NURSERY DISEASE NOTES USDA FOREST SERVICE NORTHERN REGION

No. 115 January 1991

COLONIZATION OF ENGELMANN SPRUCE AND LODGEPOLE PINE SEED WITH *MORTIERELLA* SPP. - USDA FOREST SERVICE NURSERY COEUR D'ALENE, IDAHO

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INTRODUCTION

Conifer seed usually requires stratification in cool, moist environments prior to sowing in forest tree nurseries. During periods of stratification, fungal mycelium may develop over the surfaces of seedcoats from spores carried on seed. In some cases this fungal growth increases and becomes profuse during stratification because of cool, moist conditions which favors growth of some fungi. Since most fungi are carried externally on seed, amount of fungal growth usually varies greatly among different seedlots.

During May 1990, several lots of Engelmann spruce (***Picea engelmanni*** Parry) and lodgepole pine (***Pinus contorta*** Dougl.) seed were extensively colonized with mold during stratification at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Fungal mycelium grew rapidily in the cool stratification temperatures and spread extensively over many seeds. Growers were concerned about this excessive molding and wondered if the associated fungi were potential pathogens that might reduce germination or cause disease of young germinants. Therefore, an evaluation was conducted to identify fungi colonizing seedcoats and determine extent of fungal penetration of seedcoats and colonization of endosperms.

MATERIALS AND METHODS

Molded seed were initially examined under the microscope to determine if infecting fungi were sporulating. Tufts of representative fungi were aseptically transferred to plates of several different media including potato dextrose agar (PDA), 2% water agar, a selective medium for ***Fusarium*** and closely-related fungi (Komada 1975), and a selective medium for water mold fungi composed of V-8 juice agar amended with pimaricin and several other antibiotics. One hundred randomly-selected Engelmann spruce and lodgepole pine seed taken directly from stratification were placed on both Komada's and V-8 juice agar to quantify colonization of seedcoats. Fifty lodgepole pine seed were randomly selected and rinsed thoroughly under running tap water for a few minutes to dislodge superficial mycelium. They were then aseptically dissected with their seedcoat carefully removed.

Seed embryos were aseptically placed on both Komada's and V-8 juice agar. Plates of Komada's medium were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 5-7 days. Plates with V-8 juice agar were incubated in the dark at about 23°C for 3 days. Fungi emerging from seeds and embryos were identified to genus using the taxonomic descriptions of Barnett and Hunter (1972). Selected isolates were transferred to PDA slants and identified using descriptions by Domsch and others (1980) and Nelson and others (1983).

Selected isolates of ***Mortierella*** obtained from seed were grown on PDA within a growth chamber at two temperatures: 5°C and 24°C. Plates with fungi were incubated for 7 days at these temperatures under continuous darkness. Linear colony growth was measured daily and growth rates (mm/day) calculated.

RESULTS AND DISCUSSION

Most isolations on V-8 juice agar yielded three distinct isolates of ***Mortierella*** (Table 1). Small levels of ***Fusarium*** spp. as well as ***Trichoderma***, ***Penicillium***, ***Alternaria***, and ***Mucor*** were isolated on Komada's medium. ***Mortierella*** was isolated at low levels from lodgepole pine seed embryos (Table 1). ***Penicillium*** and ***Trichoderma*** spp. also colonized embryos at low levels.

Using descriptions by Domsch and others (1980), three species of ***Mortierella*** were identified from spruce and lodgepole pine seed isolates (see Appendix for species descriptions). The most common species were ***M. alpina*** Peyronel and ***M. ramanniana*** (Moller) Linnem. The other species, ***M. nana*** Linnem., was isolated less frequently. Although all three species are frequent inhabitants of forest soil (Domsch and others 1980), ***Mortierella*** spp. are not listed as common colonizers of conifer seed (Anderson 1986).

Mortierella alpina has been described as a common soil inhabitant under pine and spruce trees (Balasooriya and Parkinson 1967; Gray and Baxby 1968; Hayes 1965a). This species also occurs within the rhizosphere of conifer tree roots (Lihnell 1939). It is well adapted to cool, temperate forest regions and grows fairly rapidly even at 1°C (Domsch and others 1980). Some reports (Domsch 1960; Maciejowskz 1962) indicate that ***M. alpina*** exhibits antagonism toward root pathogenic fungi such as ***Rhizoctonia solani*** and ***Gaeumannomyces graminis***.

Mortierella ramanniana is one of the most common species of ***Mortierella*** found in forest soils (Domsch and others 1980; Sewell and Brown 1959). It prefers cold, temperate areas and particularly conifer forest soils (Domsch and others 1980). It commonly occurs within soil under pine (Badura 1960; Ellis 1940; Kendrick 1963) and spruce (Soederstroem 1975) trees and has also been isolated from the roots of spruce trees (Meyer 1960). This species common occurs in pine (Hayes 1965a; 1965b) and spruce (Meyer 1960; Saito 1956) litter and the rhizosphere of many different types of forest plants. It has also been isolated from forest nursery soil. Like, ***M.*** *alpina*, ***M. ramanniana*** grows best at low temperatures (Franz 1975) and is well adapted to acidic soil conditions (pH 3-4). ***Mortierella ramanniana*** may also be an important antagonist toward other soil microorganisms. It produces a potent toxin (ramycin) that is quite restrictive to development of soil bacteria (Dijck 1969).

Table 1. Fungal colonization of stratified Engelmann spruce and lodgepole pine seed - USDA Forest Service Nursery, Coeur d'Alene, Idaho.[1]

	Percent Colonization		
Fungal Species	**Engelmann Spruce**	**Lodgepole Pine**	
	Seedcoats	**Seedcoats**	**Embryos**
Mortierella[2]			
M. alpina	42	63	10
M. ramanniana	36	19	6
M. nana	9	3	0
All *Mortierella*	87	85	16
Fusarium[3]			
F. oxysporum	3	1	0
F. proliferatum	2	3	0
F. acuminatum	1	0	0
All *Fusarium*	6	4	0
Trichoderma[3]	46	37	20
Penicillium[3]	65	43	16
Alternaria[3]	17	3	0
Mucor[3]	9	2	0

[1] One hundred seed of each species incubated on both Komada's and V-8 juice agar for seedcoat colonization. Fifty lodgepole pine seed embryos incubated on both Komada's and V-8 juice agar.

[2] Assay on V-8 juice agar.

[3] Assay on Komada's medium.

Mortierella nana is found in many temperate forest soils, particularly in cool environments (Domsch and others 1980). It has been described in spruce (Soederstroem 1975) and pine (Zycha and others 1969) forest soils and isolated from the rhizospheres of pine (Kuhlman 1969) and several other types of forest trees (Zycha and others). This species has not been encountered nor studied as much as the previous two.

All three species of ***Mortierella*** tested grew faster at 15°C than at 24°C (Table 2). They seemed well adapted to cool temperatures and could probably grow quite well during seed stratification. ***Mortierella*** spp. are usually not considered plant pathogenic (Domsch and others 1980). Although they were found colonizing seed embryos at low levels, their colonization did not cause necrosis of embryo tissues.

Table 2. Linear growth rates of ***Mortierella*** spp. from Engelmann spruce and lodgepole pine seed - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Linear Growth Rate (mm/day)[1]	
Mortierella Species	15°C	24°C
M. alpina	5.5	4.2
M. ramanniana	5.4	4.6
M. nana	2.9	1.4
Average	4.6	3.4

[1] Isolates grown on PDA and incubated in the dark at the appropriate temperature for 7 days.

Beds sown with infected seedlots did not exhibit poor seed germination nor greater than usual amounts of disease on young germinants. It is possible that infected seed were somewhat protected from attack by potential pathogens such as ***Fusarium***, ***Pythium*** and ***Rhizoctonia*** which cause damping-off. However, this was not determined in this evaluation.

Growers often become worried when they find excessive growth of fungal mycelium on seed. Such fungal growth may be a problem if potentially pathogenic fungi are involved. However, occurrence of fungi on seed should not necessarily cause concern. In some cases, if the right fungal species are on seed, some seed colonization may actually be beneficial from a disease potential standpoint. In this evaluation, ***Mortierella*** spp. were aggressively colonizing Engelmann spruce and lodgepole pine seed during stratification. The fungus caused clumps of seed to form, but otherwise caused no noticeable damage to seed. Once seed was washed to remove superficial mycelium and dried, visible fungal growth no longer occurred. These fungi probably contaminated deed during collection or processing, particularly when cones came into contact with forest soil and litter. However, they did not adversely affected germination and seedling establishment. Therefore, the general conclusion that moldy seed are always bad and to be avoided was not applicable in this particular case. However, it is important that growers have problem seedlots evaluated for types of fungal colonizers so that they can predict seed performance after sowing.

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APPENDIX

Mycological Descriptions

Mortierella alpina Peyronel

Subgenus: ***Mortierella***
Section: ***Alpina***
Synonymy:
Mortierella renispora Dixon-Stewart
Mortierella thaxteri Bjorling
Mortierella monospora Linnem.
Mortierella acuminata Linnem.

Colonies reaching 2.5 cm in diameter in 5 days at 20°C on PDA.

Sporangiophores simple (unbranched), short (maximum 120u long), with a widened and often irregularly swollen base.

Sporangia containing numerous ellipsoidal spores, although the entire sporangium may fall of without separating into individual spores.

Chlamydospores are rare.

Produces naken zygospores in the aerial mycelium after mating of compatible strains.

Produces hyphal "knots" in the mycelium which may be pre-zygospores.

Mortierella ramanniana (Moller) Linnem.

Subgenus: ***Micromucor***
Synonymy:
Mucor ramannianus Moller

Colonies reaching 2.4 -2.7 cm in diamter in 5 days at 20°C on PDA; velvety with vinaceous-brown coloration from the sporangia.

Sporangiophores simple or branched, long (300-700u) with a small, definite columella visible after dehiscence of the sporangium wall.

Sporangia containing numerous oval to ellipsoidal spores which are released after dehiscence of the sporangium wall.

Chlamydospores abundant, thick walled, round to irregular shaped and contain dense, oily materials.

Mortierella nana Linnem.

Subgenus: ***Micromucor***
Synonymy:
Mortierella alba Manka & Gierczak

Colonies slow growing, reaching 0.7 - 1.4 cm diameter in 5 days at 20°C on PDA; velvety and pure white in color.

Sporangiophores short (30-80u long) with short, verticillate branches arising close together from a slightly swollen node.

Sporangia consistently one-spored and smooth walled.

Chlamydospores absent.