



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

Revision History: Version 1.0 November 20, 2001
Revised 20.11.2001 J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.0.1, 01 January 2005, editorial changes
Version 1.1 2008-01-01
“Treated seed” revised; “Reporting results” revised

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 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 & Koenraad, 2007) with the recommendation to accept for a further five years.

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.

Treated Seed

This method has not been validated for the determination of *Caloscypha fulgens* 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 | - 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 15 ± 2 °C. |

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 H₂O₂ per one volume of seeds).

CCP *The 30 min surface sterilization with 30% H₂O₂ 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 min.

Drain off the hydrogen peroxide and agitate the seeds for 5 min 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 per 9 cm Petri

dish. 25 seeds are placed in a Petri dish.

3. Incubation

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

4. Examination

Examine every 3 days and remove seeds exhibiting blue stain (produced by *C. fulgens*) in agar (Fig. 3) or typical coarse varrucose, right angle branched hyphae (Fig. 2), usually covered with water droplets. Characteristics are identifiable at $\times 100$ using a stereomicroscope. Seeds that germinate do not yield the fungus.

The conidiophores (Fig. 4) which arise from aerial hyphae are 200–550 μm high, smooth pale yellow to yellow brown below, 8–17 μm in diameter below and taper to 3.6–6 μm above; unbranched up to 220 μm , then more or less dichotomously branched; hyaline above. Conidia (Fig. 4) are 4.6–7.6 $\mu\text{m} \times 3.2$ –4.0 μm ; 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. 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 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, p 644).

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

1.5% Water Agar

Compound	g/L
Agar	15.0
Distilled/de-ionized water	1000 mL

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 mL of distilled/de-ionized water.
3. Dissolve powdered Agar in distilled/de-ionized water by stirring.
4. Autoclave at 15 p.s.i. and 121 °C for 15 min.
5. Allow agar to cool to approx. 50 °C.

6. Pour 15 mL of molten agar into 90 mm Petri plates and allow to solidify at room temperature before use.

Storage

Prepared plates may be stored at 4 °C for up to 6 weeks.

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

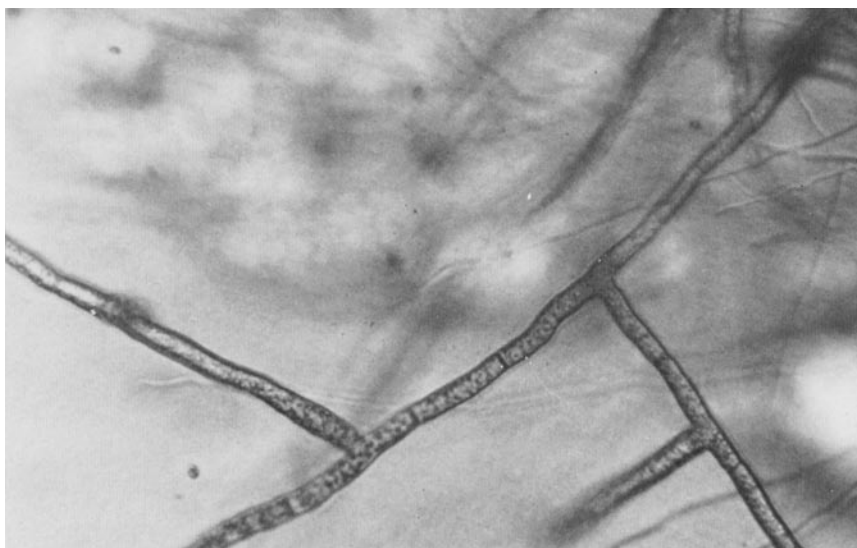


Fig. 2. Coarse, verrucose, right-angle branching hyphae of *C. fulgens*.

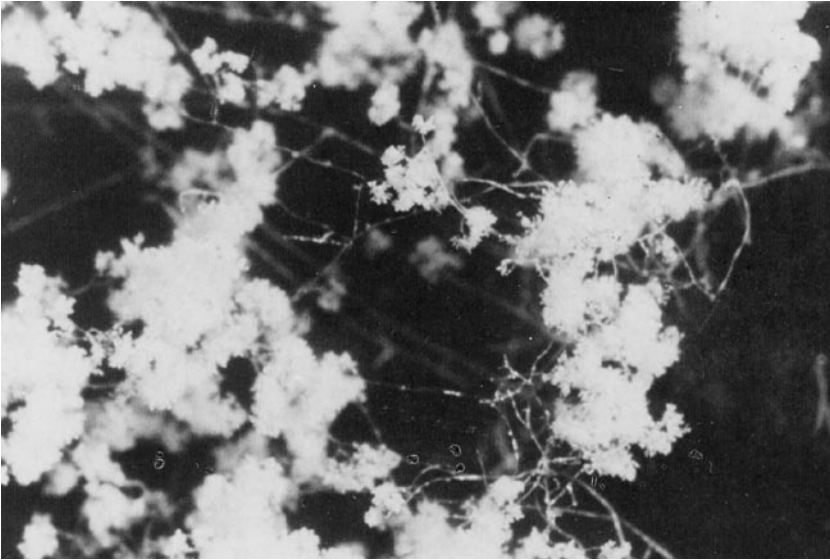


Fig. 3. Conidia produced on Sitka spruce seed incubated on water agar

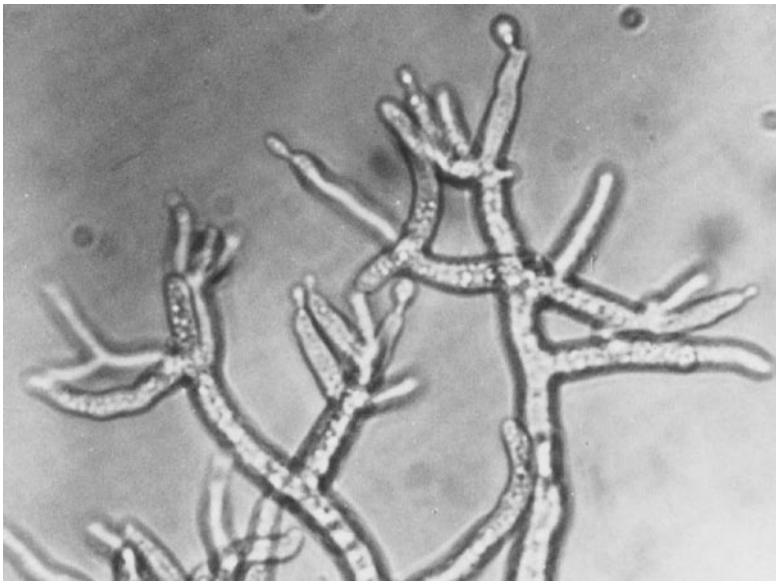


Fig. 4. Conidiophores and conidia of *C. fulgens*

