International Rules for Seed Testing 2024

Validated Seed Health Testing Methods

7-008: Detection of *Caloscypha fulgens* in *Picea engelmannii* and *P. glauca* (spruce) seed

Including changes and editorial corrections adopted at the Ordinary General Meeting 2023 in Verona, Italy

Effective from 1 January 2024

Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at ista.office@ista.ch.

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

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 or damage, etc. resulting from the use of this method.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during weighing out of ingredients. It is assumed that persons carrying out this test are in a laboratory suitable for carrying out microbiological procedures and familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic techniques. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French, German and Spanish versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.

Published by
The International Seed Testing Association (ISTA)
Richtiarkade 18, CH-8304 Wallisellen, Switzerland

Online ISSN 2310-3655

7-008: Detection of *Caloscypha fulgens* in *Picea engelmannii* and *P. glauca* (spruce) seed

Host: *Picea engelmannii* Engelm.; *Picea glauca* (Moench) Voss
Pathogen(s): *Caloscypha fulgens* (Pers.) Boud. (Imperfect state *Geniculodendron pyriforme* Salt)

Submitted by: ISTA-PDC Method Validation Sub-committee
Authors: ISTA-PDC Method Validation Sub-committee

Revision history

Version 1.0, 2001-11-20
Revised 2001-11-20: J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.0.1, 2005-01-01: Editorial changes
Version 1.1, 2008-01-01: Treated seed revised; Reporting results revised
Version 1.2, 2014-01-01: Addition of positive control
Version 1.3, 2017-01-01: Reporting results revised
Version 1.4, 2021-01-01: Sample preparation changed to Sample size and paragraph revised; addition of sodium hypochlorite solution in Media and solutions
Version 1.5, 2024-01-01: Sample size revised

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

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.)

Sample size

The sample size (total number of seeds to be tested) depends on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The minimum sample size should be 400 seeds.

Materials

Reference material: reference cultures or other appropriate material
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

Methods

1. Pretreatment: Surface sterilise the seeds for 30 min. in 30 % hydrogen peroxide (three volumes of the H_2O_2 per one volume of seeds).
   CCP**:** The 30 min surface sterilisation with 30 % H_2O_2 significantly reduces contamination from other fungi and bacteria and allows better detection of *C. fulgens*; surface sterilisation for periods longer than 30 min. 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. Plating

2.1 Medium: Place the surface-sterilised seed onto 1.5 % water agar, about 15 ml per 90 mm Petri dish. 25 seeds are placed in a Petri dish.

2.2 Positive control (reference material): Aseptically place seeds pretreated in the same way as 1., in an appropriate number of plates 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.

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 ×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 µm × 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). Compare with positive control.

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are 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 Table 5B Part 1 of Chapter 5 of the ISTA Rules, or Table G1 in Miles (1963).

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'.
The report must indicate the number of seeds tested.
In the case of a negative result (pathogen not detected), the results must be reported as 'not detected'.
In the case of a positive result, the report must indicate the percentage of infected seeds.

Quality assurance

Critical control points (CCP)

Where the wording of the original Working Sheet suggests that an action is critical this has been marked with CCP.

Media and solutions

Sodium hypochlorite solution

Sodium hypochlorite for pretreatment of seed can be prepared from commercial bleach diluted to 1 % available chlorine. The concentration of chlorine in commercial bleach varies considerably and declines with storage. Use the formula:

$$V_{stock} = V_{final} \times C_{final} / C_{stock}$$

(where V = volume and C = % available chlorine) to calculate the volume of commercial bleach stock solution required to prepare sodium hypochlorite solutions for use in seed pretreatment.

To prepare a 1 l solution of sodium hypochlorite containing 1 % chlorine from a stock of commercial bleach containing 12 % available chlorine:

$$V_{stock} = 1 \times 1/12 = 0.083$$

Thus add 83 ml of the 12 % stock to 917 ml water.

The percentage of active chlorine decreases rapidly in solution so, NaClO 1 % solution must be stored in the dark and used within 3 days of preparation. It is possible to check chlorine concentration with chlorine strip tests.

1.5 % water agar

Agar: 15.0 g
Distilled/deionised water: 1000 ml

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Dissolve powdered Agar in distilled/deionised 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 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.

Figure 1. Indigo-coloured mycelium of *C fulgens* growing from a Sitka spruce seed on water agar.

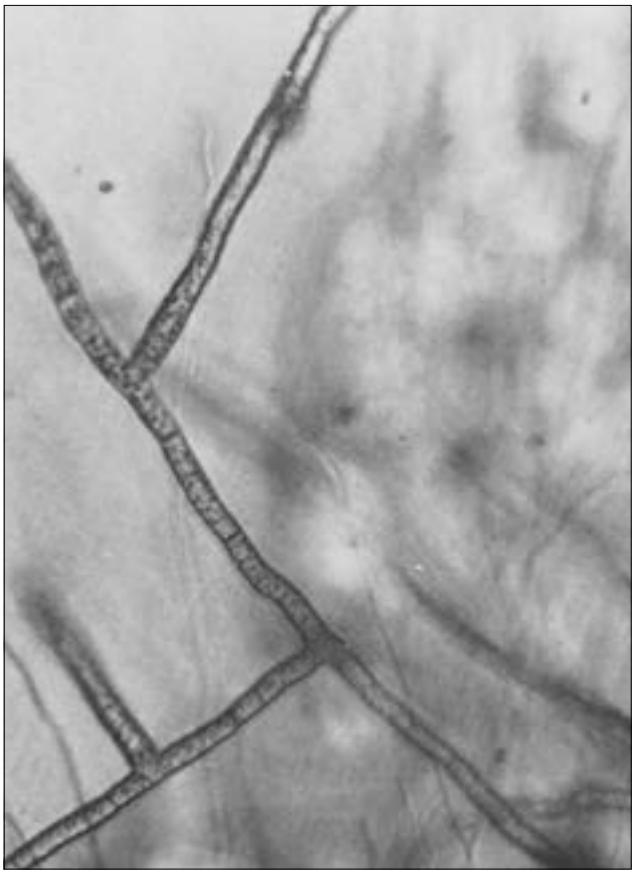

Figure 2. Coarse, verrucose, right-angle branching hyphae of *C. fulgens*.

References

The following references are extracted from the *ISTA Handbook of Seed Health Testing*, Working Sheet No. 63, J.R. Sutherland,1987.

Miles, S. R. (1963). Handbook of tolerances and of measures of precision for seed testing. *Proceedings of the International Seed Testing Association*, **28** (3), 525–686.

Paden, J. W. Sutherland, J. E. & 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. & 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, *BiMonthly Research Notes,* **34** (4), 20–21.

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

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

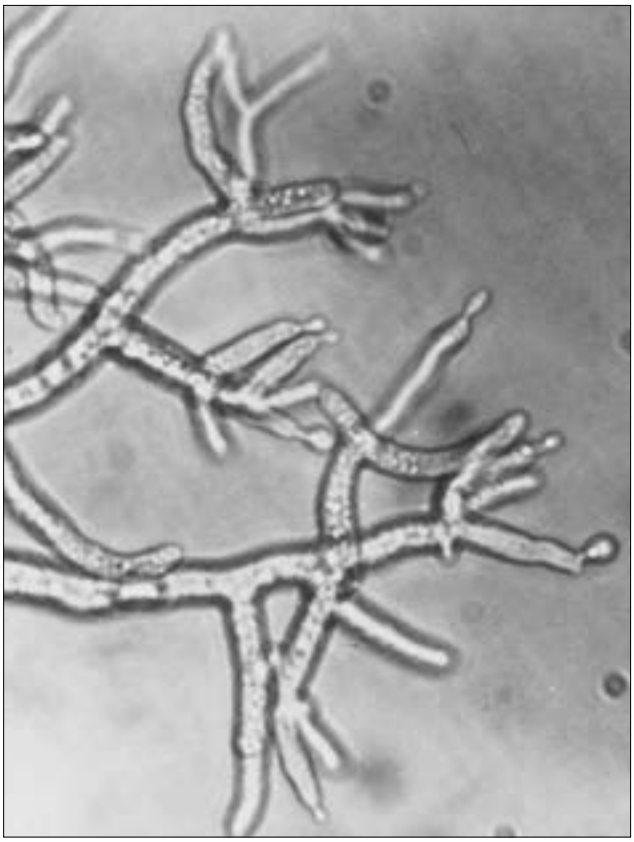

Figure 4. Conidiophores and conidia of *C. fulgens.*