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# Validation of methods/protocols for routine detection and quantification of *Spongospora subterranea* in field soils and in production and storage facilities

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## INTRODUCTION

In France, occasional powdery scab infections may occur in potato plants grown in infested soils or substrates, and strict preventive measures are taken to avoid dissemination of Sss. The objective of this study was to test the reliability of our tools to detect preventively in routine Sss 1) in different soil textures where potato is grown in France, 2) in different production facilities where first seed generation has been grown and stored.

## Reliability of Sss detection in different soil textures

Seven soils with different textures collected from seed potato fields of different geographical areas were tested. Five replicates of 1 g of soil were inoculated with tree spore concentrations (moderate, medium and high). DNA extractions were done using Macherey-Nagel NucleoSpin® Soil (MN) and real time PCR was done using the markers developed by Maldonado *et al*, 2013 (*Plant Pathology*).

### Comparison of Sss detection on different soil textures using Real Time PCR

| Soils     | Soil textures    | Spore concentrations (spores/ g soil) |              |              |              |
|-----------|------------------|---------------------------------------|--------------|--------------|--------------|
|           |                  | 0                                     | 30           | 300          | 3000         |
|           |                  | Ct Mean ± SD                          | Ct Mean ± SD | Ct Mean ± SD | Ct Mean ± SD |
| Soil n1   | Sandy-clay-loam  | 40 ± 0,0                              | 30,0 ± 0,5   | 27,1 ± 0,2   | 23,6 ± 0,4   |
| Soil n°2  | Sandy-loam-clay  | 40 ± 0,0                              | 31,8 ± 1,0   | 28,3 ± 0,4   | 25 ± 0,4     |
| Soil n°3  | Clayey-sand-loam | 38,1 ± 0,6                            | 28,2 ± 0,7   | 25,5 ± 0,2   | 22,4 ± 0,1   |
| Soil n°4  | Loamy-clay-sand  | 39,4 ± 0,6                            | 30,1 ± 0,7   | 27,1 ± 0,3   | 24 ± 0,3     |
| Soil n°5  | Clay-loam        | 39,5 ± 0,9                            | 28,5 ± 1,0   | 25,0 ± 0,2   | 21,9 ± 0,2   |
| Soil n°6  | Loamy            | 39,7 ± 0,5                            | 29,8 ± 0,5   | 25,7 ± 0,3   | 22,9 ± 0,5   |
| Soil n°7  | Sandy            | 40 ± 0                                | 30,1 ± 0,8   | 25,7 ± 0,3   | 23,9 ± 0,3   |
| P-value   |                  |                                       | 0.0002084    | 6.967e-07    | 4.779e-06    |
| Detection |                  | -                                     | +            | ++           | +++          |

- None of the tested soils was naturally contaminated by Sss
- Inoculated soils showed accurate DNA quantification,
- Sss was detected in all inoculated samples whatever the soil texture,
- Very low variability between the 5 replicates of the same soil
- Small but non significant differences were observed between the different soil structures whatever the contamination level

This study showed the reliability of this detection tool whatever the soil texture. It has been transferred to seed potato organisation to detect Sss preventively.

## Detection in dust : materiel & methods

In this experiment, we compared different types, different quantity of swabs and different DNA extraction kits. Dusts were collected from Sss-free facilities (greenhouses and stores). Extracted DNA was amplified by real time PCR markers described above

| Sampling | Swab types      | Number swabs | DNA Kits                 |
|----------|-----------------|--------------|--------------------------|
|          | Supermarket<br> |              | NucleoSpin Plant II (MN) |
|          | Dutscher<br>    |              | NucleoSpin Tissue (MN)   |
|          | Biolog<br>      |              | NucleoSpin Soil (MN)     |

## Detection in dust : main results

|                             | Number of samples <sup>1</sup> | Swab 2+ NucleoSpin Soil Kit (MN)<br>Ct Mean ± SD | Swab + Plant II Kit (MN)<br>Ct Mean ± SD | Swab + Tissue kit(MN)<br>Ct Mean ± SD |
|-----------------------------|--------------------------------|--|--|---------------------------------------|
| Non contaminated dust       | 7                              | 40 ± 0<br>=> Négatif                             | 40 ± 0<br>=> Négatif                     | 40 ± 0<br>=> Négatif                  |
| Inoculated dust             | 8                              | 25,2 ± 2,2<br>=> Positif                         | 29,9 ± 1,5<br>=> Positif                 | 30,5 ± 0,2<br>=> Positif              |
| Naturally contaminated dust | 7                              | 25,5 ± 0,8<br>=> Positif                         | 36,6 ± 3,3<br>=> Négatif                 | 40 ± 0<br>=> Négatif                  |

<sup>1</sup> five replicates were tested for each sample  
<sup>2</sup> results of 1 swab/tube, the results of 2 swabs/tube didn't improve the results (data not shown)

- We selected DNA extraction method based on:
  - NucleoSpin Soil (MN)
  - Supermarket swabs, 1 swab/tube
- This method was also evaluated on other pathogens in samples collected from several potato storage facilities (data not shown).

## Automatisation of DNA extraction

For routine and rapid detection of pathogens including Sss, automatized DNA extraction was evaluated on soils and culture substracts using the machine Magnépure-96 of Macherey Nagel. This method was compared to the manual DNA extraction (NucleoSpin Soil MN).



|                                | Manual NucleoSpin Soil extraction         |              | Automatized NucleoMag Microbiome extraction |              |
|--------------------------------|---|--------------|---|--------------|
|                                | Number of Sampls                          | Ct Mean ± SD | Number of Sampls                            | Ct Mean ± SD |
| Sss contaminated substracts    | 30  | 24,0 ± 1,0   | 10  | 24,7 ± 0,6   |
| Sss contaminated sols          | 30  | 26,9 ± 1,3   | 10  | 26,8 ± 0,7   |
| Rhizoctonia contaminated soils | 10  | 31,8 ± 0,9   | 10  | 32,9 ± 0,2   |
| Pythium contaminated soils     | 15  | 25,8 ± 0,5   | 10  | 26,3 ± 0,3   |
| Extraction cost                | 3,38€/extraction<br>2h for 24 extractions |              | 4,52€/extraction<br>3h for 96 extractions   |              |

- The sensitivity of automatized DNA extraction is almost equal to that of the manual method whatever the matrices and pathogens
- The automatized method allow to quadruple the number of tested samples and thus reduce the cost
- This automatized extraction is ongoing on a large quantity of soil, more than 1 g/sample.