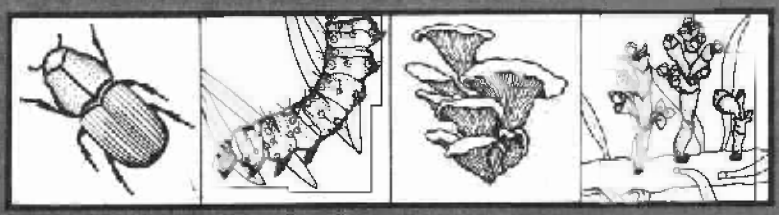
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FUNGI ON DOUGLAS-FIR AND PONDEROSA PINE CONES FROM THE USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

by

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ABSTRACT

Cones of Douglas-fir and ponderosa pine with extensive mold on external portions of scales were sampled for presence of fungi potentially pathogenic to conifer seedlings in nurseries. The major colonizers of cone scales and external seedcoats for both conifer species were *Trichoderma* spp. *Penicillium* spp. were also very common. The only potentially pathogenic organisms found on moldy cones were low levels of *Fusarium* (*F. acuminatum* and *F. oxysporum*), *Cylindrocarpon* (*C. tenue*, *C. didymum*, and *C. gracile*), and *Botrytis cinerea*. Levels of these potential pathogens were only a fraction of that of the common saprophytes, *Trichoderma* and *Penicillium*. Tests to evaluate effects of these fungi on seed germination and seedling establishment are needed. Cones with high levels of external moldiness were not extensively colonized with potentially pathogenic fungi and need not be discarded because of extensive fungal growth.

INTRODUCTION

Cones of several conifer species are collected each fall and shipped to the USDA Forest Service Nursery in Coeur d'Alene, Idaho for seed extraction and processing. This seed is used to grow either bareroot or container seedlings which are planted in areas within "seed zones" from which the cones were initially collected. Growers at the nursery have often been concerned about possible roles of fungi residing within and on seed in limiting germination and seedling establishment. Occasionally cones are delivered to the nursery with extensive external moldiness. This superficial growth of fungi indicates colonization of cones at some point either during development, collection, or storage. Cones are routinely stored in burlap bags which are reused several times. These bags may harbor fungal spores from previously collected cones that could potentially infect new cones.

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Growers at the Coeur d'Alene Nursery are concerned that moldy cones may be infected with potentially pathogenic fungi which could affect seed germination and seedling establishment. Their concern is that such cones may contain seed infected with higher than normal levels of pathogens. If pathogens are present at high levels on seed from moldy cones, additional steps, such as special seed treatments, are required to reduce pathogen levels. In some cases, excessively moldy cones may have to be discarded.

METHODS

Cones from three seedlots each of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) and ponderosa pine (*Pinus ponderosa* Laws.) were sampled. Ten cones with extensive superficial fungal mycelium growing on external scales were selected from each seedlot. Cones were aseptically dissected to extract winged seed; scales were also extracted from cones using a sterile scalpel. Ten scales from each cone were randomly selected for sampling. Twenty-five seeds with attached wings were also randomly selected per seedlot following cone dissection. Pieces of cone scale were placed on a selective agar medium used to isolate species of *Fusarium* and closely related pathogenic fungi (Komada 1975). Wings were aseptically detached from seed and both were separately plated on the selective agar medium. Several seeds from each seedlot were also placed on another selective agar medium used to isolated species of *Pythium* and related water mold fungi. This medium was made from V-8 juice agar amended with several antibiotics (James 1993).

Plates of Komada's medium were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-10 days. Plates of V-8 juice agar were incubated in the dark at about 24°C for 3 days. Selected fungi emerging from cone scales, seed, and wings were transferred to potato dextrose agar for identification. Percentage colonization by several groups of fungi was calculated.

To identify fungi present on cones but incapable of growing on either of the selective media used, representative cone scales from each seedlot were incubated in moist chambers for 5-7 days. Fungi sporulating on scales were then identified to genus.

RESULTS

Fungi that have traditionally been most troublesome as pathogens at the Coeur d'Alene Nursery are readily isolated on Komada's selective medium (James 1991; James and others 1990). The two major pathogenic fungal groups obtained on this medium are *Fusarium* and *Cylindrocarpon* (Komada 1975). In addition, species of *Trichoderma*, which are common inhabitants of soil, growing media, and nursery seedlings, are also readily isolated on Komada's medium. These latter fungi are usually not considered pathogenic; rather, they may exert antagonistic influences on pathogens such as *Fusarium* (Papavizas 1985). As indicated in tables 1 and 2, levels of *Fusarium* and *Cylindrocarpon* were quite low on both Douglas-fir and ponderosa pine cone scales obtained from moldy cones. However, levels of *Trichoderma* were very high on cone scales of both conifer species. Two other groups of fungi were isolated from cone scales onto Komada's medium: *Penicillium* and *Botrytis*. Although *Penicillium* spp. do not grow rapidly on the medium, they were found at fairly high levels, particularly on ponderosa pine cone scales. *Botrytis* (*B. cinerea* Pers. ex Fr.) was not found on Douglas-fir, but was isolated at low levels from two seedlots of ponderosa pine.

Fusarium and *Cylindrocarpon* spp. were likewise found at low levels on sampled seed and wings from the same moldy cones (tables 3 and 4). *Fusarium* spp. were found on two of the Douglas-fir seedlots, but not isolated from any of the sampled ponderosa pine seed or wings. *Trichoderma* spp. were the most common inhabitants of both Douglas-fir and ponderosa pine seed and wings assayed on Komada's medium, although species of *Penicillium* were also common. *Botrytis* was located on a few ponderosa pine seed and wings.

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Table 1--Infection of Douglas-fir cones with selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Percent Cone Infection [1]				
Seedlot	Fusarium[2]	Cylindrocarpon[3]	Trichoderma	Penicillium	Botrytis
6833	50 (10)	10 (1)	100 (99)	70 (15)	0
6838	20 (2)	0	100 (100)	80 (18)	0
6839	10 (1)	0	100 (100)	0	0
All Seedlots	26.7 (5.3)	3.3 (0.3)	100 (99.7)	50 (11.0)	0

[1] Ten cones sampled per seedlot; each cone was dissected and 10 randomly selected cone scales were sampled. Figures in parentheses indicate percent of sampled cone scales colonized with appropriate fungus.

[2] Includes *F. acuminatum* on 20.0 percent of the cones (4.6 percent of sampled cone scales) and *F. oxysporum* on 6.7 percent of the cones (0.7 percent of sampled cone scales).

[3] *C. tenue*.

Table 2--Infection of ponderosa pine cones with selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Percent Cone Infection [1]				
Seedlot	Fusarium[2]	Cylindrocarpon[3]	Trichoderma	Penicillium	Botrytis
6830	10 (2)	80 (21)	100 (79)	100 (67)	30 (4)
6837	30 (4)	20 (3)	100 (100)	100 (54)	0
6841	20 (2)	10 (1)	100 (100)	100 (29)	10 (1)
All Seedlots	20 (2.7)	37 (8.3)	100 (93)	100 (32.2)	13 (1.7)

[1] Ten cones sampled per seedlot; each cone was dissected and 10 random cone scales were sampled. Figures in parentheses indicate percent of sampled cone scales colonized with appropriate fungus.

[2] Includes *F. acuminatum* on 13 percent of the cones (1.7 percent of sampled cone scales) and *F. oxysporum* on 10 percent of the cones (1.0 percent of sampled cone scales).

[3] Includes *C. tenue* on 33 percent of the cones (6.0 percent of sampled cone scales), *C. didymum* and *C. gracile* each on 3 percent of the cones (0.3 percent of sampled cone scales).

Table 3--Infection of Douglas-fir seed and wings with selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

SEED

	Percent Cone Infection [1]		
Seedlot	Fusarium[2]	Trichoderma	Penicillium
6833	0	100	72
6838	12	96	12
6839	0	100	20
All Seedlots	4.0	98.7	34.7

WINGS

Seedlot	Fusarium[2]	Trichoderma	Penicillium
6833	0	100	76
6838	4	96	36
6839	4	100	72
All Seedlots	2.7	98.7	61.3

[1] Twenty-five seeds were sampled per seedlot; sampled seeds were extracted from moldy cones. Wings were aseptically removed from sampled seed and incubated spearately.

[2] *F. acuminatum* and *F. oxysporum*, were both isolated from seed (2.7 percent and 1.3 percent, respectively); only *F. acuminatum* was isolated from wings.

Table 4--Infection of Douglas-fir seed and wings with selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

SEED

	Percent Cone Infection [1]		
Seedlot	Fusarium[2]	Trichoderma	Penicillium
6830	72	68	16
6837	100	92	0
6841	100	24	0
All Seedlots	90.7	61.3	5.3

WINGS

Seedlot	Trichoderma	Penicillium	Botrytis	Cylindrocarpon[2]
6830	72	76	0	4
6837	100	100	0	0
6841	96	24	4	0
All Seedlots	89.3	66.7	1.3	1.3

[1] Twenty-five seeds were sampled per seedlot; sampled seeds were extracted from moldy cones. Wings were aseptically removed from sampled seed and incubated spearately.

Two *Fusarium* spp were isolated in this evaluation. The most prevalent was *F. acuminatum* Ell. & Ev.; the other species was *F. oxysporum* Schlecht. Both species have commonly been associated with diseased seedlings at the nursery (James 1985; James and others 1990). *Fusarium oxysporum* is especially important in causing damping-off of both bareroot and container-grown seedlings and root disease of bareroot stock (James and others 1987; 1990). Isolates of *F. acuminatum* are generally not as aggressive as *F. oxysporum* in causing seedling diseases, although individual isolates may be very pathogenic (James and Gilligan 1984).

Isolations from Douglas-fir and ponderosa pine seeds on V-8 juice agar failed to consistently yield water mold fungi. *Mortierella* spp., which grow well on this medium, were isolated from a few seeds, but no *Pythium* and/or *Phytophthora* were obtained from any of the sampled seed. These latter two groups of fungi may occasionally cause disease problems at the nursery (James 1982, 1993), although they are not nearly as important as other pathogens such as *Fusarium* spp.

Cone scales incubated in moist chambers yielded *Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., *Rhizopus* sp. and *Mucor* spp. These latter four genera do not grow well on the selective media used in this evaluation and may have been important colonizers of cones.

DISCUSSION

Fungi are common colonizers of conifer seed; some of these fungi may cause disease to either the seed itself or germinating seedlings following sowing. It is difficult to predict importance of specific groups of fungi colonizing seed because many different organisms may cause disease under varying environmental conditions. Growers have traditionally been concerned about specific groups of fungi known to cause problems with germination and seedling establishment, such as *Fusarium*, *Cylindrocarpon*, *Pythium*, and *Phytophthora* (Bloomberg 1966; James and others 1991). However, other fungi usually considered "saprophytic" may also cause seed decay and reduce germination (pre-emergence damping-off) (Gibson 1957; Harvey and Carpenter 1975).

In this evaluation of moldy cones, traditional groups of pathogens did not make up a large proportion of the fungal growth. Rather, it appeared that *Trichoderma* spp. were the most commonly encountered organisms on both Douglas-fir and ponderosa pine cones. Other fungi usually considered saprophytic were also present at relatively high levels. Of these, *Penicillium* spp. have been implicated as possible seed decayers in the past (Anderson 1986; Harvey and Carpenter 1975; James 1989). The other fungi found on cones, such as *Aspergillus*, *Rhizopus*, and *Mucor* spp. are commonly reported on conifer seed (Anderson 1986; James 1984; James and Genz 1981, 1981), but their role as potential pathogens of conifer seed is largely unknown.

Pathogenic fungi, such as *Fusarium* spp., commonly colonize external portions of conifer seedcoats (James and others 1987). Such fungi may be introduced into growing operations and account for severe losses, especially early in the crop cycle. If seed infection by pathogens exceeds 10 percent of particular seedlots, high levels of seed decay and damping-off losses can be expected (James and others 1987). Therefore, when seed infection exceeds this threshold, growers are usually advised to chemically treat seed prior to stratification. Treatments include surface sterilization with sodium hypochlorite (bleach), hydrogen peroxide, or treatment with fungicides. Most growers routinely rinse seed in running water before and after stratification to reduce level of seedcoat contamination with fungi and to prime seeds for germination (James 1986; James and others 1987). This helps reduce levels of pathogens, such as *Fusarium* spp., on seed. However, if very high infection levels occur, treatments with surface sterilizing chemicals are advised.

In this evaluation of Douglas-fir and ponderosa pine cones with extensive moldiness, levels of potentially pathogenic fungi were very low on either the cone scales themselves or on seed within cones. Levels of *Fusarium* encountered on seed were well within what is considered "normal" (James 1986). Therefore, it would probably not be advisable for growers to discard cones solely on the basis on external moldiness. Most moldiness was caused by *Trichoderma* and *Penicillium* spp. which are usually non-pathogenic. However, tests to evaluate effects of these molds on seed germination and seedling establishment should be conducted. Since many different fungi are capable of eliciting seed decay and/or adversely affecting germination, it is possible that these saprophytic molds may be undesirable when present at high levels. On the other hand, species of *Trichoderma* may be antagonistic toward common pathogens such as *Fusarium* spp. (Papavizas 1985). Therefore, presence of *Trichoderma* and other saprophytes on seed may be desirable to help limit infection by pathogens after sowing (Kommedahl and Windels 1978). Some investigations (Bloomberg 1969; Kliejunas 1985; Salisbury 1955) have shown that seed colonization by common saprophytic mold fungi such as *Trichoderma* and *Penicillium* was not deleterious to germination.

Molds contaminating cones come from many different sources. If cones come into contact with soil or are collected from squirrel caches, they are likely to become infected with a number of different fungi (James 1986). Also, burlap bags that are reused without adequate cleaning may harbor mold spores that can cause infection. Generally, the longer cones are stored, the greater their chance of becoming contaminated with molds (Harvey and Carpenter 1975; Shea 1960). Long storage periods allow fungi to penetrate through cones and reach seed which may then become infected. Storage at high temperatures (above 25-30°C) is especially conducive for rapid colonization by certain molds, particularly species of *Trichoderma*, *Penicillium*, and *Aspergillus* (Bloomberg 1969; Harvey and Carpenter 1975; Shea 1960). *Fusarium* spp. are also favored by these higher temperatures (James and others 1991). Therefore, it is recommended that cones be collected

when mature and stored for as short a time as possible before seed extraction. Also, cones need not necessarily be discarded just because they exhibit external moldiness. If growers are concerned about introducing pathogens into growing operations from moldy cones, it is recommended that seed extracted from affected seedlots be screened for presence of pathogens prior to sowing. This way, seedlots extensively colonized by pathogens can be identified and either treated or discarded.

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