International Rules for Seed Testing
Annexe to Chapter 7: Seed Health Testing Methods

7-025: Detection of *Aphelenchoides besseyi* on *Oryza sativa*

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Crop: *Oryza sativa* L. (rice)

Pathogen: *Aphelenchoides besseyi* Christie

Prepared by: Remeeus, P.M.[1] and Pelazza, N.[2]
[1]Naktuinbouw, P.O. Box 40, 2370 AA Roelofarendsveen, Netherlands; e-mail: p.remeeus@naktuinbouw.nl;
[2]Ente Nazionale delle Sementi Elette, Via F.lli Laviny, 35, 13100 Vercelli, Italy; e-mail: milano-labvercelli@ense.it

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Background

White tip disease of rice (*Oryza sativa* L.) caused by *Aphelenchoides besseyi* Christie is widely distributed in all rice-growing areas (Fortuner and Williams, 1975). *A. besseyi* is a seed-transmitted nematode and therefore important from the point of view of quarantine (Gergon and Mew, 1991). The European and Mediterranean Plant Protection Organization (EPPO) has published a simple method to test rice seeds in order to detect *A. besseyi* in seed lots for quarantine purposes (EPPO, 1998). Until now a standardized method for detecting and estimating numbers of *A. besseyi* has never been presented to ISTA. Using dehulled seeds for the extraction of the nematodes resulted in an increased number of nematodes compared to the existing EPPO method (Giudici et al., 2003). The suitability of this method for the detection of *A. besseyi* was confirmed in the peer validation study for this method.

Validation studies

Remeeus, P.M. 2007. Proposal for a new method for the detection of *Aphelenchoides besseyi* Christie in *Oryza sativa* L. seeds.

Copies are available by e-mail from ista.office@ista.ch, or by mail from the ISTA Secretariat.

Please send comments, suggestions or reports of problems relating to this method to the ISTA Seed Health Committee, c/o ISTA Secretariat.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation of media, autoclaving, and weighing out of ingredients. It is assumed that this procedure is carried out in a microbiological laboratory by persons familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic technique. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

Treated seed

This method has not been validated for the determination of *A. besseyi* on treated seed. Seed treatments may affect the performance of this method.

(Definition of treatment: any process, physical, biological or chemical, to which a seed lot is submitted. See 7.2.3).

Sample and subsample size

The sample (total number of seeds tested) and subsample size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). In any case, the maximum subsample size should be 100 seeds.

Materials

Mill: Husker TR-120 (Kett Electric Laboratory, Japan)
Containers: beakers 45 mm diameter
Counting dish: any standard nematode counting dish (e.g. De Grisse dish 90 mm diameter)
Sieves: nylon, with meshes of 0.25 mm
Incubator: operating at 25 ± 2 °C
Microscopes: dissecting microscope, magnification ×50; high-power microscope, magnification ×1000

Sample preparation

The test is carried out on a working sample obtained as described in section 7.4.1 of the International Rules for Seed Testing.

Method

[Critical control points are indicated by **CCP**]

1. Extraction
   1.1 Dehull the seeds by using a mill with a 1 mm distance between the rolls (CCP).
   1.2 Fit a nylon sieve, with a mesh of 0.25 mm, into a beaker of 45 mm diameter and transfer kernels and hulls onto the nylon sieve. Fill this beaker with 20 mL of water.
   1.3 Leave the beaker undisturbed for 24 h at 25 ± 2 °C.
   1.4 Remove the sieve from the beaker and squeeze gently.
2. Examination
   2.1 Pour water sample from the beaker into a counting dish.
   2.2 Allow the sample to stand for at least 20 min to allow any nematodes to settle to the bottom of the counting dish.
   2.3. Count both juveniles and adults of *A. besseyi*, in the counting dish under the dissecting microscope (magnification ×50) (see General Methods).
3. Confirmation/identification of suspect nematodes
   3.1 Confirm the identification at a higher magnification of ×1000.
   3.2 *A. besseyi* is a bisexual nematode: females (0.62–0.88 mm) are usually longer than males (0.44–0.72 mm). The body is slender with lip region expanded, wider than neck at base of lips, stylet (spear) 10 µm long. Tail tapering conoid, armed with four mucronate points (Fig. 1). Vulva typically postmedian, usually between 60% and 75% of the body length (EPPO, 2004).

General methods (common to many test procedures)

Reporting results

The result of a seed health test should indicate the scientific name of the pathogen and the test method used. When reported on an ISTA Certificate, results are entered under *Other Determinations*.

In the case of a negative result (pathogen not detected in any subsamples), the results should be reported in terms of the tolerance standard and detection limit. The tolerance standard depends on the total number of seeds tested, n, and is approximately 3/n (p = 0.95) (Roberts *et al.,* 1993); the detection limit per subsample is equal to the detection limit per ml multiplied by the volume of extract.

In the case of a positive result, the report should indicate the mean number of nematodes per subsample and either the number of positive subsamples out of

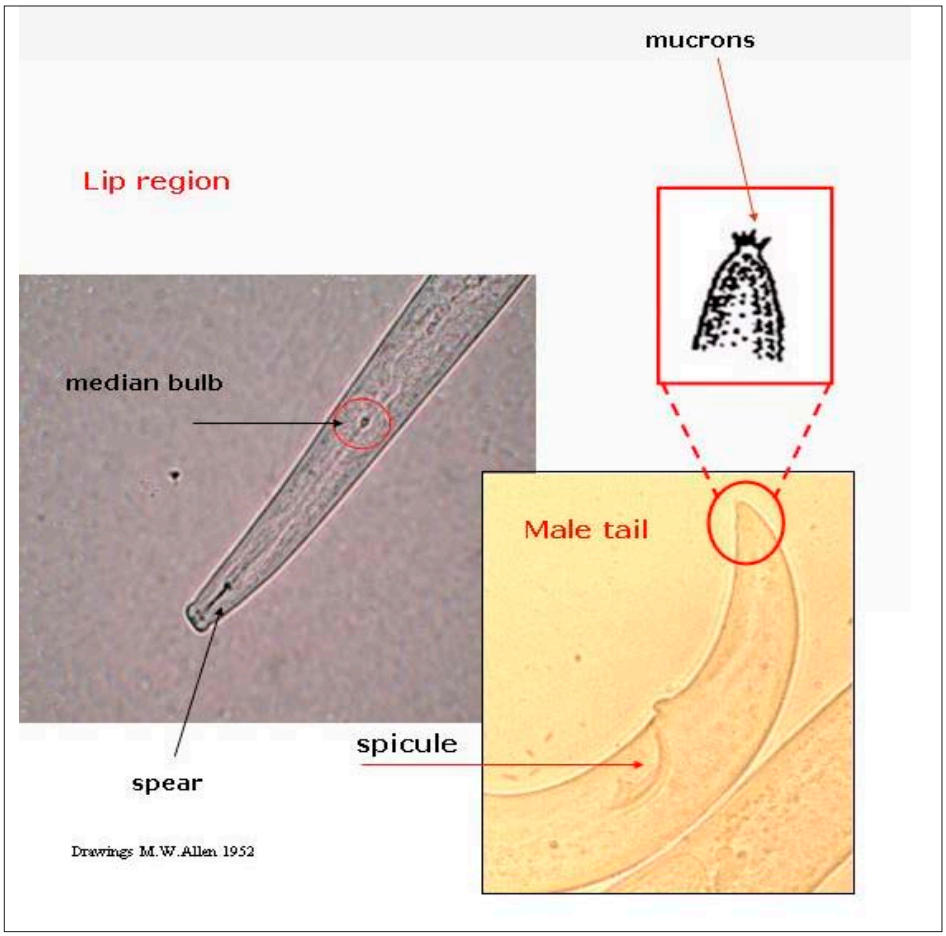

Figure 1. *A. besseyi* showing details of the lip region and male tail (taken from Allen, 1952).

the total number tested and the sample size or the maximum likelihood estimate of the proportion of infested seeds.

Quality assurance

Critical control points

[Identified by CCP in the methods]

Clean the mill between each sample to prevent cross contamination (Step 1.1).

References

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