Fungal contaminants on selected conifer seed, J. Herbert Stone Nursery

Katy M. Mallams, Plant Pathologist
Southwest Oregon Forest Insect and Disease Service Center
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Seeds of sugar pine, western white pine and Douglas-fir were evaluated for the presence of fungal contamination. J. H. Stone Nursery has been concerned about pre-emergence damping-off and the level of post-emergence mortality among some lots of bareroot sugar and western white pine seedlings. Fungal pathogens including several species of *Fusarium* are often associated with the dead, dying and low vigor seedlings. Diseases caused by *Fusarium* are one of the main causes of damping-off. Spores of *Fusarium* and other pathogenic fungi can be seed or soil borne. To control diseases effectively, it is important to know the source of the inoculum. This evaluation was conducted to determine if pathogenic fungi were present on seeds used in an evaluation of the biocontrol agent T-22 (*Trichoderma harzianum* Rifai Strain T-22) at J. H. Stone Nursery during the spring of 2003. This product was tested to determine its efficacy as a treatment to reduce losses caused by *Fusarium* and other pre- and post-emergence fungal pathogens.

Methods

Three seedlots were selected by the nursery; one each of sugar pine, western white pine and Douglas-fir. After the seeds were removed from the thaw box I randomly selected forty seeds from each lot. The remaining seeds were treated with bleach by seed lab personnel. Their treatment consisted of soaking the seeds in 40% bleach solution (4 parts household bleach and 6 parts water) for ten minutes, followed by rinsing in running tap water. Then I randomly selected forty of the bleached seeds from each lot. The remaining seeds were stratified. Western white pine seeds were stratified by soaking 48 hours in running tap water, followed by 120 days in peat in the stratification cooler at 33 to 35° F. Sugar pine seeds were stratified by soaking 72 hours in running tap water, followed by 90 days in the stratification cooler. Douglas-fir seeds were stratified by soaking 48 hours in running tap water, followed by 80 days at 33 to 35° F. When stratification was complete, I selected forty seeds at random from the Douglas-fir and western white pine lots. I did not sample the sugar pine seeds after stratification. For my evaluation I placed the seeds directly onto plates of Komada's medium, which is selective for *Fusarium* and several other related fungi. Half of the seeds were cut in half and half were plated whole. After about ten days, fungi that appeared on these plates were transferred to new media for species identification (potato dextrose agar, carnation leaf agar and Spezieller-Nährstoffermer agar). Several cultures were sent to Bob James (Plant Pathologist, USDA Forest Service, Coeur d'Alene, Idaho), Jean Juba (Fusarium Research Center, Pennsylvania State University) and Jeff Stone (Research Associate Professor, Oregon State University, Corvallis) for identification.

Results and Discussion

No *Fusarium* was isolated from seeds taken from the thaw box or after bleach treatment, but after stratification *Fusarium sporotrichioides* was isolated from 100 percent of the Douglas-fir seeds, and *F. acuminatum*, *F. avenaceum* and *F. culmorum* were isolated from 15, 13 and 15 percent, respectively, of the western white pine seeds (Table 1 and Figures 1-3). One explanation for the seemingly sudden appearance of *Fusarium* after stratification is that it was actually present at low levels in the stored and bleached seeds, but was not detected due to the

small number of seeds sampled. However, once the seeds were in stratification, the conditions were favorable for rapid spread and infection of many more of the seeds from even a small amount of inoculum. Another possible explanation is that the seeds were not contaminated, but became infected by inoculum already present in the stratification chamber. *F. solani* and *F. oxysporum* are the two *Fusarium* species that have previously been isolated from diseased seedlings in fields at Stone Nursery. Since these species were not isolated from any of the seeds I evaluated, if they are found later on seedlings in the field it would suggest that infection occurred during or after sowing.

In many cases, seedborne diseases often increase after seeds are stored for long periods (James 1987). The fact that *Fusarium* was not detected until after stratification is evidence that even though it may have been present on a few seeds, conditions in the seed storage facility were not conducive for the population to build up and contaminate a large number of seeds. However, even surface sterilization with bleach or hydrogen peroxide after storage often does not remove all *Fusarium* spores because they can adhere tightly to the seedcoats, and can penetrate seedcoats and colonize the tissue inside (James and Burr 2000). In the stratification chamber where the cool, moist conditions are very favorable for the fungus, it can build up from a small amount of inoculum to infect a large number of seeds.

According to the literature, *F. acuminatum* and *F. avenaceum* commonly colonize conifer seed. However, James (2000) found that the vast majority of F. *acuminatum* isolates he studied were not pathogenic on Douglas-fir seedlings. Most colonized only epidermal or cortical tissues and did not normally induce disease. In other studies James (1985, 1993) found *F. acuminatum* and *F. avenaceum* in association with pre- and post-emergence damping-off of conifer germinants and young seedlings, possibly as a result of seed borne inoculum. Further research is needed to understand the significance of these species on conifer seed and their role in seedling diseases.

F. culmorum is occasionally reported in association with conifer seeds (Hoefnagels and Linderman 1999). They found that *F. culmorum* did not decrease germination of Douglas-fir seed or increase post-emergence mortality. *F. sporotrichioides* is also found occasionally on conifer seed. James and Perez (1999) found that most isolates of *F. sporotrichioides* they tested were not pathogenic to conifer germinants. More recently however, this species has been found associated with mortality in containerized conifer seedlings (Bob James, Plant Pathologist, USDA Forest Service, Coeur d'Alene, personal communication). Although these species of *Fusarium* are usually considered non-pathogenic because they rarely cause disease in conifers, they might be capable of damaging seed with poor germination capacity or cause damping-off under favorable environmental conditions.

Hoefnagels and Linderman (1999) and James (2000) suggest that colonization of seedling root tissues by non-pathogenic *Fusarium* species might actually prevent colonization later by other, more pathogenic *Fusarium* species. More research is needed to confirm the extent to which this might occur. However, the rapid spread of non-pathogenic species on the seeds during stratification also suggests that a small amount of seed contaminated with pathogenic species could lead to large numbers of infected seed by the time stratification is complete, if pathogenic species spread at the same rate as non-pathogenic ones.

Several other fungi were also isolated from the seeds; species of *Arthrobotrys, Botrytis, Cladosprium, Penicillium, Rhizopus* and *Trichoderma* (Table 1 and Figures 1-3). Although

several have the potential to reduce germination, none except *Botrytis* is known to cause disease in seedlings. *Arthrobotrys* is common in soil and decaying plant debris. *Botrytis* was isolated from pine seeds at all three sampling times. According to Bob James (personal communication) *Botrytis* is found on seed occasionally. It is not known to adversely affect germination. However, it probably remains on the seedcoats and may infect and cause disease in seedlings later. *Cladosporium* is a weak pathogen. It has been linked to reduction in germination of conifer seed (Littke and Browning 1990). *Penicillium* was isolated from seeds of all three species after storage and bleach treatment, and from western white pine seeds after stratification. *Penicillium* is commonly found on conifer seed and spreads readily during stratification (James 1999). It is generally saprophytic, but some species are known to reduce germination of conifer seed (Littke and Browning 1990). *Rhizopus* is a ubiquitous airborne saprophyte or weak parasite. It is a common contaminant of stored plant parts (Agrios 1988). *Trichoderma* is another saprophytic fungus that readily colonizes conifer seed. It is antagonistic to many other fungi and may prevent colonization by other species, including *Fusarium*. However, there is evidence that *Fusarium* may survive bleach treatment better than other species of fungi like *Trichoderma*. In a study by James et al (1987) seeds treated with bleach had higher levels of *Fusarium* and lower levels of *Trichoderma* than untreated seeds. They hypothesized that *Trichoderma* was more readily killed, leaving the surviving *Fusarium* with less competition. More work would be needed to determine if and when seed treatments other than bleach might be more effective in reducing *Fusarium* populations.

Recommendations and Conclusion

The purpose of this study was to identify fungi on seed used in a study of T-22 as a soil amendment for biocontrol of pre- and post-emergence seedling diseases, especially those caused by *Fusarium*. The species of *Fusarium* I found are not considered major pathogens of conifer seed and were not species associated with diseased seedlings from fields at Stone Nursery in the past. If, later in the study, diseased seedlings are found infected with pathogenic species of *Fusarium* it would suggest that the source of inoculum might be sowing equipment or soil, rather than the seed.

This study also provided an opportunity for me to learn about seed handling practices at the nursery. After studying recent literature related to seed handling and pathogenic fungi associated with conifer seeds, I would offer the following recommendations:

- Continue to surface sterilize all seed prior to stratification to remove as many fungal contaminants as possible.
- Consider testing seedlots with low germination rates for *Fusarium* prior to stratification. Poor germination is often associated with *Fusarium* infection inside the seedcoat. Testing would identify contaminated lots so they could be treated to prevent additional losses due to disease.
- Use the minimum stratification time to reduce the potential for spread of pathogenic fungi. If seeds are stratified for more than 30 days, consider surface-drying, and then returning to stratification (Campbell et al 1990).
- Clean seed storage, stratification, and sowing equipment as often as is practical.
- Continue testing and use of biocontrol agents such as T-22 for seed and soil treatments.

This study has demonstrated the ubiquitous nature of fungi on conifer seed. It highlights the importance of proper seed storage and handling, and monitoring of seed quality.

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Tables and Figures

Table 1. Percent of conifer seeds infected by fungi after storage, bleach and stratification

	PILA		PSME		PIMO	
	N	percent	N	percent	N	percent
After storage						
total number of seeds	40		40		40	
Arthrobotrys	1	3				
Botrytis	6	15				
Cladosporium					14	35
Penicillium	18	45	40	100	39	98
Rhizopus	10	25	6	15	5	13
After bleach						
total number of seeds	40		40		38	
Botrytis	2	5			4	11
Cladosporium					22	58
Penicillium	1	3	6	15	16	42
Rhizopus	9	23			3	8
Trichoderma			1	3	7	18
After stratification						
total number of seeds	0		40		40	
Botrytis					2	5
F. sporotrichioides			40	100		
F. acuminatum					6	15
F. avenaceum					5	13
F. culmorum					6	15
Penicillium					24	60

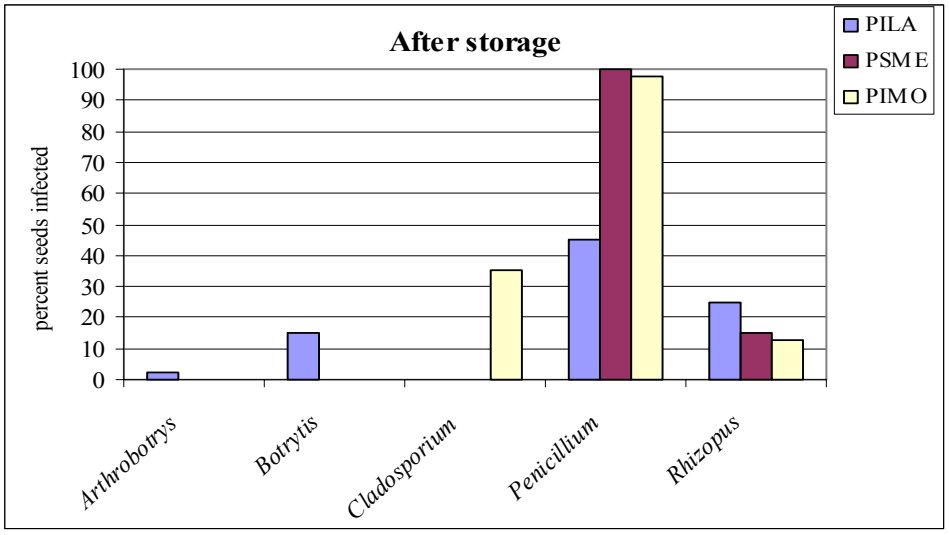

Figure 1. Percent of seeds infected by fungi after storage

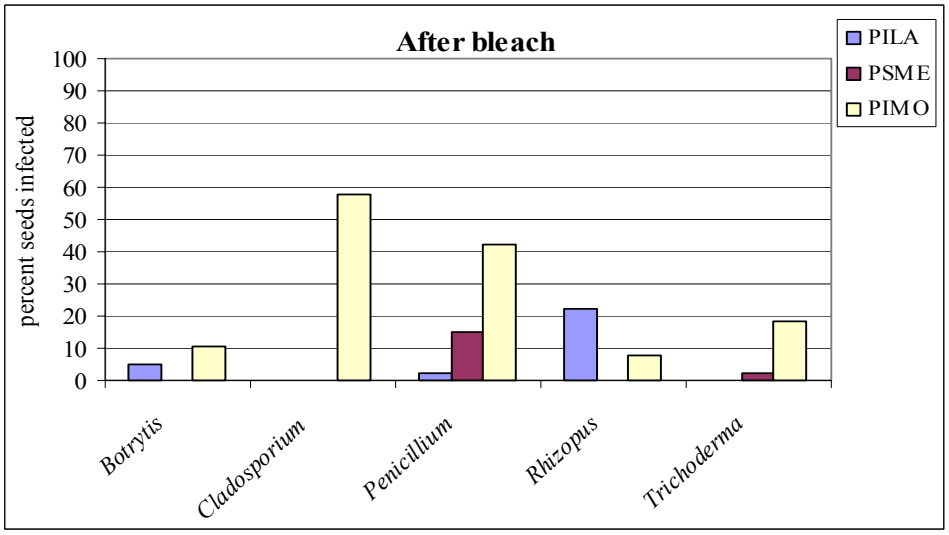

Figure 2. Percent of seeds infected by fungi after bleach treatment

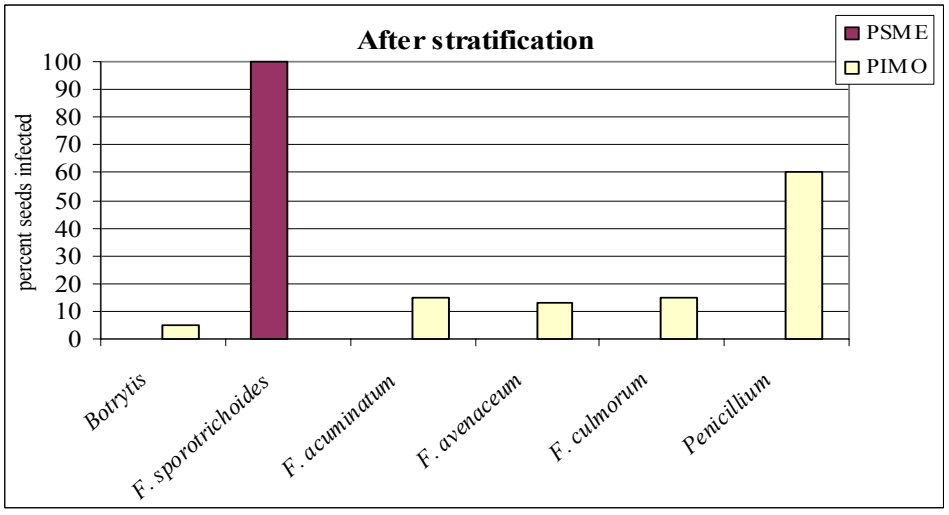

Figure 3. Percent of seeds infected by fungi after stratification