OCCURRENCE OF FUNGI ON WESTERN WHITE PINE SEED, PLUM CREEK NURSERY, PABLO, MONTANA

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Use of western white pine (Pinus monticola Dougl.) seed to grow seedlings in forest tree nurseries has always caused problems for growers. In order to have adequate germination, lengthy stratification periods are usually required. Even after stratification, some seedlots do not germinate adequately. Factors such as embryo maturation, seedcoat non-permeability, and colonization by fungi may all contribute to poor germination.

Seedlot MA-85, collected from the 1985 crop at the Moscow Arboretum (Idaho), was distributed to several nurseries in northern Idaho and western Montana for the 1986 growing season. Portions of this seedlot used at the Plum Creek Nursery, Pablo, Montana, were covered with extensive mold growth following 120 days of stratification. Also, germination was extremely poor (less than 10 percent). Post-stratification seed samples were tested by Penisulab® for occurrence of fungi. A portion of the seed tested were surface sterilized with 30 percent hydrogen peroxide for 1 hour and compared with untreated seed. Results indicated that levels of Pythium in both treated and untreated seed were extremely high, although amounts were not reported. Also, 10 percent of the treated and 33 percent of the untreated seed were infected with Fusarium. Other fungi found on the seed included Alternaria, Aspergillus, Penicillium, Trichoderma, and Rhizopus. It was concluded that Pythium and Fusarium infections significantly contributed to the poor germination of this seedlot.

The Nursery had about 50 pounds of seed from this lot left and was concerned that much of it might not be usable because of fungal infection problems. Therefore, an analysis of unstratified seed from this lot was conducted to confirm results from the Penisulab® evaluation and determine if fungal infection was a problem before stratification.

Five hundred unstratified seed were sampled for occurrence of fungi on their seedcoats. One hundred of these were also aseptically dissected to evaluate occurrence of fungi within their endosperm. Half of each sample was placed on a selective medium for Fusarium spp. (Komada 1975) and half on a selective medium for Pythium, composed of V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and PCNB. Twenty non-dissected and five dissected seed were placed on each plate. Those seed on the Fusarium media were incubated for 7 days at 22°C under cool fluorescent light and those on the Pythium media

were incubated in the dark for 5 days. Fungi emerging from seedcoats and endosperms were identified and colonization percentages calculated. Occurrence of different fungi were compared with an analysis of variance and Tukey's multiple range comparison test. Relationships between occurrence of Pythium and Fusarium with Trichoderma, a common antagonistic competitor, were compared with a simple regression.

Evaluation results are summarized in table 1. Species of Pythium occurred on about 30 and 58 percent of the seedcoats and endosperms sampled, respectively. Fusarium spp. were found on only 1.2 percent of the seedcoats sampled, but on 6 percent of the endosperms. Other common fungi found either on or within seed included Penicillium, Trichoderma, Aspergillus, Rhizopus, Alternaria, and Aureobasidium. Occurrence of several of these fungi was significantly different from each other (P=0.05) (table 1). There was no significant relationship between occurrence of Pythium or Fusarium with Trichoderma (regression $r^2 = 0.01$).

Table 1.--Occurrence of selected fungi on western white pine seedcoates and endosperms from the Plum Creek Nursery

Organism	Percentages	
	Seedcoats[1]	Endosperms[2]
Penicillium	100.0 A[3]	80.0 A[3]
Trichoderma	68.8 B	4.0 B
Pythium	30.4 C	58.0 A
Fusarium	1.2 D	6.0 B
Aspergillus	3.6 D	2.0 B
Rhizopus	--	12.0 B
Alternaria	--	8.0 B
Aureobasidium	--	8.0 B

[1] Sample total = 250 seeds
[2] Sample total = 50 seeds
[3] Within each column, means followed by the same capital letter are not significantly different (P = 0.05) using Tukey's Multiple Range Comparison Test.

These results confirm the extensive colonization of white pine seed by Pythium spp. These water-mold organisms are common soil inhabitants, but usually are not found at high levels on conifer seed (James and Genz 1982). Pythium spp. were located at higher levels within the seed (endosperm) than on their surfaces, probably indicating that the fungi readily penetrated seed at some point during seed development or processing.

Fusarium spp. were located on very few of the unstratified seed sampled. However, stratified seed apparently were colonized by these fungi to a much greater degree. Therefore, these fungi probably spread from infected seed during the long stratification period. Occurrence of Fusarium within seed was also greater than on seed surfaces.

The other fungi colonizing white pine seed were common seed inhabitants (James and Genz 1982). Penicillium readily penetrated seedcoats to colonize endosperms. However, Trichoderma spp. were ususally restricted to seedcoats.

Occurrence of such high levels of Pythium on white pine seed from a seed orchard is disturbing. It is unlikely that infection occurred during seed development, especially since several other nurseries which received this same seedlot did not encounter similar problems with Pythium infection. It is more likely that contamination occurred somewhere during extraction or processing. In any event, there was very high infection levels prior to stratification.

LITERATURE CITED

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Komada, H. 1975. Development of a selective medium for quantitative isolation of Fusarium oxysporum from natural soil. Rev. Plant Protec. Res. 8:114-125.