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

7-013: Detection of *Ustilago nuda* on *Hordeum vulgare* (Barley)

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Crop: *Hordeum* spp.
Pathogen: *Ustilago nuda* (Jens.) Rostr.

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Method Abstract

This method was originally published in the ISTA Handbook of Seed Health Testing in November 1964 as S.3. No. 25 and revised in 1988 by W J Rennie, Agricultural Scientific Services, East Craigs, Edinburgh, Scotland. The method appears in Annexe 7.4.3.A.7 of the ISTA Rules (1999). It has been incorporated into the new Annexe to Chapter 7, Seed Health Testing Methods as method 7-013 and is subject to review before 2006.

Summary of Validation Study

Studied in International Comparative Testing 1960, 1963, 1964, 1979

Comparative tests organised by the ISTA Plant Diseases Committee gave reasonable agreement between stations when samples with more than 1.0 per cent infection were tested by stations experienced in the test procedure (Rennie,1978; Tempe,1976).

If an accurate assessment of loose smut is required for a seed lot with less than 1.0 per cent infection, it may be necessary to increase the number of embryos examined to 2000 or 3000.

Safety Precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation of sodium hydroxide and handling of lactophenol. It is assumed that this procedure is being carried out in microbiological laboratory by persons familiar with the principles of Good Laboratory Practice, and Good Microbiological Practice. Dispose of all waste materials in an appropriate way and in accordance with local safety regulations.

The extraction and preparation procedure involves the use of potentially dangerous chemicals. Analysts should familiarise themselves with the appropriate material safety data sheets. Appropriate protective clothing should always be worn during the extraction process and at all times when handling sodium hydroxide and lactophenol.

Lactophenol should only be handled within the fume cupboard.

When preparing the aqueous sodium hydroxide solution, it is essential that the operation is carried out in a well-ventilated room; the analyst should wear full protective clothing.

Treated Seed

This procedure may involve the handling of chemically treated seed. Analysts should familiarise themselves with the risks attributed to chemical treatments by reference to the material safety data sheets. Contact by inhalation, skin absorption or ingestion must be avoided.

Materials

Reference material	-	Seed known to be infected or other appropriate material.
Incubator	-	Operating at 20 ± 2°C.
Brass sieves	-	1mm mesh, 3 required.
Microscope	-	with sub-stage illumination, x25 and x50 magnification.
5% sodium hydroxide	-	See page 4-5.
Lactophenol (1:1:1)	-	See page 5, water free.
Fume cupboard		
Glycerol		

Sample Preparation

The test is carried out on a working sample of 200-240 gm depending upon the 1000 seed weight as described in ISTA Rules Chapter 10. This test can be completed on both treated and untreated seed.

Method

1. Embryo Method Working sample
   1.1 Two replicates of 100-120 g containing, depending on 1000 seed weight, 2000-4000 seeds.
2. Extraction and clearing of embryos
   2.1 Place the working sample in one litre of a freshly prepared 5% aqueous solution

of sodium hydroxide (NaOH) and maintain at 20ºC for 24 hours.

CCP *A weaker solution of NaOH or a lower temperature makes extraction difficult.*

2.2 After soaking, the entire sample should be transferred to a suitable container and washed in warm water to separate the embryos, which appear through the softened pericarps.

2.3. Collect the embryos in a sieve of 1 mm mesh. Additional sieves of larger mesh can be used to collect pieces of endosperm and chaff.

2.4. Transfer the embryos to a mixture of equal quantities of glycerol and water in which further separation of the embryos and chaff can be made.

2.5. Transfer the embryos to a beaker containing 75 ml of fresh water-free lactophenol and clear them by maintaining the lactophenol at boiling point for approximately 30 seconds in a fume cupboard.

2.6. Transfer the embryos to fresh, slightly warm glycerol for examination.

3. Examination

3.1. Examine 1000 embryos from each replicate at x16-25 magnification with adequate substage illumination for the characteristic golden brown mycelium of *Ustilago nuda*.

3.2. Mycelium is approximately 3µ thick, is golden brown in colour and visible without a stain (Fig. 1). Infection may vary from a few strands of short hyphae to complete invasion of the scutellum tissues. Occasionally fungi other than *Ustilago nuda* occur in the scutellum but are usually darker in colour and quite distinct. When cell walls become discoloured they may be confused with mycelium of *U. nuda*, but this can be checked by examination at x50 or higher magnification (Fig. 2).

General Methods (common to many test procedures)

1. Reporting Results

The result of a seed health test should indicate the scientific name of the pathogen detected and the percentage of infected seeds. When reported on an ISTA Certificate results are entered under Other Determinations. The results should be accompanied by information on the test method used, including any pretreatment.

Preparation of Chemicals

Preparation of 5% Sodium Hydroxide Solution

The exact concentration of sodium hydroxide solution is not critical.

Option A:

Compound	g/l	g/500 ml
Sodium Hydroxide pellets	50	25
Cold tap water	1l	500 ml

Preparation

1. Weigh 50 grams Sodium Hydroxide pellets.
2. Dissolve sodium hydroxide pellets in 1L of cold tap water It is important to ensure that all the pellets are completely dissolved and this necessitates constant stirring with a metal rod.

Option B: *(Sodium Hydroxide can be purchased as 50% stock solution)*

Compound	g/l	g/500 ml
Sodium Hydroxide 50% solution	100 ml	50 ml
Cold tap water	900 ml	450 ml

Preparation

1. Add 100 ml of 50% stock solution to 900 ml Cold tap water.

Lactophenol (1:1:1)

Compound	g/l	g/500 ml
Glycerine	333.3 ml	166.6 ml
Lactic Acid	333.3 ml	166.6 ml
Phenol	333.3 ml	166.6 ml

Preparation

1. Phenol may be crystalline. To liquefy place in a water-bath and heat gently.
2. Phenol is extremely corrosive, wear rubber gloves and avoid breathing fumes.
3. Add equal parts of glycerin, lactic acid and phenol.
4. Final solution should be clear and almost colorless. The solution will turn yellow with age and exposure to light. Store in amber bottle and avoid exposure to light.

Quality Assurance

Critical Control Points

Where the wording of the original Working Sheet suggests that an action is critical this has been marked with ***CCP***.

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 25, W. J. Rennie, 1988.

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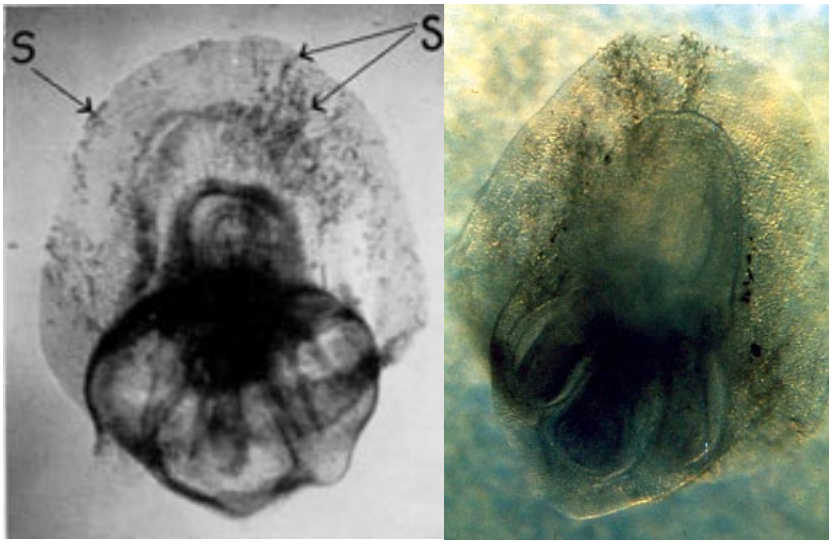

Fig. 1. Infected embryo, smut mycelium at S in scutellum

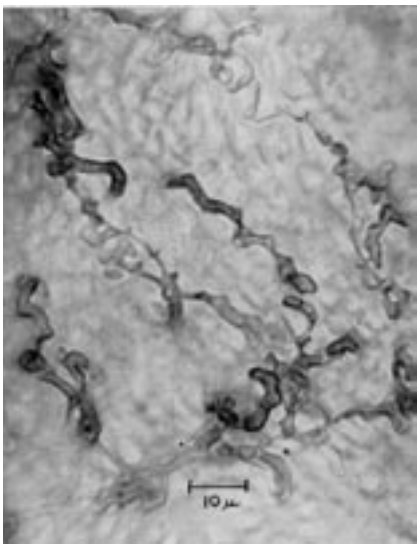

Fig. 2. Smut mycelium in scutellum