

Improvement of the detection of *Aphelenchoides besseyi* on rice seeds

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Background



Rice (*Oryza sativa* L.) is an important crop for human nutrition (500 Mt per year).

The white tip disease in rice caused by the nematode *Aphelenchoides besseyi* can reach severe yield losses. *A. besseyi* is a seed-borne plant-parasite that can survive in a dehydrated state between the glumes and seeds of rice.

Seed health assessment is essential to limit the spread of *A. besseyi*. An international detection method (ISTA¹) is available.

The objective of this project is to study the performance criteria and suggest improvements in the ISTA method.

Sources : EPPO & GEVES

¹ : International Seed Testing Association

GEVES, a NRL specialized on seed health

French National Reference Laboratory

Participate in limiting the spread of pests by implementing European policies

Develop, improve, and evaluate official detection methods

Manage approved laboratories, organize proficiency test and train laboratories

Perform official analyses

Characterisation and improvement and of the ISTA 7-025 method

ISTA 7-025 method

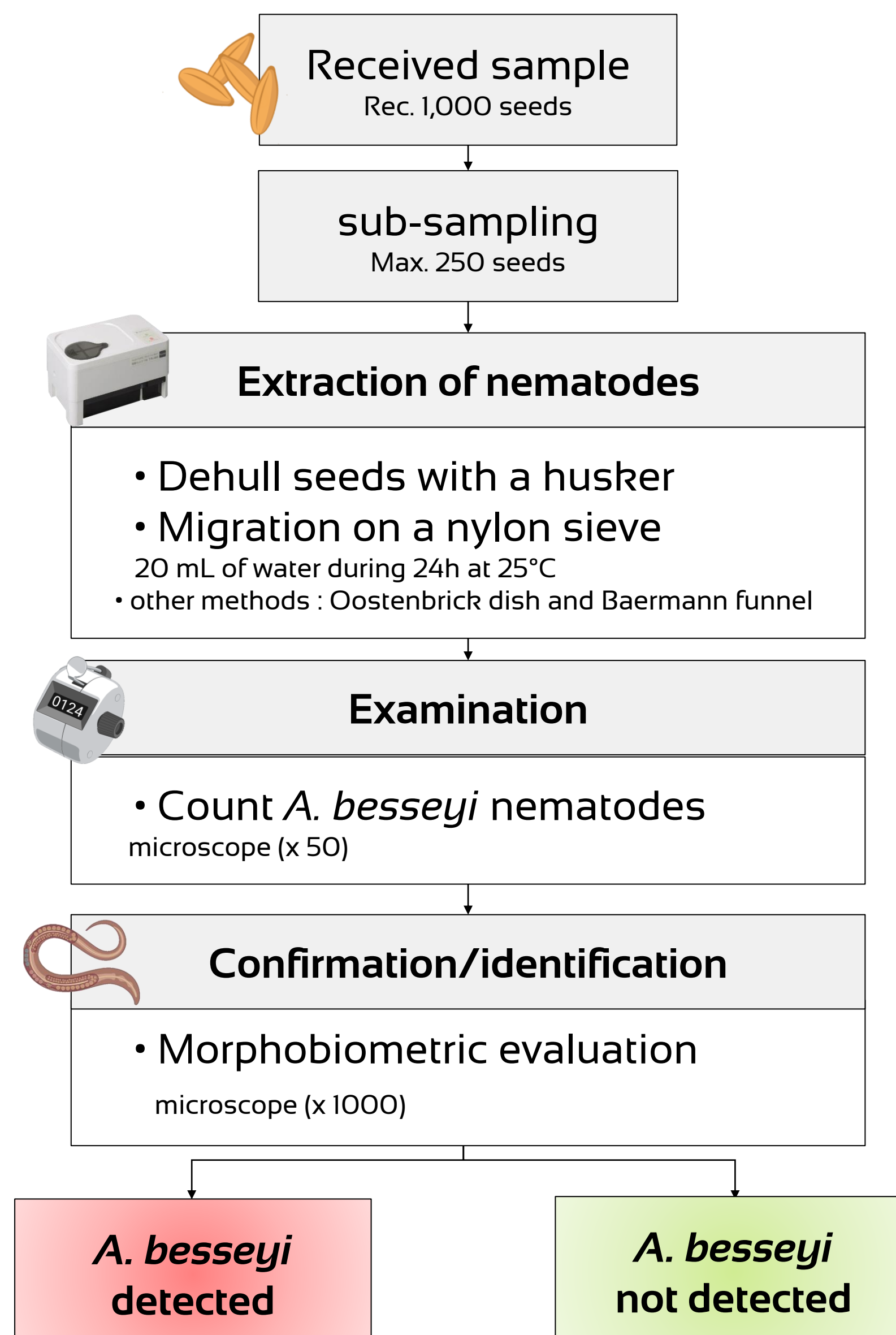
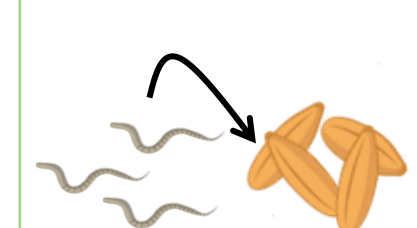


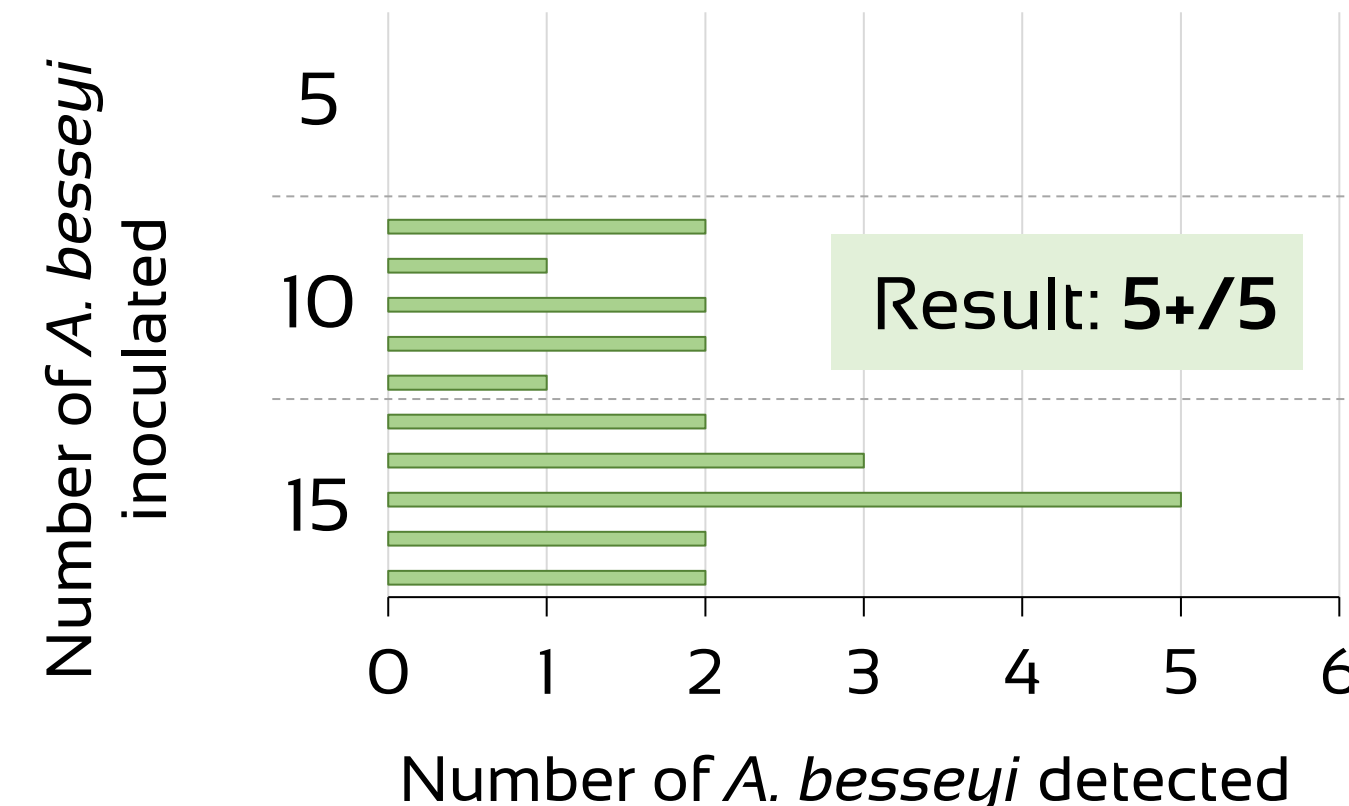
Figure 1 – Process diagram of the ISTA 7-025 method.

Reliability of the ISTA method



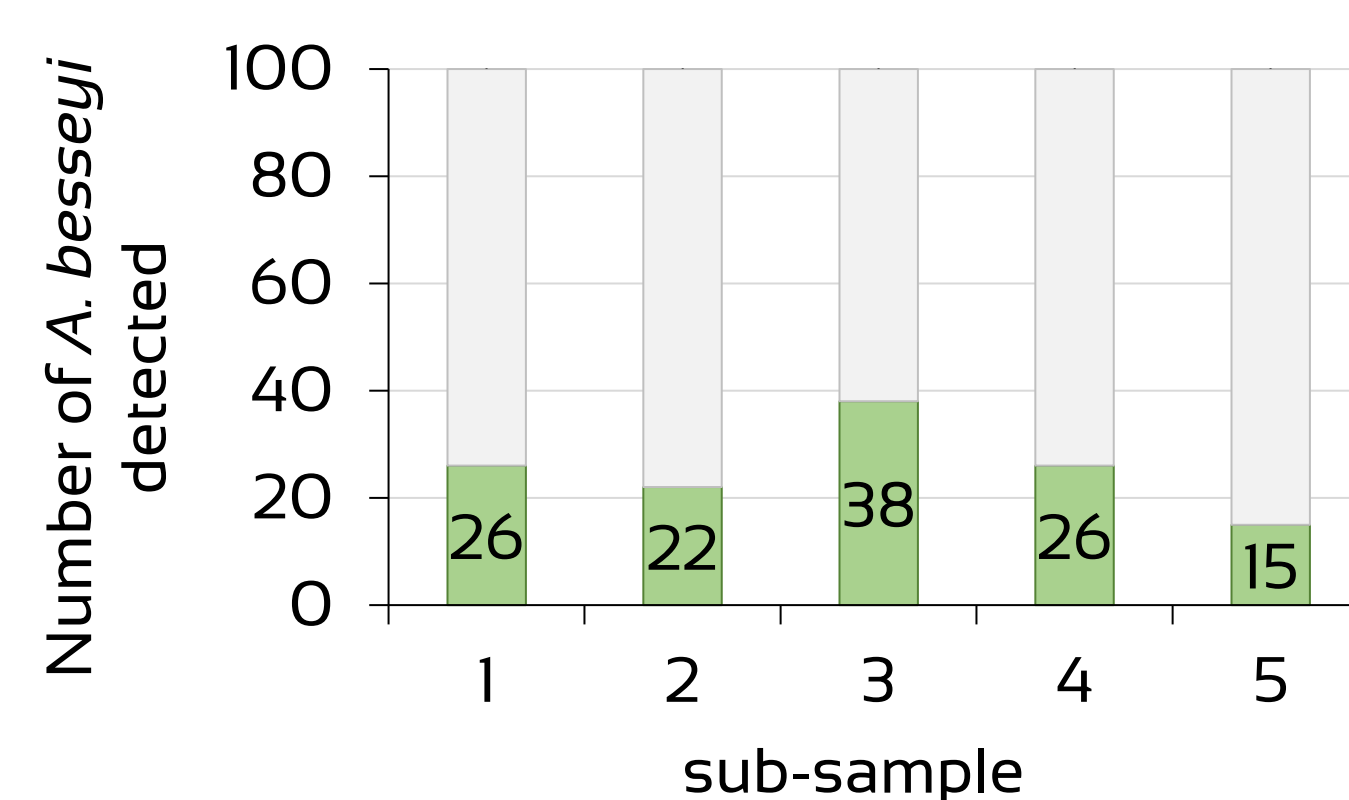
Inoculation of a precise number of *A. besseyi* and analysis of samples according to the ISTA method

Analytical sensitivity



Detection threshold:
10 *A. besseyi* per sub-sample

Recovery rate

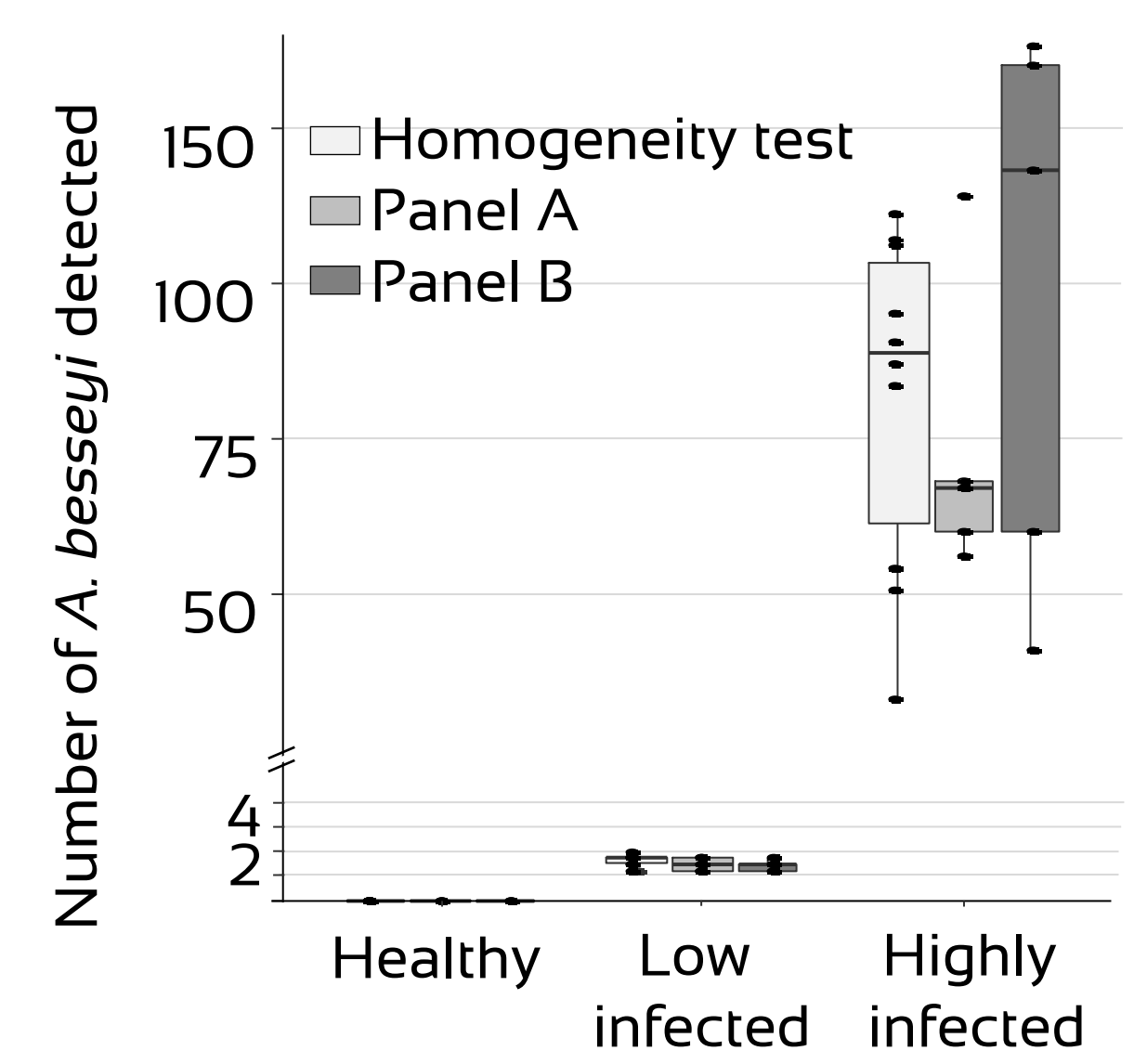


Recovery rate:
23.2%

Diagnostic sensitivity, specificity, repeatability and reproducibility

3 healthy
5 low infected
5 highly infected

Panel A Panel B



No significant difference between test results
Kruskal-Wallis test

Diagnostic sensitivity, specificity, repeatability and reproducibility validated

Proposition of improvements in the ISTA method

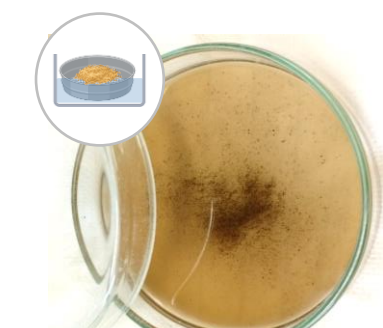


4 sub-samples per sample: time-consuming
Difficult-to-read macerate: clarification of the macerate required
Improvement 1: nematode extraction by centrifugation

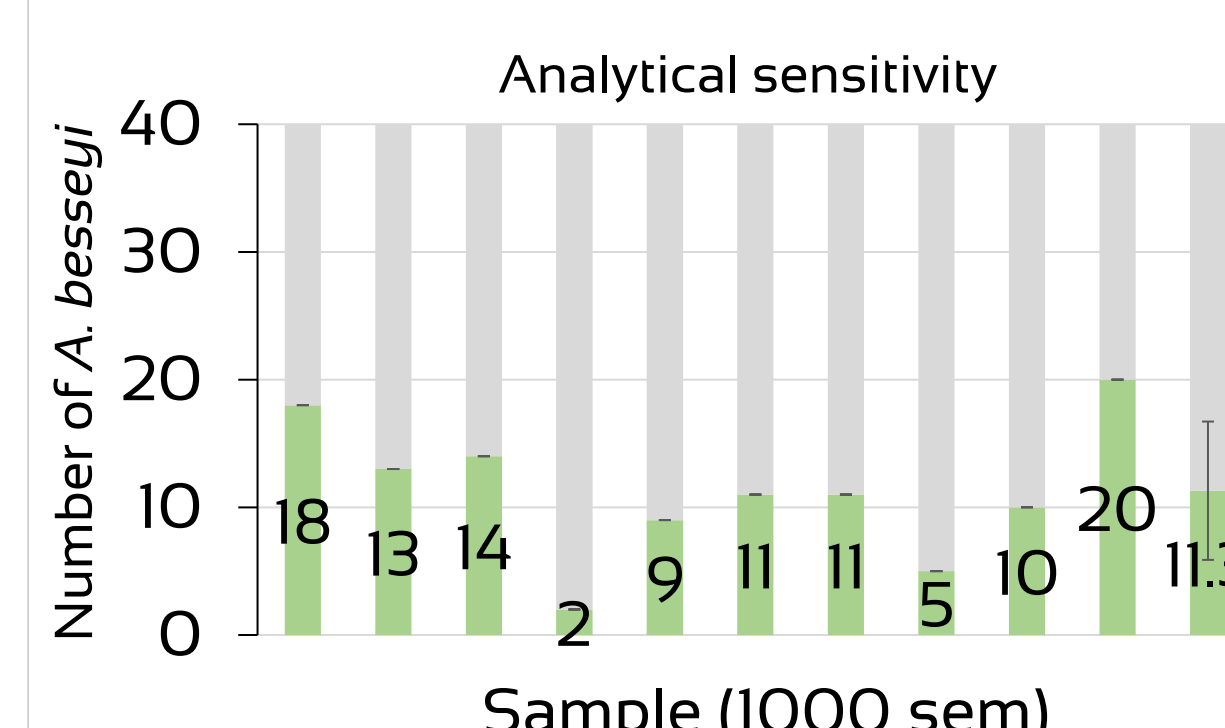


Confusion possible between *A. besseyi* and *A. fujianensis*
Improvement in the specificity of the method necessary
Improvement 2: confirmation of the identity by qPCR

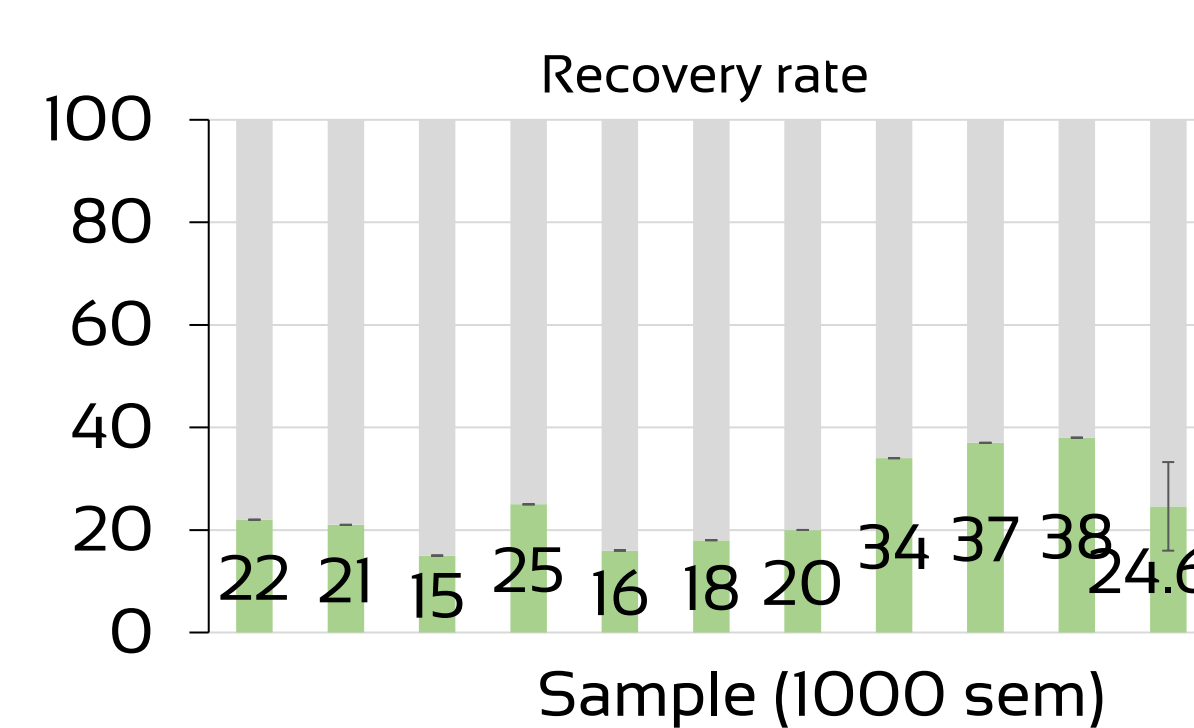
Analytical sensitivity and recovery rate



Inoculation of a precise number of *A. besseyi* and extraction of nematodes by centrifugation



Detection threshold validated:
40 *A. besseyi* / sample



Recovery rate validated:
24.6%

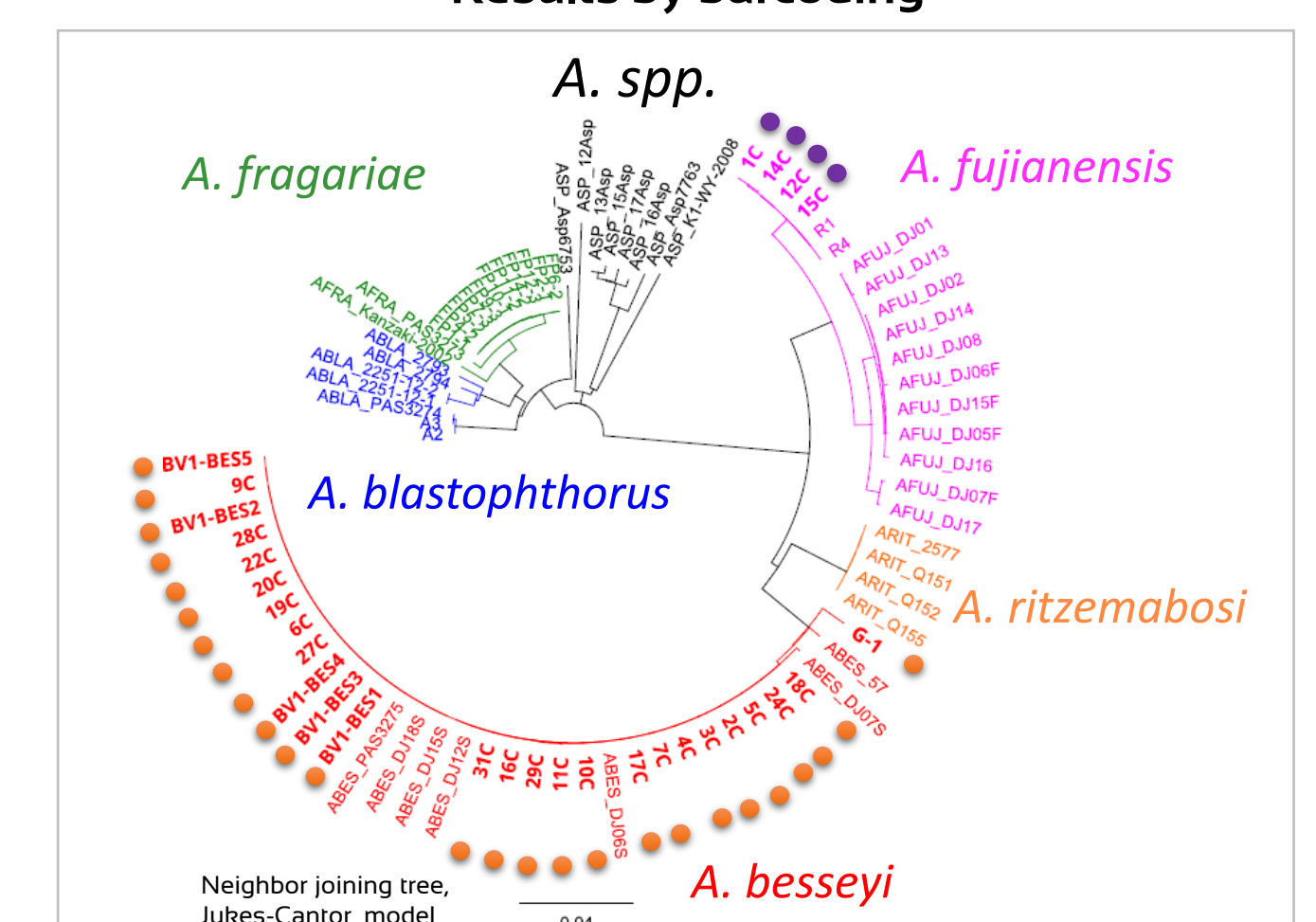
Comparison of the analytical specificity ISTA morphobiometry VS qPCR



Isolation of nematodes, extraction of DNA, qPCR and barcoding
qPCR: EPPO PM 7/39 Appendix 2, Rybarczyk-Mydlowska et al., 2012
Barcoding: EPPO PM 7/129 (2)

	Results by morphobiometry	
	<i>A. besseyi</i>	Other
Results by qPCR	26	0
Other	4	31

Results by barcoding



Morphological confusion possible between *A. besseyi* and *A. fujianensis*
qPCR permits to discriminate *A. besseyi* to other nematodes

Outlook

- Validation of method improvements with other laboratories
- Propose the method improvements at ISTA



The main official missions of GEVES are:

- to conduct DUS and VCUS studies for the Registration of new varieties in the Official Catalogue
- to conduct DUS studies for the Legal protection of varieties (PBR)
- to evaluate the quality and the varietal identity of seed lots and for the Certification of seeds, for species requiring statutory certification.