RELATIONSHIPS BETWEEN EXTENT OF ROOT COLONIZATION AND FOLIAR SYMPTOM PRODUCTION IN CONTAINERIZED DOUGLAS-FIR, PONDEROSA PINE, AND LODGEPOLE PINE SEEDLINGS INFECTED WITH FUSARIUM AT THE MONTANA STATE NURSERY, MISSOULA

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ABSTRACT

Containerized Douglas-fir, ponderosa pine, and lodgepole pine seedlings with different levels of foliar necrosis at the Montana State Nursery in Missoula were evaluated for amount of root infection and soil mix colonization by Fusarium. Fusarium oxysporum was the only Fusarium species isolated from seedlings. Although all sampled seedlings were infected with F. oxysporum, severity of foliar necrosis and level of root colonization seemed only related in ponderosa pine. Both the organic and mineral components of soil mixes were colonized by F. oxysporum in all tested seedlings.

INTRODUCTION

Fusarium spp. are important root pathogens of containerized conifer seedlings grown in greenhouses. These fungi are often seedborne (James 1985a) and may cause needletip dieback during the later phases of the crop cycle that may progress to seedling mortality (James 1984a; James 1984b). Possible sources of secondary inoculum and periods of infection are unknown. Studies were conducted to determine if amount of root infection by Fusarium could be predicted by level of foliar symptom production. This information will help growers estimate amount of damage done by Fusarium at various stages of crop production.

METHODS

Four containerized Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), five ponderosa pine (Pinus ponderosa Laws.), and five lodgepole pine (Pinus contorta Dougl.) seedlings with various levels of needletip dieback and foliar necrosis (figures 1-14 in Appendix) were selected for study from the Montana State Nursery in Missoula. Selected seedlings were given a foliage symptom rating based on extent of needle chlorosis/necrosis present (table 1).

Table 1.--Foliage symptom scheme for containerized conifer seedlings infected with Fusarium.

0 =	No foliar symptoms.containers
1 =	Slight needletip dieback on either lateral or seedling tops
2 =	Needle necrosis spread throughout at least half the crown; may be at several different locations or concentrated in areas.
3 =	Needle necrosis general - more than half the crown affected.

Seedlings were carefully removed from their containers, and their root systems,with attached soil particles, were placed in beakers of distilled water. Roots were agitated to remove loose soil particles. The soil-water solution was filtered through two layers of cheesecloth to remove large organic particles. The organic component was resuspended in distilled water; samples of this component were randomly selected and placed on a selective medium for Fusarium spp. (Komada 1975). Portions of the mineral component of the soil mix which had passed through the cheesecloth were also placed on the selective medium.

Root systems were washed for several minutes under tap water and surface sterilized in a solution of 0.21 percent aqueous sodium hypochlorite (4.0 percent bleach) for 4 minutes. Following sterilization, roots were washed thoroughly in distilled water to remove excess bleach. Ten lateral roots were randomly selected and cut from the main tap root. Root tips about 0.5 cm in length were cut from selected roots and aseptically placed on the selective medium. Also, a piece from the top of each selected root (where it joined the main tap root) was placed on the selective medium.

All plates were incubated for 7 days at about 24°C under a 12-hour diurnal light-darkness regime. The number of root or organic soil mix component pieces infected with Fusarium were counted to determine percentage of infection. Colonization of the mineral soil mix component was classified using a numerical scale based on relative number of Fusarium colonies which grew from the soil (see tables 2-4 for more detailed descriptions).

RESULTS AND DISCUSSION

Colonization of roots and soil mixes by Fusarium in seedlings with different levels of foliar symptoms are summarized for Douglas-fir, ponderosa pine, and lodgepole pine in tables 2, 3 and 4, respectively. Only one species of Fusarium, F. oxysporum Schlect., was isolated from all sampled seedlings. This species was obtained from the roots of all sampled seedlings. Douglas-fir and lodgepole pine seedlings with the more severe foliar symptoms did not necessarily have a greater portion of their root system colonized by Fusarium (tables 2 and 4). However, for ponderosa pine (table 3) there appeared to be positive relationships between foliar symptoms and level of root colonization, although samples were not large enough to develop statistical relationships through regressions.

Table 2.--Colonization of roots and adjacent soil mix by Fusarium oxysporum in containerized Douglas-fir seedlings with different levels of foliar symptoms.

Seedling number	Foliar symptom rating[1]	Root colonization[2]			Soil mix colonization	
		Tips	Joints	All	Organic component[2]	Mineral component[3]
1	3	100	90	95	100	2
2	1	100	100	100	100	3
3	2	80	100	90	67	2
4	2	100	100	100	100	3

[1]See table 1 for descriptions of foliar symptom ratings.

[2]Values are percentages of samples colonized by Fusarium oxysporum. Joints are where lateral roots meet the main tap root.

[3]Mineral component colonization: 3 = completely colonized; 2 = partially (about half) colonized; 1 = very little colonized (only a few Fusarium colonies); 0 = no colonization.

Table 3.--Colonization of roots and adjacent soil mix by Fusarium oxysporum in containerized ponderosa pine seedlings with different levels of foliar symptoms.

Seedling number	Foliar symptom rating[1]	Root colonization[2]			Soil mix colonization	
		Tips	Joints	All	Organic component[2]	Mineral component[3]
1	2	90	70	80	100	2
2	1	90	50	70	67	1
3	3	100	100	100	63	2
4	2	100	60	80	88	3
5	2	100	70	85	100	3

[1]See table 1 for descriptions of foliar symptom ratings.

[2]Values are percentages of samples colonized by Fusarium oxysporum.

[3]Mineral component colonization: 3 = completely colonized; 2 = partially (about half) colonized; 1 = very little colonized (only a few Fusarium colonies); 0 = no colonization.

Table 4.--Colonization of roots and adjacent soil mix by Fusarium oxysporum in containerized lodgepole pine seedlings with different levels of foliar symptoms.

Seedling number	Foliar symptom rating[1]	Root colonization[2]			Soil mix colonization	
		Tips	Joints	All	Organic component[2]	Mineral component[3]
1	2	50	60	55	100	2
2	2	80	80	80	100	3
3	3	100	100	100	0	1
4	1	100	80	90	100	3
5	2	100	90	95	100	2

[1]See table 1 for descriptions of foliar symptom ratings.

[2]Values are percentages of samples colonized by Fusarium oxysporum.

[3]Mineral component colonization: 3 = completely colonized; 2 = partially (about half) colonized; 1 = very little colonized (only a few Fusarium colonies); 0 = no colonization.

In most cases, root tips were colonized by F. oxysporum to a greater degree than joints, probably indicating most initial root infection occurring toward the tips of lateral roots. Both the organic and mineral components were often greatly colonized by Fusarium. Some seedlings with only slight disease symptoms had much of their soil mix colonized.

These studies did not evaluate the virulence of the F. oxysporum isolates colonizing seedlings. It is well known that this fungal species often colonizes roots of many types of plants without causing disease (Armstrong and Armstrong 1948; Bloomberg 1966). At other times, F. oxysporum appears to be an aggressive pathogen capable of attacking and killing its host quickly (James and Gilligan 1984; James 1985b). Factors affecting the ability of this fungus to elicit disease of containerized conifer seedlings are largely unknown, but may be related to inoculum potential and host vigor. If there are multiple sources of inoculum within greenhouses, such as seed and crop debris and if secondary spread is important, there may be multiple periods of seedling infection. This may result in more disease expression than if only one period of host infection is common. Also, if seedling stress is important in symptom expression, practices such as withholding water and nutrients during hardening off and bud set may contribute to appearance of disease symptoms.

Although the number of seedlings sampled in this investigation was small, it appears that the level of foliar necrosis may not be correlated to amount of root system infection by F. oxysporum. Other environmental or genetic factors may have more influence on disease symptom production.

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APPENDIX

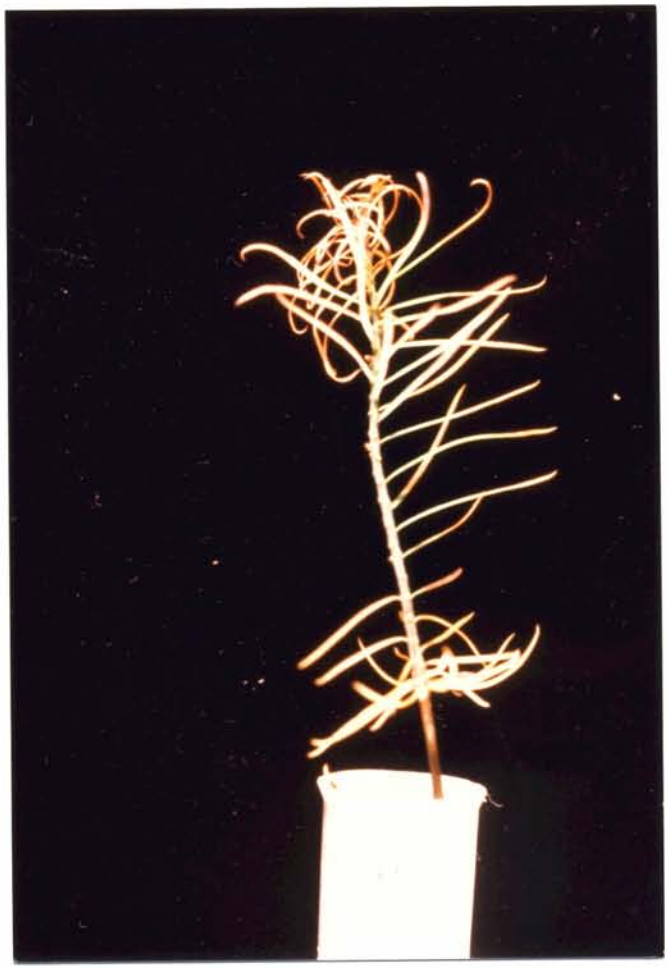

Figure 1.--Containerized Douglas-fir seedling (no. 1) with foliar necrosis (rating 3-table 1).

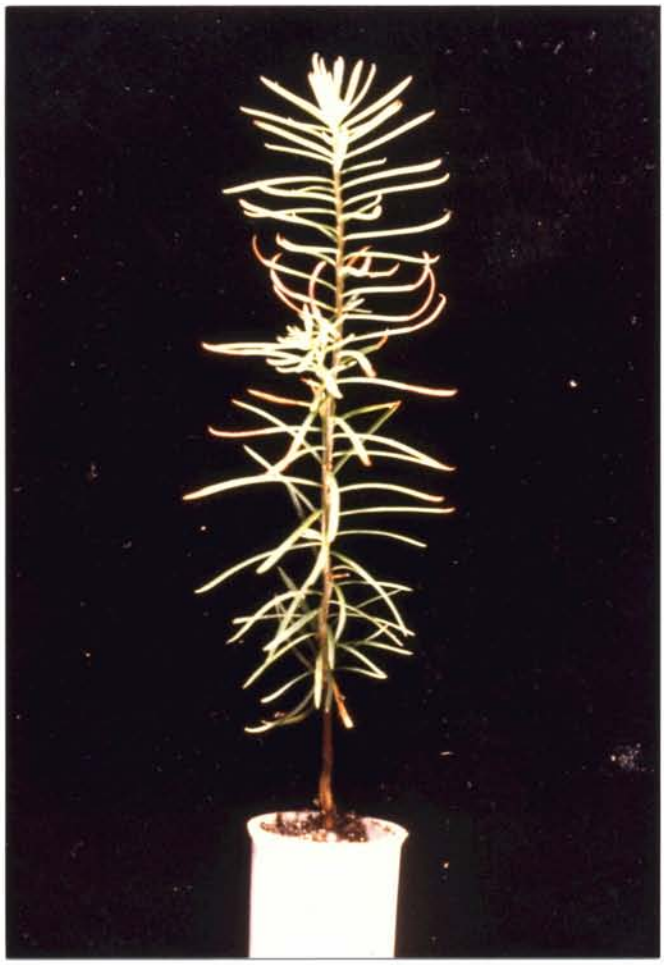

Figure 2.--Containerized Douglas-fir seedling (no. 2) with foliar necrosis (rating 1-table 1).

Figure 3.--Containerized Douglas-fir seedling (no. 3) with foliar necrosis (rating 2-table 1).

Figure 4.--Containerized Douglas-fir seedling (no. 4) with foliar necrosis (rating 2-table 1).

Figure 5.--Containerized ponderosa pine seedling (no. 1) with foliar necrosis (rating 2-table 1).

Figure 6.--Containerized ponderosa pine seedling (no. 2) with foliar necrosis (rating 1-table 1).

Figure 7.--Containerized ponderosa pine seedling (no. 3) with foliar necrosis (rating 3-table 1).

Figure 8.--Containerized ponderosa pine seedling (no. 4) with foliar necrosis (rating 2-table 1).

Figure 9.--Containerized ponderosa pine seedling (no. 5) with foliar necrosis (rating 2-table 1).

Figure 10.--Containerized lodgepole pine seedling (no. 1) with foliar necrosis (rating 2-table 1).

Figure 11.--Containerized lodgepole pine seedling (no. 2) with foliar necrosis (rating 2-table 1).

Figure 12.--Containerized lodgepole pine seedling (no. 3) with foliar necrosis (rating 3-table 1).

Figure 13.--Containerized lodgepole pine seedling (no. 4) with foliar necrosis (rating 1-table 1).

Figure 14.--Containerized lodgepole pine seedling (no. 5) with foliar necrosis (rating 2-table 1).