7-027: Osmotic method for the detection of *Pyrenophora teres* and *Pyrenophora graminea* on *Hordeum vulgare* (barley)

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

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7-027: *Pyrenophora* on *Hordeum vulgare*

Crop: *Hordeum vulgare* (barley)

Pathogen: *Pyrenophora teres* Drechsler (anamorph: *Drechslera teres* (Sacc.) Shoem.) *P. graminea* Ito & Kurib., in Ito (anamorph: *D. graminea* (Rabenh. ex Schlecht.) Shoem.)

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Revision history:

Version 1.0, 2011-01-01
Version 1.1, 2014-01-01: Addition of positive control; addition of minimum sample size
Version 1.2, 2014-01-01: Common name of host added

Submitted by: Nordic Seed Pathology Working Group and ISTA Seed Health Committee

Background

Pyrenophora teres and *P. graminea* are seed-transmitted fungi in barley. Plants infected with *P. graminea* will not give any yield. Infection with seed-borne *P. teres* contributes to yield reduction, especially if plants are infected early.

Previous methods published by ISTA for the detection of these pathogens were the freezing blotter method (1964a) in S. 3. No. 6 (barley leaf stripe), revised as Working Sheet No. 6 (2. ed) in 1984 (Rennie and Tomlin, 1984), and Working Sheet S. 3. No. 7 (barley net blotch) (ISTA, 1964b).

The osmotic method was invented by Joelson in the 1980s (Joelson, 1983). He found that by using a method that is not based on morphological characteristics, costs were lowered as staff input was reduced and throughput increased. With this method, seeds are incubated on filter paper moistened by a sugar solution. The osmotic pressure from the sugar inhibits the germination of the seeds (giving the method its name). The method is based on the ability of *Pyrenophora* spp. to produce brick-red pigments (anthraquinones) on the filter paper by incubation of seeds under certain conditions (correct temperature, bright light and adequate moisture). However, the method cannot distinguish between *P. teres* and *P. graminea* because they produce the same pigment – catenarin (Engström *et al*., 1993). The pigments turn from brick-red to violet when a weak solution of NaOH is added.

During the 1990s, a Nordic working group on seed pathology organized meetings and comparative tests to harmonize procedures and performance of the osmotic method for detection of *P. graminea/P. teres* in barley seed (Brodal *et al.,* 1994; Brodal, 1995). In 1994–1995, a comparative test with the osmotic method was organized by a sub-working group of the ISTA Plant Disease Committee (Brodal, 1997).

Validation studies

Karin, S. and Brodal, G. (2011).
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Safety precautions

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

Treated seed

This method has not been validated for the determination of *Pyrenophora teres* or *P. graminea* on treated seed. Seed treatments may affect the performance of the method. (Definition of treatment: any process, physical, biological or chemical, to which a seed lot is subjected, including seed coatings. See 7.2.3)

Sample size

The total number of seeds to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infected). In any case, the minimum sample size should be 400 seeds.

Materials

Reference material: infected seeds of *Pyrenophora* spp. with a known infection level.
Filter paper: Munktell Quality 1731, size 162 mm in diameter (for 50 seeds) or filter paper of a corresponding quality. For 100 seeds, a larger size is needed.
Sugar: ordinary table sugar, as used for human consumption.
Plastic plates: with transparent tightly-fitting tops.
1 % NaOH: the exact concentration is not critical.
Impression tool: equipment that can press 50 or 100 wells (indentations) of approximately 3 mm depth in moist filter paper.
Oven: capable of operating at 90 ± 5 °C.
Incubator: capable of operating at 22 ± 2 °C during the dark phase and at 26 ± 2 °C during the light phase.
Fluorescent lamp: daylight lamp capable of an illumination of at least 4000 lux (**CCP**).

Sample preparation

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

Method

Pretreatment

1. Place the seeds in open trays or dishes in a thin layer and heat for 2 hours in an oven at 90 °C to reduce the growth of saprophytes.

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Preparation of substrate

2. Quickly dip the filter paper in the sugar solution (170 g sugar per litre) and drain off surplus solution.
3. Punch 50 or 100 hollows in the filter paper.
4. Put the punched filter paper in a plate with a tight-fitting transparent lid.

Plating and incubation

5. The seeds are placed on the paper, one seed per well, by hand or by vacuum counter (if available)
6. Incubate samples for 7 days with alternating bright light (at least 4000 lux) for 16 hours at 26 ± 2 °C and darkness for 8 hours at 22 ± 2 °C.
7. A control sample of seed with known infection must be incubated under the same conditions as the test samples or other suitable control.

Examination

8. Remove the seeds and pour 1 % NaOH solution onto the filter paper. Approximately 15 mL is used for a paper with 50 wells, and double that amount for a paper with 100 wells. The brick-red pigment will immediately change colour to violet. Count the violet-coloured pigmented spots under a magnifying lamp. Very faint spots (i.e. smaller spots with no distinct violet colour) should not be recorded (Figs. 1–3). Compare with positive control (reference material).

General methods

(common to many test procedures)

1. Checking tolerances

Tolerances provide a means of assessing whether or not the variation in result within or between tests is sufficiently wide as to raise doubts about the accuracy of the results. Suitable tolerances, which can be applied to most direct seed health tests, can be found in Tables 5B of Chapter 5 of the ISTA Rules, or in Miles (1963).

2. Reporting results

The result of a seed health test should indicate the scientific name of the pathogen detected 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), the results should be reported in terms of the tolerance standard. The tolerance standard depends on the total number of seeds tested (n) and is approximately 3/n (p = 0.95) (see Roberts et al., 1993).

In the case of a positive result, the report should indicate the percentage of infected seeds.

Quality assurance

This test should only be performed by persons who have been trained in the method or under direct supervision of someone who has.

Critical control points

(Identified in the methods by CCP)

– It is essential that the lamps provide at least 4000 lux.

Preparation of chemicals

Preparation of sugar solution:

Dissolve 170 g of table sugar (sucrose) in 1 L deionized or tap water. Distilled water is not suitable, due to some hydrolysis of the sugar, which leads to acidification.

The sugar solution should not be stored for more than one week, and the temperature during storage should not exceed 25 °C. If there is any suspicion of growth of microorganisms, the sugar solution must not be used.

Preparation of sodium hydroxide:

Dissolve 10 g NaOH pellets in 1 L of tap water.

References

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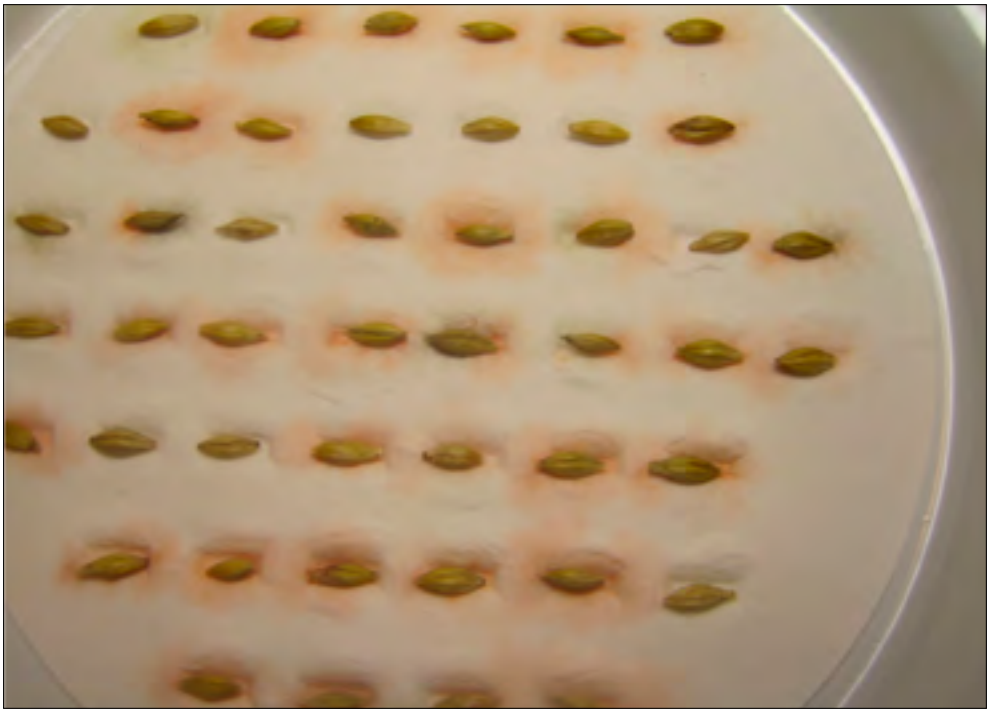

Figure 1. A dish after seed incubation.

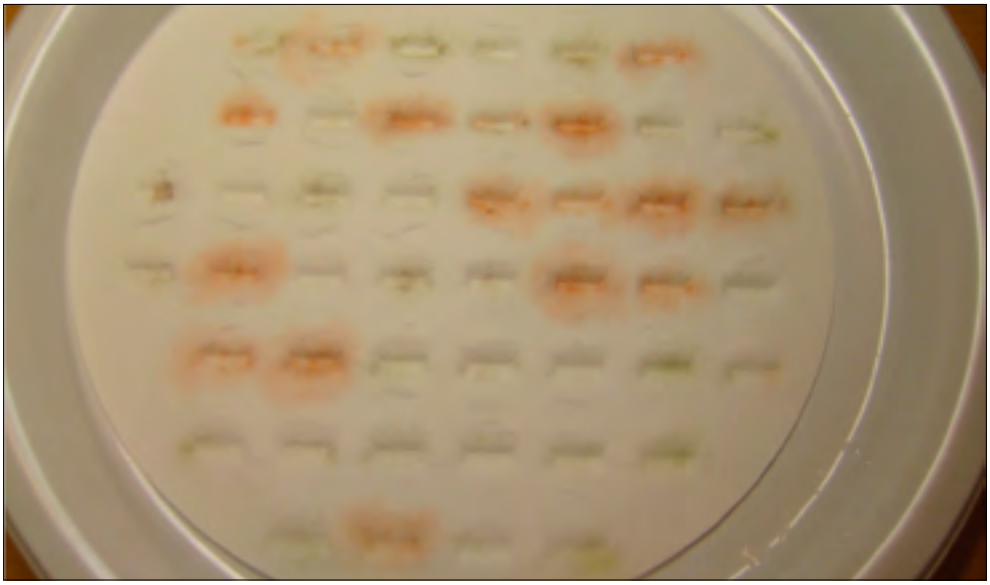

Figure 2. A dish after removal of incubated seeds, before addition of NaOH solution.

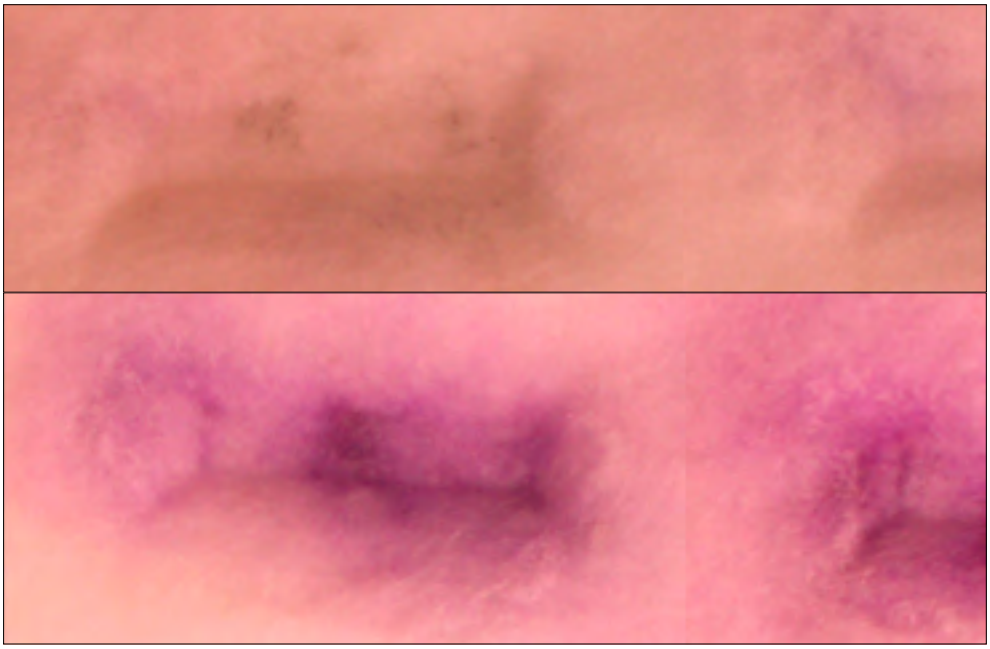

Figure 3. Enlargement of spots visible on the blotters after addition of NaOH solution. Faint spots not to be recorded (above) and normal spots to be recorded (below).