CONTAINER-GROWN LODGEPOLE PINE SEEDLING DISEASE USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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Nursery Disease Notes #100

January 1990

During production of the second 1989 crop of container-grown seedlings at the USDA Forest Service Nursery in Coeur d'Alene, Idaho, several lodgepole pine (***Pinus contorta*** Dougl.) seedlings displayed symptoms indicative of root disease about 4-5 weeks after sowing. Affected seedlings had foliar dieback that started at the tips of cotyledons and progressed until all cotyledons were necrotic. Several seedlings were killed; these were often bent over, similar to symptoms of post-emergence damping-off. When symptoms were first noticed, growers applied benomyl and captan through the overhead irrigation system in an effort to restrict disease spread. These fungicide applications successfully prevented increases in disease.

Several diseased seedlings with different symptom severity were analyzed for associated organisms on their roots and cotyledons. Seedlings were carefully extracted from their pine cell containers so that the entire taproot was removed intact (this usually involved cutting the container longitudinally to expose roots). Seedlings were carefully washed under running tap water to remove adhering particles of growing media. They were then numerically rated for severity of root disease symptoms using a scheme designed to evaluate crown necrosis, root decay, and watersoaking of roots which may be indicative of root invasion by organisms (table 1). Cotyledons were aseptically severed from the stem, surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite) for 1 minute, rinsed with sterile distilled water, and placed on potato dextrose agar (PDA). Tap roots were surface sterilized and rinsed as described above and aseptically cut into 10-20mm length pieces .Pieces were sequentially placed on an agar medium selective for ***Fusarium*** spp. and related root disease fungi (Komada 1975). All agar plates were incubated at about 26°C under diurnal cycles of cool fluorescent light for 7 days. Emerging fungi were transferred to PDA and carnation leaf agar and identified using standard taxonomic guides (Barnett and Hunter 1972; Booth 1966; Nelson and others 1983).

Table 1. Numerical ratings of root disease symptoms on container-grown lodgepole pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Disease Criteria	Numerical Rating[1] Seedling Number 1	2	3	4	5	6	7	8	9	10
Crown necrosis	1	2	3	1	2	1	2	1	2	3
Root decay	0	0	0	1	2	2	3	0	2	3
Root watersoak	1	1	1	1	1	1	1	1	1	1
Total rating[2]	2	3	4	3	5	4	6	2	5	7

[1] Based on the following criteria:

Percent crown necrotic: 0-10 = 0; 11-40 = 1; 41-60 = 2; 61-100 = 3.

Root decay (evidenced by sloughing of epidermis): none = 0; decay limited to root tips = 1; decay on root tips and located on some intercalary roots = 2; decay common throughout root system = 3.

Root watersoaking (evidenced by darkening and transparency of roots): not present = 0; present = 1.

[2] Summary of ratings for severity of root disease symptoms: Severe = 6-7; Moderately severe = 5; Moderate = 3-4; Slight = 1-2; None = 0.

Several different fungi were found colonizing cotyledons of diseased seedlings (table 2). The most commonly isolated included ***Alternaria*** spp. and two species of ***Phoma*** (***P. eupyrena*** Sacc. and ***P. herbarum*** Westend). Although both these ***Phoma*** species may potentially cause seedling diseases (James 1985; James and Hamm 1985), they were not consistently isolated from most diseased lodgepole pine seedlings. Other common saprophytic fungi isolated from cotyledons (***Alternaria***, ***Trichoderma***, and ***Penicillium***) were most likely secondary colonizers of necrotic tissues. Many of the necrotic cotydedons were not colonized with any fungus that was detected using isolation procedures outlined above (table 2); therefore, the cotyledon necrosis was most likely due to pathogenic problems elsewhere on seedlings.

Results of root isolations are summarized in table 3. Several groups of potentially pathogenic fungi were commonly isolated from roots of diseased seedlings. The most consistently isolated group was ***Cylindrocarpon***, comprised of two species: ***C. tenue*** Bugn. and ***C. didymum*** (Hartig)Wollenw. These fungi were isolated more frequently near the top of taproots (nearer the soil line) (table 4.) Both species have been implicated in diseases of forest tree seedlings (Booth 1966; Houten 1939; James 1987, 1988; James and Gilligan 1985) and are frequently isolated from the roots of seedlings exhibiting disease symptoms. However, their ability to cause disease has not been evaluated and it is not currently known if they are capable of eliciting the type of symptoms seen on these lodgepole pine seedlings.

Table 2. Fungal colonization of diseased lodgepole pine seedling cotyledons - USDA Forest Service Nursery, Coeur d'Alene, ID[1].

Fungi	Seedling No.[2] 1	2	3	4	5	6	7	8	9	10	Aver-age
Phoma											
P. eupyrena	0	75	0	0	25	0	50	0	0	0	14.3
P. herbarum	0	0	0	33	0	0	0	0	0	0	5.7
Trichoderma	17	0	33	0	0	0	0	0	0	0	5.7
Penicillium	0	25	0	0	0	0	0	0	0	0	2.9
Alternaria	0	0	33	17	50	50	50	50	100	33	28.6
None	83	25	33	50	25	50	0	50	0	67	45.7

[1] Numbers in table are percent of sampled cotyledon pieces colonized by appropriate fungus.

[2] See table 1 for descriptions of root disease symptom severity of sampled seedlings.

Fusarium spp. (***F. oxysporum Schlect.*** and ***F. acuminatum*** Ell. & Ev.) were isolated from the roots of some seedlings (nos. 1, 3 & 9 - table 3), but were not as consistently isolated as were ***Cylindrocarpon*** spp. Although these root disease organisms are common on container-grown seedlings at the Coeur d'Alene Nursery (James 1985b), they were not involved in eliciting disease symptoms in many of these lodgepole pine seedlings.

Phoma herbarum Westend. is commonly isolated from container-grown seedlings and capable of causing disease under certain circumstances (James 1985a). However, this fungus was isolated from the roots of only two seedlings (table 3) and can therefore probably not be considered important in causing disease of lodgepole pine seedlings. The other organisms isolated from roots of diseased seedlings (***Trichoderma, Penicillium,*** and several types of unidentified bacteria) were common saprophytes.

Table 4 outlines distribution of four groups of fungi (***Cylindrocarpon, Fusarium, Phoma,*** and ***Trichoderma***) along taproots of sampled diseased lodgepole pine seedlings. These data show that ***Cylindrocarpon*** and ***Phoma*** colonized the tops of taproots (near the soil line) while the bottom of roots were colonized more commonly with ***Fusarium*** and ***Trichoderma***. Previous investigations (James 1989; James and Gilligan 1988) have shown that ***Fusarium*** spp. are usually detected at higher levels at the bottom of containers, while the other three groups of fungi are more evenly distributed throughout plugs. Therefore, it is not surprising to find greater seedling colonization by ***Fusarium*** at the bottom of plugs.

In conclusion, disease of container-grown lodgepole pine seedlings was most likely due to root colonization and decay by either ***Cylindrocarpon*** or ***Fusarium***. These fungi apparently decayed the roots to the point where seedlings began to wilt as evidenced by dieback of cotyledon tips. As deterioration of root systems progressed, seedlings died. It is likely that these pathogenic fungi were introduced on contaminated containers (James 1989; James and Gilligan 1988). Adequate sterilizing of containers before they are reused is important to reduce future losses. Several procedures for improving cleanliness of containers are currently being developed at the nursery.

Table 3. Root colonization of container-grown lodgepole pine seedlings with selected fungi and bacteria at the USDA Forest Service Nursery, Coeur d'Alene, ID[1].

	Seedling Number[2]										
Organisms	1	2	3	4	5	6	7	8	9	10	Average
Cylindrocarpon											
C. tenue	0	18	0	0	0	0	0	0	0	13	3.1
C. didymum	0	0	18	40	20	80	29	38	0	34	28.1
Fusarium											
F. oxysporum	8	0	37	0	0	10	0	0	37	0	9.2
F. acuminatum	0	0	0	0	0	0	0	0	26	0	2.3
Phoma herbarum	0	0	0	0	21	0	31	0	0	0	5.4
Trichoderma	10	70	9	47	34	10	12	62	25	53	31.1
Penicillium	0	23	9	0	14	0	0	0	0	0	4.6
Bacteria	64	21	54	26	33	39	59	13	18	26	36.5
None	28	0	0	0	0	0	0	0	0	0	2.9
Root length (mm)	131	120	126	116	142	183	129	85	115	137	128.4

[1] Numbers in table are percent of taproot length colonized by appropriate fungi.

[2] See table 1 for descriptions of root disease symptom severity of samples seedlings.

Table 4. Distribution of selected fungi colonizing taproots of container-grown lodgepole pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Selected Fungi	Location Along Taproot		
	Bottom	Middle	Top
Cylindrocarpon	27.5	25.0	47.5
Fusarium	40.5	27.0	32.5
Phoma	0	26.0	74.0
Trichoderma	52.1	37.8	10.1

[1] Figures in table are percentages of linear colonization by appropriate fungi within the bottom, middle, and top one-third of seedling taproots.

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