Investigations of the Cause of First-Year Stunting of Douglas-fir Seedlings in Bareroot Conifer Nurseries

R.G. Linderman, K.W. Russell, and Y. Tanaka

Research Plant Pathologist, USDA-Agricultural Research Service, Horticultural Crops Research Laboratory, Corvallis, OR; Plant Pathologist, formerly Washington State Department of Natural Resources, Forest Health, Olympia, WA; Forest Nursery Ecologist, deceased, formerly Weyerhaeuser Company, Western Forestry Research Center, Centralia, WA

Abstract

We investigated several possible causes of 1-0 stunt in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] in two fumigated bareroot nurseries. The results suggest that (1) delayed development of a root system contributes to seedling nutrient stress; (2) lack of ectomycorrhizae did not cause stunt; (3) root diseases were not involved; and (4) some soil microorganisms on root surfaces may have produced toxins that prevented nutrient-absorbing hair root development. We conclude that some combination of factors limits root system development, leading to nutrient deficiency, bud set, and stunting.

Introduction

Stunting of first-year conifer seedlings (1-0 stunt) in bare-root seedling nurseries in the Pacific Northwest (PNW) has been a concern for many years. Stunted seedlings cease growth early in the growing season. They thus fail to reach commercial size and are a potential loss to the nursery. The pattern of stunted trees is often a mosaic (Landis 2001) in which areas within beds have stunted trees next to areas where trees are not stunted (figure 1). Frequently, however, individual trees are stunted immediately adjacent to trees that are not (figure 2). The cause of this abnormal growth pattern has been hypothesized to be either a phosphorus (P) deficiency or lack of mycorrhizae that could help seedlings acquire P more effectively. In many cases, adding higher rates of preplant P fertilizer has solved the problem. Sometimes, however, the fertilizer is not thoroughly or uniformly mixed into the planting bed, and P is not mobile enough in soil to even out the P level and availability (Landis 2001). Thus, some trees could become stunted because of localized P deficiency and prematurely set bud for the season. Because mycorrhizal fungi are eliminated by soil fumigation in many nurseries, mycorrhizae may not be in place in time to improve P uptake by young seedlings.

Nutrient deficiency in conifer seedlings during the first 2 mo of growth, resulting from lack of mycorrhizae, low levels of available soil P, or both, could stop shoot growth and cause terminal bud set in the middle of the growing season. This stunting of seedlings during the first growing season (figure 1) occurs, but the cause has not been thoroughly

Figure 1. Mosaic stunting pattern of Douglas-fir seedlings in a bare-root nursery showing a mixture of stunted and normal seedlings.

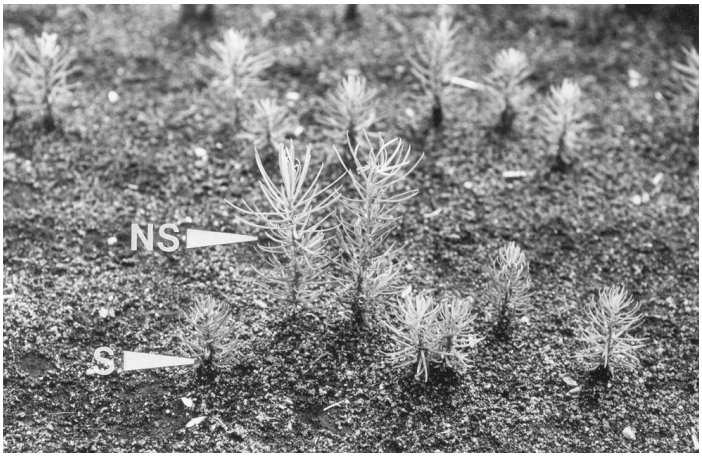

Figure 2. (A) Stunted (S) first-year Douglas-fir seedlings, grown in nursery soil fumigated with methyl bromide/chloropicrin, adjacent to unstunted seedlings (NS).

investigated to determine whether lack of mycorrhizae (Trappe and Strand 1969), P deficiency, root disease, some other cause, or a combination of factors, is involved.

Soil fumigation has been practiced for many years as a means of combating weed, insect, and disease problems. In the PNW, fumigation is generally done in late summer to early fall when conditions are optimum, rather than in spring, when conditions are generally too cold and wet. This practice results in a lengthy fall-winter fallow period before spring sowing in early May, during which soils can become colonized by both deleterious and beneficial microbes. Root pathogens of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], such as species of *Pythium* and *Fusarium* (Hansen and others 1990), may readily invade the fumigated soil, either as mycelium growing from below the fumigation layer or as spores in soil carried by moving water, wind, or machinery. At the time of sowing, pathogens of Douglas-fir, such as *F. oxysporum* Schlect., also may be introduced to the fumigated beds as seedborne inoculum (Graham and Linderman 1983; Hoefnagels and Linderman 1999). Species of *Fusarium* and *Pythium* commonly encountered in plate counts of soil from PNW nurseries are F. *oxysporum*, F. *roseum* (LK.), P. *debaryanum* Auct. non R. Hesse, P. *ultimum* Trow, and P. *sylvaticum* W. A. Campbell and J. W. Hendrix (K. W. Russell, unpublished data).

While soil fumigation is an effective means of combating soilborne pests and pathogens, the concomitant reduction or elimination of beneficial mycorrhizal fungi is a concern (Campagna and White 1969; Henderson and Stone 1970; Carpenter and Boyd 1980; Kough and others 1985; Linderman 1987; Riffle 1980) because mycorrhizae aid seedlings in acquisition of water and nutrients, especially immobile nutrients such as P, copper and zinc. Commercial bareroot nurseries in the PNW rely on natural recolonization of fumigated beds by mycorrhizal fungi via vegetative growth of residual mycelium up through the fumigation layer, or by airborne spores from fruiting bodies elsewhere in the nursery or surrounding areas.

We observed that first-year (1-0) stunting occurs at greater frequency in unfumigated areas of fumigated nurseries. This observation suggested that a biotic agent may be involved. If deleterious microorganisms are involved, and fumigation seems to eliminate these agents temporarily, then one must assume that they reestablish during the period between fall fumigation and planting in the spring or, possibly, are introduced at seeding as seed contaminants. Furthermore, if mycorrhizal establishment were to prevent stunting by improving seedling nutrient uptake capacity, that symbiotic relationship would have to be fully functional before mid-July of the first growing season when the stunt syndrome appears.

The purpose of this study, therefore, was to examine possible causes of 1-0 stunt on Douglas-fir, including the time of development of feeder roots on seedlings, the time of development of ectomycorrhizae that could aid in nutrient uptake, and the possible role of deleterious microbes, including root rot pathogens, that could reduce nutrient uptake by seedlings. An earlier report described some of the results (Tanaka and others 1986).

Materials and Methods

Treatments, plot design, and soil assays. Fumigation treatments were applied at two Washington nurseries (designated A and B) in a randomized block design with three plot replications per treatment. For comparative purposes, soils were fumigated or not with methyl bromide/chloropicrin or dazomet (Basamid). Treatments were conducted at both nurseries in early September 1985, as follows:

Treatment 1: Fumigation with methyl bromide/chloropicrin (MBC) (2:1) at 404 kg ha^{-1} (360 lb ac^{-1}) rate; plots were covered with a polyethylene tarpaulin, which was removed after 2 d at Nursery B and 1 mo at Nursery A (normal operational practice). MBC was injected at the 15 cm (5.9 in) depth and tarped.

Treatment 2: Fumigation with dazomet (DAZ) at 393 kg ha^{-1} (350 lb ac^{-1}). Dazomet fumigation was done by rototilling the granules into the top 15 cm (5.9 in) of soil and sealing by irrigation but without tarping.

Treatment 3: Unfumigated control (C). Each plot consisted of three 4-m (13.1-ft) long sections of 1.3-m (4.3-ft) wide seedling beds included in an approximately 7 × 7 m^2 (23 x 23 ft^2) area.

Soil samples were collected from the three replicate plots for each treatment at 6 and 8 mo (presow), and 10 mo (postsow) after fumigation, the last sampling being just before expected onset of stunt symptoms. Three 2.5-cm (0.98-in) diameter core soil samples from each plot were separately pooled from 0-30 cm (11.8-in) depth. The core

sampler was flamed between plots to prevent cross contamination. Combined samples weighing approximately 500 g were screened (1 cm/0.39 in) to eliminate large particles and debris, and refrigerated until plated, usually within 3 d.

Standard soil dilution plating techniques on selective media were used to determine populations of *Pythium* and *Fusarium* species. The *Pythium*-selective cornmeal, antibiotic medium was a modification of that used by Mircetich (1971). The only change made was to substitute 250 mg of ampicillin sodium salt (Sigma Chemical Co., St. Louis, MO) for the 300 mg of vancomycin hydrochloride antibiotic. The *Fusarium*-selective medium was that developed by Komada (1975).

Soils for *Pythium* dilution plating first were screened through a 4-mm (0.16-in) sieve; then 5.0 g was mixed into 100 ml of 0.5 percent water agar, swirled sufficiently to suspend the soil, and allowed to stand for 30 s to settle the larger particles. One milliliter of the suspension was ladled onto each of five Petri plates containing 25 ml of selective medium and carefully spread with a sterile spoon, taking care to keep the suspension away from the plate edge. After incubating in the dark at 20 °C for 60–72 hours, inoculated plates were washed with running tap water to remove the surface water agar and soil to facilitate counting of colonies. *Pythium* species were not differentiated, and populations were expressed as propagules g^{-1} moist soil.

Fusarium populations were assayed as in the *Pythium* assays, except that the soil was suspended in a 0.3 percent water agar. Plates were incubated for 5 d at 22–24 °C in natural light, and colonies were counted as described by Komada (1975).

Seedling sampling and assays. Seed was sown by machine at both nurseries in early May 1986. Measurements of growth and mycorrhizal colonization were made on 10 seedlings from each replicate plot on July 15, 1986. Seedlings were carefully washed to remove adherent soil, bagged, and shipped on ice to the Horticultural Crops Research Laboratory in Corvallis, OR, where each was examined for presence of ectomycorrhizae. We determined total number of short roots per seedling, percentage of short roots with mycorrhizae per seedling (as indicated by the presence of a fungal mantle), and number of seedlings with any mycorrhizae. Measurements were made on photocopy images of each root system, and number of short roots was determined from the photocopies.

During the 1986 growing season, incidence of 1-0 stunting within the trial plots also was determined on the basis of the percent of stunted seedlings within a 0.557-m^2 (6-ft^2) area of bed. Based on previous years' observations, a seedling was considered stunted if it was less than 7 cm (1.5 in) tall from ground to apical bud.

In a supplemental experiment, soil was collected from areas in Nursery A where stunted seedlings occurred. Soils were sent to the Horticultural Crops Research Laboratory in Corvallis, OR, pasteurized with air-steam (60 °C, 30 min) or not pasteurized, and used to fill pine cell (55 cm^3/3.356 in^3) Leach tubes (Steuve and Sons, Corvallis, OR). The stunt soils, pasteurized or not, were seeded with Douglas-fir, and seedlings were grown under greenhouse conditions without additional fertilizer beyond the residual in the soil collected from the nursery. Soil was washed from seedling roots after 2-mo growth, and roots were observed under a dissecting microscope.

All data were analyzed statistically as means of replicate plots with analysis of variance (Steel and Torrie 1960). When differences between treatments were significant at the 5 percent level of probability, means were compared with Duncan's new multiple range test. Percentages were analyzed after arcsin transformation.

Results

Plant responses. *First year stunting*. The incidence of first-year stunting was very low at both nurseries in the blocks used for this study. The highest level for any treatment was in the unfumigated controls, being 0.14 percent for Nursery A and 2.5 percent for Nursery B (table 1). In general, seedlings grown in soil fumigated with MBC or DAZ were significantly larger (root weight and shoot height) at the end of the growing season than were those grown in unfumigated soil.

Root development. In general, MBC (and, to a lesser extent, DAZ) fumigation increased the root mass and length and the number of short feeder roots (table 1). At the end of the growing season, the number of short roots was greater on seedlings grown at Nursery B than Nursery A. Careful removal of stunted and unstunted trees from Nursery A and visual examination showed a striking difference in the size of the root systems, as well as in the adherence of soil to the roots. With stunted seedling roots, the soil fell off easily, leaving largely bare roots; in contrast, soil ad-

hered to the roots of unstunted seedlings (figure 3). Washing the soil off the unstunted seedlings revealed abundant hair roots to which the soil had adhered; hair roots were mostly missing from the stunted seedlings. Seedlings from Nursery B were not examined in this manner.

Mycorrhiza formation. Examination of short roots for mycorrhizae on seedlings in Nursery A on July 15, 1986, revealed that more mycorrhizae generally occurred on seedlings from the DAZ fumigated plots than on those from the MBC-fumigated or unfumigated control plots (table 1). The occurrence of seedlings with mycorrhizae was scattered and on relatively few seedlings. In Nursery B, seedlings in plots fumigated with either MBC or DAZ also had more mycorrhizae than in the unfumigated control, comparable to Nursery A. Over all treatments, 16–57 percent of the seedlings at Nursery A and 16–50 percent at Nursery B had some mycorrhizae at the end of the growing season. The percentage of short roots with mycorrhizae at that sampling time, however, was only 1–9 percent at Nursery A and 5–17 percent at Nursery B, with control treatments generally having a lower level of mycorrhizae than fumigation treatments. Thus, less than half of the seedlings had any ectomycorrhizae; the incidence on short roots of those that did was very low on both stunted and unstunted seedlings.

Root rot pathogen population dynamics. Population counts of *Fusarium* and *Pythium* were determined for samples taken, at the same times from both nurseries, from the top 30 cm (11.8 in) of soil (table 2). The data clearly show the effectiveness of MBC fumigation in reducing *Fusarium* and *Pythium* populations. Dazomet was statistically as effective in reducing *Fusarium* populations as MBC at all sampling dates in Nursery A, but generally did not reduce them to the extent of the MBC treatments. The same trend was found for *Fusarium* at Nursery B.

Figure 3. Left: stunted (right) and unstunted (left) seedlings after washing. Right: stunted (left) and unstunted (right) seedlings just after removal from nursery soil. Seedlings were immediately adjacent in the nursery bed; note the difference in the soil adherence to the two root systems.

Table 1. Effects of soil fumigation with methyl bromide/chloropicrin (MBC) or dazomet (DAZ) compared to with untreated controls (C) on development of shoots, roots, and mycorrhizae (MR) in relation to the incidence of stunting on Douglas-fir seedlings grown at two Pacific Northwest bare-root nurseries, as measured on July 15 following seeding in May 1986.

Treatment	Shoot height (cm)	Root weight (mg)	Root length (cm)	No. short roots/ seedling	Short roots with MR(%)	Seedling roots with MR(%)	Stunting (%)
				Nursery A			
MBC	17.8a [x]	185a	89a	155a	1b	23 [y]	0b
DAZ	13.8b	134b	63b	109b	9a	57	0b
C	13.1c	105c	66b	112b	2b	16	0.14a
				Nursery B			
MBC	13.3a	211a	112a	264a	17a	50	0b
DAZ	12.1b	181b	103a	189b	12b	43	0.8b
C	11.5c	137c	84b	178b	5c	16	2.5a

[x]Data are means of means of 3 replicate plots per treatment, 10 seedlings per plot. Means in a column followed by the same letter are not significantly different ($p<0.05$; Duncan's new multiple range test).

[y]Data are means of 3 replicate plots, each derived from the average of 10–15 seedlings sampled

Table 2. Effects of soil fumigation with methyl bromide/chloropicrin (MBC) or dazomet (DAZ) compared with untreated controls (C) on populations of *Fusarium* and *Pythium* in the top 30 cm of soils assayed 6 and 8 m (presow) and 10 mo (postsow) after fumigation and prior to the expected onset of Douglas-fir seedling stunting in Pacific Northwest nurseries A and B.

	SAMPLE (TIME) AFTER FUMIGATION IN MONTHS											
	Fusarium						Pythium					
	6 (March)		8 (May)		10 (July)		6 (March)		8 (May)		10 (July)	
TREATMENT	A	B	A	B	A	B	A	B	A	B	A	B
MBC	20b	7b	0b	0b	0b	20b	0b	2b	45b	0b	1b	4b
DAZ	247b	13b	133b	13b	0b	133ab	8b	0b	53b	7b	4b	3b
C	3100a	300a	687a	553a	13a	180a	117a	80a	277a	97a	36a	56a

Data presented are mean numbers of propagules/g moist soil taken from three replicate plots. Data in columns followed by the same letter are not significantly different ($p<0.05$; Duncan's new multiple range test).

Fusarium population fluctuations at both nurseries only occurred to any extent in the C treatment in the March to May samples taken before sowing in the spring. populations were consistently greater at Nursery A than at Nursery B until the July sampling, when populations were inexplicably greater in B than A.

Pythium populations were effectively reduced by either fumigation treatment at both nurseries and remained low throughout the study (table 2). In the unfumigated control (C), the populations began to increase in March, peaked by the May sampling, and then declined in the July sample; the effect was more pronounced at Nursery A than B. A similar trend occurred in the May samplings from the MBC and DAZ treatments, but to a much lesser extent than in C.

In the supplemental experiment comparing pasteurized and unpasteurized stunt soil, pasteurization appeared to reduce the incidence of seedling stunting. Even seedlings grown in pasteurized soil however, did not grow well and were generally stunted and chlorotic, indicating nutrient deficiency in the small tubes. Nevertheless, seedlings grown in pasteurized stunt soil had more hair roots, similar to the unstunted seedlings observed in the bareroot nursery (figure 3). Roots of seedlings grown in the unpasteurized stunt soil exhibited the lack of hair roots as seen in nursery A. No data on seedling growth or hair root development were taken, however. Also, no microbial isolations were performed to determine what possible deleterious microbes might have been involved.

Discussion

Several possible causes were investigated to explain stunting of conifer seedlings that can occur during the first year of growth in PNW bareroot nurseries. The first was that there was a delay in the development of feeder roots. Our growth data on seedling roots indicated that there were fewer short feeder roots on stunted than on unstunted seedlings at the time of stunt onset, which could have reduced nutrient uptake during a critical time of seedling growth. Nutrient deficiency at that time would lead to growth cessation and bud set. There was also a lack of hair roots on stunted seedlings, compared with unstunted seedlings, which also would help in nutrient uptake to sustain growth following exhaustion of seed reserves.

The second possible cause of first-year stunting that was investigated, that fumigation had eliminated mycorrhizae that could aid nutrient uptake and seedling growth, was not confirmed by our study. Mycorrhizae were at low levels before and during the time when stunting usually appears (mid-July), yet very little stunt occurred in our fumigated study plots. In other sites in previous years, as in this study, stunted seedlings had no fewer mycorrhizae than nearby (often immediately adjacent) unstunted seedlings (figure 2). Furthermore, the incidence of stunt in this study was highest in the unfumigated plots, as we had observed in previous years. However, early formation of mycorrhizae might have prevented stunting. In some cases, lack of mycorrhizae can reduce seedling growth (Campagna and White 1969; Trappe and Strand 1969; Ridge and Theodorou 1972; Sinclair and others 1975; Riffle 1980; Hung and others 1982; Kough and others 1985; Linderman 1987), especially under nutrient-limiting conditions. Inoculation of such soils with mycorrhizal fungi can often correct growth

problems by increasing the nutrient-capturing capacity of seedlings.

The third cause that we investigated was that root pathogens might damage the root system enough to impair nutrient uptake. The hypothesis that root rot caused stunting would be supported by our observations that stunting was greater in unfumigated control soil, where deleterious microbes might have occurred, than in fumigated soil. However, stunt has occurred in soil previously fumigated with MBC in the fall. If some deleterious microbes caused the stunt, they must have reinvaded the soil after fall fumigation and before spring sowing or been introduced on the seed (Graham and Linderman 1983; Hoefnagels and Linderman, 1999). It is possible that such microbes affect seedlings at sublethal levels, as has been shown by Sinclair and others (1975). At the time of stunt occurrence, we saw no signs of infection by pathogens causing root rot, so we discounted them as the cause of stunt. We did, however, isolate at the end of the season and found that the roots of most seedlings in both nurseries, regardless of soil treatment or occurrence of stunt, were heavily infected with species of *Fusarium* and *Pythium*, although root rot was not obvious (data not presented). One could, however, hypothesize the presence of deleterious microbes on the root surface that functioned under cool wet conditions, such as some species of *Pythium* or bacteria, producing toxic substances that inhibited feeder root or hair root development. Our observations that rhizosphere soil adhered more readily to unstunted seedlings than to stunted (figure 3) indicated that there were differences in hair root development and probably rhizosphere microbial activity, even between adjacent stunted and unstunted seedlings.

In the supplementary study, growing seedlings in pasteurized stunt soil, we observed a slight reduction of the stunting effect. Pasteurization of soil eliminates most deleterious microbes. Seedlings grown in pasteurized stunt soil also developed abundant hair roots to which soil adhered; roots of seedlings grown in unpasteurized stunt soil were devoid of hair roots. These observations were similar to those made in the nurseries. Unfortunately, no attempt was made to isolate the hypothetically responsible microbes that air-steam pasteurization would have eliminated. Their potential to deleteriously affect seedling root development therefore could not be determined. Reports by Suslow and Schroth (1982), Nehl and others (1997), and Li and others (2002) indicate that specific deleterious rhizobacteria can suppress root hair development. We can only speculate, however, that such bacteria were present and involved in the 1-0 stunt phenomenon. Future research would shed more light on this hypothesis.

Conclusions and Application

Stunted conifer seedlings occur, often forming a mosaic pattern, in many PNW bareroot nurseries during the first growing season. Lack of ectomycorrhizae under nutrient stress conditions, presence of root pathogens, or both have been hypothesized to explain the mid-season cessation of top growth. We examined stunting in two nurseries in relation to fumigation, root development and formation of ectomycorrhizae, and population dynamics of potential *Fusarium* and *Pythium* root pathogens or other deleterious microbes. We found that (1) root growth on stunted seedlings, compared to unstunted seedlings, was retarded with fewer short roots and less hair root development; (2) seedlings exhibit stunting symptoms before ectomycorrhizae become significantly established, and unstunted seedlings generally had no more ectomycorrhizae than stunted, leading to the conclusion that lack of mycorrhizae in fumigated nursery soils does not cause this type of first-year stunting; (3) there were no apparent signs of root rot disease, and (4) elimination of potentially deleterious microbes by soil pasteurization with aerated steam appeared to correct the stunt problem. We therefore hypothesize that some microbial causal agent or agents of stunt reinvade fumigated soil during the winter fallow period or on contaminated seed and colonize the roots of some young seedlings, subtly affecting the developing roots. Their distribution may be scattered and not associated with previous sites where stunting had occurred. Their capacity to induce stunting may be closely linked with soil conditions in the immediate vicinity of individual seedlings, since adjacent seedlings within a few centimeters frequently are not stunted. Analysis of the fertility and microbial composition in the rhizosphere soil of adjacent stunted and unstunted seedlings could provide insight as to the identity of causal agents and the nutrient elements that limit shoot growth when root system function is reduced. For some nurseries, increasing soil P levels has decreased or eliminated the 1-0 stunt phenomenon (W. Littke, personal communication), making P deficiency less likely, even if root development is delayed or inhibited.

Address correspondence to: R.G. Linderman, USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, OR, 97331; email: lindermr@science.oregonstate.edu; Tel. 541-738-4062. Fax. 541-738-4025

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