from 20 different countries have participated in ILPTs for the identification of the *G. pallida* and *G. rostochiensis* species (Figure 2).

Organisation of ILPTs dedicated to *Globodera* genus nematodes

The key stages of the methodology used in 2013 are summarised chronologically in Table 1. The analysis stage is described in detail below.

Analytical methods

The analyses undertaken by French laboratories are described in the official methods for the “Detection of *Globodera* genus nematodes” (Anonymous, 2011) and the “Identification of *Globodera pallida* and *G. rostochiensis* by morpho-biometric and biomolecular analysis” (Anonymous, 2012). It should be noted that French participants, and particularly accredited laboratories and laboratories applying for accreditation, are required to strictly follow these methods. Foreign participants can use the method of their choice, generally the one that is routinely used in their laboratory. The requirements specific to each ILPT are described below:

- ILPT for the “Detection of the *Globodera* genus”: regardless of the origin of the participating laboratories, soil samples undergo an extraction process usually using a Seinhorst elutriator or a Schuiling centrifuge (or any other similar device).

(1) Each participating laboratory has been counted only once for the entire period in question.



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Table 1. The main organisational stages for the 2013 “Detection of the *Globodera* genus” and “Identification of *Globodera pallida* and *G. rostochiensis*” ILPTs

Stage	Details
Call for applications	The participants are: <ul style="list-style-type: none"> • in France, laboratories accredited by the Ministry of Agriculture and laboratories applying for accreditation; • in Europe, National Reference Laboratories, regional and professional laboratories.
Preparation of comparative media	The panel subject to analysis is made of 10 samples identified by a unique code. Each sample contains: <ul style="list-style-type: none"> • for the “Detection of the <i>Globodera</i> genus” ILPT, soil that is free from or has been artificially infested with <i>Globodera</i> sp. cysts (several contamination levels); • for the “Identification of <i>Globodera pallida</i> and <i>G. rostochiensis</i>” ILPT, 3 isolated cysts.
Analyses (See § on Analytical methods)	The method used is: <ul style="list-style-type: none"> • for French laboratories, the official analysis method (MOA019); • for foreign laboratories, the method of their choice, which is generally the one they routinely use.
Test report: notification of compliance or non-compliance of results	Expected performance levels: <ul style="list-style-type: none"> • 100% sensitivity (all positive samples are found positive by the participating laboratory); • 100% specificity (all negative samples are found negative by the participating laboratory); • 100% accuracy (summary of the above two criteria).
Satisfaction survey	The results of the satisfaction survey are processed so as to continuously improve the organisation of ILPTs.

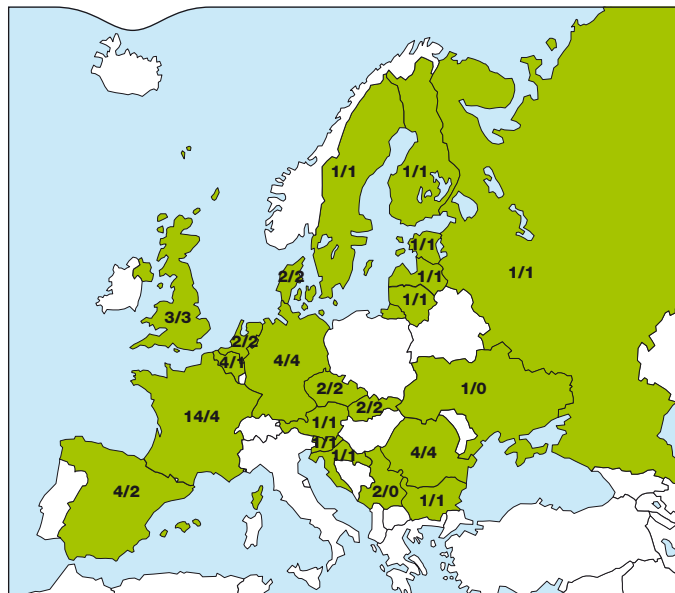


Figure 2. Distribution and number of laboratories participating in the “*Globodera*” ILPTs organised by the LSV’s Nematology Unit. Left-hand figure: total number* of laboratories that participated in the ILPT for the “Detection of *Globodera* genus nematodes” from 2003 to 2013; right-hand figure: total number* of laboratories that participated in the ILPT for the “Identification of *G. pallida* and *G. rostochiensis* species” from 2006 to 2013.

* Each participating laboratory has been counted only once for the entire period in question.

The extract is then examined with a stereomicroscope to screen for cysts without vulval cones. Morphological criteria are taken into consideration for this (presence or absence of vulval cones among other things) to identify the genus. Therefore, for each sample, the participating laboratories must decide whether or not there are cysts without vulval cones and then return the detected cysts to the organiser for confirmation of the genus.

- ILPT for the “Identification of *G. pallida* and *G. rostochiensis*”: French laboratories, applying the official method, base their identification on morpho-biometric criteria applicable to cysts (Granek’s ratio which is the distance from the vulva to the anus divided by the diameter of the vulva; number of cuticular ridges found between the vulva and the anus) and larvae (shape of stylet knobs and stylet size). This identification is supplemented by a molecular analysis (DNA amplification by conventional PCR) of the larvae. The results obtained with these two techniques are then compared and a conclusion on the final status of the sample is issued. Foreign laboratories can use the method of their choice (morpho-biometric and/or biomolecular). Like above, for each sample, the laboratories decide as to the presence or absence of *G. pallida* and/or *G. rostochiensis*.

Feedback on ILPTs dedicated to *Globodera* genus nematodes

The organiser’s feedback

The organisation of ILPTs first requires the production and maintenance of biological reference materials. To that end, the Nematology Unit has been maintaining a collection of species and species populations since 2003. The growing number of participants in the two ILPTs has required the production of appropriate reference materials for the preparation of comparative media.



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To ensure the conformity of these materials, the Nematology Unit has improved its verification processes over the years. Specifically for the ILPT for the “Detection of the *Globodera* genus”, this involves conducting preliminary tests in the matrix (soil) to guarantee the absence of target organisms (cysts without vulval cones such as *Globodera* sp. and *Punctodera* sp.). Moreover, to prevent the risk of cross contamination, the preparation of samples at various levels of contamination is separated over time and/or space (preparation of healthy samples and then contaminated samples in dedicated areas of the laboratory). Furthermore, 20 samples per contamination level undergo homogeneity tests before the panels are sent to the participating laboratories. Stability tests are not necessary since *Globodera* sp. cysts can persist for several decades (Wright and Perry, 2006). Lastly, dual verifications, undertaken by different operators, are performed to ensure (i) each sample’s compliance with the level of contamination and (ii) agreement between the reference (code) assigned to each sample and its status.

Other practical aspects can be raised, related to the development of procedures, specific documentation and data input. For example, each participating laboratory signs a participation contract setting forth each party’s commitments, among other things. Starting in 2013, to limit errors in the reproduction of results, the organising laboratory will ask the participants to submit their results electronically, preferably on a form (Word document) sent by email.

An analysis of the results obtained through the various ILPT sessions for the “Detection of the *Globodera* genus” (Ladevèze and Anthoine, 2010) showed that the likelihood of detecting a cyst in a sample is not related to the total number of cysts occurring. Therefore, the likelihood of detecting at least one cyst in a sample containing n cysts is defined by the following formula: $P = 1 - (1-p)^n$. This relationship has been used to determine more appropriate levels of contamination above the limit of detection to be submitted to the participating laboratories.

Participant feedback

For French laboratories, the main objectives are to comply with (i) the official method in force when undertaking analyses and (ii) the deadlines for conducting analyses and submitting results. An analysis of the results obtained from the ILPT for the “Detection of the *Globodera* genus” showed that some participants mistook nematode cysts for other elements (seeds, propagules other than nematode cysts, etc.). Although this sorting error was then corrected in the identification stage, it shows that it is difficult to delegate methods based on morphological criteria. The ILPTs for the “Identification of *Globodera pallida* and *G. rostochiensis*” that have been organised since 2006 have confirmed the benefits of combining morpho-biometric and biomolecular analysis techniques rather than using just one analysis technique (biomolecular or morpho-biometric), to guarantee more reliable results (Ladevèze and Anthoine, 2010).

Conclusion and outlook

Like the Nematology Unit, all of the Plant Health Laboratory’s units now organise Inter-Laboratory Proficiency Tests in their areas of expertise (see 2013 timetable given in this issue of *EuroReference*). This has occurred slightly more recently, with the delegation of official analyses to the network of accredited laboratories. Again, just like in the unit used as an example in this article, participation in these ILPTs is gradually opening up to European laboratories, particularly the reference laboratories of other European Union Member States and countries with which the laboratory collaborates. Documents are gradually being translated into English alongside the implementation of the quality approach in accordance with the ISO 17043 standard to allow these foreign countries to participate. These countries have responded extremely favourably to this type of proposal, as there are very few ILPT providers (or next to none for certain disciplines such as mycology and entomology) in the area of plant health in Europe.

It should however be noted that the participating laboratories must be authorised, by their official services, to receive samples containing quarantine pests.

While the laboratories participating under an accreditation for the French authorities are required to adhere to the official methods, in most cases, foreign participants can use the detection protocol of their choice for the proposed samples. These protocols rely on different approaches (morphometric, biomolecular, serological, etc.).

References

- Anonymous. 2000. 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. The Council of the European Union, L169/1-L169/106.
- Anonymous. 2011. Detection of *Globodera* genus nematodes. MOA019 part A version 1a. 23 pp. [accessed on 25 March 2013, in French] <http://www.anses.fr/sites/default/files/documents/LABO-Ft-MOA019-Globo.pdf>
- Anonymous. 2012. Identification of *Globodera pallida* and *G. rostochiensis* by morphobiometric and biomolecular analysis. MOA019 part B version 1a, 28 pp. [accessed on 25 March 2013, in French] <http://www.anses.fr/sites/default/files/documents/LABO-Ft-MOA019B-ident-Globo.pdf>
- COFRAC. 2012. LAB REF 02: Requirements for the accreditation of laboratories in accordance with the NF EN ISO/CEI 17025 standard. Revision 07: 54 pp. [accessed on 25 March 2013, in French] <http://www.cofrac.fr/documentation/LAB-REF-02>
- Greco N, Di Vito M, Brandonisio A, Giordano I, De Marinis G. 1982. The effect of *Globodera pallida* and *G. rostochiensis* on potato yield. *Nematologica*, 28: 379-386
- ISO. 2010. ISO/IEC 17043 – General requirements for proficiency testing: 41 pp.
- Ladevèze L, Anthoine G. 2010. Outcomes of a seven-year proficiency test for the detection and identification of potato cyst nematodes. Proceedings of the 3rd symposium on potato cyst nematodes, Harper Adams University College, UK, 14-15 September 2010. *Aspects of Applied Biology*, 103: 1-9
- Picard D, Plantard O, Scurrah M, Mugniéry D. 2004. Inbreeding and population structure of the potato cyst nematode (*Globodera pallida*) in its native area (Peru). *Molecular Ecology* 13: 2899-2908
- Wright DJ, Perry RN. 2006. Reproduction physiology and biochemistry. In: *Plant Nematology*. CABI publishing, Wallingford, UK: 187-209