A lateral flow immunoassay "*Sss* AgriStrip" as a tool for specific and rapid detection of *Spongospora subterranea* on potato tubers

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***Spongospora subterranea*, the causative agent of powdery scab is mainly spread through infected seed and can survive in contaminated soil for many years. Visual inspection of seed tubers risks misidentification between common scab (due to *Streptomyces* spp.) and powdery scab symptoms (due to *S. subterranea*). To avoid these problem, a rapid test tool "Sss AgriStrip", using monoclonal antibodies which are specific to resting spores of Sss has been developed (BIOREBA, Switzerland). The objective of this study was to assess it's accuracy and sensitivity in the routine diagnostics of *Sss* using tubers showing different types of symptoms (typical and atypical=suspicious lesions) and compared with other validated test methods**

Comparison of the detection ability of the *Sss* AgriStrip with microscopy, DAS-ELISA PCR and real-time PCR

Methods

General Methodology

AgriStrip: based on lateral flow immunochromatography using monoclonal antibodies specific to resting spores of *Sss*

Bands start developing after 1-2 min and reach maximum intensity after 10-15 min.

Other tested Method

- DAS-ELISA : performed using a monoclonal antibody developed against the sporosori of Sss (Merz et al. 2005)
- PCR : performed using Sss specific primers Sps1 and Sps2 (Bell et al. (1999)
- Real-time PCR (TaqMan Tm) : performed using the primers and probes designed on the ITS1 and ITS2 regions (Bouchek-Mechiche et al., 2004)
- Microscopy: powder scrapped from lesions were observed under light microscope at 10 and 40X magnification.

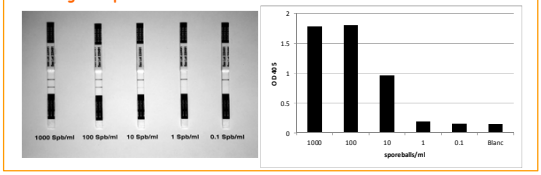
Study of the sensitivity of the Sss AgriStrip (1)

The sensivity of the *Sss* AgriStrip was compared to that of DAS-ELISA with a dilution series of Sss sporosori in buffer

Performance comparison between Sss AgriStrip and other validated method (2)

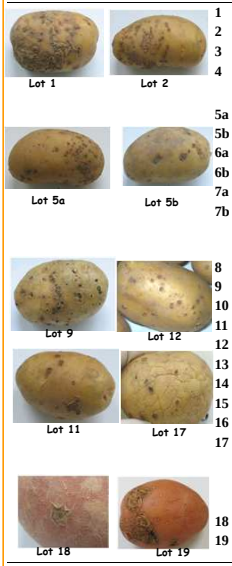
Nineteen tuber lots with different types of scab symptoms were analysed by different methods

Results : 1) Sensitivity of Sss AgriStrip



The *Sss* AgriStrip is as sensitive as the DAS-ELISA with a detection limit between 1 and 10 sporosori per ml buffer

Results : 2) Comparison of the detection ability of the *Sss* AgriStrip with other validated lab methods



Lots	Symptoms	Sss AgriStrip	Microscopy	ELISA	PCR	qPCR	pg DNA/µl[1]
1	TPL	+	+	+	+	+	6300
2	TPL	+	+	+	+	+	700
3	TPL	+	+	+	+	+	2600
4	TPL	+	+	+	+	+	1410
5a	TPL	+	+	+	+	+	1120
5b	ATL	+	+	+	+	+	242
6a	TPL	+	+	+	+	+	150
6b	ATL	+	+	+	+	+	52
7a	TPL	+	+	+	+	+	176
7b	ATL	+	+	+	+	+	58
8	ATL	-	-	-	-	-	0
9	ATL	-	-	-	-	-	0
10	ATL	-	(+)	-	-	-	0
11	ATL	-	-	-	-	-	0
12	ATL	-	-	-	-	-	0
13	ATL	-	-	-	-	-	0
14	ATL	-	-	-	-	-	0
15	ATL	-	-	-	-	-	0
16	ATL	-	-	-	-	-	0
17	ATL	-	(+)	-	-	-	0
18	TCL	-	-	-	-	-	0
19	TCL	-	-	-	-	-	0

[1]mean data from two replicates ; TPL: typical powdery scab lesions ; ATL: atypical lesions (suspicious); TCL: typical common scab lesions ; (+): few doubtful sporosori-like bodies

Results of the *Sss* AgriStrip were highly consistent with DAS-ELISA, PCR, real-time PCR, and microscopy. *Sss* was detected in all tubers with typical symptoms but only in a few lots with tubers showing atypical (suspicious) lesions. The appearance of these atypical lesions positive for the presence of Sss was similar: a diffuse brown necrotic tissue under the periderm and the absence of the dark brown *Sss* sporeballs (photo lot 5b). *Sss* was not detected in most of the atypical lesions with the *Sss* AgriStrip as well as with the other methods. The tubers with the atypical lesions free of *Sss* were analyzed for the presence of *Streptomyces*.

Search for the presence of *Streptomyces* spp. in samples detected free of *S. subterranea*

Methods

Streptomyces isolation

***Streptomyces* spp. were isolated from atypical lesions on tubers as described by Bouchek-Mechiche et al. (1998)**

PCR analysis of genes encoding thaxtomin synthetase (txtAB)

Performed using the primers *Txt*AB1 and *Txt*AB2 specific to the *txt*AB genes encoding the pathogenicity determinant thaxtomin in *Streptomyces* spp.

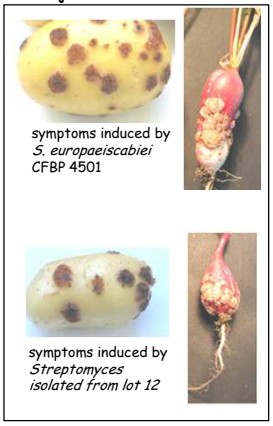
Pathogenicity testing

Pathogenicity of isolated *Streptomyces* spp. was assessed on potato and on radish in the growth chamber at 22°C. radish scoring data on a scale of 0 to 5. Potato scab index on a scale 0 to 100%. Isolates were considered pathogenic on radish: scab index above treshold of pathogenicity of 1.0 and on potato :scab index > 5%.

Results : Evaluation of pathogenicity, the presence of the txtAB operon, for isolated *Streptomyces*

Lot	Isolate	pathogenicity	*Streptomyces* 16 S rDNA[a]	*Streptomyces txtAB genes*
8	8a	+	+	+
9	9a	+	+	+
	9b	+	+	+
10	10a	+	+	+
	10b	+	+	+
	10c	+	+	+
	10d	+	+	+
11	11a	+	+	+
	11b	-	+	-
	11c	+	+	+
12	12a	+	+	+
13	12b	+	+	+
	12c	+	+	+
	12d	+	+	+
14	14a	+	+	+
15	14b	+	+	+
	14c	+	+	+
	15a	+	+	+
	15b	+	+	+
16	16a	-	+	-
17	17a	-	+	-
	17b	-	+	-
Pos. control	CFBP 4501	+	+	+

Symptoms induced in pot test with isolated *Streptomyces* spp. On potato cv. Bintje and radish cv. Polka



symptoms induced by *S. europaeiscabiei* CFBP 4501

symptoms induced by *Streptomyces isolated from lot 12*

***Streptomyces* spp. could be identified as the cause of most of the atypical lesions negative to the presence of *Sss* through isolation and subsequent pathogenicity tests and amplification of the *txt*AB genes.**

CONCLUSION

This data demonstrates the simplicity, robustness and sensitivity of the *Sss* AgriStrip, which makes it ideally suited for rapid detection of *Sss* on farms and at border-inspection points. This test will substantially increases the accuracy of inspection procedures and field scoring based on visual assessment