

Managing Seedborne Diseases in Western Forest Nurseries

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Fungi that contaminate conifer seed frequently cause seed rot and seedling disease. In this paper, we discuss criteria for seed treatment, assay procedures for the seedborne fungus Fusarium, and safe and effective seed treatments. Subjects needing further investigation are summarized. Tree Planters' Notes 41(4):3-7; 1990.

The Problem

Conifer seeds are normally contaminated with a variety of different microorganisms, including potentially pathogenic fungi. The fact that many seedling diseases can be caused by seedborne pathogens has been recognized by forest nursery pathologists for many years. The true scope of the problem was not realized, however, until conifer seedlings began to be grown in container nurseries, where the losses are readily apparent (fig. 1).

Because both containers and growing areas are cleaned and "sterilized" between crops, and artificial growing media are also considered disease-free, contaminated seed is the most likely means of disease introduction (7). In container nurseries, fungi frequently can be seen fruiting on the seed coat and often are associated

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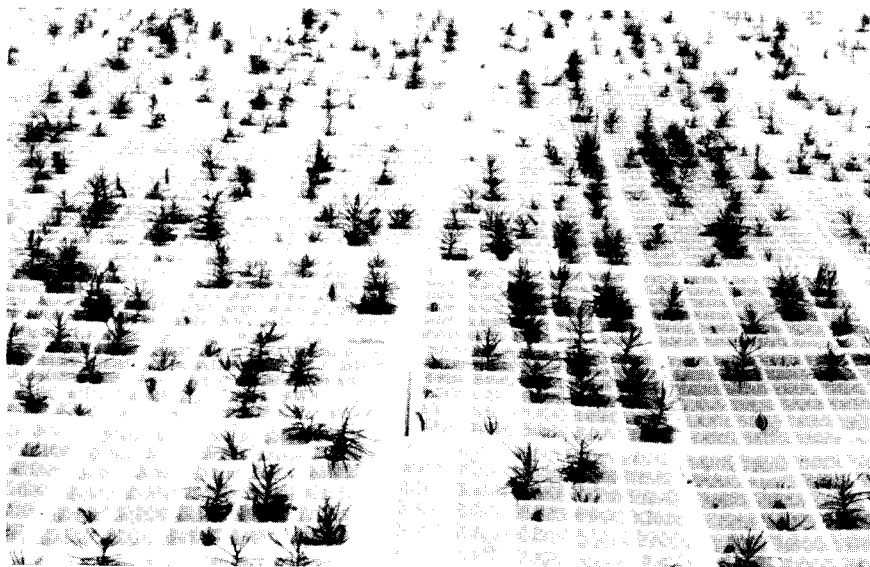


Figure 1—Seedborne disease problems are particularly evident in container nurseries.

with damping-off, cotyledon blight (fig. 2), and root rots.

A number of different pathogenic fungi have been isolated from conifer

seed (table 1), including *Alternaria* spp., *Cladosporium* spp., *Cylindrocarpon* spp., *Fusarium* spp., *Penicillium* spp., and *Tricho-*

Table 1—Common genera of seedborne fungi in Pacific Northwest conifers

Fungus	Appearance in culture	Associated disease
<i>Alternaria</i>	Gray-green to black; septate, club-shaped spores	Radicle and cotyledon disease of white spruce
<i>Caloscypha</i>	Beige, whitish-gray, orange to pale yellowish brown; smooth, colorless round spores	Seed rot, reduced germination
<i>Cladosporium</i>	Dark velvety green; sickle-shaped spores	Reduced vigor of germination
<i>Fusarium</i>	White, salmon to pinkish-red; sickle-shaped spores with 3 to 4 septations	Reduced germination; cotyledon, hypocotyl, and root disease
<i>Penicillium</i>	Green to bluish-green; small, round spores	Reduced vigor of germination
<i>Trichoderma</i>	White turning to green; small, round spores	None; this is usually a beneficial fungus
<i>Trichothecium</i>	Pinkish; 2-celled spores	Reduced germination

Source: Modified from Littke (1990).



Figure 2—Cotyledon blight is one fungal disease that is obviously seedborne. In this picture, the fungus can be seen growing from the seedcoat to the cotyledons.

thecium spp. (2,8). These fungi can, under certain conditions, cause seed or seedling disease. Other fungi are frequently isolated from seed but either are not harmful (e.g., *Trichoderma* spp.) or their pathogenic abilities are unknown. Fungal contamination of conifer seed can occur while the cone is still on the tree, during collection and storage, during seed extraction and processing, or during stratifica-

tion. Cones collected from squirrel caches are particularly vulnerable (9). Because it is impractical to try to protect the seed from contamination throughout the entire period between cone harvest and seed sowing, it is logical to treat seed for pathogens before stratification or sowing.

Three general methods have been used to treat seed; they are

listed in order of increasing severity:

1. Surface cleansing (washing the seed with running water and/or detergents to remove contaminants).
2. Surface disinfecting (treating the seed coat with heat or chemical disinfectants, such as sodium hypochlorite or hydrogen peroxide).
3. Coating the seed with fungicides (such as thiram or captan).

In the past, nursery managers routinely treated their seed with fungicides before sowing. However, this practice has come under increasing scrutiny because of possible toxicity to the germinating seed as well as adverse effects on human health and the environment. Cleansing or disinfecting the surface of the seed is becoming increasingly popular as a way to control seedborne pathogens.

Steps for Managing Seed Disease

The following recommendations, based on the collective practical experience and operational trials of western nursery pathologists, will be refined and improved as new information becomes available. They are listed in sequential order:

Determine if seed treatment is warranted. Because any seed treatment is a potentially damaging operation, nursery managers should first attempt to determine if

a seedborne disease problem exists and assess the potential seriousness of the problem. We recommend seed treatments for the following situations:

- Seeds to be grown in container nurseries, where pathogens can be introduced into a relatively sterile environment and the value of seedlings is high.
- Seedlots showing problems, such as low germination rates, visible mold, or a history of poor germination or seedling disease (fig. 1).
- Seeds of high-risk species that typically exhibit high levels of disease in the nursery, which could be caused by seedborne pathogens (for example, sugar pine (*Pinus lambertiana* Dougl.), western white pine (*Pinus monticola* Dougl.), western larch (*Larix occidentalis* Nutt.), and Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco.]).
- High-value seeds such as those used for tree improvement, especially those of high-risk species.

Assay seeds and identify species of pathogens.

Before considering any seed treatment, suspected problem lots should be assayed for the presence of pathogenic fungi. Because it is impossible to identify contaminated seed lots simply by looking at them, nursery managers should send samples of seed from problem lots to a plant pathogen laboratory that offers assays for specific seed pathogens.

Characteristics of colonies and spores of common seedborne fungi are described in table 1. Currently, *Fusarium* spp. are the only seedborne pathogens that are frequently assayed in the Pacific Northwest, and the following procedure is recommended:

1. For each seedlot to be analyzed, randomly select 500 seeds; if the lot is extremely small, a representative sample is sufficient. Do not wash or surface sterilize this sample.
2. Place up to 25 seeds (depending on seed size) on a petri dish of Komada's medium (6), using forceps or a specially prepared template.
3. Incubate the plates, agar side down, under constant light for 5 to 7 days, at room temperature [20 to 25 °C (68 to 77 °F)] .
4. Identify and count the number of colonies of *Fusarium* spp. associated with seed under a compound microscope (fig. 3).
5. Report results as a percentage of seeds with *Fusarium* spp. present on them.

Treat seed lots that show

contamination. Once a problem lot has been identified, seeds should be cleansed with running tap water or disinfected with bleach, ethanol, or hydrogen peroxide. Currently registered seed fungicides are not recommended for use.

1. *Cleansing seed surfaces* with a running water rinse is recommended for all species. A

running water rinse is more effective than a standing water soak for the pre-stratification imbibing treatment. The following tap water rinse has no phytotoxic potential, has been effective in reducing pathogen loads (5,10), and can be easily implemented in any nursery operation:

Place seeds loosely in mesh bags that have twice the volume of the seed. Run cold tap water over seeds with periodic gentle agitation for at least 48 hours before stratification.

2. *Disinfecting seed surfaces* can be phytotoxic, but the following seed treatments have been effective in reducing pathogen loads on problem seedlots. Several chemical disinfectants have proven effective for different species, but any such treatment should be tested on a small scale before being implemented operationally:

Bleach treatments have been tested with several species of pine [ponderosa (*Pinus ponderosa* Dougl.), lodgepole (*P. contorta* Dougl.), western white, Scotch (*P. sylvestris* L.), and Austrian (*P. nigra* Arnold)] and Douglas-fir (10). Soak the seeds for 10 minutes in a 40% solution of household bleach [2 parts bleach (5.25% sodium hypochlorite) in 3 parts tap water], then rinse thoroughly in running water for at least 48 hours. Place seeds in mesh bags

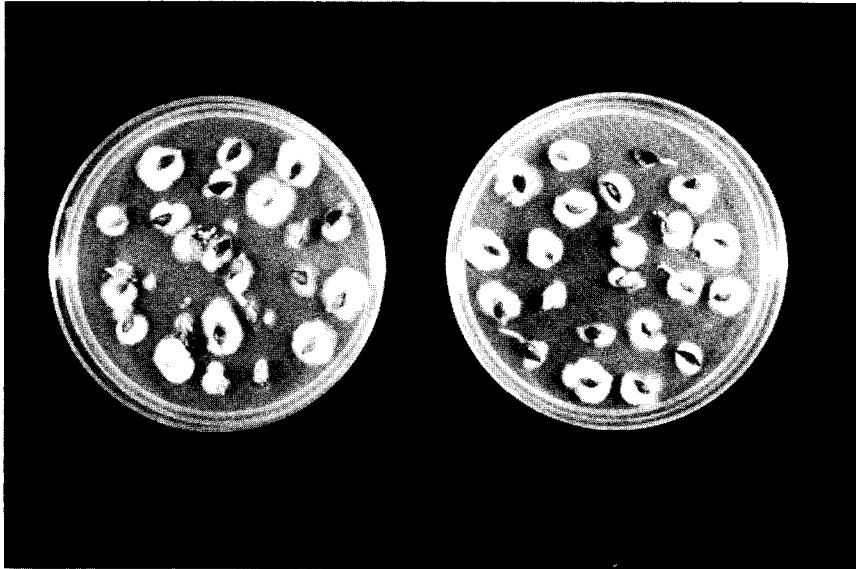


Figure 3—Pathogenic assays of suspected problem seedlots confirm the presence of seed-borne fungal pathogens. In this photograph, the mycelia of *Fusarium* spp. can be seen growing from the seeds.

that have twice the volume of the seed. Seeds can be treated before or after stratification.

Ethanol treatments have been tested with Douglas-fir. Dumroese and others (4) found that placing seeds in 95% ethanol for 15 seconds reduced levels of pathogens. A trial with a 10-minute soak in 70% ethanol, followed by a 48-hour rinse with running tap water reduced pathogens but also inhibited seed germination (1). Seeds should be placed in mesh bags that have twice the volume of the seed. Seeds can be treated before or after stratification.

Hydrogen peroxide treatments have been tested with sugar pine and Douglas-fir (4). Soak seeds in a 3% hydrogen peroxide solution for 3 to 5 hours, then rinse them thoroughly in running water for at least 48 hours. Seeds should be placed loosely in mesh bags that are twice the volume of the seed. Seeds have been treated before (sugar pine) or after (Douglas-fir) stratification.

Surface-dry seed during extended stratification. Any seed lot stratified for more than 30 days should be surface-dried and then put back into stratification. The

objective of this procedure is to reduce or prevent mold development, which is more likely to develop in the high humidity conditions that exist in long-term stratification. This procedure is currently used by Federal nurseries in Oregon and Washington.

Ideas For Future Work

Because so little is known about seedborne pathogens and their effect on forest nursery production, additional work is needed on this subject. We present the following ideas for future research:

1. Determine the pathogenicity of fungi, other than *Fusarium* spp., that are associated with the seeds of western conifers. Identify the types and modes of injury as well as the potential for economic damage.
2. Determine the importance of seed-contaminating fungi versus seed-infecting fungi, and the effect that seed quality has on the impact of each.
3. Develop biological control organisms, such as *Trichoderma* spp. or *Gliocladium* spp., that can be applied to seed to reduce or prevent infection of seeds or seedlings by seedborne pathogens.
4. Run operational trials on conifer seed with commercial formulations of biocontrol organisms produced for other crops.
5. Test new seed treatments [for example, microwaving, hot

water soaks, use of kelp formulations (Kelpak 66), use of oil] for their effectiveness at controlling pathogenic seedborne fungi.

6. Retest and refine seed treatments that are currently used operationally or have been previously tested, including household bleach, ethanol, and hydrogen peroxide. These treatments should be tested on other conifer and hardwood species, both before and after-stratification.
7. Test seed fungicides and new methods of applying fungicides to seed. New, untested chemicals may prove effective and safe. New technology exists for applying fungicides that may minimize phytotoxicity and maximize effectiveness; both old and new fungicides may be more effective if appropriately applied to seed.

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