



# **EUPHRESCO Final Report**

## for Non-Competitive research projects

Please send the final report to all your project partners, to the NC topic coordinators and to the EUPHRESCO Secretariat ([euphresco@fera.gsi.gov.uk](mailto:euphresco@fera.gsi.gov.uk)).

### ***Potato cyst nematodes:***

Ring testing methods for identification and resistance testing

#### **Project Duration:**

|                    |          |
|--------------------|----------|
| <b>Start date:</b> | 01/09/08 |
| <b>End date:</b>   | 31/12/09 |

## 1. Research Consortium Partners

| Coordinator 1 – Partner 1               |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Plantenziektenkundige Dienst   |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Loes den Nijs  | <b>Gender:</b> | F |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Postbus 9102 NL-6706 EA Wageningen, The Netherlands                        |                |   |
| <b>E-mail</b>                           | <a href="mailto:l.j.m.f.den.nijs@minlnv.nl">l.j.m.f.den.nijs@minlnv.nl</a> |                |   |
| <b>Phone</b>                            | +31 317-496911   |                |   |

| Coordinator 2 - Partner 2               |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Institute for Agricultural and Fisheries Research (ILVO)                               |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Nicole Viaene  | <b>Gender:</b> | F |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Burgemeester Van Gansberghelaan 96 bus 2, B-9820 Merelbeke, Belgium                    |                |   |
| <b>E-mail</b>                           | <a href="mailto:nicole.viaene@ilvo.vlaanderen.be">nicole.viaene@ilvo.vlaanderen.be</a> |                |   |
| <b>Phone</b>                            | +32 92722425   |                |   |

| Partner 3                               |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Austrian Agency for Health and Food Safety (AGES), Institute of Plant Health (PGH) |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Ines Gabl  | <b>Gender:</b> | F |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Spargelfeldstr. 191, A-1220 Vienna, Austria  |                |   |
| <b>E-mail</b>                           | <a href="mailto:ines.gabl@ages.at">ines.gabl@ages.at</a>                           |                |   |
| <b>Phone</b>                            | + 43 0 50555-33326   |                |   |

| <b>Partner 4</b>                        |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Plant Protection Institute, Central Laboratory of Plant Quarantine |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Olia Karadjova   | <b>Gender:</b> | F |
| <b>Job Title</b>                        | Director   |                |   |
| <b>Postal Address</b>                   | 35 P. Volov St., 2230 Kostinbrod, Bulgaria                         |                |   |
| <b>E-mail</b>                           | <a href="mailto:oliakaradjova@abv.bg">oliakaradjova@abv.bg</a>     |                |   |
| <b>Phone</b>                            | + 359 72166061   |                |   |

| <b>Partner 5</b>                        |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Laboratoire National de la Protection des Végétaux   |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Géraldine Anthoine   | <b>Gender:</b> | F |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Domaine de la Motte, BP35627, F-35653 LE RHEU cedex, France  |                |   |
| <b>E-mail</b>                           | <a href="mailto:geraldine.anthoine@agriculture.gouv.fr">geraldine.anthoine@agriculture.gouv.fr</a> |                |   |
| <b>Phone</b>                            | + 33 2 23 48 52 08   |                |   |

| <b>Partner 6</b>                        |   |                |   |
|---|---|----------------|---|
| <b>Organisation</b>                     | GEVES-SNES (Station Nationale d'Essais de Semences)   |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Valérie Grimault                                  | <b>Gender:</b> | F |
| <b>Job Title</b>                        |   |                |   |
| <b>Postal Address</b>                   | Rue G Morel, BP90024, F-49071 Beaucouzé Cedex, France |                |   |
| <b>E-mail</b>                           | valerie.grimault@geves.fr                             |                |   |
| <b>Phone</b>                            | + 33 2 41 22 58 50                                    |                |   |

| <b>Partner 7</b>                        |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen - Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Björn Niere  | <b>Gender:</b> | M |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Messeweg 11/12, DE-38104 Braunschweig, Germany   |                |   |
| <b>E-mail</b>                           | <a href="mailto:bjoern.niere@jki.bund.de">bjoern.niere@jki.bund.de</a>   |                |   |
| <b>Phone</b>                            | + 49 531-299 3382  |                |   |

| <b>Partner 8</b>                        |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Central Agricultural Office Directorate of Plant Protection, Soil Conservation and Agri-environment, Central Laboratory for Pest Diagnosis |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Ms. Ágnes TÓTH   | <b>Gender:</b> | F |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | 1118 Budapest, Budaörsi út 141-145, Hungary  |                |   |
| <b>E-mail</b>                           | <a href="mailto:Toth.Agnes@ntai.ontsz.hu">Toth.Agnes@ntai.ontsz.hu</a>   |                |   |
| <b>Phone</b>                            | + 36 1 309 1000 / 1814   |                |   |

| <b>Partner 9</b>                        |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | C.R.A. - Istituto Sperimentale per la Zoologia Agraria                   |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Beatrice Carletti  | <b>Gender:</b> | F |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Via di Lanciola 12/a, I-50125 Firenze, Italia                            |                |   |
| <b>E-mail</b>                           | <a href="mailto:beatrice.carletti@isza.it">beatrice.carletti@isza.it</a> |                |   |
| <b>Phone</b>                            | + 39 055-2492.250  |                |   |

| <b>Partner 10</b>                       |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Dpto: Protección Vegetal, Grupo de Patología Vegetal: hongos y nematodos<br>INIA |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Antonieta De Cal   | <b>Gender:</b> | F |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Ctra de La Coruña, km 7.500, ES-28040 Madrid, Spain                              |                |   |
| <b>E-mail</b>                           | <a href="mailto:cal@inia.es">cal@inia.es</a>                                     |                |   |
| <b>Phone</b>                            | + 34 91 347 6846   |                |   |

| <b>Partner 11</b>                       |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Plant Protection Central Research Institutes   |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Mr. Emre Evlice  | <b>Gender:</b> | M |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Gayret Mah. Fatih Sultan Mehmet Bulvarı No: 66 P.K. 49, TR-06172<br>Yenimahalle/ANKARA, Turkey |                |   |
| <b>E-mail</b>                           | <a href="mailto:emreevlice@gmail.com">emreevlice@gmail.com</a>                                 |                |   |
| <b>Phone</b>                            | + 90 312 3445994/145   |                |   |

| <b>Partner 12</b>                       |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | State Phytosanitary Administration, Diagnostics dpt.           |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Vladimir Gaar  | <b>Gender:</b> | M |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Ztracena 10/1099, 161 00 Prague 6, Czech Republic              |                |   |
| <b>E-mail</b>                           | <a href="mailto:vladimir.gaar@srs.cz">vladimir.gaar@srs.cz</a> |                |   |
| <b>Phone</b>                            | + 420 235010310  |                |   |

| <b>Partner 13</b>                       |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Science and Advice for Scottish Agriculture, Scottish Government           |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Jon Pickup   | <b>Gender:</b> | M |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Roddinglaw Road , Edinburgh EH12 9FJ, UK                                   |                |   |
| <b>E-mail</b>                           | <a href="mailto:Jon.pickup@sasa.gsi.gov.uk">Jon.pickup@sasa.gsi.gov.uk</a> |                |   |
| <b>Phone</b>                            | + 44 131-2448859   |                |   |

| <b>Partner 14</b>                       |  |                |   |
|---|--|----------------|---|
| <b>Organisation</b>                     | Agricultural Institute of Slovenia                         |                |   |
| <b>Name of Contact</b><br>(incl. Title) | Dr. Gregor Urek  | <b>Gender:</b> | M |
| <b>Job Title</b>                        |  |                |   |
| <b>Postal Address</b>                   | Hacquetova 17, 1000 Ljubljana, Slovenija                   |                |   |
| <b>E-mail</b>                           | <a href="mailto:gregor.urek@kis.si">gregor.urek@kis.si</a> |                |   |
| <b>Phone</b>                            | + 386 1 280 52 62  |                |   |

## 2. Executive Summary

### Project Summary

Please provide a summary suitable for web publication and which is understandable to the intelligent non-scientist.

Include: Title, main objectives, appropriate Methods, Results and Conclusions. (max. 2 pages)

### **Potato cyst nematodes: ring testing methods for identification and resistance testing.**

#### **Introduction:**

Potato cyst nematodes (PCN) *Globodera pallida* and *Globodera rostochiensis* cause serious damage to potato crops world-wide by restricting root growth and uptake of nutrients, leading to losses in tuber yield. They are considered quarantine organisms in many countries. Management of the populations to undamaging levels is priority number one and eradication is a major aim although hardly feasible. In the EU, a control directive is active against these nematodes: the EU directive 2007/33/EC. The do's and don'ts are described in this directive, especially for seed potatoes. This document also describes how the resistance level of potatoes against these nematodes has to be assessed before cultivars are allowed on the market and be used as a management tool.

Resistant cultivars play the most important role in the management of PCN. Many potato cultivars are totally resistant to *G. rostochiensis*, but none are fully resistant to *G. pallida*. Moreover, the choice of cultivars with resistance to *G. pallida* is rather restricted as not many cultivars are available. Careful and correct identification of the species of *Globodera* present in a field is therefore very important. This can be done using morphological characteristics and biochemical methods. Protocols for species identification based on biochemical techniques include immunology, protein electrophoresis, IEF techniques and DNA-based methods. Of the latter, several PCR-protocols with species-specific primers have been published. Identification methods are described in an EPPO diagnostic protocol.

Next to differentiation into species, different populations of the same species of potato cyst nematodes can show different multiplication patterns depending on the potato variety or clone. When populations can multiply on resistant cultivars or clones, they are virulent and often called a certain pathotype. Virulence of a cyst population is measured by its multiplication rate (final population density Pf/ initial population density Pi) on a specific potato variety or clone. The two terms (pathotype and virulence group) are often used together, in the EU directive 2007/33/EC as well.

Standard cyst populations, belonging to known pathotypes, have to be used to test the resistance of new potato cultivars. They are listed in the Council Directive 2007/33/EC, where a resistance testing procedure is described to evaluate new potato accessions for their resistance against different PCN pathotypes/virulence groups

#### **Main objective**

The main goal of the project was to investigate if the joining countries can identify the potato cyst nematode to species level and that the standard resistance testing method, described in the new control directive EU directive 2007/33/EC, can be performed. The aim of this project is thus twofold: to learn and validate test protocols for (1) identification and for (2) resistance testing.

#### **Methods**

A proficiency test for species identification was held:

Four different species of cysts (*Globodera pallida*, *G. rostochiensis*, *G. tabacum*, *Heterodera schachtii*) were sent in two-fold to each participant for identification up to species level. The eight coded vials each contained 5 cysts of a same species. The diagnostic EPPO-protocol PM 7-40 was used; this protocol allows each country to perform its own identification method within the limits of the protocol. A form was sent to the participants to fill in the details of the identification method performed.

A ring test for resistance testing was held:

Appendix V of the Control Directive 2007/33/EC was taken as an initial starting point for the writing of an extended manual in which all procedures used in the screening test are described in detail. In a general (start) meeting, held on the 6<sup>th</sup> of October 2008 in Ghent (Belgium), this manual was walked

through and some points were stressed to assure good performance. However, each country was allowed to adapt within the given freedom of the appendix V of 2007/33/EC.

The inoculum, *Globodera pallida* Pa3 Chavornay, was provided by the organizer, the Dutch Plant Protection Service (PPS). The potato tubers, also provided by the Dutch PPS, consisted of the susceptible variety Desiree, the *G. pallida* resistant variety Innovator and 3 unknown varieties with various resistance levels. The aim of the test was to determine the resistance levels of these unknown varieties O, Y and Z to the standard pathotype 3 (Chavornay). Cysts and tubers were sent to all and each participant received a form to record conditions and all other relevant information of the test. A final meeting was held to discuss all results on 9 October 2009 in Ghent (Belgium).

## Results:

### Species identification test:

In total 15 participants from 13 countries joined the proficiency test. Almost all participants used PCR methods (13 out of 15), additionally or solely. They all obtained good results with some exceptions. One participant had identified the potato cyst nematodes correctly using morphology but mentioned that the ITS fragments did not react at all. The two participants that used only morphology were able to identify the potato cyst nematode species with one exception. The *G. tabacum* cysts however created some more difficulties; the identification was more often wrong than for the potato cysts. The bad state of the cyst contents was obviously not helpful. For the statutory point of view, the only thing that matters is to make a distinction between the potato cyst nematodes and the non-potato cyst nematodes. The positive success rate was 87% for *G. rostochiensis* and 90% for *G. pallida*, no false positives were found for both species. When PCR was used, the non-potato cyst nematodes gave correctly negative results; all potato cyst nematodes were identified (with the exceptions as mentioned above). The morphological identification gave four times an incorrect result on the *G. tabacum* identity, making it 23% false positive results for the potato cyst nematodes. The Heterodera cysts were identified correctly as being not *Globodera* cysts, all were identified to be *H. schachtii* or suggested to be this species.

### Resistance test:

All participants (14 from 12 countries) fulfilled the demand of sending the results at the end of September 2009 with one (late) exception, but finally all results were analyzed. The final results showed that all participants, except one, fulfilled the official criteria mentioned in the EU directive: Pf/Pi-values of 20 or more on the susceptible combination (all countries), overall variance of coefficient below 35% (all except one).

The resistance levels found by each participant for the three unknown varieties are expressed as % relative susceptibility compared to Desirée, and with the scoring according to the standard scoring rate (1 to 9) of the EU Council Directive 2007/33/EC. The three unknown varieties were Santé, Simply Red and Mondial and their resistance under Dutch testing conditions with Pa3-population E400 (Rookmaker) are respectively 3, 6 and 2. The mean value of the relative susceptibility of the different varieties compared to Desiree did not differ much when calculated based on cyst counts or based on counts of eggs+juveniles, being for Innovator 1.5 and 1.1 (cysts versus eggs), for Santé 33.2 and 28.8, for Simply Red 10.7 and 7.7 and for Mondial 85.7 and 89.0%. The translation to the scoring gave the same results, resp. 3, 6 and 2. It is clear that some countries have deviating results, and these outlier values influence the mean considerably. This is important for each country to realize as in practice tests are only replicated twice (in two separate years), whereas now it was performed 14-fold. The results on Mondial were much more variable in general but specific outliers were not recognizable. Effects of differences in parameters were not very strong, as one of the expected variances could have been the inoculum type such as cysts or eggs suspension. In the results these effects were not noticeable. Also the circumstances in which the experiment were performed, e.g. greenhouse, climate chamber,... did not have an apparent effect on the outcome. In general, a clear reason for the outliers could not be given and the results did not point to an obvious cause. Therefore it can be concluded that the method described in the control directive, with the freedom of choosing certain parameters and performed properly, gives rise to adequate results.

## Conclusions:

As one of the aims of Euphresco is to expand networks and cooperate with NPPOs, this project fulfilled its purpose as a network of nematologists in NPPOs has been developed by performing these two ring tests. The tests gave good results and cooperation in future is planned.

Testing *Globodera* populations for their virulence should now be possible for every country as the basic techniques for this kind of tests were practiced.



### 3. Report

This report is to provide EUPHRESKO and others with the outputs of the research project to allow EUPHRESKO to publish details of the outputs.

This report does not preclude contractors from publishing results in scientific journals or elsewhere. Also, this report does not release research partners from any national reporting obligations required by national contracts.

The report should be in Arial Font size 12 for normal text and size 14 bold for headings. It should consist of maximal 20 pages.

The report to EUPHRESKO should include:

- **Objectives and tasks of the project**, as stated in the work plan, with degree of achievement
- **Methods used and Results obtained**, including statistical analysis (if appropriate)
- **Discussion of results and their reliability**
- **Main conclusions**, including:
  - The expected benefits and usability of results (technology transfer)
  - Implication for stakeholders and policy
  - Recommendations for future work (on the activities or other steps that may be taken to further develop, disseminate or to uptake the results of the project)
- Papers, other publications and dissemination activities done.
- Acknowledgements
- References
- Possible Appendices: problems encountered, delays and corrective actions taken (if any), publications and (planned) dissemination activities, and possibly terms and definitions, abbreviations, protocols.

The report of this project (Potato cyst nematodes: ring testing methods for identification and resistance testing) consists of three parts:

- the report on the ring test on identification (I),
- the report on the ring test on resistance testing (II) and
- the main conclusions with recommendations for future work (III).

# I. Potato cyst nematodes: ring testing of methods for identification.

PROJECT COORDINATORS: Loes den Nijs and Nicole Viaene

PARTICIPANTS: Austria, Belgium, Bulgaria (2x), Czech republic, France, Germany, Hungary, Italy, The Netherlands (2x), Spain, Slovenia, Turkey, United Kingdom (see appendix 1 for details)

## **INTRODUCTION:**

Potato cyst nematodes (PCN) *Globodera pallida* and *Globodera rostochiensis* cause serious damage to potato crops world-wide by restricting root growth and uptake of nutrients, leading to losses in tuber yield. PCN are included in the EPPO A2 list and are the scope of specific Council Directive 69/465/EEC, repealed by the new directive 2007/33/EC of June 11, 2007, on the control of potato cyst nematodes.

For taking the correct control measures careful and correct identification of the species of *Globodera* in a field is very important. This can be done using morphological characteristics and biochemical methods. Protocols for species identification based on biochemical techniques include immunology, protein electrophoresis, IEF techniques and DNA-based methods. Of the latter, several PCR-protocols with species-specific primers have been published. Identification methods are described in an EPPO diagnostic protocol (PM 7/40, in revision).

A proficiency test for species identification was held. This test is part of the *Globodera* ring test project within Euphresco.

## **Material and methods:**

Four different species of cysts (*Globodera pallida*, *G. rostochiensis*, *G. tabacum*, *Heterodera schachtii*) were sent in two-fold to each participant for identification up to species level. The eight coded vials each contained 5 cysts of a same species. The diagnostic EPPO-protocol PM 7-40 was used; this protocol allows each country to perform its own method within the limits of the protocol.

A form was sent to the participants to fill in the details of the identifications performed.

## **Results:**

In total 15 participants from 13 countries joined the proficiency test. Results are shown in Table 1 and in detail in appendix 2. Letters correspond with a participant; all participants are informed about the letter they correspond with.

Almost all participants used PCR methods (13 out of 15), additionally or solely. They all gave good results with the exception of participants G, K and D. We suspect that participant D switched the samples as the cysts were identified wrongly. After informing participant K, they declared that mistakes were made in the laboratory by the performance of the test resulting in wrong identifications. Participant G had identified the potato cyst nematodes correctly using morphology but mentioned that the ITS fragments did not react at all.

The two participants (A and I) that used only morphology were able to identify the potato cyst nematode species with one exception.

The *G. tabacum* cysts however created some more difficulties; the identification was more often wrong than for the potato cysts. The bad state of the cyst contents was obviously not helpful. In addition, one participant did not identify up to species level as they thought that excluding PCN was enough.

For the statutory point of view, the only thing that matters is to make a distinction between the potato cyst nematodes and the non-potato cyst nematodes. The positive success rate was 87% for *G. rostochiensis* (table 1A) and 90% for *G. pallida* (table 1B), no false positives were found for both species. When PCR was used, the non-potato cyst nematodes gave correctly negative results; all potato cyst nematodes were identified (with the exceptions as mentioned above). The morphological identification gave four times an incorrect result on the *G. tabacum* identity, making it 23% false positive results (table 1 C) for the potato cyst nematodes.

The Heterodera cysts were identified correctly as being not Globodera cysts, all were identified to be *H. schachtii* or suggested to be this species.

**Table 1: General results proficiency test *Globodera* spp. 2008**

Number of participating labs: 15  
 Date preparation of samples: 26/9/2008  
 Date sending of samples : 6/10/2008  
 Date receipt first analysis report:17/11/2008  
 Date receipt last analysis report: 03/03/2009

A.

| Lab code | Identification method used | main test for <i>Globodera rostochiensis</i> |                     |                     |
|----------|----------------------------|--|---------------------|---------------------|
|          |                            | success rate (%)                             | false positives (%) | false negatives (%) |
| A        | m                          | 100  | 0                   | 0                   |
| B        | m + PCR                    | 100  | 0                   | 0                   |
| C        | m + PCR                    | 100  | 0                   | 0                   |
| D        | m + PCR                    | 0  | 0                   | 100                 |
| E        | m + PCR                    | 100  | 0                   | 0                   |
| F        | m + PCR + RFLP             | 100  | 0                   | 0                   |
| G        | m + PCR + RFLP             | 100  | 0                   | 100 (PCR)           |
| H        | m+PCR+RFLP+O               | 100  | 0                   | 0                   |
| I        | m                          | 100  | 0                   | 0                   |
| J        | m + PCR                    | 100  | 0                   | 0                   |
| K        | PCR                        | 0  | 0                   | 100                 |
| L        | m + PCR + RFLP             | 100  | 0                   | 0                   |
| M        | m + PCR                    | 100  | 0                   | 0                   |
| N        | PCR                        | 100  | 0                   | 0                   |
| O        | m + PCR                    | 100  | 0                   | 0                   |
| average  |                            | 87%  | 0                   | 20%                 |

B.

| Lab code number | main test for <i>Globodera pallida</i> |                     |                     |
|-----------------|--|---------------------|---------------------|
|                 | success rate (%)                       | false positives (%) | false negatives (%) |
| A               | 100                                    | 0                   | 0                   |
| B               | 100                                    | 0                   | 0                   |
| C               | 100                                    | 0                   | 0                   |
| D               | 0                                      | 0                   | 100                 |
| E               | 100                                    | 0                   | 0                   |
| F               | 100                                    | 0                   | 0                   |
| G               | 100                                    | 0                   | 100 (PCR)           |
| H               | 100                                    | 0                   | 0                   |
| I               | 50                                     | 0                   | 50                  |
| J               | 100                                    | 0                   | 0                   |
| K               | 100                                    | 0                   | 0                   |
| L               | 100                                    | 0                   | 0                   |
| M               | 100                                    | 0                   | 0                   |
| N               | 100                                    | 0                   | 0                   |
| O               | 100                                    | 0                   | 0                   |
| average         | 90%                                    | 0                   | 17%                 |

C.

| Lab code number | optional test for <i>Globodera tabacum</i> |                                 |                              |
|-----------------|--|---------------------------------|------------------------------|
|                 | success rate (%)                           | false positives (%)             | false negatives (%)          |
| A               | 0  | 50 ( <i>G. pallida</i> )        | 50                           |
| B               | 0  | 0                               | 0 (no identification)        |
| C               | 0  | 0                               | 0 (no identification)        |
| D               | 0  | 0                               | 100 ( <i>G. artemisiae</i> ) |
| E               | 100  | 0                               | 0                            |
| F               | 100  | 0                               | 0                            |
| G               | 0  | 100 ( <i>G. pallida</i> )       |                              |
| H               | 100  | 0                               | 0                            |
| I               | 0  | 100 ( <i>G. pallida</i> )       | 0                            |
| J               | 100  | 0                               | 0                            |
| K               | 0  | 100 ( <i>G. rostochiensis</i> ) | 0                            |
| L               | 100  | 0                               | 0                            |
| M               | 0  | 0                               | 100 ( <i>G. artemisiae</i> ) |
| N               | 0 (100)                                    | 0                               | 0                            |
| O               | 0  | 0                               | 0 (no identification)        |
| average         | 33%  | 23%                             |                              |

**Conclusion:**

The identification of potato cyst nematodes was generally performed to a satisfactory level. However, care has to be taken not to mistaken *G. tabacum* for a potato cyst nematode. Using both morphological and molecular identification methods is recommended. Participating in a proficiency test seems a valuable idea as mistakes or systematic errors show up in the process.

## Appendix 1

### Participants ID test 2008

| country      | Participant                |  | ID test   |
|--------------|----------------------------|--|-----------|
| AT-AGES      | Ines Gabl                  | Institute for Plant Health (AGES), Vienna  | 1         |
| BE-ILVO      | Nicole Viaene              | Institut for Agricultural and Fisheries research (ILVO), Merelbeke                 | 1         |
| BG-NSPP      | Dr. Zhenya Ilieva          | Plant Protection Institute - Kostinbrod  | 1         |
|              | Denitsa Staneva            | Central Laboratory Plant Quarantine - Sofia  | 1         |
| FR-DGAL      | Géraldine Anthoine         | Laboratoire national de la protection des végétaux (LNVP), Le Rheu                 | 1         |
| DE           | Björn Niere                | Julius Kühn institute, Münster   | 1         |
| HU           | Agnes Toth                 | Central Agricultural office, Central laboratories for pest Diagnoses, Budapest     | 1         |
| IT-MPAF      | Beatrice Carletti          | Istituto Sperimentale per la Zoologia Agraria (CRA), Firenze                       | 1         |
| NL-PPS       | Loes den Nijs              | Plant Protection Service, Wageningen   | 2         |
| ES-INIA      | Antonieta de Cal y Cortina | Proteccion Vegetal Grupo de Patologia Vegetal (INIA), Madrid                       | 1         |
| TR-GDAR      | Emre Evlice                | Plant protection Central research Institute, Ankara                                | 1         |
| CZ           | Vladimir Gaar              | State Phytosanitary Administration, Diagnostics department, Olomouc                | 1         |
| UK-SASA      | Jon Pickup                 | Science and Advice for Scottish Agriculture, Scottish Government (SASA), Edinburgh | 1         |
| SL           | Gregor Urek                | Agricultural Institute of Slovenia, Ljubljana                                      | 1         |
| <b>TOTAL</b> |                            |  | <b>15</b> |

Appendix 2:

| Participant | identification method*       | <i>G. tabacum</i> | <i>G. tabacum</i> | <i>H. schachtii</i> | <i>H. schachtii</i> | <i>G. pallida</i> | <i>G. pallida</i> | <i>G. rostochiensis</i> | <i>G. rostochiensis</i> |
|-------------|------------------------------|-------------------|-------------------|---------------------|---------------------|-------------------|-------------------|-------------------------|-------------------------|
| A           | m                            | x                 | -                 | x                   | x                   | x                 | x                 | x                       | x                       |
| B           | m+PCR                        | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
| C           | m+PCR                        | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
| D           | m+PCR                        | x/-               | x/-               | x                   | x                   | -                 | -                 | -                       | -                       |
| E           | m+PCR                        | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
| F           | m+PCR-RFLP                   | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
| G           | m+PCR-RFLP                   | -                 | -                 | x                   | x                   | x/-               | x/-               | x/-                     | x/-                     |
| H           | m+PCR-RFLP+O                 | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
| I           | m                            | -                 | -                 | x                   | x                   | x                 | -                 | x                       | x                       |
| J           | m+PCR                        | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
| K           | PCR                          | -                 | -                 | x                   | x                   | x                 | x                 | -                       | -                       |
| L           | m+PCR-RFLP                   | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
| M           | m+PCR                        | x/-               | x/-               | x                   | x                   | x                 | x                 | x                       | x                       |
| N           | PCR                          | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
| O           | m+PCR                        | x                 | x                 | x                   | x                   | x                 | x                 | x                       | x                       |
|             |                              |                   |                   |                     |                     |                   |                   |                         |                         |
|             | total correct identification | 12                | 11                | 15                  | 15                  | 14                | 13                | 13                      | 13                      |

X means that the method identified correctly ( meaning that when *G. pal* or *G. ros* was present this was correctly identified and with other cysts it gave a negative result for the identification of *Globodera pallida* or *G.rostochiensis* ,which is ok, hence a "x"),  
X/- means correctly identified with one method but wrong with other  
- means a wrong identification or no identification  
m means morphology  
0 means other technique (ELISA)

## II. **Potato cyst nematodes: proficiency test for resistance testing.**

PROJECT COORDINATORS: Loes den Nijs and Nicole Viaene

PARTICIPANTS: Austria, Belgium, Bulgaria (2x), Czech Republic, France (2x), Germany, the Netherlands (2x), Spain, Slovenia, Turkey, United Kingdom (see Appendix 1 for details)

### **Introduction:**

Cultivar resistance plays an important role in the management of potato cyst nematodes (PCN). Many potato cultivars are totally resistant to *G. rostochiensis*, but none are fully resistant to *G. pallida*. Moreover, the choice of cultivars with resistance to *G. pallida* is rather restricted as not many cultivars are available.

Standard cyst populations, belonging to known pathotypes, have to be used to test the resistance of a new potato accession. They are listed in the Council Directive 2007/33/EC, where a resistance testing procedure is described to evaluate new potato accessions for their resistance against different PCN pathotypes/virulence groups.

This proficiency test was part of the “EUPHRESCO ring test for Globodera” and is based on the resistance testing procedure of the Council Directive 2007/33/EC.

### **Methods:**

Appendix V of the Control Directive 2007/33/EC was taken as an initial starting point for the writing of an extended manual in which all procedures used in the screening test are described in detail. In a general (start) meeting, held on the 6<sup>th</sup> of October 2008, this manual was walked through and some points were stressed to assure good performance. However, each country was allowed to adapt within the given freedom of the appendix V of 2007/33/EC.

To keep the proficiency test feasible for all participants, a minimum number of potato varieties and only one potato cyst pathotype was included. The inoculum, *Globodera pallida* Pa3 Chavornay, was provided by the organizer, the Dutch Plant Protection Service (PPS). The potato tubers, also provided by the Dutch PPS, consisted of the susceptible variety Desiree, the *G. pallida* resistant variety Innovator and 3 unknown varieties with various resistance levels. The aim of the test was to determine the resistance levels of these unknown varieties O, Y and Z.

Cysts and tubers were sent on the 27<sup>th</sup> of January 2009 to all participants with a accompanying letter with information on the test population being the Pa3 Chavornay with 87% viability and cyst contents of 410 eggs and juveniles/cyst. Each participant received also a form to record conditions and all other relevant information of the test (Appendix 2).

Results were expected before the end of September, so that analysis of the data could be performed in October 2009.

### **Results:**

All participating countries fulfilled the demand of sending the results at the end of September 2009 with one (late) exception, but finally all results were analyzed.

Data of various parameters that were recorded during the test can be found in Table 1, while the final results on nematode multiplication and resistance are shown in Table 2.

The final results (Table 2) show that all participants, except one, fulfilled the official criteria mentioned in the EU directive:

- Pf/Pi-values of 20 or more (all countries),
- overall variance of coefficient below 35% (all except country m).

The resistance levels found by each participant for the three unknown varieties are expressed as % relative susceptibility compared to Desirée, and with the scoring according to the standard scoring rate (1 to 9) of the EU Council Directive 2007/33/EC (Table 2). The three unknown varieties were O= Santé, Y= Simply Red and Z= Mondial and their resistance under Dutch testing conditions with Pa3-population E400 (Rookmaker) are:

| Code | Name       | Resistance | Susceptibility | Score* |
|------|------------|------------|----------------|--------|
| O    | Santé      | Pa2        | Pa3            | 3      |
| Y    | Simply Red | Pa3        | Pa2            | 6      |
| Z    | Mondial    | -          | Pa2 and Pa3    | 2      |

\* under Dutch testing conditions with Pa3-population E400 (Rookmaker)

The raw data of nematode multiplication on each potato variety are not shown in this report, but can be requested by any of the participants.

The mean value (of all 14 participants) of the relative susceptibility of the different varieties compared to Desirée (Table 2) did not differ much when calculated based on cyst counts or based on counts of eggs+juveniles:

| Variety    | Relative susceptibility (%) |                                    |
|------------|-----------------------------|------------------------------------|
|            | based on number of cysts    | based on numbers of eggs+juveniles |
| Innovator  | 1.5                         | 1.1                                |
| Santé      | 33.2                        | 28.8                               |
| Simply Red | 10.7                        | 7.7                                |
| Mondial    | 85.7                        | 89.0                               |

The relative susceptibility of every variety (except Desirée of course) reported by all 14 countries are also shown in graphs in Figs. 1 to 4. It is clear that some countries have deviating results, such as country n for Innovator, countries h, j and n for Simply Red and country n for Santé. These outlier values influence the mean considerably. The results on Mondial were much more variable in general but specific outliers were not recognisable.

Effect of differences in parameters are not very strong, as one of the expected variances could have been the inoculum type such as cysts or eggs suspension. In the results these effects are not noticeable. Also the circumstances in which the



experiment was performed, e.g. greenhouse, climate chamber or outside, did not have an apparent effect on the outcome.

In general, a clear reason for the outliers could not be given and the results did not point to an obvious cause. Therefore it can be concluded that the method described in the control directive, with the freedom of choosing certain parameters and performed properly, gives rise to adequate results.

**Table 1: Compilation of all parameters used by the participating countries**

| EUPHRESCO- GLOBODERA: RING TESTING RESISTANCE OF POTATO CULTIVARS 2009 | a                                | b                       | c   | d                         | e                  | f              | g   | h                           | i                             | j                        | k                                   | l                          | m  | n                    |
|--|----------------------------------|-------------------------|---|---------------------------|--------------------|----------------|---|-----------------------------|-------------------------------|--------------------------|-------------------------------------|----------------------------|--|----------------------|
| PARTICIPANT:   | 1                                | 2                       | 3   | 4                         | 5                  | 6              | 7   | 8                           | 9                             | 10                       | 11                                  | 12                         | 13   | 14                   |
| <b>DATES</b>   |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| set-up of pots:  | 8/04/2009                        | 20/03/2009              | 5/02/2009                                       | 21/04/2009                | 13/03/2009         | 25/03/2009     | 19/03/2009  | 6/04/2009                   | 25/03/2009                    | 5/03/2009                | 5/03/2009                           | 17/04/2009                 | 15/06/2009                                 | 11/05/2009           |
| planting of tubers   | 10/04/2009                       | 20/03/2009              | 29/05/2009                                      | 21/04/2009                | 24/03/2009         | 26/03/2009     | 19/03/2009  | 6/04/2009                   | 5/03/2009                     | 5/03/2009                | 5/03/2009                           | 20/04/2009                 | 16/06/2009                                 | 12/05/2009           |
| infestation with Globodera   | 10/04/2009                       | 20/03/2009              | 29/05/2009                                      | 21/04/2009                | 24/03/2009         | 26/03/2009     | 19/03/2009  | 6/04/2009                   | 10/03/2009                    | 5/03/2009                | 5/03/2009                           | 20/04/2009                 | 16/06/2009                                 | 12/05/2009           |
| stop watering:   | 13/07/2009                       | 19/03/2009              | 20/08/2009                                      | 20/08/2009                | 20/08/2009         | 26/03/2009     | 24/06/2009  | 17/07/2009                  | 8/07/2009                     | 5/06/2009                | 5/06/2009                           | 14/07/2009                 | 14/08/2009                                 | 14/08/2009           |
| extraction of cysts  | 28/07/2009                       | 17/08/2009              | 9/07/2009                                       | 31/07/2009                | 27/08/2009         | 17/08/2009     | 10/07/2009  | 25/07/2009                  | 17/08/2009                    | 20/07/2009               | 24/08/2009                          | 28/07/2009                 | 28/07/2009                                 | 31/08/2009           |
| counting of cysts, eggs, J2  | 12/08/2009                       | 19/08/2009              | 31/08/2009                                      | 10/08/2009                | 3/09/2009          | 13/08/2009     | 26/08/2009  | 18/08/2009                  | 24/08/2009                    | 27/07/2009               | 24/08/2009                          | 10/09/2009                 | 21/09/2009                                 | 21/09/2009           |
| <b>TIME FRAME (deducted from dates)</b>                                |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| days between planting and infestation                                  | no                               | 0                       | 0   | 0                         | 1                  | 0              | 0   | 0                           | 0                             | 0                        | 0                                   | 0                          | 1  | 0                    |
| days between infestation and stop watering                             | 94                               | 91                      | 84  | 91                        | 100                | 92             | 96  | 102                         | 119                           | 92                       | 92                                  | 84 or 120                  | 120  | 94                   |
| days between infestation and extraction of cysts                       | 109                              | 109                     | 90  | 102                       | 150                | 105            | 111   | 110                         | 159                           | 137                      | 137                                 | 98 or 135                  | 111  | 111                  |
| <b>SET UP OF EXPERIMENT</b>  |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| environment  |                                  |                         | growth chamber                                  | climate chamber           | greenhouse         |                | greenhouse  | greenhouse                  | climate chamber               | greenhouse               | outside                             | climate chamber            | climate chamber                            | greenhouse           |
| pot size (in litre)  | 2                                | 1.5 l                   | 1.5 liter                                       | 2                         | 21                 | 1.5 litre      | 21 with 1500 ml soil                                    | 1.4                         | 1                             | 1.5 l                    | 1                                   | 2 with 1650 ml             | 1  | 2                    |
| colour of pots   | brown                            | transparent             | brown   | black                     | white              | black          | black   | black                       | tested pots; orange - control | transparent              | black                               | terra cotta                | white                                      | black                |
| substrate (as in protocol, different)                                  | protocol                         | different (sandy soil)  | and 1/3 hydroculture gra                        | protocol                  | protocol           | protocol       | : 40% sand and 60% pathog                               | loess                       | soil and sand                 | soil and sand            | Different                           | protocol                   | protocol                                   | as in protocol       |
| number of pots per cultivar (replications)                             | 5                                | 5                       | 5   | 5                         | 5                  | 5              | 5   | 7/8                         | 6                             | 5 (7 for Desiree)        | 5                                   | 5                          | 5  | five                 |
| lay-out (CR: completely randomized, B: blocking, O: other-des)         | CR                               | CR                      | CR  | B                         | CR                 | Blocking       | blocking  | blocks (cultivar)           | B                             | B                        | B                                   | CR                         | B  | CR                   |
| rotation of pots: yes or no  | yes                              | no                      | yes   | Y                         | yes                | Yes            | no  | no                          | yes                           | No                       | No                                  | yes                        | yes  | yes                  |
| <b>POTATO TUBERS</b>   |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| pregerminated tubers used: Y/N   | Y                                | yes                     | Y   | Y                         | yes                | Yes            | (but with very small sprou                              | yes                         | N                             | Y                        | N                                   | Y                          | yes  | Y                    |
| removed all but one sprout before planting: Y/N                        | N                                | no                      | Y   | Y                         | yes                | No             | yes   | yes                         | Y                             | N                        | No Sprouts Present                  | Y                          | yes  | Y                    |
| <b>INFESTATION WITH GLOBODERA</b>                                      |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| time of pre-soaking cysts (hrs)  | 24 h                             | 0                       | 1   | 2                         | 60                 | 0              | 0   | 0                           | 0                             | 240                      | 3                                   | 24                         | 24   | 24                   |
| inoculum: cysts (C) or eggs + J2 (E+J2)                                | E+J2                             | C                       | 5000  | E+J2                      | C                  | C              | Cysts   | C                           | C                             | C                        | C                                   | E+J2                       | eggs + J2 (E+J2)                           | E+J2                 |
| viability (%)  | no                               | 87%                     | 98%   | 87%                       | 85                 | 87%            | 87%   | 87                          | 87%                           | 87                       | 87%                                 | 87                         | 87   | 82                   |
| hatching test: yes or no, if yes: % hatch                              | no                               | no                      | no  | N                         | 85                 | No             | but before start we crush                               | no                          | no                            | No                       | No                                  | no                         | no   | no                   |
| viability of provider taken into account: yes or no                    | no                               | yes                     | Y   | yes                       | Yes                | Yes            | we visually observed dur                                | yes                         | no                            | no                       | yes                                 | 5                          | yes  | no                   |
| PI: number of viable eggs +J2 per ml soil applied                      | 5.12                             | 4.9 6                   | 5   | 5.12                      | 5.27               | 6,291          | if juveniles hatched                                    | approx. 8                   | 34                            | 4962,94=15 cysts         | 10                                  | 12                         | 8250 p. pot                                | 5                    |
| if viability not taken into account: number of eggs+J2/ml              | n/a                              | 20                      | 20  | 20                        | 20                 | 20             | yes   | yes                         | 4                             | 4962,94=15 cysts         | 10                                  | 12                         | 8250 p. pot                                | 5                    |
| PI: number of cysts per pot (if cysts used)                            | n/a                              | 20                      | 20  | 20                        | 20                 | 20             | yes   | yes                         | 4                             | 4962,94=15 cysts         | 10                                  | 12                         | 8250 p. pot                                | 5                    |
| coefficient of variance on PI (%)                                      | 10%                              | /                       | /   | /                         | 11                 | 6,97           | 6,97  | 7,14                        | no                            | no                       | 0%                                  | 0%                         | 1,1  | no                   |
| biotest of infectivity: yes or no                                      | no                               | no                      | no  | no                        | yes                | no             | no  | no                          | no                            | no                       | no                                  | no                         | no   | no                   |
| mode of infestation: describe  |                                  |                         | cysts in a bag put near the tuber - mesh 250 µm | as in protocol            |                    |                | cysts were placed in half-filled                        | cysts were spread around    | bag                           | 1                        | cysts added in 'teabag' at planting | protocol                   |  |                      |
| number of holes  | 8                                | 6                       | 6   | 6                         | 6                  | 30             | all replicates with 30 cysts                            | pot was filled up with sand | 10 cm                         | 8                        | 5,5 cm                              | 6                          | 10 cm                                      | 6                    |
| depth of holes   | 10 cm                            | 6-8 cm                  | pipet   | 15-20cm                   | needles            | no             | no  | no                          | 10 cm                         | 7cm                      | needle                              | 10cm                       | needles                                    | needles              |
| using needles or pipet or other  | needles                          | pipet                   | pipet   | needles                   | needles            | no             | no  | no                          | 10 cm                         | 7cm                      | needle                              | 10cm                       | needles                                    | needles              |
| <b>MAINTENANCE OF EXPERIMENT</b>                                       |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| TEMPERATURE (°C)   |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| measuring device   | air (A) or soil (S) thermometer  | A                       | growth chamber thermometer                      | min-max thermometer AS    | logger+thermometer | A              | controlled climatic glass house conditions air and soil | logger                      | A - labguard; B - thermometer | Logger A                 | logger A                            | logger A                   | logger A and B (Both)                      |                      |
| (if both were measured, note both)                                     | A & S                            | A                       | A   | AS                        | A                  | A              | air and soil  | A                           | A - labguard; B - thermometer | A                        | A                                   | A                          | A and B (Both)                             |                      |
| minimum  | 15                               | 15 °C                   | 21  | 12                        | 12                 | A              | 13.1°C (air), 19°C (soil - 1 pot)                       | 13°C                        | 10                            | 8,8°C                    | 8,9                                 | 11,9°C                     | 17,9 (soil)                                |                      |
| duration of minimum (hrs)  | 10                               | /                       | 11  | 12                        | 12                 | A              | 6 h (air)   | 10                          | <3hrs                         | 0,5                      | 1                                   | 0,5                        | 1  |                      |
| maximum  | 20                               | 25 °C                   | 23  | 20                        | 20                 | A              | 35,2°C (air), 24°C (soil - 1 pot)                       | 20°C                        | 20°C                          | 40,3°C                   | 26,5                                | 31,5°C                     | 19,8 (soil)                                |                      |
| duration of maximum (hrs)  | 14                               | /                       | 13  | 12                        | 12                 | A              | 1 h (air)   | 14                          | <3hrs                         | 0,5                      | 1                                   | 0,5                        | 1  |                      |
| temperature > 25 °C in first 8 weeks after infestation with Gloc       | no                               | no                      | N   | no                        | no                 | no             | times, not more than 2 hours                            | no                          | no                            | 1 day                    | yes, 4,5 hours (average 28°C)       | no                         | no   | no                   |
| average day  | 19,5                             | /                       | 23  | 19                        | 20°C               | 20°C           | 21°C (air)  | 22,7°C                      | 22,7°C                        | 20,25°C                  | 19                                  | 20,25°C                    | 19   |                      |
| average night  | 16                               | /                       | 21  | 17                        | 20°C               | 20°C           | 21°C (air)  | 16,7°C                      | 16,7°C                        | 13,50°C                  | 16,2                                | 13,50°C                    | 16,2                                       |                      |
| <b>LIGHT</b>   |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| natural or artificial  | artificial                       | natural                 | artificial                                      | natural                   | artificial         | Artificial     | natural and artificial                                  | natural                     | artificial                    | Natural                  | Natural                             | artificial                 | artificial                                 | artificial           |
| hours day  | 14                               | /                       | 13  | 14                        | 14                 | 14             | 16 h  | 8 h                         | 10                            | /                        | 10                                  | 14                         | 14   | 16                   |
| hours night  | 10                               | /                       | 11  | 14                        | 8                  | 10             | 8 h   | 10                          | 10                            | /                        | 10                                  | 14                         | 14   | 8                    |
| <b>FERTILIZER</b>  |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| type (liquid, slow-release, NPK,...)                                   | liquid NPK 20-20-20 every 2nd wk | liquid SN SP 5X 4 times | liquid twice/week                               | liquid NPK as in protocol | liquid+NPK 2       | 2              | leaf fertilizer, WUXAL Super, NPK 2 x:week 6 & 8        | Osmocote at beginning       | Kristalon 1x14 days           | Liquid Phostrogen Weekly | liquid weekly                       | NPK per week               | liquid NPK 18-18-18 every week (wk 4 - 10) |                      |
| frequency  | every 2nd wk                     | 4 times                 | twice/week                                      | as in protocol            | 2                  | 2              | 2 x:week 6 & 8  | at beginning                | 1x14 days                     | Weekly                   | weekly                              | per week                   | every week (wk 4 - 10)                     |                      |
| <b>WATERING</b>  |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| by weight (W), by sight (S)  | W                                | S                       | S   | W                         | W                  | W              | S+W (as control)  | sight                       | S                             | /                        | S                                   | W                          | W  | w                    |
| other: describe  | N/A                              | /                       | /   | as in protocol            | 12-20%             | As in protocol |   |                             |                               | every 2 days             | /                                   |                            |  |                      |
| soil moisture level (%) aimed for                                      | 20%                              | /                       | 15%   | as in protocol            | 12-20%             | As in protocol |   |                             |                               | /                        |                                     | 12 to 20                   | protocol                                   | 20                   |
| <b>PLANTS</b>  |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| removed all but one stems during growth: Y/N                           | Y                                | no                      | Y   | Y                         | Y                  | N              | Y   | no                          | Y                             | N                        | N                                   | Y                          | Y  | Y                    |
| <b>PESTICIDE USE</b>   |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| when   | no                               | no                      | N   |                           | no                 |                | 2 x (week 7&8)  |                             | no                            | No                       | N                                   | no                         | no   |                      |
| what   | /                                | /                       | /   |                           | /                  |                | Chrysopa carnea (biocontrol agent)                      |                             | /                             | /                        | N                                   | /                          | /  |                      |
| why  | /                                | /                       | /   |                           | /                  |                | against aphids  |                             | /                             | /                        | N                                   | /                          | /  |                      |
| <b>HARVEST</b>   |                                  |                         |   |                           |                    |                |   |                             |                               |                          |                                     |                            |  |                      |
| visual inspection of root ball of cyst maturity: yes/no                | no                               | no                      | Y   | Y                         | yes                | Yes            | yes   | yes                         | no                            | Yes                      | No                                  | yes                        | yes  | yes                  |
| extraction method  | Fenwick cane                     | schulling centrifuge    |   | Schulling centrifuge      | Fenwick            | Fenwick        | MEKU-soil extractor                                     | sieving method              |                               |                          | Fenwick Can                         | Seinhorst-Stairring method |  | seinhorst elutriator |
| wet or dried soil  | dried soil                       | dried                   | dried   | dried soil                | dried              | dried          | dry   | dry soil                    | dried                         | dried soil               | dried                               | dried                      | dried                                      | dried                |
| separate examination of roots: yes/no                                  | yes                              | no                      | dried   | N                         | Yes                | Yes            | no  | no                          | Yes                           | No                       | Yes                                 | Yes                        | Yes  | no                   |
| if infestation with cysts: old cysts subtracted: yes/no                | N/A                              | yes                     | yes   | Yes                       | Yes                | Yes            | yes   | yes                         | yes                           | Yes                      | Yes                                 | Yes                        | Yes  | Yes                  |
| cysts counted on all replicates: yes/no (should be yes)                | yes                              | yes                     | yes   | Y                         | yes                | Yes            | yes   | yes                         | yes                           | Yes                      | Yes                                 | Yes                        | Yes  | yes                  |
| PI: cyst content determined on all replicates: yes/no (should be yes)  | yes                              | yes                     | yes   | Y                         | yes                | Yes            | yes   | yes                         | yes                           | Yes                      | Yes                                 | Yes                        | Yes  | yes                  |
| coefficient of variation (%) on standard susceptible control Desiree   | 21 (cysts)                       | 6,3                     | 6,3   | 24,5                      | 15,89%             | 9,7            | 9,7   | 26,1                        | 19,1                          | 23,5                     | 22%                                 | 9,8                        | 14,7                                       | 14,7                 |
| coefficient of variation (%) eggs                                      | 19,08                            | 17 (eggs/J2)            | 10,9  | 32,16                     | 20,1               | 16,1           | 7,8   | 7,8                         | 26,1                          | 23,5                     | 22%                                 | 9,8                        | 14,7                                       | 14,7                 |

**Table 2:** Coefficient of variation, reproduction on the susceptible reference Desiree and relative susceptibility and scores of the unknown varieties O, Y, Z, to the tested Pa3 population Chavorney, for each of the 14 participants. A: Calculations based on the number of eggs and juveniles and B: based on the number of cysts.

A

| Number of eggs and juveniles |                     |                  | Relative susceptibility followed by score (1-9) |     |           |     |           |     |           |     |
|------------------------------|---------------------|------------------|---|-----|-----------|-----|-----------|-----|-----------|-----|
| Participant                  | coeff. of variation | Pf/Pi on Desiree | Innovator                                       |     | Variety O |     | Variety Y |     | Variety Z |     |
| a                            | 19,1                | 53,0             | 0,1   | 9,0 | 0,7       | 9,0 | 0,2       | 9,0 | 56,9      | 2,0 |
| b                            | 17,0                | 34,0             | 2,1   | 8,0 | 26,7      | 3,0 | 4,1       | 7,0 | 82,6      | 2,0 |
| c                            | 10,9                | 37,2             | 0,6   | 9,0 | 24,1      | 4,0 | 7,8       | 7,0 | 100,6     | 1,0 |
| d                            | 32,2                | 34,6             | 0,2   | 9,0 | 14,6      | 5,0 | 4,6       | 7,0 | 19,2      | 4,0 |
| e                            | 24,5                | 100,8            | 0,7   | 9,0 | 48,5      | 3,0 | 9,7       | 6,0 | 75,7      | 2,0 |
| f                            | 16,1                | 83,5             | 0,3   | 9,0 | 23,0      | 4,0 | 1,3       | 8,0 | 38,7      | 3,0 |
| g                            | 7,8                 | 101,6            | 0,5   | 9,0 | 19,6      | 4,0 | 6,0       | 6,0 | 57,9      | 2,0 |
| h                            | 27,0                | 35,2             | 0,5   | 9,0 | 2,0       | 8,0 | 16,0      | 4,0 | 177,8     | 1,0 |
| i                            |                     |                  | 1,1   | 8,0 | 15,3      | 5,0 | 3,2       | 7,0 | 161,4     | 1,0 |
| j                            |                     |                  |   |     |           |     |           |     |           |     |
| k                            |                     |                  | 0,2   | 9,0 | 32,0      | 3,0 | 11,0      | 5,0 | 86,0      | 2,0 |
| l                            | 9,8                 | 104,8            | 0,1   | 9,0 | 13,8      | 5,0 | 5,2       | 6,0 | 75,1      | 2,0 |
| m                            | 45,6                | 24,0             | 0,4   | 9,0 | 49,9      | 3,0 | 6,6       | 6,0 | 68,5      | 2,0 |
| n                            | 24,0                | 32,4             | 8,0   | 6,0 | 103,7     | 1,0 | 24,7      | 4,0 | 156,8     | 1,0 |
| mean                         | 21,3                | 58,3             | 1,1   | 8,6 | 28,8      | 4,4 | 7,7       | 6,3 | 89,0      | 1,9 |
| min                          | 7,8                 | 24,0             | 0,1   | 6,0 | 0,7       | 1,0 | 0,2       | 4,0 | 19,2      | 1,0 |
| max                          | 45,6                | 104,8            | 8,0   | 9,0 | 103,7     | 9,0 | 24,7      | 9,0 | 177,8     | 4,0 |

B

| Number of cysts |                     |                  | Relative susceptibility followed by score (1-9) |     |           |     |           |     |           |     |
|-----------------|---------------------|------------------|---|-----|-----------|-----|-----------|-----|-----------|-----|
| Participant     | coeff. of variation | Pf/Pi on Desiree | Innovator                                       |     | Variety O |     | Variety Y |     | Variety Z |     |
| a               |                     |                  | 0,2   | 9   | 3,6       | 7   | 0,6       | 9   | 35,4      | 3   |
| b               | 21                  | 48,0             | 0,6   | 9   | 29,4      | 3   | 4,6       | 7   | 83,7      | 2   |
| c               | 6,3                 |                  | 0,9   | 9   | 21,9      | 4   | 8,6       | 6   | 101,5     | 1   |
| d               |                     |                  | 0,5   | 9   | 21,4      | 4   | 5,9       | 6   | 55,3      | 2   |
| e               | 20,1                |                  | 0,9   | 9   | 49,1      | 3   | 9,9       | 6   | 73,8      | 2   |
| f               | 15,9                | 91,1             | 0,5   | 9   | 28,3      | 3   | 4,1       | 7   | 60,6      | 2   |
| g               | 9,7                 | 105,2            | 1,0   | 9   | 28,7      | 3   | 10,3      | 5   | 71,4      | 2   |
| h               | 26,1                | 51,1             | 1,8   | 8   | 13,6      | 5   | 28,6      | 3   | 130,8     | 1   |
| i               | 19,1                | 42,0             | 2,7   | 8   | 17,2      | 4   | 4,4       | 7   | 86,3      | 1   |
| j               | 23,5                | 71,9             | 3,0   | 8   | 53,0      | 2   | 24,0      | 4   | 122,0     | 1   |
| k               | 22,2                | 95,4             | 0,4   | 9   | 31,0      | 3   | 13,0      | 5   | 105,0     | 1   |
| l               | 8,8                 |                  | 0,3   | 9   | 21,8      | 4   | 8,2       | 6   | 81,7      | 2   |
| m               | 49,1                |                  | 0,5   | 9   | 57,4      | 2   | 6,4       | 6   | 67,6      | 2   |
| n               | 14,7                |                  | 7   | 6   | 89,0      | 2   | 21,0      | 4   | 125,0     | 1   |
| mean            | 19,7                | 72,1             | 1,5   | 8,6 | 33,2      | 3,5 | 10,7      | 5,8 | 85,7      | 1,6 |
| min             | 6,3                 | 42,0             | 0,2   | 6,0 | 3,6       | 2,0 | 0,6       | 3,0 | 35,4      | 1,0 |
| max             | 49,1                | 105,2            | 7,0   | 9,0 | 89,0      | 7,0 | 28,6      | 9,0 | 130,8     | 3,0 |

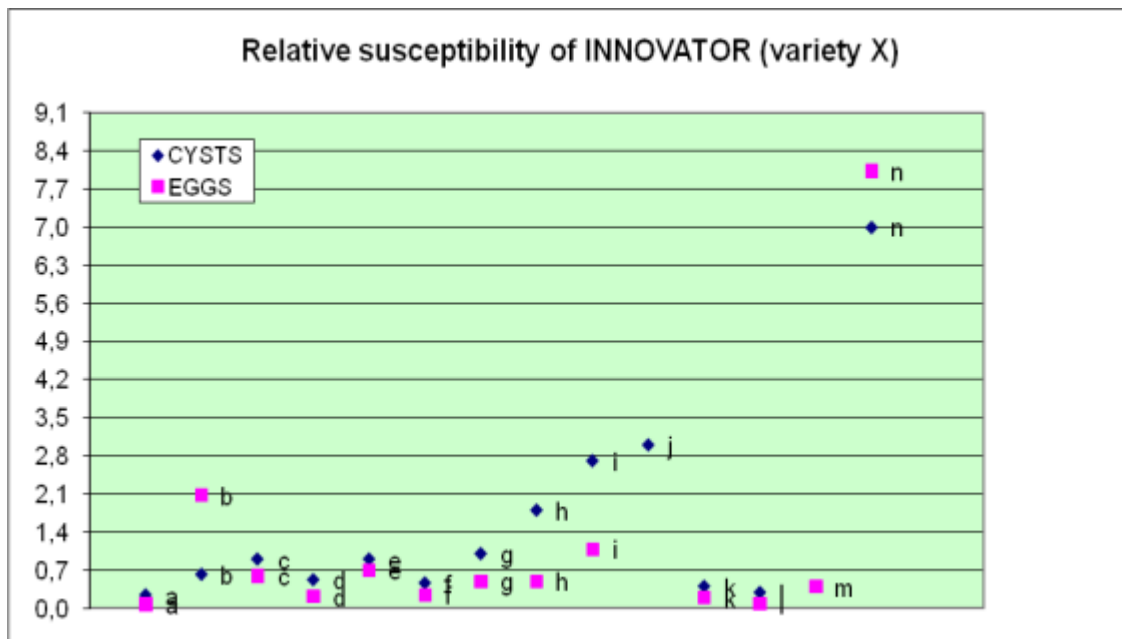


Fig. 1 Relative susceptibility of Innovator (variety X) recorded by each participant (a to n) based on cysts or egg counts.

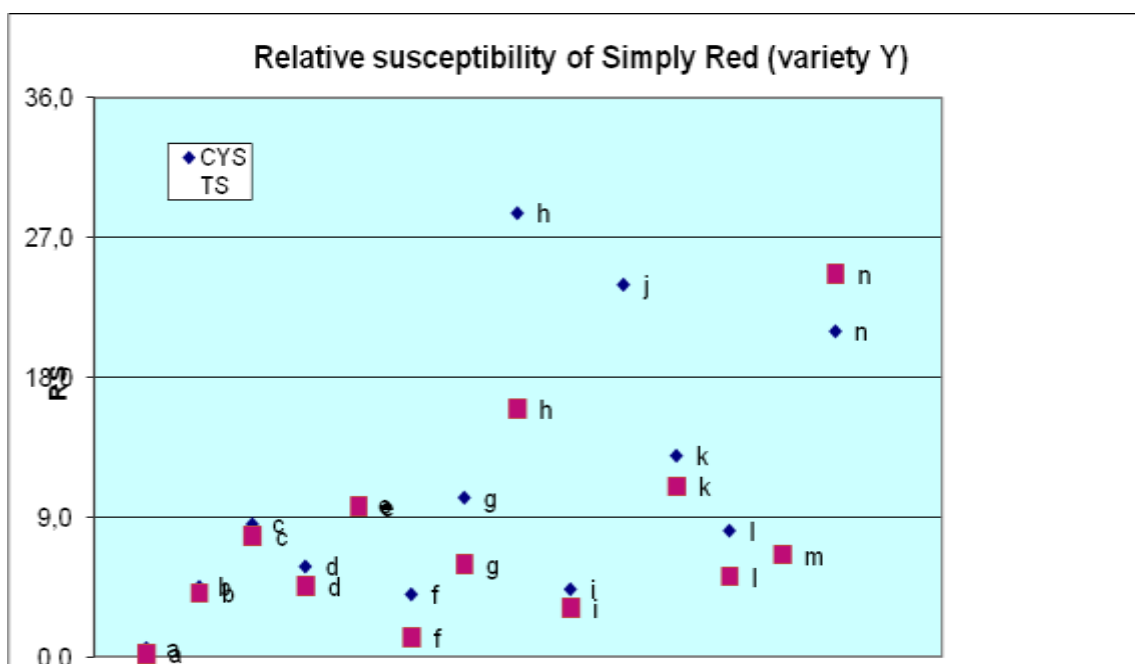


Fig. 2 Relative susceptibility of Simply Red (variety Y) recorded by each participant (a to n) based on cysts or egg counts.

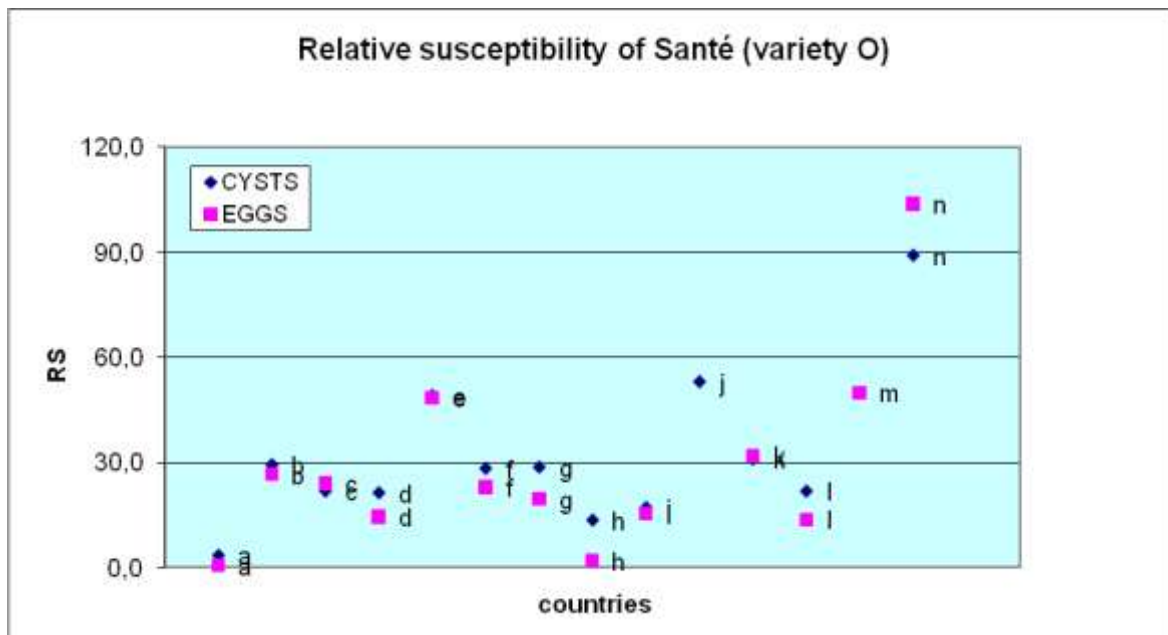


Fig. 3 Relative susceptibility of Santé (variety O) recorded by each participant (a to n) based on cysts or egg counts.

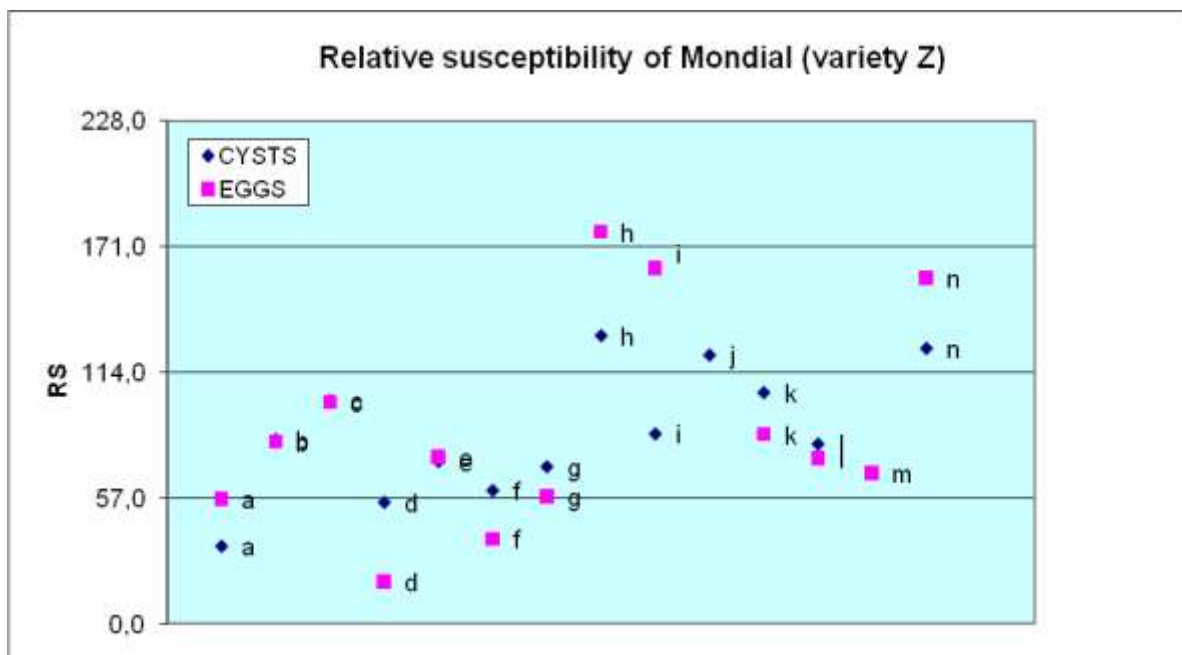


Fig. 4 Relative susceptibility of Mondial (variety Z) recorded by each participant (a to n) based on cysts or egg counts.

**Appendix 1.**  
**Participants Resistance test 2009**

| country      | Participant                |  |           |
|--------------|----------------------------|--|-----------|
| AT-AGES      | Ines Gabl                  | Institute for Plant Health (AGES), Vienna  | 1         |
| BE-ILVO      | Nicole Viaene              | Institut for Agricultural and Fisheries research (ILVO), Merelbeke                 | 1         |
| BG-NSPP      | Zhenya Ilieva              | Plant Protection Institute - Kostinbrod  | 1         |
|              | Denitsa Staneva            | Central Laboratory Plant Quarantine - Sofia  | 1         |
| FR-DGAL      | Géraldine Anthoine         | Laboratoire national de la protection des végétaux (LNVP), Le Rheu                 | 1         |
| FR           | Valerie Grimault           | GEVES/SNES, Beaucauzé Cedex  | 1         |
| DE           | Björn Niere                | Julius Kühn institute, Münster   | 1         |
| NL-HLB       | Wijnand Saathof            | HLB, Wijster   | 1         |
| NL-PPO       | Thea van Beers             | Applied Arable Research, Lelystad  | 1         |
| ES-INIA      | Antonieta de Cal y Cortina | Proteccion Vegetal Grupo de Patologia Vegetal (INIA), Madrid                       | 1         |
| TR-GDAR      | Emre Evlice                | Plant protection Central research Institute, Ankara                                | 1         |
| CZ           | Vladimir Gaar              | State Phytosanitary Administration, Diagnostics department, Olomouc                | 1         |
| UK-SASA      | Jon Pickup                 | Science and Advice for Scottish Agriculture, Scottish Government (SASA), Edinburgh | 1         |
| SL           | Gregor Urek                | Agricultural Institute of Slovenia, Ljubljana                                      | 1         |
| <b>TOTAL</b> |                            |  | <b>14</b> |

**Appendix 2. Form to fill in for resistance test by each participant  
EUPHRESKO-GLOBODERA: RING TESTING RESISTANCE OF POTATO  
CULTIVARS 2009**

*fill in het grey blocks*

*feel free to add any relevant information between the provided space*

**PARTICIPANT:**

**DATES**

|                             |      | day | month | year |
|-----------------------------|------|-----|-------|------|
| set-up of pots              | e.g. | 5   | 2     | 2009 |
| planting of tubers          |      |     |       |      |
| infestation with Globodera  |      |     |       |      |
| stop watering               |      |     |       |      |
| extraction of cysts         |      |     |       |      |
| counting of cysts, eggs, J2 |      |     |       |      |

**TIME FRAME (deducted from dates)**

|  |  |
|--|--|
| days between planting and infestation            |  |
| days between infestation and stop watering       |  |
| days between infestation and extraction of cysts |  |

**SET-UP OF EXPERIMENT**

|  |  |
|--|--|
| environment (greenhouse, outside, climate chamber...)              |  |
| pot size (in litre)  |  |
| colour of pots   |  |
| substrate (as in protocol, different)                              |  |
| number of pots per cultivar (replications)                         |  |
| lay-out (CR: completely randomized, B: blocking, O:other-describe) |  |
| rotation of pots: yes or no  |  |

**POTATO TUBERS**

|  |  |
|--|--|
| pregerminated tubers used: Y/N                   |  |
| removed all but one sprout before planting : Y/N |  |

**INFESTATION WITH GLOBODERA**

|   |  |
|---|--|
| time of pre-soaking cysts (hrs)                           |  |
| inoculum: cysts (C) or eggs + J2 (E+J2)                   |  |
| viability (%)   |  |
| hatching test: yes or no, if yes: % hatch                 |  |
| viability of provider taken into account: yes or no       |  |
| Pi: number of <u>viable</u> eggs +J2 per ml soil applied  |  |
| if viability not taken into account: number of eggs+J2/ml |  |
| Pi: number of cysts per pot (if cysts used)               |  |
| coefficient of variance on Pi (%)                         |  |



[Please insert Project acronym]



biotest of infectivity: yes or no  
 mode of infestation: describe  
     number of holes  
     depth of holes  
     using needles or pipet or other

|  |  |
|--|--|
|  |  |
|--|--|

**MAINTENANCE OF EXPERIMENT**

**TEMPERATURE**

(°C)

measuring device (logger, minx-max thermometer,...)

air (A) or soil (S) temperature

(if both were measured, note both)

minimum

duration of minimum (hrs)

maximum

duration of maximum (hrs)

temperature > 25 °C in first 8 weeks after infestation with Globodera?

average day

average night

|  |
|--|
|  |
|--|

**LIGHT**

natural or artificial

hours day light

hours night

|  |
|--|
|  |
|--|

**FERTILIZER**

type (liquid, slow-release,NPK,..)

frequency

|  |
|--|
|  |
|--|

**WATERING**

by weight (W), by sight (S)

other- describe

soil moisture level (%) aimed for

|  |
|--|
|  |
|--|

**PLANTS**

removed all but one stems during growth : Y/N

|  |
|--|
|  |
|--|

**PESTICIDE USE**

when

what

why

|  |
|--|
|  |
|--|

**HARVEST**

visual inspection of root ball of cyst maturity: yes/no

|  |
|--|
|  |
|--|

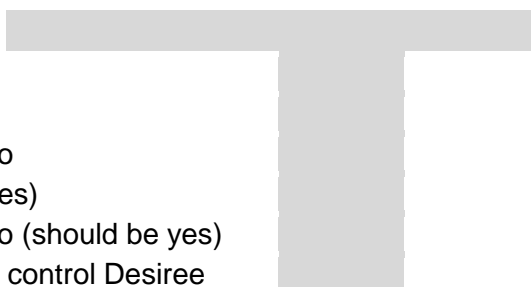




[Please insert Project acronym]



extraction method for cyst  
 wet or dried soil  
 separate examination of roots: yes/no  
 if infestation with cysts: old cysts subtracted: yes/no  
 cysts counted on all replicates: yes/no (should be yes)  
 Pf: cyst content determined on all replicates: yes/no (should be yes)  
 coefficient of variation (%) on standard susceptible control Desiree



## RESULTS

### MEAN VALUES

|                             | Desiree | Innovator | variety O | variety Y | variety Z |
|-----------------------------|---------|-----------|-----------|-----------|-----------|
| Pi cysts (if cysts used)    |         |           |           |           |           |
| Pi eggs and J2              |         |           |           |           |           |
| Pf cysts                    |         |           |           |           |           |
| Pf eggs and J2              |         |           |           |           |           |
| Pf/Pi cysts (if cysts used) |         | *         |           |           |           |
| Pf/Pi eggs and J2           |         | *         |           |           |           |

\* should be at least 20

### DETAILS Pf cysts

|      | Desiree | Innovator | variety O | variety Y | variety Z |
|------|---------|-----------|-----------|-----------|-----------|
| rep1 |         |           |           |           |           |
| rep2 |         |           |           |           |           |
| rep3 |         |           |           |           |           |
| rep4 |         |           |           |           |           |
| rep5 |         |           |           |           |           |

### DETAILS Pf eggs +J2

|      | Desiree | Innovator | variety O | variety Y | variety Z |
|------|---------|-----------|-----------|-----------|-----------|
| rep1 |         |           |           |           |           |
| rep2 |         |           |           |           |           |
| rep3 |         |           |           |           |           |
| rep4 |         |           |           |           |           |
| rep5 |         |           |           |           |           |



### III. Main conclusions:

As one of the aims of Euphresco is to expand networks and cooperate between NPPOs the start and final meeting were considered as good opportunities for sharing experiences and provide this interaction.

Overall, the Euphresco –Globodera project was evaluated as a very useful learning experience where there was also an opportunity to meet and discuss about the topic Globodera and exchange valuable information.

The original aim of the Euphresco Globodera project was to teach the participants to assess pathotypes of Globodera present in their country and to perform such tests, so that each country knows which pathotypes they have to deal with. The current project was much too short to do this (1 year), so the content was adapted: countries had to get acquainted with identification and resistance testing. The follow up could be that each participant can perform this survey in their own country, now the tools are provided and one has learned to use them.

Specific remarks about the identification ring test:

- It was decided that the results of this ring test and the conclusion, perhaps together with conclusions of other ring tests, will be put in a paper. In this paper the application of morphological and molecular identification will be discussed. In the EPPO diagnostic protocol, no consideration of the purpose of the test is made. In the paper, different cases (routine, import, survey, control seed potatoes) will be considered and the + and - of each situation and method will be described.
- It was suggested that our findings and all information available on Globodera identification can be put on internet, on a Globodera forum.
- The identification test will not be repeated, as there are ring test available in the EU. In ring tests, it is recommended to ask for separate results of the molecular and the morphological test, together with the conclusion made by the lab. Now both are considered together and reported as one result. This way, a better understanding of the results of both tests is possible.
- A meeting with our group will be organized at the next meeting of the European Society of Nematologists (ESN) in September 2010 in Vienna to keep up with the proposed activities.

Specific remarks about the resistance ring test:

- The EU guideline on resistance testing should be evaluated in a few years.
- The ring test on resistance should be repeated. About 7 participants want to join immediately. It was suggested to first analyze the results of the current test in more detail and learn how the test can be improved and to add local populations. This could be achieved by 2011.
- Recommendations for further analysis of the test results:
  - compare ranking of varieties between countries,
  - calculate Z-scores
  - compare with the Rookmaker classification
- Testing for virulence: Testing Globodera populations for their virulence should now be possible for every country as the basic techniques for this kind of tests



[Please insert Project acronym]



were practiced. Virulence characteristics can be discussed in the “forum” that will be put on the internet. This forum could be considered as the extension of the current project. A reference population for each pathotype and cultivars to be tested can be suggested so that everybody uses the same cultivars. Care should be taken that the cultivar is “pure” (from clean stock, can be checked with DNA fingerprinting). It was suggested that each participant takes care of the multiplication of one variety, preferably by tissue culture technique, before they are released to all participating countries.

- Possibilities for ring testing in Europe: Possibilities for ring testing for resistance are not present, as far as we know. This project might have been the first.