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

7-009: Detection of *Fusarium moniliforme* var. *subglutinan* on *Pinus taeda* and *P. elliotii* (Pine)

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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.

Crop: *Pinus taeda* and *P. elliotii* (Pine)
Pathogen: *Fusarium moniliforme* var. *subglutinans* Wollenw. & Reinke

Prepared by: ISTA-PDC Method Validation Sub-committee

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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 blotter method appears in annex 7.4.3.A.6 of the ISTA Rules (1999). It has been incorporated into the new Annexe to Chapter 7, Seed Health Testing Methods as method 7-009 and is subject to review before 2006.

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.

Materials

Reference Material	-	The use of reference cultures or other appropriate material is recommended when ever possible.
Media	-	Blotters
PCNB	-	Liquid solution.
Plastic containers	-	133 x 133 x 32 mm.
Incubator	-	Capable of operating in the range 20ºC ± 2ºC. 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*

   On blue blotter paper in plastic containers (133 mm x 133 mm x 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.
4. *Incubation*

   10–16 days at 20ºC 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 x100-400 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).

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. A tolerance table, which can be applied to most direct seed health tests, can be found in Table 5.1 of Annex 15 of the ISTA Rules or in Table G1 of the Handbook of Tolerances and Measures of Precision for Seed Testing by S. R. Miles (Proceedings of the International Seed Testing Association 28 (1963) No 3, pp 644).

2. 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 Media and Solutions

PCNB Liquid Solution (Anderson 1986; Blakeslee et al 1980).

Compound	g/l	g/500 ml
Peptone	15	7.5
$MgS0_4 \cdot 7H_20$	5	2.5
KH_2P0_4	1	0.5
75% wettable powder (PCNB) (Terraclor 75% wettable powder)	1	0.5
Deionized/Distilled Water	1l	500 ml
Streptomycin Sulphate	1	0.5
Neomycin Sulfate	0.12	0.06

Preparation

1. Weigh out ingredients (except streptomycin sulphate and neomycin sulfate) into a suitable autoclavable container.
2. Add 1000 (or 500) ml of distilled water.
3. Dissolve powdered agar, peptone, $MgS0_4 \cdot 7H_20$, KH_2P0_4 and PCNB in deionized H_2O by stirring.
4. Autoclave at 15 PSI and 121°C for 15 min.
5. Cool to room temperature.
6. Add streptomycin sulfate and neomycin sulfate.
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.

Anderson, R.L. (1986). A new method for assessing contamination of slash and loblolly pine seeds by *Fusarium moniliforme* var. *subglutinans* Plant Disease 70(5), 452-453.

Anderson, R.L., Belcher, Earl, and Miller, T. (1984). Occurrence of seed fungi inside slash pine seeds produced in seed orchards in the United States. Seed Science and Technology 12, 795-799.

Anderson, R.L., Miller, T., Mistretta, P. Starkey, D., Affeltranger, C., Covington, S., Knighten, J., and Gentry, T. (1983). Occurrence of seed fungi from 37 loblolly seedlots collected in 19 seed orchards. Report 83 1-15. Atlanta, Georgia: United States Department of Agriculture, Forest Service, Southern Region, Forest Pest Management. 8 pp.

Blakeslee, G.M., Dwinell, L.D., and Anderson, R.L. (1980). Pitch canker of southern pines. Forest Report SA FR11. Atlanta, Georgia: United States Department of Agriculture, Forest Service, State & Private Forestry, Southeastern Area. 27 pp.

Miller, T. and Bramlett, D. (1977). Damage to reproductive structures of slash pine by two seedborne pathogens: *Diplodia gossypina* and *Fusarium moniliforme* var. *subglutinans*. Pages 347-355. In: Proceedings, flowering and seed development in trees: A symposium; New Orleans, LA: USDA Forest Service, Southern Forest Experiment Station. Unnumbered report.

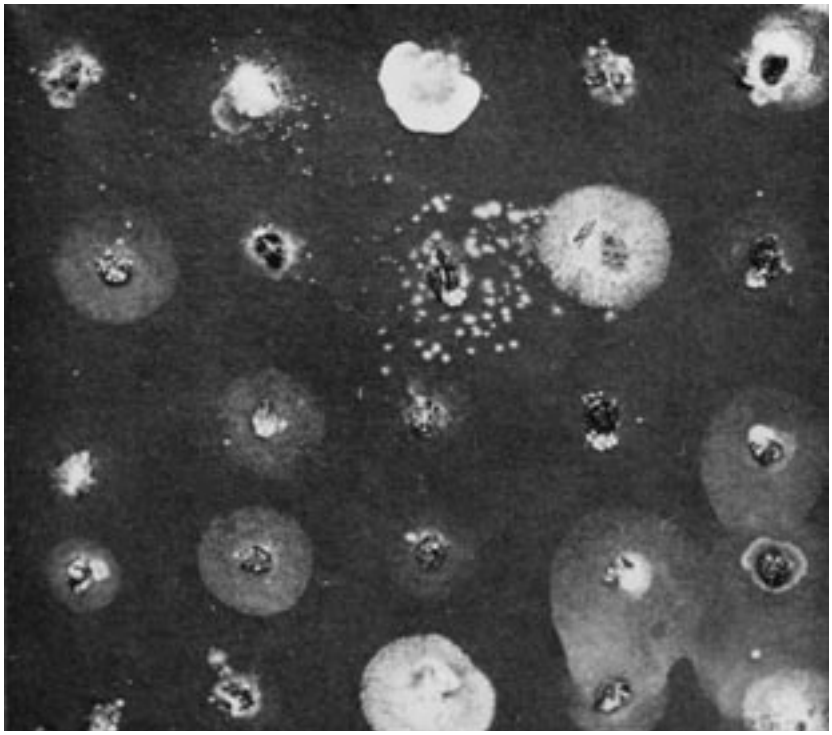

Fig. 1. Colonies of *Fusarium moniliforme* var. *subglutinans* on blotters.

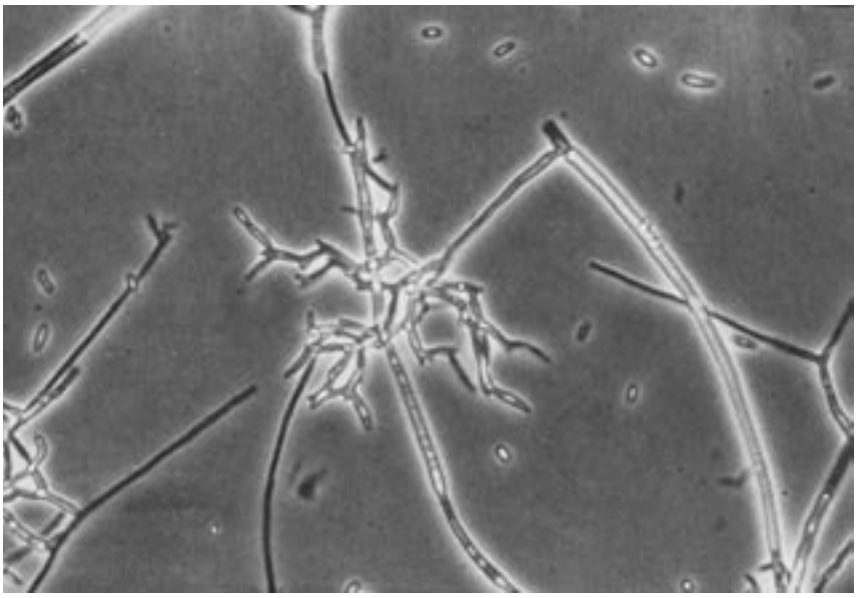

Fig. 2A Polyphilades of *F. moniliforme* var. *subglutinans* (Magnification x1200).

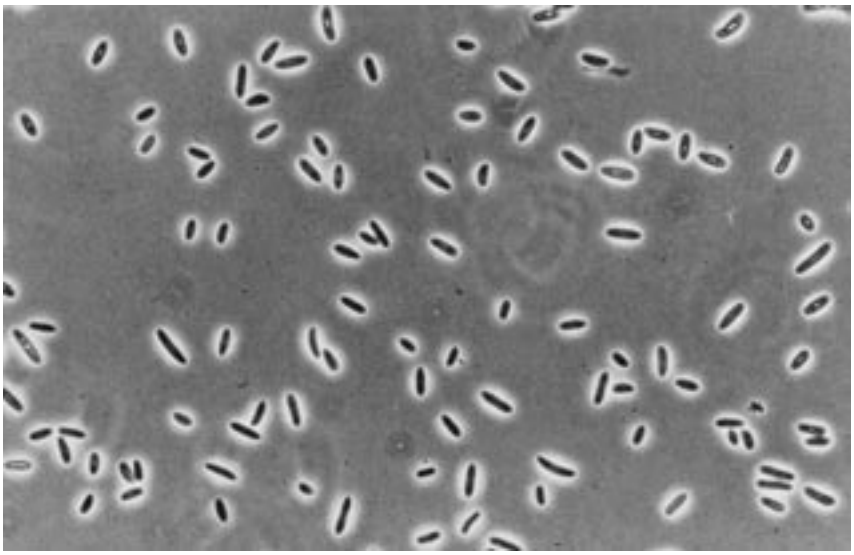

Fig. 2B. Microconidia of *F. moniliforme* var. *subglutinans* (Magnification x1200).

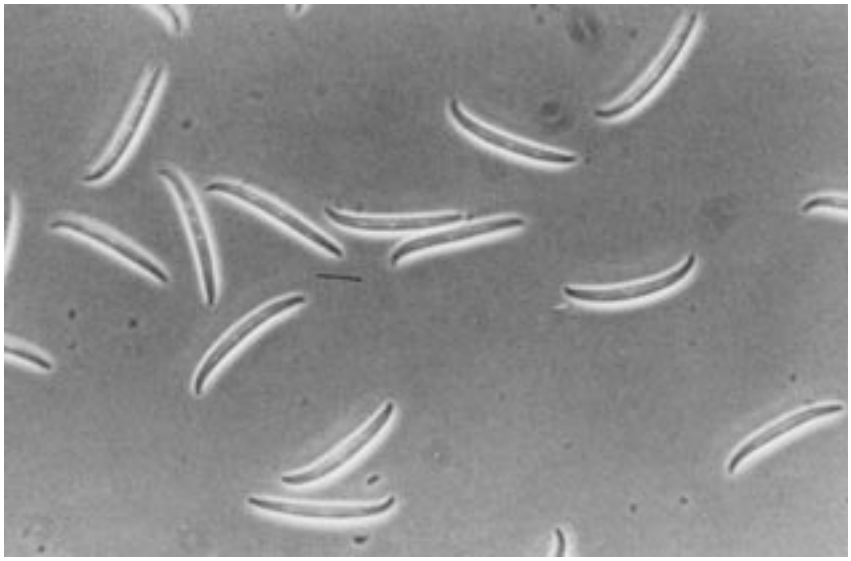

Fig. 2C. Macroconidia of *F. moniliforme* var. *subglutinans* (Magnification x1200).