

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**

		Spore concentrations (spores/g soil)							
Soils	Soil textures	0		30		300		3000	
		Ct Mea	n ± SD	Ct Mea	an ± SD	Ct Mea	in ± SD	Ct Mea	n ± 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				0.000	2084	6.967	7e-07	4.779	e-06
	Detection	_		+		++		+++	

- > None of the tested soils was naturally contaminated by Sss
- > Inoculeted 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 transfered to seed potato organisation to detect *Sss* preventively.

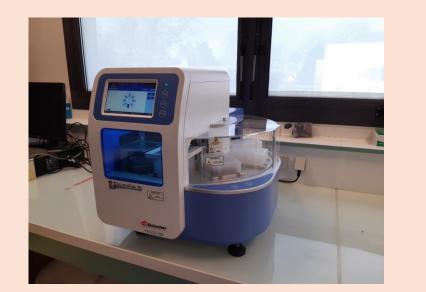
Detection in dust : materiel & methods

Automatisation of DNA extraction

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 typ	es Number	r swabs DN	DNA Kits				
	Supermarket			NucleoSpin Plant II (MN)				
	Dutse	cher		NucleoSpin Tissue (MN)				
	Biolog			NucleoSpin Soil (MN)				
Detection in dust : main results								
		L.IIIaIII	ICJUILS					
	Number of	Swab ² + NucleoSpin Soil Kit (MN)	Swab + Plant II Kit (MN)	Swab + Tissue kit(MN)				
		Swab ² + NucleoSpin	Swab + Plant	Tissue				
Non contaminated dust	Number of	Swab ² + NucleoSpin Soil Kit (MN)	Swab + Plant II Kit (MN)	Tissue kit(MN)				
Non contaminated	Number of samples ¹	Swab ² + NucleoSpin Soil Kit (MN) Ct Mean ± SD 40 ± 0	Swab + Plant II Kit (MN) Ct Mean ± SD 40 ± 0	Tissue kit(MN)Ct Mean ± SD40 ± 0				

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





Magnétapure-96

Sss

Py

96 samples

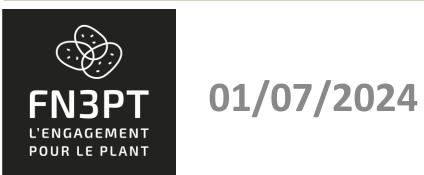
		NucleoSpin Soil Atraction	Automatiezd NucleoMag Microbiome extraction		
	Number of Sampls	Ct Mean ± SD	Number of Sampls	Ct Mean ± SD	
s contaminated substracts	30	24,0 ± 1,0	10	24,7 ± 0,6	
Sss contaminated sols	30	26,9 ± 1,3	10	26,8 ± 0,7	
<i>hizoctonia</i> contaminated soils	10	31,8± 0,9	10	32,9 ± 0,2	
thium contaminated soils	15	25,8 ± 0,5	10	26,3 ± 0,3	
EVTRACTION COST		€/extraction 24 extractions	4,52€/extraction 3h for 96 extractions		

¹ five replicates were tested for each sample ² 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).

- 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.

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