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INVESTIGATIONS OF POTENTIAL DISEASE-CAUSING ORGANISMS ASSOCIATED WITH PRODUCTION OF CONTAINER-GROWN BITTERBRUSH SEEDLINGS USDA FOREST SERVICE LUCKY PEAK NURSERY BOISE, IDAHO

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ABSTRACT

Container-grown bitterbrush seedlings exhibited foliage disease symptoms characterized by leaf margin necrosis that extended to entire leaves and petioles of some seedlings. Isolations made from necrotic leaves and healthy-appearing roots of affected seedlings yielded primarily *Alternaria alternata* and several species of *Phoma*, particularly *P. eupyrena.* Small amounts of *Fusarium* (*F. proliferatum* and *F. oxysporum*) were also isolated from diseased seedlings. The disease was best controlled by reducing duration of leaf wetness and nitrogen fertilizer amendments during early seedling growth. Fungicides also helped reduce disease severity. Future disease is best prevented by manipulating environmental conditions under which seedlings are grown, making them less conducive to potential pathogens common at the nursery.

INTRODUCTION

The USDA Forest Service Lucky Peak Nursery near Boise, Idaho has traditionally produced bare root conifer seedlings for reforestation requirements on Forest Service lands within the Intermountain Region (Nevada, Utah and southern Idaho). However, in recent years conifer production has been reduced because of greatly lowered demand for seedlings for reforestation. Reduced conifer seedling production has been replaced by growing other plant species, particularly brush species for enhancement of wildlife habitat. High demand for bitterbrush (*Purshia tridentata* [Pursh] DC.) has resulted in increasing production of this species at the nursery. To provide the type of stock desired within the time frames necessary, growers have been growing bitterbrush within Super Cell® containers. Unfortunately, not all crops have been successfully grown. One major production limitation is disease which may result in extensive seedling mortality and adversely affect seedling growth and quality.

Very little is known about diseases of bitterbrush in general and problems within nurseries in particular (Farr et al. 1989; Furniss and Krebill 1971). Also, growing any plant in containers presents unusual problems because the conditions under which seedlings are grown are often ideal for certain pathogens (James 1984c). Previous container crops of bitterbrush have been grown outside, where environmental conditions, such as temperature, cannot be adequately controlled. However, future plans call for construction of a large greenhouse where bitterbrush and other plant species will be grown.

Because of recent bitterbrush production problems and the likelihood of increased production of this species in the future, investigations were conducted to elucidate association of potentially-pathogenic organisms with young bitterbrush seedlings displaying foliar disease symptoms. With this background information, improved strategies for reducing future disease-associated losses are possible.

MATERIALS AND METHODS

During the summer of 2001, several container-grown bitterbrush seedlings displayed leaf margin necrosis (figure 1) that progressed until entire leaves became necrotic. In some cases, necrosis extended down leaf petioles and into branches. Symptoms appeared rather quickly rather than being developed over time. Leaves of affected seedlings were detached, surface sterilized in 10% bleach solutions (0.525% aqueous sodium hypochlorite), rinsed in sterile water, and incubated in moist chambers for several days. Emerging fungi were aseptically transferred to 2% water agar and potato dextrose agar (PDA) for identification. Organisms were identified to genus using the taxonomy of Barnett and Hunter (1998). Other taxonomic keys (Dorenbosch 1970; James and Hamm 1985; Jolly 1967) were used to delineate particular species.

Although roots of affected seedlings generally appeared healthy, they were also tested for presence of potentially-pathogenic fungi. Roots from extracted seedlings were washed thoroughly to remove adhering particles of growing media. They were then cut into pieces approximately 5 mm in length. Root pieces were surface sterilized as described above, rinsed in sterile water, blotted dry, and placed on a selective agar medium for *Fusarium* and closely-related species (Komada 1975). Plates with roots were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7 days. Selected *Fusarium*

Figure 1. Bitterbrush leaf with margin necrosis from container-grown seedlings from the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

isolates were transferred to PDA and carnation leaf agar (Fisher et al. 1982) for identification of species using the taxonomy of Nelson et al. 1983. Percentages of sampled root systems colonized by particular fungi were calculated.

RESULTS AND DISCUSSION

Eighteen fungal isolates were obtained from detached necrotic leaves. Forty percent of these were identified as *Alternaria alternata* (Fr.) Keissler (Jolly 1967; Simmons 1967). More than 50% of the isolates were species of *Phoma*. By far the most common was *P. eupyrena* Sacc.; other species isolated less frequently included *P. glomerata* (Corda) Wollenw. & Hochapf. and *P. pomorum* Thum. *Fusarium proliferatum* (Matsushima) Nirenberg was isolated once from necrotic leaves.

Phoma spp. were also isolated frequently from the roots of affected seedlings (table 1). *Fusarium* spp. were isolated much less frequently and common saprophytic *Penicillium* and *Trichoderma* spp. were commonly associated with roots.

None of the organisms (*Alternaria, Phoma*, and *Fusarium*) isolated from diseased foliage have previously been reported on bitterbrush (Farr et al. 1989; Furniss and Krebill 1971). Several of these organisms may be capable of causing diseases of young seedlings, particularly under conducive environmental conditions such as high moisture and moderate temperatures

Table 1. Colonization of roots of container-grown bitterbrush seedlings with selected fungi - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Seedling Number	Percent Root Colonization[1]			
	Fusarium[2]	*Trichoderma*	*Penicillium*	*Phoma*[3]
1	10	20	80	10
2	10	10	90	10
3	10	0	20	80
4	0	20	10	70
5	10	0	100	10
6	0	40	60	50
7	0	20	20	70
8	0	0	60	60
9	0	60	80	70
10	0	10	60	50
Average	4	18	58	48

[1] Ten randomly-selected root pieces sampled per seedling.
[2] Included *Fusarium proliferatum* (seedling #1) and *F. oxysporum* (seedlings 2, 3, & 5).
[3] Included mostly *Phoma eupyrena* with lower levels of *P. glomerata*, *P. pomorum* and *P. herbarum*.

(Dorenbosch 1970; James and Hamm 1985).

Alternaria alternata is a fairly common pathogen on a wide range of agricultural hosts, often implicated as causing leaf spot diseases (Atilano 1983; Bashan et al. 1991; Chandrashekar and Ball 1980; McRoberts and Lennard 1966; Mortensen et al. 1983) and is a possible pathogen of seedlings in forest nurseries (James and Woo 1987). This fungus produces phytotoxins that can readily kill host tissues (Abbas and Vesonder 1993; Brandwagt et al. 2000; Fuson and Pratt 1988; Kohmoto et al. 1984); the pathogen develops rapidly and can spread quickly throughout a crop during periods of high moisture (Bashan et al. 199l; Thomas 1983).

Phoma spp. are common saprophytes on plant organic material, but can also sometimes be important pathogens (Boerema 1976; Dorenbosch 1970). *Phoma eupyrena*, the most commonly encountered *Phoma* species in this evaluation, is often a soilborne pathogen in forest nurseries (James and Hamm 1985; Morgan-Jones and Burch 1988) and is important in causing tip dieback diseases or young seedling mortality (Cooley 1983, 1984, 1985; Cordell et al. 1988; James 1979, 1980, 1983, 1984a, 1984b, 1986, 1987, 1990). When inoculum and moisture are high, this fungus can be an aggressive pathogen, quickly attacking and killing susceptible host tissues (James and Cooley 1987; James and Schwandt 1989; Kliejunas 1984; Kliejunas and Allison 1983; Kliejunas et al. 1985). The other *Phoma* species isolated from bitterbrush leaf or root tissues are considered potentially less important pathogens (Dorenbosch 1970; James and Hamm 1985). *Phoma glomerata* has been found occasionally within forest nurseries and on a wide range of r agricultural crops (Boerema et al. 1965, 1971; Chohan and Chand 1980; Danquah 1975; Hosford 1975; Luedemann 1959; Srago et al. 1989).

Phoma pomorum and *P. herbarum* are encountered less frequently in forest nurseries, but can sometimes be associated with foliar or tip blight disease symptoms (Boerma 1964, 1970; James 1985; Johnston 1981; Jones 1976; Swift 1932).

Although *Fusarium* spp. are very important pathogens at the Lucky Peak Nursery (James 1996; James and Beall 1999, 2000), their low level of occurrence on diseased bitterbrush seedlings probably indicates that they were not important in eliciting this disease. These fungi are common in nearby bareroot fields (James 1996; James and Beall 1999, 2000) and probably contaminated the bitterbrush seedlings.

Disease symptoms on bitterbrush symptoms were associated with prolonged periods of wet foliage. When steps were taken to reduce foliage wetness and fungicides were applied to the crop, disease levels were greatly reduced and seedlings grew quickly. Therefore, organisms isolated from diseased seedlings probably exhibited low levels of virulence and could not elicit disease symptoms on hosts when foliage moisture was reduced and seedlings became less stressed. Another possible contributing factor to disease was high nitrogen applied early in the growth phase. High nitrogen makes plant tissues more succulent and increases susceptibility to some fungal pathogens (James 1997), especially fairly weak pathogens that require high host susceptibility for eliciting disease. When growers reduced nitrogen, as well as leaf wetness, and applied fungicides, the disease was greatly reduced. Apparently, bitterbrush seedlings do not need large amounts of nitrogen early in the growth cycle like conifer seedlings. Therefore, controlling fertilizers and limiting duration of foliage wetness will be necessary to reduce future disease problems on container-grown bitterbrush seedlings at the Lucky Peak Nursery. Hopefully, disease intensity can be limited by manipulating environmental factors rather than relying on chemical fungicides.

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