International Seed Federation
Seed is Life

Detection of *Peronospora valerianellae* in Corn Salad Seed

APRIL 2025

Developed by ISHI

Detection of *Peronospora valerianellae* in Corn Salad Seed

Crop: Corn salad (*Valerianella locusta*)

Pathogen(s): *Peronospora valerianellae*

Version: 1 (April 2025)

PRINCIPLE

Detection of viable *Peronospora valerianellae* in seed of corn salad is done by growing out seeds under environmental conditions highly conducive to producing disease symptoms, followed by morphological examination of the seedlings.

The method process workflow is presented in Figure 1.

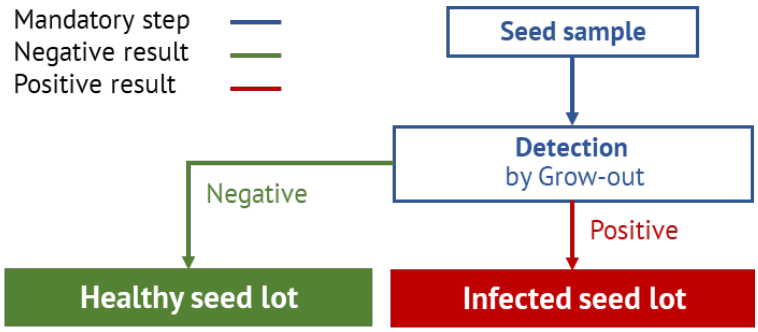

Figure 1. Method process workflow.

METHOD VALIDATION

The validation report for the detection of *P. valerianellae* in corn salad seed (Orgeur, 2025) can be found on the ISF website.

RESTRICTIONS ON USE

Before using this protocol routinely, it is necessary to verify its performance, especially when material and consumables from different suppliers are used. Technical details on the reagents/material used in the validation study (e.g., supplier's information) are provided in the protocol and the validation report.

This test method is suitable for untreated seed and seed that has been treated using physical (e.g., hot water) or chemical (acid extraction, calcium or sodium hypochlorite, tri-sodium phosphate, etc.) processes with the aim of disinfestation/disinfection, provided that any residue, if present, does not influence the assay. It is the responsibility of the user to check for inhibition by experimental comparisons or other means.

This test method has not been validated for seed treated with protective chemicals or biological substances. If treated seed is tested using this method, it is the responsibility of the user to

determine empirically (through analysis, sample spiking, or experimental comparisons) whether the protective chemicals or biological substances have an effect on method results.

METHOD EXECUTION

To ensure process standardization and valid results, it is strongly recommended to follow the Best Practices for Seed Health Tests developed by ISHI.

SAMPLE AND SUBSAMPLE SIZE

The recommended minimum sample size is 400 seeds.

REVISION HISTORY

Version	Date	Changes (minor editorial changes not indicated)
1	April 2025	First version of the protocol

Protocol for detecting *P. valerianellae* in corn salad seed

I. DETECTION BY GROW-OUT

Materials

- Containers: potting trays/boxes with a lid
- Potting soil substrate (containing up to 1/3 sand)
- Ethanol 70% (v/v) or equivalent disinfectant
- Controls (See Table I.1)
- Growth chamber (12 ± 3 °C) supplemented with light
- Water

Table I.1. Types of controls used.

Control type	Description
Positive process control (PPC)	Known *P. valerianellae* infected seed sample
Negative process control (NPC)	Known healthy seed sample

1. Sowing

1.1. Disinfect the containers with 70% (v/v) ethanol or equivalent.

1.2. Fill containers with potting soil substrate (containing up to 1/3 sand). Different sizes of containers with lids can be used. Add between one-third to one-half of the container's volume to allow for seed germination and growth during 21 to 28 days.

1.3. Sow 400 seeds evenly at the rate of 1 seed / 1.5 to 2 cm per cm^2. The number of containers required for 400 seeds will depend on their dimensions and the prescribed sowing density.

1.4. Water the containers taking into account their dimension (e.g., 100 mL for a 10×15 cm box).

Note: Optimising the quantity of water required during sowing and during plant growth is essential. The relative humidity in the containers can greatly influence the outcome of the test and must be controlled for and monitored for the duration of each test.

1.5 Close the container with the lid.

2. Positive process control (PPC)

2.1. Prepare another container for the infected seed in the PPC using the same conditions as for the test samples.

3. Negative Process Control (NPC)

3.1. Prepare a container for the healthy seed sample, the NPC, using the same conditions as for the test samples. Preferably prepare the NPC after the samples to be tested and PPC.

4. Incubation

4.1. Incubate the sample and controls at 12 ± 3 °C and 12 h light for 21 days. Record the temperature in the closed container with a temperature probe. Check relative humidity regularly (optimal 100%), condensation should be present on the complete lid of the container during the entire duration of the test.

5. Examination of the seedlings

5.1. The results are indicated as the percentage of seeds infected by *P. valerianellae* calculated as the number of infected seedlings against the total number of seeds sown.

5.2. Examine PPC containers. Seedlings from the PPC seeds should show cotyledon curling and/or white sporulation on cotyledons (Figure I.1). The number of seedlings of the PPC that are infected is dependent on the rate of infection of the seeds.

5.3. Examine the seedlings from the test samples for typical sporulation by comparing them with the PPC seedlings. If only curling cotyledons are observed, prolong the incubation for one extra week.

5.4. At the end of the sample observation, examine the NPC containers. No disease symptoms should be observed on the NPC seedlings.

Note: Test results are only valid when all included controls presented in Table I.1 give the expected results. If more than 25-30% of the corn salad seedling show damping-off or do not germinate, test results are considered to be inconclusive

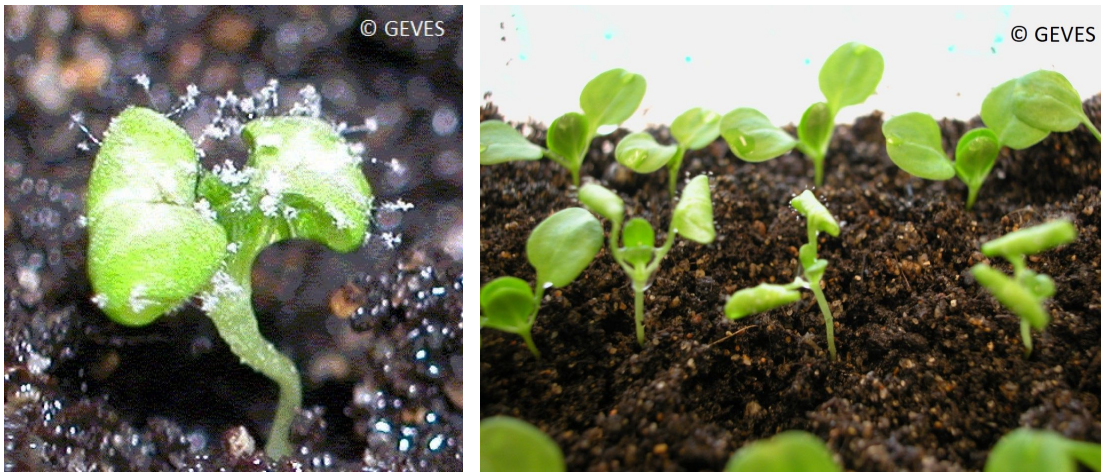

Figure I.1. Typical sporulation of *P. valerianellae* (left and right) and cotyledon curling (right) on infected corn salad seedlings.

REFERENCES

Orgeur, G. (2025). Detection of *Peronospora valerianellae* in corn salad (*Valerianella locusta*) seed. Validation report, International Seed Federation (ISF), Nyon, Switzerland. https://www.worldseed.org/our-work/seed-health/ishi-method-development-and-validation/