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

7-008: Detection of *Caloscypha fulgens* on *Picea engelmannii* and *glauca* (Spruce)

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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: *Picea* spp., Spruce (White, Sitka, Engelmann, or their hybrids)

Pathogen: *Caloscypha fulgens* (Pers.) Boud. Imperfect state: *Geniculodendron pyriforme* Salt

Prepared by: ISTA-PDC Method Validation Sub-committee

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Revised 20.11.2001 J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.0.1, 01 January 2005, editorial changes

Submitted by: ISTA-PDC Method Validation Sub-committee

Background

This method was originally published in the ISTA Handbook of Seed Health Testing in 1987 as Working Sheet No.63 prepared by Jack R. Sutherland, Pacific Forestry Centre, Canadian Forestry Service, 506 W. Burnside Road, Victoria, B.C., V8Z 1M5, Canada. The 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 7-008 and is subject to review before 2006.

Safety Precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation and handling of hydrogen peroxide (a strong oxidizing agent), media, 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	-	Water Agar.
Hydrogen Peroxide	-	(30%) for seed disinfection.
Petri dishes	-	when sowing density is given by a number of seeds per petri dish, a diameter of 90 mm is assumed.
Incubator	-	capable of operating in the range 15ºC ± 2ºC.

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*

   Surface sterilize the seeds for 30 min. in 30% hydrogen peroxide (three volumes of the H_20_2 per one volume of seeds).

   CCP *The 30 min. surface sterilization with 30%* H_20_2 *significantly reduces contamination from other fungi and bacteria and allows better detection of C. fulgens; surface sterilization for periods longer than 30 minutes decreases incidence of the pathogen (Sutherland, et al., 1978)*

   Stir the seeds once or twice during the 30 minutes.

   Drain of the hydrogen peroxide with and agitate the seeds for 5 minutes in sterile, distilled water; then drain off.

   Surface dry the seeds on sterile paper in a sterile environment.

2. *Medium Water Agar*

   Place the surface sterilised seed onto 1.5% water agar, about 15 ml/9 cm petri dish. 25 seeds are placed in a petri dish.

3. *Incubation*

   Store the plates in plastic bags at 15ºC for 5 weeks under fluorescent light for 8-12 hour intervals of alternation with darkness.

4. *Examination*

   Examine every 3 days and remove seeds exhibiting blue stain (Fig. 3) (produced by

C. fulgens) in agar or typical coarse varrucose, right angle branched hyphae (Fig. 2) usually covered with water droplets. Characteristics are identifiable at x100 using a stereomicroscope. Seeds that germinate do not yield the fungus.

The conidiophores (Fig. 4) which arise from aerial hyphae are 200- 550µ high, smooth pale yellow to yellow brown below, 8-17µ in diameter below and taper to 3.6 6µ above; unbranched up to 220µ, then more or less dichotomously branched; hyaline above. Conidia (Fig. 4) are 4.6-7.6µ x 3.2-4.0µ; holoblastic, smooth, hyaline, ovate, dry. More details on the characteristics of the hyphae, conidiophores and conidia of *C. fulgens* are given by Paden et al. (1978) and Salt (1974).

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

1.5% Water Agar

Compound	g/l	g/500 ml
Agar	15	7.5
Deionized/Distilled Water	1l	500 ml

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 (or 500) ml of deionized/distilled water.
3. Dissolve powdered Agar in deionized/distilled water by stirring.
4. Autoclave at 15 PSI and 121°C for 15 min.
5. Allow agar to cool to approx. 50°C.
6. Pour 15ml of molten agar into 90 mm Petri plates and allow to solidify at room temperature before use.

Plates may be stored at room temperature in a dry cabinet or at 4ºC.

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. 63, J. R. Sutherland,1987.

Paden, J.W. Sutherland, J.E. and Woods, T.A.D. (1978). *Caloscypha fulgens* (Ascomycetidae, Pezizales): the perfect state of the conifer seed pathogen *Geniculodendron pyriforme* (Deuteromycotina, Hyphomycetes). Canadian Journal of Botany 56(19): 2375-2379.

Salt, G.A. (1974) *Geniculodendron pyriforme* gen. et sp. nov., a pathogen of conifer seeds. Transactions of the British Mycological Society 63(2): 339-351.

Sutherland, J.R., Woods, T.A.D., Lock, W. and Gaudet, D. A. (1978). Evaluation of surface sterilants for isolation of the fungus *Geniculodendron pyriforme* from Sitka spruce seeds. Canada Department of Fisheries and Environment, Canadian Forestry Service, Bi Monthly Research Notes 34(4): 20-21.

Thomson, A.J., Sutherland, J.E., Woods, T.A.D., and Moncrieff, S.M. (1983). Evaluation of seed disease effects in container sown Sitka spruce. Forest Science 29(1): 59-65.

Fig. 1. Patches of hard, whitish grey mycelium on Stika spruce seeds (ca. x50)

Fig. 2. Coarse, verrucose, right-angle branching hyphae of *C. fulgens.*

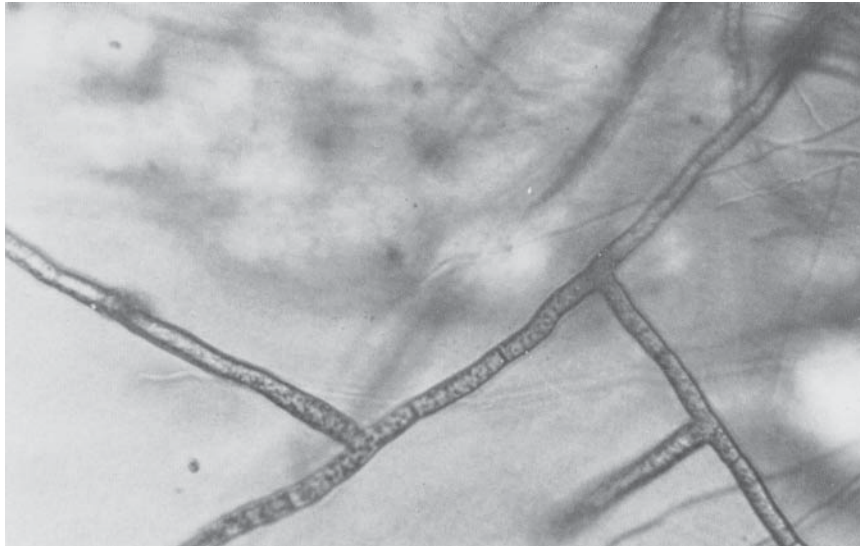
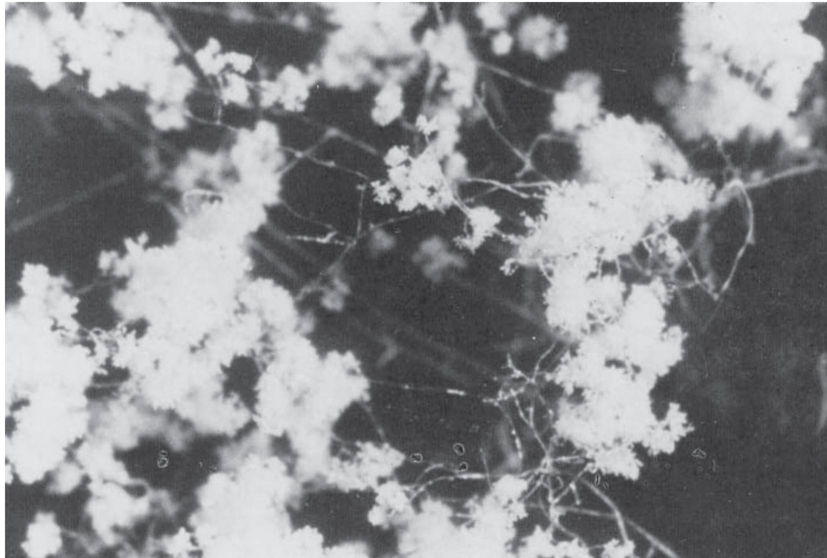


Fig. 3. Indigo-coloured mycelium of *C fulgens* growing from a Stika spruce seed on water agar.



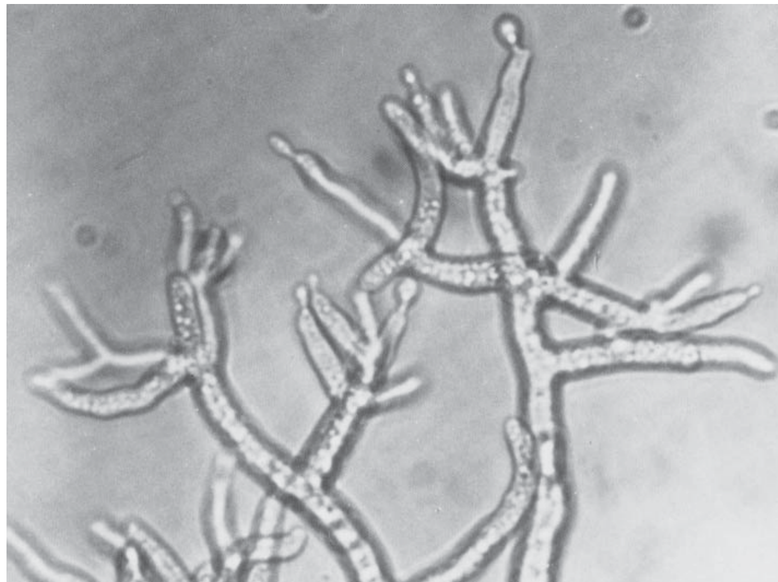


Fig. 4. Conidia (top) produced on Stika spruce seed incubated on water agar; (bottom) conidiophores and conidia of *C. fulgens*