TESTA: Validating a detection method of *Ditylenchus dipsaci* and *D. gigas*.

ORGEUR. G.[1]; DELAUNAY. A.[1]; AVRILLON. M.[1]; BEDUNEAU. H.[1]; ANDRO. C.[1]; ROLLAND. M.[1]; THOMAS. J.[2] WOOD. T.[2] and GRIMAULT. V.[1]

1: GEVES, 25 rue Georges Morel, CS 90024, 49071 Beaucouzé – France ; 2: NIAB, Huntingdon Road, Cambridge, CB30LE – England

Background

The stem and bulb nematode, *Ditylenchus dipsaci*, is responsible of many crops losses (Esquibet *et al,* 2003). A race, distinguished from *D. dipsaci*, owing to a greater body size was named "giant races" 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 (the 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). A EPPO protocol exist and is currently under review.

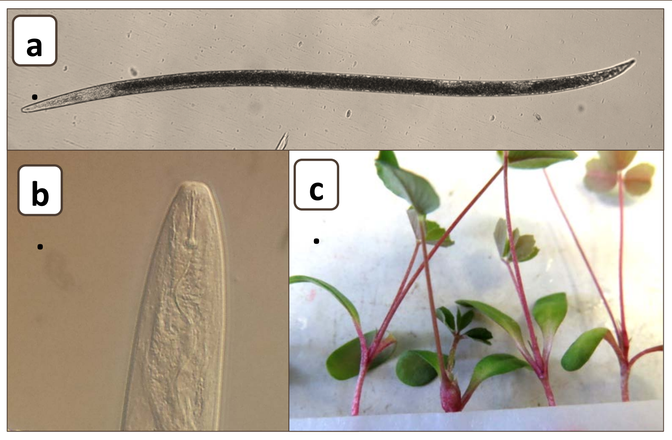

Fig.1. Stem and bulb nematode

a : *Ditylenchus dipsaci/*b : Head of *Ditylenchus gigas/*c : artificial inoculation of plantlets.

Objectives

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

Methods

1- Detection by morphological characteristics

The filtration (Fig.2.) and decantation protocols are compared on 3 alfalfa seed lots with 5 subsamples per seed lot. The detection threshold of each method is studied by artificial inoculation of alfalfa seed samples.

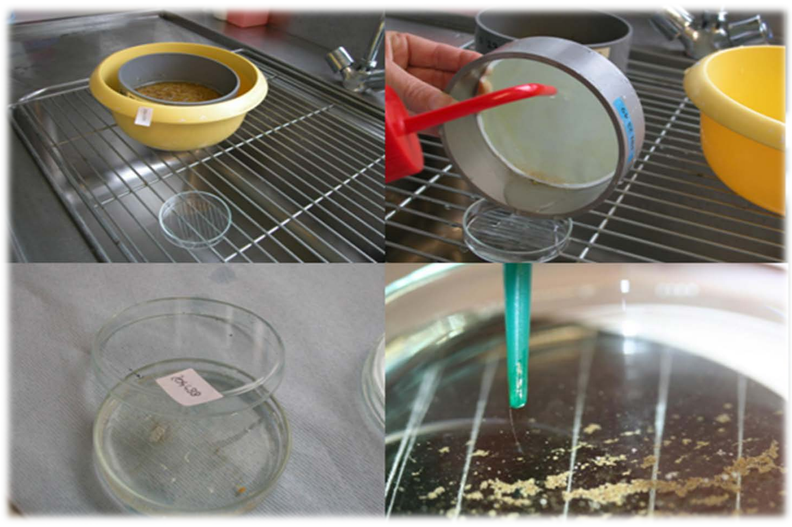

Fig.2. Filtration protocol used for nematodes detection

2-PCR confirmation

Three PCR protocols are tested on a nematodes collection of 60 invidious isolated from plants, seeds and coming from different countries. These protocols are compared according to their trueness and repeatability.

Table 1: Primers used for *Ditylenchus dipsaci* and *Ditylenchus gigas*

Primers	*Ditylenchus dipsaci*		*Ditylenchus gigas*	
Kerkoud *et al* 2007	DdpS1 (F)	rDNA2 (R)	DdpS2 (F)	rDNA2 (R)
Esquibet *et al* 2003	H05 (F)	H06 (R)	D09 (F)	D10 (R)
Wood *et al* 2013	Dd (F)	Dd (R)	Dg (F)	Dg (R)

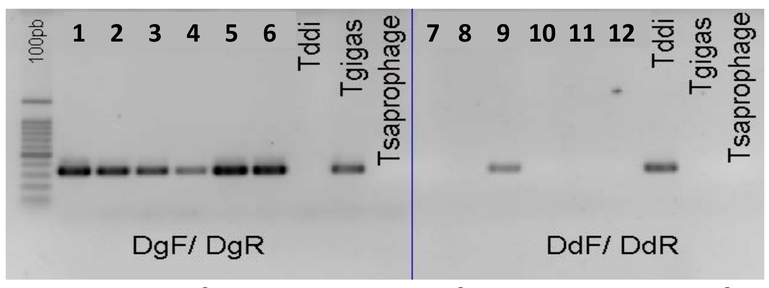

Fig.3. Wood primers tested on 12 nematodes

Results

1- Detection by morphological characteristics

Risk of false negative sample when using decantation method on seed lot with low contamination rate due to the risk of non detection.

Table 2: Comparison between sieving and decantation protocol

Alfalfa sample		Filtration method		Decantation method			
				Bottom		Supernatant	
		No positive/ No samples	Counting	No positive/ No samples	Estimation	No positive/ No samples	Estimation
Seed Lot 1	Sample 1	5/5	5000	5/5	> 500	5/5	> 500
	Sample 2		490		> 500		> 500
	Sample 3		1755		> 500		> 500
	Sample 4		1180		> 500		> 500
	Sample 5		1765		> 500		> 500
Seed Lot 2	Sample 1	4/5	30	4/5	50 to 500	4/5	15 to 50
	Sample 2		0		15 to 50		15 to 50
	Sample 3		5		0		0
	Sample 4		19		15 to 50		1 to 15
	Sample 5		400		50 to 500		15 to 50
Seed Lot 3	Sample 1	5/5	38	4/5	50 to 500	5/5	50 to 500
	Sample 2		4		15 to 50		15 to 50
	Sample 3		20		15 to 50		15 to 50
	Sample 4		15		**0**		**1 to 15**
	Sample 5		47		50 to 500		50 to 500

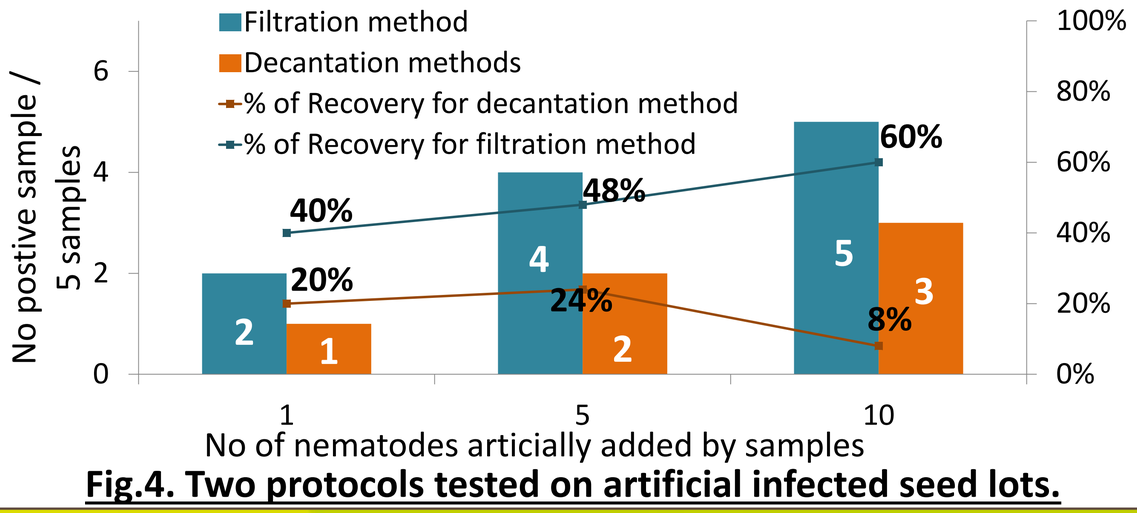

Fig.4. Two protocols tested on artificial infected seed lots.

2-PCR confirmation

Kerkoud primers gave the best results. With a 89 % accuracy, the method developed by NIAB provides a good identification level. Compared to the Kerkoud method, it has the advantage of allowing the identification of both species in mixed samples.

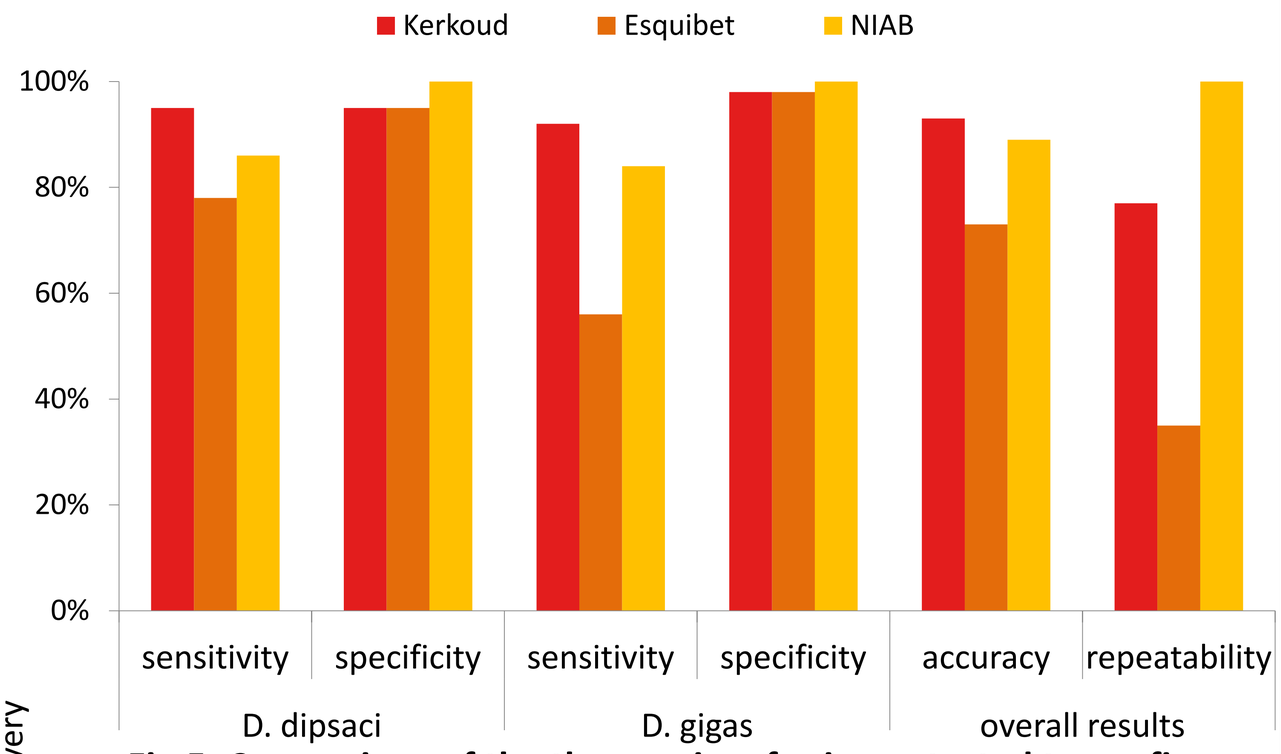

Fig.5. Comparison of the three pairs of primers tested to confirm identity of *D.dipsaci* and *D.gigas*.

Higher recovery capacity with filtration protocol compared to decantation protocol. The decantation protocol has a limit of detection up to 10 nematodes

Conclusion

The comparison showed the weakness of decantation methods especially when samples have low contamination rate. Limit of detection of the filtration method is at 10 nematode whereas the decantation method is up to 10 nematodes. Two out of the three PCR methods are able to identify either *D. dipsaci* or *D. gigas*. However, only one method (Wood from NIAB) is able to identify *D. dipsaci* and *D. gigas* when these species are present in more complex samples.

Contact GEVES : Geoffrey ORGEUR - Laboratoire de pathologie - 25 rue Georges Morel 49071 Beaucouzé – 02.41.22.58.56 - geoffrey.orgeur@geves.fr