No. 114 December 1990

NEEDLE NECROSIS OF CONTAINER-GROWN DOUGLAS-FIR SEEDLINGS - CAL-FOREST NURSERIES, ETNA, CA

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During production of container-grown Douglas-fir (***Pseudotsuga menziesii*** Dougl.) seedlings in 1990 at Cal-Forest Nurseries in Etna, California, growers noticed several young seedlings displaying disease symptoms during the latter part of May and early June. Affected seedlings had needle tip necrosis that sometimes extended down to encompass entire needles (figure 1). Needle necosis spread throughout greenhouses in a pattern similar to a disease-causing agent. However, needle symptoms became evident relatively quickly and did not usually progress to the point of seedling mortality. Since growers believed the cause of the disorder was pathogenic organisms that incite root diseases, applications of benomyl were made shortly after symptoms were first noticed.

Seedlings with needle-tip necrosis were sampled for presence of potential root pathogens because prior experience had indicated that pathogenic organisms may be associated with these types of symptoms on Douglas-fir (James 1984a, 1984b, 1986b). Eighteen seedlings with various levels of needle necrosis were selected for isolations. Seedlings were carefully extracted from styroblock containers and their roots washed thoroughly under running tap water to remove particles of growing media. Root pieces (2-3 mm in length) were aseptically excised and surface sterilized in a 10% bleach (0.525% aqueous sodium hypochlorite) solution for 1 minute, rinsed with sterile water and placed on two selective media. Ten root pieces from each seedling were placed on both a medium selective for ***Fusarium*** and closely associated organisms (Komada 1975) and one selective for ***Pythium*** and similar organisms (V-8 juice agar amended with pimaricin and several other antibiotics). Plates with Komada's medium were incubated at about 24°C under diurnal cycles of cool, fluorescent light for 7-10 days and those with V-8 juice agar were incubated in the dark at the same temperature for 3 days. Fungi emerging from root pieces were identified to genus using the taxonomic guide of Barnett and Hunter (1972). Selected isolates were transferred to potato dextrose agar slants for identification to species. ***Fusarium*** isolates were identified using procedures described by Nelson and others (1973). ***Cylindrocarpon***, ***Phoma***, and ***Mortierella*** spp. were identified using taxonomic descriptions of Booth (1966), Dorenbosch (1970), and Domsch and others (1980), respectively.

Results of root isolations are summarized in Table 1. Half the sampled seedlings with needle necrosis symptoms had roots colonized with *Fusarium* spp., which are potential pathogens of conifer seedlings (James and others 1988). However, extent of *Fusarium* root colonization on these seedlings was probably not sufficient to cause above-ground disease symptoms (James 1986c), i. e., only 8.9% of the root pieces sampled were colonized (Table 1). Past experience indicated that seedlings with needle necrosis caused by *Fusarium* have root systems that are nearly 100% colonized with these fungi (James 1986c).

Figure 1. Container-grown Douglas-fir seeling with needle necrosis from the Cal-Forest Nursery, Etna, California.

Table 1. Fungal colonization of roots from container-grown Douglas-fir seedlings - Cal Forest Nurseries.

Fungus	Percent Seedlings Infected[1]	Root Colonization Percentage[2]
Fusarium		
oxysporum	5.5	0.5
sporotrichioides	5.5	1.1
acuminatum	3.3	6.7
proliferatum	5.5	0.5
All *Fusarium*	50.0	8.9
Cylindrocarpon		
destructans	77.8	25.0
Phoma		
eupyrena	44.4	10.5
herbarum	5.5	1.7
All *Phoma*	50.0	12.2
Trichoderma spp.	100.0	66.1
Penicillium spp.	88.9	51.7
Mortierella		
alpina	50.0	21.3
ramanniana	37.5	16.3
All *Mortierella*	87.5	37.5

[1] 18 seedlings sampled.

[2] Percent of root pieces colonized with appropriate fungus (10 sampled per seedling).

Isolation results indicated presence of at least four different ***Fusarium*** species (Table 1). The most common was ***F. acuminatum*** Ell. & Ev.; others included ***F. sporotrichioides*** Scherb., ***F. oxysporum*** Schlecht., and ***F. proliferatum*** (Matsushima) Nirenberg. Some of these species, especially ***F. acuminatum*** and ***F. oxysporum***, are capable of being pathogenic, particularly when environmental conditions are conducive for disease development (James and others 1985). However, there are also saprophytic strains of all four species that may colonize root cortical cells or are present in the rhizosphere and do not cause disease. It is suspected that most of the ***Fusarium*** isolates obtained in this investigation were probably saprophytes, although pathogenicity tests to confirm this conclusion were not conducted.

Another potentially pathogenic fungus isolated from these Douglas-fir seedlings was ***Cylindrocarpon destructans*** (Zins.)Scholten. This organism is a common colonizer of conifer seedling roots and is capable of being either pathogenic or saprophytic (Booth 1966; James 1988a). The fungus may be a problem when seedlings are stressed, particulary when grown with too much or too little water. Under these conditions, ***C. destructans*** will progressively decay roots until most of the root system is gone. In container seedling operations, seedlings severely infected with ***Cylindrocarpon*** do not often show above-ground disease symptoms, but root decay symptoms are discovered when seedlings are extracted from containers at the end of the growing season (James and Gilligan 1990). Although most Douglas-fir seedlings assayed from the Cal-Forest Nursery had roots colonized with ***Cylindrocarpon***, very little root necrosis was found and it is unlikely that this fungus was acting as an aggressive pathogen.

Other fungi colonizing roots of Douglas-fir seedlings included the common saprophytes ***Trichoderma*** and ***Penicillium***. Some roots were colonized with species of ***Phoma*** (***P. eupyrena*** Sacc. and ***P. herbarum*** Westend.). Both of these fungi are potential pathogens, particularly of above-ground portions of seedlings(Domsch and others 1980; Dorenbosch 1970). However, they are usually not important causes of root disease.

Another common group of isolated fungi was ***Mortierella*** spp. These fungi were frequently isolated on V-8 juice agar. Two species were frequently encountered: ***M. alpina*** Peyronel and ***M. ramanniana*** (Moll.) Linnemann. Both species are common saprophytes and colonizers of organic debris, particularly under more acidic conditions in which conifer seedlings are grown (Domsch and others 1980; Kuhlman 1969, 1975).

It was interesting that other possible root pathogenic fungi, such as ***Pythium*** spp. and ***Rhizoctonia*** spp., were not isolated. Another potential pathogen, ***Mariannaea clavispora*** Samson & Bigg, was also not isolated. This fungus was recently described from diseased roots of Douglas-fir seedlings (Samson and Bigg 1988). It produces symptoms similar to ***Fusarium*** root disease and extensive root decay. However little root decay was found in the current investigation.

Several abiotic factors may have contributed to production of necrotic needle tissues. Fertilizer toxicity may occur in continer seedling operations when too much fertilizer is added to irrigation water and applied when seedlings are stressed, particularly during periods of high greenhouse temperatures (James 1988b; Landis and others 1989). Also, high temperatures for prolonged periods or rapidly increasing temperatures may elicit foliar necrosis of conifer seedlings (James 1986a). This may be especially prevalent when foliage tissues are succulent during rapid growth phases. Pesticides may also induce foliar "burn" if applied at improper concentrations or during periods of high tissue succulence.

Based on the results of root isolations from symptomatic seedlings, it is concluded that pathogenic fungi were probably not responsible for needle necrosis of container-grown Douglas-fir seedlings at the Cal-Forest Nursery. This conclusion is also substantiated by the facts that root systems looked healthy with very little decay and an abundance of growing root tips and that fungicide applications had no effect on symptom production and spread. Also, symptoms appeared quite suddenly rather than developing gradually over time. In addition, several seedlings with necrotic needle symptoms were grown outside for several weeks after symptoms became evident and, in most cases, developed new healthy terminal and lateral growth beyond the necrotic zones. Therefore, all evidence points to a one-time damaging agent that occurred just prior to development of needle necrosis.

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