Seed Pathogens and Seed Treatments

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INTRODUCTION

Every year, nursery growers prepare thousands of pounds of conifer seed for sowing in bare-root and container nurseries. The vigor and health of this seed is a strong determinant of the quality, uniformity and yield of the ensuing crop. To achieve the full potential from this seed and to minimize the loss from disease, growers need to be knowledgeable about seed pathogens. This is not to minimize the contribution that seed maturation level and vigor play in seedling development, but rather as an insurance of quality throughout the nursery production chain.

Occasionally things can go wrong, resulting in unacceptable seedling looses from poor germination or pre-emergence or post-emergence disease. Numerous literature citations have identified seed borne fungal pathogens as the prime or contributing factor in these losses (Littke and Browning 1990). From experience, reactive response with fungicides increases production costs and often results in marginal disease control. Therefore an understanding of the origin and nature of the association of seedborne fungi with seed may be helpful in reducing losses and capturing gains in a nursery production setting.

Today's discussion will try to capture some of our research experience on seed borne fungi. I would like to divide this subject into three areas:

1) Sources of seedborne inoculum
2) Pathogen detection methods
3) Seed treatments to control pathogen activity

ORIGINS OF SEEDBORNE INOCULUM:

Some 90+% of Weyerhaeuser Company seed for nursery use originates from seed orchards throughout Washington and Oregon. Littke and Browning (1990) reported that orchard seed can be associated with seed borne pathogens such as *Fusarium oxysporum* as well as other pathogens. We speculated that seed association with this pathogen, in particular, originated from aerial deposition on developing cones. From our work and the literature, we can deduce that three likely routes of subsequent seed contamination exist:

* physical transfer from exterior cone integument (bracts and scales) to seed coat surfaces during seed development, cone storage, and seed cleaning.
* cross contamination with "dirty" seedlots during cleaning, dewinging, imbibition, stratification, and sowing.

* contact with soil during storage or collection from squirrel caches.

This discussion will focus on the physical transfer model, since our evidence supports this as the prime origin of Fusarium in our system. Table 1 shows the seed developmental stage or processing step and our understanding of the associated disease phase.

Table 1. Physical transfer model to explain the possible associations with disease caused by seedborne fungi.

Seed Development or Process Step	Associated Disease Phase
• Deposition of airborne spores on cone flower and pollen flowers	*Contamination*
• Cone flower initiation/pollination	*Flower abortion*
• Seed development	*Seed abortion (?)*
• Mature cone at harvest (ground contact)	*Contamination*
• Mature cone in harvest bag	*Inoculum buildup*
• Ripening phase	*Seed gluing to scales*
• Cone drying and extraction	*Inoculum transfer*
• Cleaning and dewinging	*Cross-contamination*
• Freezer storage	*(too cold for activity)*
• Imbibition and stratification	*Contamination and seed-rot*
• Sowing	*Pre- and post-emergent mortality; soil inoculation*

Using this model, we have attempted to understand opportunities for control of seedborne fungi through manipulation of cone processing stages. For example, in Figure 1 we examined the levels of Fusarium prior to cone opening and following extraction. Much of the Fusarium shown in Figure 1 from closed cones is thought to be a result of contamination rather than evidence for cone infection. The important fact from this data is that seed can become contaminated during seed processing.

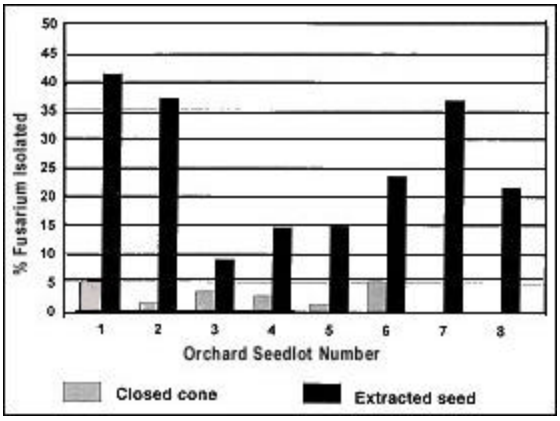

Figure 1. Levels of Fusarium prior to cone opening and after processing for Douglas-fir orchard seedlots.

We next experimented with ways to modify inoculum buildup during the cone storage phase. We placed one-bushel of cones in either a one- or two-bushel bag. In most cases, faster cone drying schedules reduced cone surface mold growth, which resulted in lower post-extraction Fusarium levels. Attempts to control cone mold using sterilants such as Clorox and have been tried unsuccessfully (Rediske and Shea 1965). Many opportunities remain in reducing seed contamination with fungal pathogens by changing cone and seed processing schedules. However, for the moment we will concentrate on detection and correction of seedborne "problems" where they exist.

PATHOGEN DETECTION METHODS

Much has been written concerning methods to detect seedborne fungi. For the purpose of this talk, I will restrict my comments to a broad generalization of seed assay methods. The main detection methodologies are shown in Table 2.

Table 2. Methods for isolating seedborne fungi.

Method	Pathogen Groups/ Benefits
* Broad Spectrum Agar Media (i.e. acidified PDA; 2%-malt agar; 1%-bacto peptone; NaCl agar)	Non-specific fungal isolation
* Fusarium Agar Media (Komada's Media; Nash and Schnieders etc.)	Fusarium groups, seed rot fungi
* Blotter media (incubate seed on moist blotters with liquid base media; used with whole, crushed or frozen\thawed seed)	Various fungal pathogens
* Serological methods	ELISA for specific pathogens
* PCR technology	DNA specific probes for pathogens

Seedborne fungi are routinely assayed in our research lab using the sampling protocol outlined below.

Seed Pathogen Assay Protocol:

1 Obtain 500 seed per seedlot to be tested.

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2. 100 seed plated directly on to Komada's Fusarium media (10/petri dish).
3. 100 seed soaked in 3% hydrogen peroxide for 30 minutes, washed 3X with water and plated as in step 2.
4. Plated seed is incubated at 25C, illuminated for 7-10 days.
5. Retest seed if post-surface sterilization Fusarium level above 10%.

6. Additionally cut 100 seed and plate seed without seed coat.
7. Report findings to seed plant operations.

Determinations of seedborne Fusarium levels are based on the difference between recovery of the pathogen in steps 2 and 3. Typically, surface sterilization with peroxide removes greater than 90% of the surface fungi. High pathogen incidence following peroxide sterilization might be indicative of seed damage, poor handling, or other seed quality problems. However, we consider these test results along with a variety of other seed quality tests, including; purity, X-ray analysis, seed size, and standard germ tests at 20-30°C and 5-15°C before making treatment recommendations. A common practice is to test seedlots with low germ (<90%) or with visible mold after stratification.

To date, our testing has confirmed that:

* potential seedling pathogens increase during cone storage and seed processing.
* Fusarium levels can vary by orchard source and year of collection.
* pathogenicity tests confirm some 60% of the Fusarium isolates from seed can cause disease.
* some 90% of the inoculum resides outside of the seed coat, and higher interior infection levels are often indicative of seed coat damage.

Seed Treatments:
Our strategy for seed treatment consists of using various agents to remove, reduce, or block the number of pathogens below a disease threshold, while not decreasing seedlot vigor.

These treatments in order of increasing treatment efficacy are;

* soaking seed in running water baths
* using chlorine or bromine agents to sanitize seed
* surface sterilization using 3% hydrogen peroxide

Water Rinse Vs. Soak:
The rinse process involves either 24 hour soak in standing water during the imbibition phase, or to use up to 7 changes of water in a 24 hour period with air agitation to stir and mix the seed. Both methods provide the needed moisture to begin stratification. In general, rinse treatments lowered recovery of *Fusarium roseum*, *Cladosporium*, *Trichothecium*, and *Penicillium*, but not *Fusarium oxysporum*. In addition, we noticed some positive benefits from the rinse treatments in terms of better overall germination. A water rinse in itself does not appear to be sufficient to reduce levels of *Fusairum oxysporum*.

Seed Coat Sterilants

A 10% Chlorox seed treatment reduced pathogen levels significantly (Figure 2). Products such as Agribrom show similar efficacy to Chlorox when supplied as a 350 ppm bromine solution. Both agents effectively sanitize surface seedborne inoculum. Seed treatment with Clorox or bromine for 10-30 minutes remove roughly +50% of the surface inoculum. These treatments appear to be more effective against seed-rot fungi type of fungi (i.e. Trichothecium, Cladosporium, Penicillium. It must be cautioned that reduced germination vigor can occur with prolonged seed exposure to
Clorox or bromine agents.

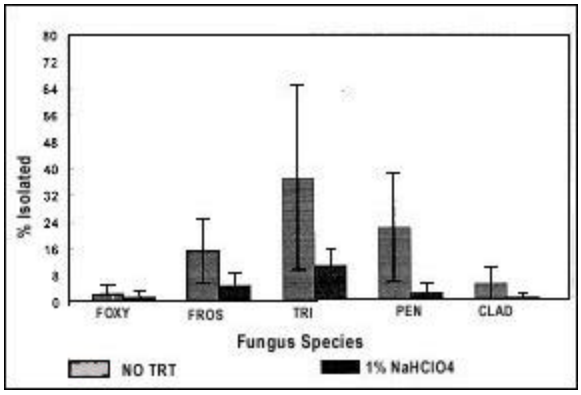

Figure 2. Reisolation of various fungi from Douglas-fir seed after 10 minutes soak in 10% Clorox solution (1% NaHClO,). Variation shown as ± 1STD. Fungus code: [FOXY -Fusarium oxysporum; FROS-Fusarium roseum complex; TRI-Trichothecium roseum; PEW Pencillium sp.; CLAD-Cladosporium.]

Seed Fungicides:

A number of fungicides have been tested as seed coat treatments. The main treatment strategy has been to inactivate potential pathogens or to reduce their numbers below a disease threshold, while not decreasing seedlot vigor. Thiram formulations for seed treatment, such as Thiram-75WP and Scram-42S have been tested across a wide range of conifer species. Typical seed treatments rates consist of 16 oz/ 100 lb of seed, plus Dow Latex Sticker (DL-24 INA). Three experimental uses of Thiram to control seedborne fungi will be briefly discussed.

Figure 3 shows the reisolation of Fusarium after treating Douglas-fir or ponderosa pine seed with sticker agent, peroxide, Thiram or Thiram+Sticker. These results clearly illustrate that Thiram is an effective fungicide against Fusarium. Similarly, Thiram, used as a seed coat treatment in non-fumigated soil can reduce post emergent mortality especially when combined with a pre-plant Subdue or post-emergent Banrot treatment.

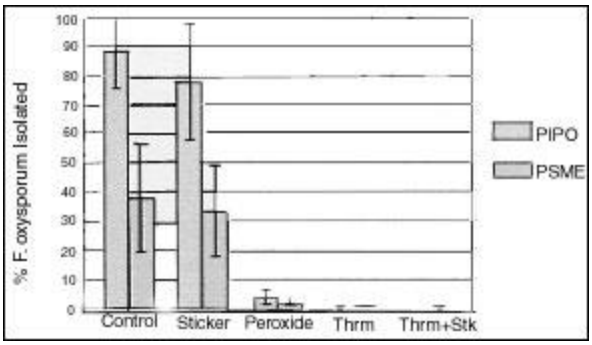

Figure 3. Reisolation of Fusarium oxysporum from ponderosa pine (PIPO) and Douglas-fir (PSME) seed after control, Sticker, 3% hydrogen peroxide for 10 minutes, Thiram or Thiram+ Sticker (label rate). Variation expressed as ± STD.

However, in Figure 4, we were able to detect some post treatment negative effects of Thirani (Arasan) on Douglas-fir seed germination performance. Germination was delayed with seed treatment, but total germination did not appear to be affected.

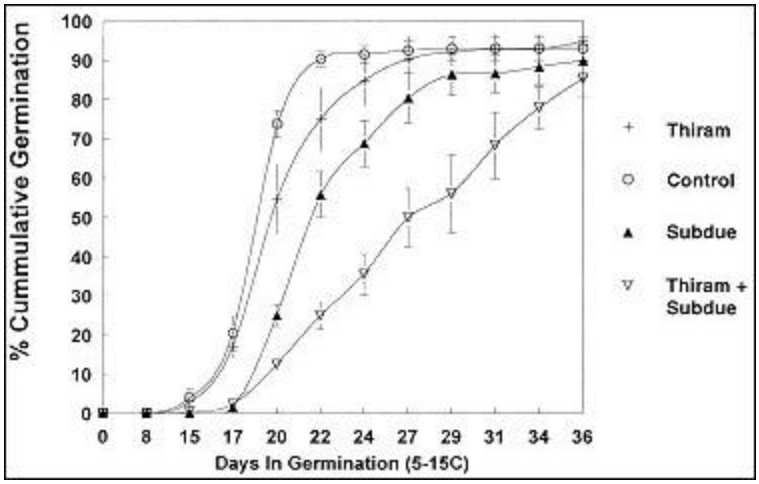

Figure 4. Germination performance of Douglas-fir with or without seed Thiram treatment with Thiram (Arasan), Control, Control Subdue, or combination of Thiram Subdue. Variation shown as ±1STD.

CONCLUSIONS/RECOMMENDATIONS

Seed treatments to negate or control potential impact of seedborne pathogenic fungi should be viewed as an important tool in integrated nursery pest management. Some of the salient points of this discussion include:

* Seed pathogen assays play a role in the IPM strategy of a nursery, and should be used in concert with operational seed germ and vigor testing.
* Most (90%) of the seedborne inoculum resides on the seed coat surface.
* Cone fungi appear to be the most likely source of seed contamination.
* Optimization of cone handling and storage procedures to facilitate drying and sanitation can reduce post-extractive seed Fusarium levels.
* Water rinses with agitation improve aeration and improve germination, but result in minimal removal of seedborne fungi such as *Fusarium oxysporum*.
* Seed coat sterilants (hydrogen peroxide, Clorox or bromine) reduce inoculum levels but do not prevent recontamination of seed, and can have a variable affect on germination performance.
* Fungicide seed treatments should only be considered after testing these chemical on seed for possible phytotoxicity.

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LITERATURE CITED:

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