COMPARISON OF DETECTION METHODS FOR *DITYLENCHUS* IN ALFALFA AND FABA BEAN SEED LOTS, AND METHOD VALIDATION

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INTRODUCTION

The stem and bulb nematode, *Ditylenchus dipsaci*, causes swelling and deformation of aerial parts and necrosis or rot of stem base. A race, on 30 biological race described, distinguished from *D. dipsaci*, owing to a greater body size was named "giant race" and described as a new species named *D. gigas* (Volvas *et al*, 2011). The detection and identification of *D. dipsaci* and *D. gigas* in seed lots is an obligatory part of the sanitary control and regulation in Europe (import, export and sale of seed lots to farmers) on alfalfa seeds. Few studies have reported the difference on morphological characters between the two pathogens. However molecular methods have been recently developed to confirm the *Ditylenchus* subsp. (Esquibet *et al,* 2003; Kerkoud *et al,* 2007; Volvas *et al,* 2011). The aim of the TESTA project was to harmonize and validate at international level a detection method of *D. dipsaci* and *D. gigas* .

GOAL

- To compare performance of the biological and molecular protocols currently used in Europe.
- To validate a method which enables the detection of *D. dipsaci* and *D.gigas* and propose it as an official ISTA and EPPO protocol.

COMPARISON OF PROTOCOLS

Filtration protocol vs Decantation protocol

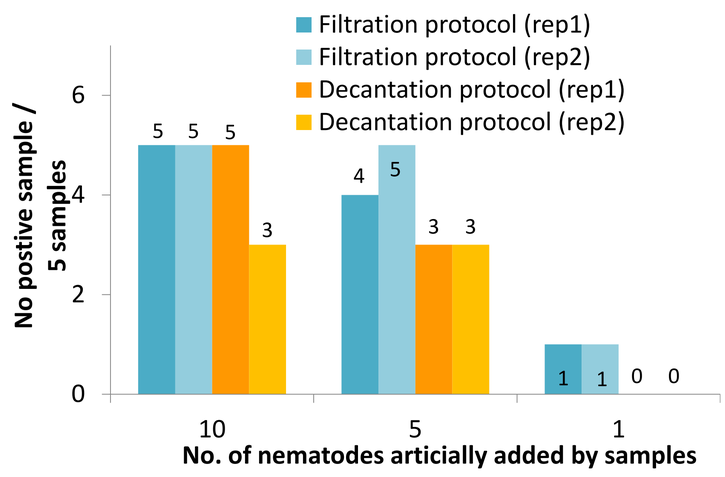
Contamination rate in seed lots

	Filtration protocol (Sieving)		Decantation protocol (bottom)		Decantation protocol (Supernatant)	
Seed lots	Nb positive/ nb samples	Couting	Nb positive/ nb samples	Estimation	Nb positive/ nb samples	Estimation
Alfaalfa 1	5/5	843	5/5	50 to 500	5/5	1 à 15
Alfaalfa 2	5/5	1204	5/5	> 500	5/5	50 à 500
Alfaalfa 3	5/5	1404	5/5	>500	5/5	50 à 500
Faba bean 1	5/5	10190	5/5	> 500	5/5	> 500
Faba bean 2	4/5	454	4/5	50 to 500	4/5	15 to 50
Faba bean 3	5/5	124	4/5	15 to 50	5/5	15 to 50

Filtration protocol = concentrate population of nematode
High capacity of detection in low infected seed lots

Decantation protocol = discarded supernatent
Detection of *Ditylenchus* sp. in supernatant
→ False negative sample

Detection limit

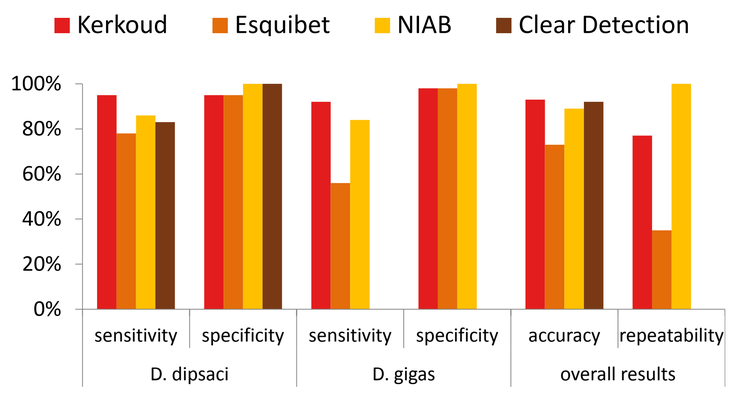


Filtration protocol
limit of detection at 10 nematodes (higher for decantation protocol)
→ **Method chosen**

Confirmation by PCR

Primers available

Accuracy and repeatability calculated for the four evaluated methods



Kerkoud primers
Best results
89 % accuracy

Wood primers
Identification of both species in mixed samples.

VALIDATION TESTS

Three seed lots (healthy, medium and high infected) of alfalfa and Faba bean were tested for homogeneitiy and confirmed by qualititive analysis.
8 participants from France, Germany, United Kingdom, Czech Republic and Slovenia tested the filtration method and the Kerkoud and Wood primers.
Performance criteria were studied : Diagnostic specificity, Diagnostic sensitivity, Accuracy, Accordance (repeatability), Concordance (reproducibility)

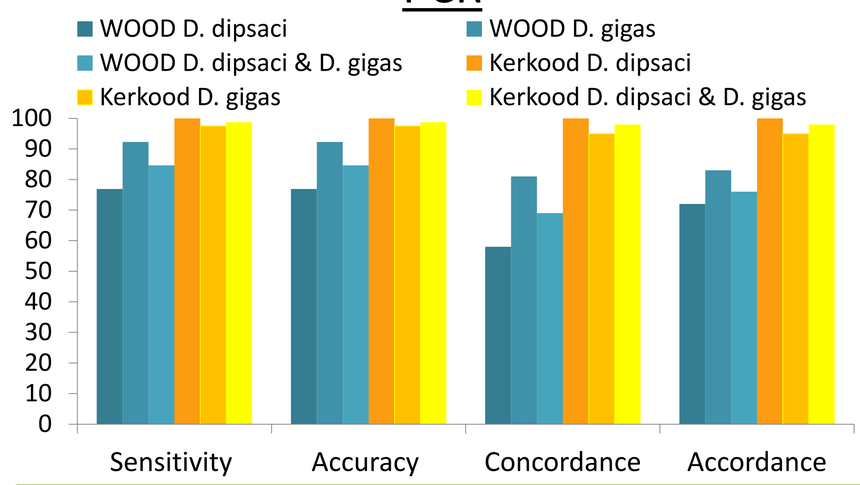
Homogeneity & Stability test

10 samples were tested per seed lot

		Homogeneity test	Stability test
Alfalfa	Lot A	0+/10	0+/10
	Lot B	10+/10	10+/10
	Lot C	10+/10	10+/10
Faba bean	Lot D	0+/10	0/10
	Lot E	9+/10	9+/10
	Lot F	8+/10	10+/10

Conform to expected results. For heterogenous seed lot E and F
No. of positive subsample conformed to expected positive subsamples out of the 10 tested at a 95% confidence

PCR

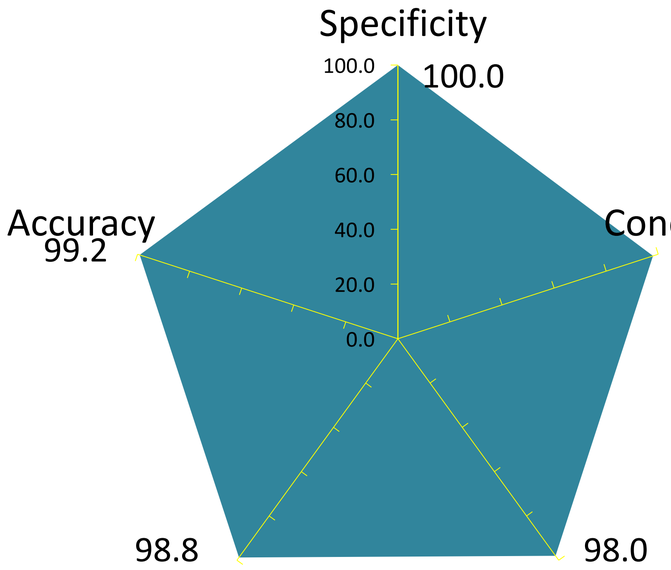


Kerkoud primers → Better results
97,5 % Accuracy / 98,8% Sensitivity
98,0% Concordance / 98,0 % Accordance

Results of the validation test

Results obtained by participants conform to expected results

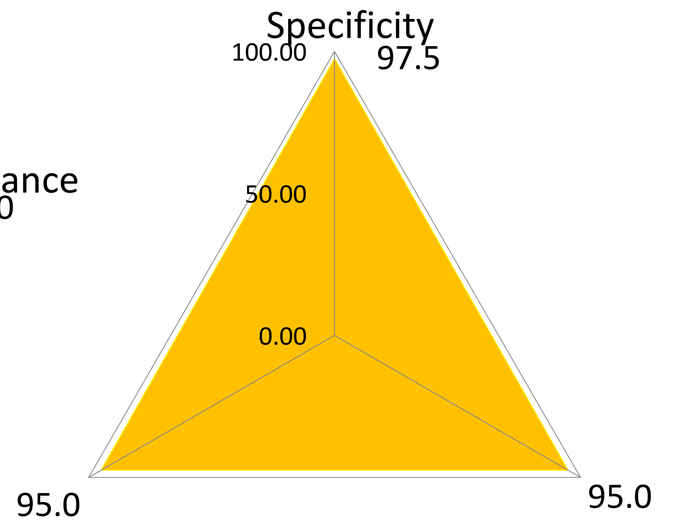
Detection protocol on alfalfa



Very good results higher than 95 %

Detection protocol on Faba bean

Due to heterogeneity of seed lots, diagnostic sensitivity and accuracy were not calculated



Very good results higher than 95 %

Combination of all these results proved that a detection method using filtration in alfalfa and faba bean seed lot was validated.

CONCLUSION

A detection method using sieving at 20µm to concentrate population of nematodes present in a alfalfa and faba bean seed lot is validated. A PCR method using Kerkoud primers is validated in order to confirm the species of *Ditylenchus* between *D. dipsaci* and *D. gigas.*

Contact GEVES

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