7-009: Detection of Gibberella circinata on Pinus taeda and Pinus elliotii

Disclaimer: whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description ISTA shall not be liable for any loss damage etc., resulting from the use of this method.

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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Crop: *Pinus taeda* and *P. elliotii* (pine)

Pathogen: *Gibberella circinata* Nirenberg & O’Donnell

Authors: ISTA-PDC Method Validation Sub-committee

Revision history:
Version 1.0 November 20, 2001
Revised 20.11.2001 J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.1 2008-01-01: “Treated seed” revised; “Reporting results” revised
Version 1.2 2011-01-01: *Fusarium moniliforme* var. *subglutinans* changed to *Fusarium cir cinatum*
Version 1.3 2012-01-01: Missing reference details added (Cockerell & Koenraadt, 2007)
Version 1.4 2014-01-01: Addition of positive control; scientific name of pathogen changed from *Fusarium circinatum*

Submitted by: ISTA-PDC Method Validation Sub-committee

Background
This method was originally published in the ISTA Handbook of Seed Health Testing in 1985 as Working Sheet No. 56 prepared by Robert L. Anderson, USDA Forest Service, Forest Pest Management, Region Asheville, North Carolina, USA. The method was incorporated into the newly revised Annexe to Chapter 7 in 2002 from the 1999 edition of the ISTA Rules. The method was reviewed by the ISTA-Seed Health Committee in 2006 (Cockerell & Koenraadt, 2007) with the recommendation to accept for a further five years. The pathogen *Fusarium circinatum*, with a teleomorph *Gibberella circinata*, was previously known as *Fusarium moniliforme* var. *subglutinans* (Nirenberg and O’Donnell, 1998).

Summary of validation study
Comparison of duplicate runs of the same seed lot from the same laboratory show a repeatability of over 0.90. That is that seed lot variation accounts for about 90 percent of the total variation. In tests run at different laboratories using the blotter method, the variation for a seed lot was ±7 % of the average for all laboratories combined (Anderson, 1986).
Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation of media, PCNB solutions, autoclaving and weighing out of ingredients. It is assumed that this procedure is being carried out in 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. autoclave, disinfect) and in accordance with local safety regulations.

Treated seed

This method has not been validated for the determination of *Gibberella circinata* 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)

Materials

**Reference material:** reference cultures or other appropriate material

**Media:** blotters

**PCNB:** liquid solution

**Plastic containers:** 133 × 133 × 32 mm.

**Incubator:** capable of operating in the range 20 ± 2 °C. The ability to alternate fluorescent light and darkness during incubation is required.

Sample preparation

The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules.

Method

1. **Pretreatment**
   None.

2. **Blotter Method**
   PCNB liquid solution.

3. **Plating**
   3.1 On blue blotter paper in plastic containers (133 mm × 133 mm × 32 mm) place 25 seeds evenly spaced in each container and crush with a sterilized piece of plastic designed to fit the container, (Anderson 1986; Anderson *et al.* 1984, 1983). Spray seed and blotter with PCNB liquid solution. Cover with transparent lid.

   3.2 Positive control (reference material): Aseptically place seeds pretreated in the same way as 1. in an appropriate number of containers to obtain the reference culture, or plate a reference culture on media. The number of plates required will depend on the level of contamination of the positive-control seed lot.

4. **Incubation**
   10–16 days at 20 °C under fluorescent light at convenient intervals of alternation with darkness until the colonies are about 2 cm in diameter (Fig. 1) (Anderson, 1986).

5. **Examination**
   Examine each colony at a magnification of ×100–400 for the presence of and appearance of polyphialides and conidia. A colony is classified as being of this species
if characteristic polyphialides (Fig. 2A), microconidia (Fig. 2B), and macroconidia (Fig. 2C) are present. Do not include colonies with microconidia in chains, pear-shaped microconidia, or with chlamydospores even if polyphialides are present (Anderson 1986; Blakeslee et al. 1980). Compare with positive control.

**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 International Seed Analysis Certificate, results are entered under Other Determinations.

**Preparation of media and solutions**

**PCNB Liquid Solution**

(Anderson, 1986; Blakeslee et al., 1980).

<table>
<thead>
<tr>
<th>Compound</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>15.0</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>5.0</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.0</td>
</tr>
<tr>
<td>75 % wettable powder (PCNB)</td>
<td>1.0</td>
</tr>
<tr>
<td>(Terraclor 75 % wettable powder)</td>
<td></td>
</tr>
<tr>
<td>Distilled/deionized water</td>
<td>1000 mL</td>
</tr>
<tr>
<td>Streptomycin sulphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Neomycin sulphate</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Preparation**

1. Weigh out ingredients (except streptomycin sulphate and neomycin sulphate) into a suitable autoclavable container.
2. Add 1000 mL of distilled water.
3. Dissolve powdered agar, peptone, MgSO₄·7H₂O, KH₂PO₄ and PCNB in deionized H₂O by stirring.
4. Autoclave at 15 psi and 121 °C for 15 min.
5. Cool to room temperature.
6. Add streptomycin sulphate and neomycin sulphate.
7. Use as described in method.
Quality assurance

Critical control points

None listed

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 56, R. L. Anderson, 1987.


Fig. 1. Colonies of Gibberella circinata on blotters.
Fig. 2A. Polyphilades of *Gibberella circinata* (Magnification x1200).
Fig. 2B. Microconidia of *F. circinatum* (Magnification x1200).

Fig. 2C. Macroconidia of *F. circinatum* (Magnification x1200).