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Influence of cultural practices on edaphic factors related to root disease in *Pinus* nursery seedlings

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Abstract

Conifer seedlings grown in bare-root nurseries are frequently damaged and destroyed by soil-borne pathogenic fungi that cause root rot. Relationships between nursery cultural practices, soil characteristics, and populations of potential pathogens in the soil were examined in three bare-root tree nurseries in the Midwestern USA. Soil-borne populations of *Fusarium* spp. and *Pythium* spp. were enumerated as a function of soil depth in the upper 42 cm; red and white pine seedling root systems were assessed visually for signs of root rot. Soil organic carbon and resistance to cone penetration (as a function of depth) were augmented by saturated hydraulic conductivity (K_{sat}), water retention characteristic, texture and pH at selected depths. Cone index (CI) provided accurate 'fingerprints' of cultural practices in each nursery. A tillage pan due to rotary tillage was detected by CI in the Minnesota and Wisconsin nurseries, but no such tillage pan was indicated in the Michigan nursery, which did not use rotary tillage. Curves of CI also indicated differing maximum depth of tillage disturbance between nurseries; maximum rooting depth based on 3 MPa CI were different among nurseries. Vertical distribution of soil-borne *Fusarium* spp. reflected the vertical incorporation pattern associated with the type of tillage implement used to incorporate cover crop residue prior to *Pinus* seedling establishment. Peak numbers of *Fusarium* spp., from 250 to 950 colony-forming units (cfu g^{-1} dry soil) were recorded between 12 – 24 cm depth in two nurseries using a moldboard plow for incorporation while steadily decreasing populations, from 1800 to 250 cfu g^{-1} dry soil, were found from 0 to 15 cm in the third nursery using a disc. Vertical distribution of the *Fusarium* spp. also correlated with organic carbon levels, which suggested that cover-crop incorporation and conifer rooting had determined the location of soil-borne *Fusarium* spp. propagules. K_{sat} suggest that tillage pans caused by rotary tillage may impede drainage during nearly daily irrigation enough to cause physiological stress to the seedlings and predispose them to disease. Low levels of mortality (from < 1% to 5%) were observed in two-year-old *Pinus* seedlings while disease severity varied by nursery and seedling species. Tillage should be used to control depth placement of biomass residue and pathogenic fungal propagules, and adjusted to prevent tillage pans within the seedling root zone. More studies are needed to determine the impact of these cultural controls on the need and application depth of fumigation for pathogen control.

Introduction

Root disease in red (*Pinus resinosa* Ait.) and white pine (*P. strobus* L.) has occurred periodically at damaging levels in bare-root forest nurseries in the North Central Region of the USA since 1945 (Riffle and Strong, 1960). Seedlings from these nurseries are the major supply for conservation plantings and reforestation in the region. Major seedling losses have been associated with *Cylindrocladium scoparium* Morgan (Thies and Patton, 1971), *C. floridanum* Sobers & Seymour (Anderson et al., 1962), and *Fusarium* spp.

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(Riffle and Strong, 1960; Ocamb and Juzwik, 1995; Juzwik and Rugg, 1996). Since about 1960, nursery managers have relied upon chemical soil fumigation to manage root diseases in these nurseries. With increasing incentive to reduce or discontinue chemical fumigation, especially with methyl bromide (USDA, 1993), there is a renewed need to understand ecological factors involved in the development and control of root diseases in these bare-root nurseries.

Cultural practices that influence root environment and the occurrence and severity of root disease in nurseries include tillage, traffic, irrigation management, mulching to increase soil organic matter, sowing of infested seed, fertilization (organic and inorganic types) and fumigation (Sutherland and Anderson, 1980; Duryea and Landis (eds.), 1984). Soil compaction associated with traffic and tillage implement operation may influence soil hydraulic properties important for water drainage (Hamblin, 1985; Horton et al., 1994), mechanical resistance to plant rooting (Hamblin, 1985; Allmaras and Logsdon, 1990), soil aeration related to O_2 and CO_2 environment of the root and pathogen microsite (Asady et al., 1985; Stepniewski et al., 1994), and interactions of soil aeration with mechanical resistance to rooting (Voorhees et al., 1975). Tillage implements used in nurseries may influence all of these environmental factors enough to favor pathogen development and to physiologically predispose seedlings to infection (Allmaras et al., 1988a). Tillage also affects the placement depth and clustering of crop residue (Staricka et al., 1991; Allmaras et al., 1996) that may subsequently harbor and favor an increase of pathogenic soil-borne fungi (Williams and Schmitthenner, 1960; Allmaras et al., 1987).

A detailed survey of pine fields in three bare-root nurseries of north central USA was conducted in 1994 and 1995 to determine how different cultural practices and associated soil characteristics may influence root disease development. The three bare-root nurseries were chosen somewhat based upon different long-term management and root disease history. Depth distributions of cone index (CI), total carbon and selected fungal populations are presented in relation to the tillage practices in each nursery. Auxiliary measurements of saturated hydraulic conductivity (K_{sat}) and root disease readings were made to support conclusions from the soil depth-related properties.

Materials and methods

Study sites

Measurements in 1994 and 1995 were made at three bare-root nurseries during the second growing season after the conifer seeds had been sown. Interviews with each nursery manager detailed the cultural practices of each nursery, such as fertilization regimes, cover cropping (or biomass production) practices, implements used, fumigation, organic amendments and irrigation.

Minnesota nursery. One red and one white pine field at the General Andrews State Forest Nursery, Willow River, MN (98° 90′ W; 46° 40′ N) were selected for measurements in 1994. The native soil, before nursery cultivation was started 40 years ago, was an Omega fine loamy sand (mixed, frigid, Typic Udipsamments). Before intensive nursery cultivation there was 5–20 g kg^{-1} organic matter in the upper 10 cm and a field capacity water content between 50 and 120 g kg^{-1} in the upper 60 cm.

Red and white pine fields were 183 m long, 10 bed rows wide (12 m), and were delimited by roads and irrigation pipes. Each field was divided into 15 plots, each with dimensions 33.5 m by two seed-bed rowwidths (2.4 m). Buffer areas of 7.6 and 8.6 m were maintained on the east and west ends of the fields, respectively, and two bed rows were maintained between plot boundaries and irrigation pipes. Red pine bed rows contained eight seedling rows, while white pine bed rows contained five seedling rows. Six plots within each red and white pine field were randomly selected for sampling.

Wisconsin nursery. One red and one white pine field at the Wilson State Forest Nursery, Boscobel, WI (90° 70′ W; 43° 10′ N) were used as study sites in 1995. The soil is a Sparta fine loamy sand (sandy, mixed, mesic Entic Hapludoll). The native soil, before intensive nursery cultivation began in 1954, had an organic matter content of 10 g kg^{-1} in the upper 20 cm, and a field capacity water content between 90 and 120 g kg^{-1} in the upper 40 cm.

Fields were delimited by overhead irrigation pipes for lateral watering. Red pine had been seeded in the two bed rows closest to and on both sides of the irrigation pipes, while white pine had been seeded in the middle of the field, furthest from the irrigation pipes. Each field had eight bed rows, two bed rows of red pine near the irrigation pipe located on one side of the

field, followed by four bed rows of white pine in the center, and ending with two more bed rows of red pine adjacent to the next irrigation pipe on the other side of the field. Fields were 168 m long. Each field was divided into 10 plots each 30.5 m long and two bed rows (2.4 m) wide. Buffers of 7.6 m on the west end of the field and 12.5 m on the east end of the field were established. To sample contiguous fields of red or white pine seedlings, white pine plots were set up across the four center bed rows in one field, and red pine plots were set up across two fields, two bed rows in one field, irrigation pipe, and two more bed rows in the adjacent field. Red and white pine bed rows each contained seven rows of seedlings. Four plots from each of the red and white pine fields were randomly chosen for sampling.

Michigan nursery. One red pine field at the Wyman State Forest Nursery, Manistique, MI (86° 40′ W; 46° 80′ N) was used as a study site in 1995. The soil is a Wallace fine sand (sandy, mixed, frigid, ortstein Typic Haplorthods). The native soil, before intensive nursery cultivation began in 1930, had an organic matter content of 5–20 g kg^{-1} in the upper 26 cm and 0–5 g kg^{-1} in the upper 26–60-cm depth; field capacity water content was 70–120 g kg^{-1} in the upper 11 cm and 10–50 g kg^{-1} in the 11–60-cm depth.

The red pine field 152 m long and 10 bed rows wide was divided into 15 plots that were each 24.4 m long and two bed rows (2.0 m) wide. Buffers of 5.6 m were maintained on both ends of the field, and two bed rows were maintained between plot boundaries and lateral ground pipes for sprinkler irrigation. Bed rows contained six rows of red pine seedlings. Six of the 15 plots were randomly chosen for sampling. White pine seedlings had previously been produced at Wyman Nursery, but the production was discontinued due to root rot damage.

Enumeration of soil-borne fungi

Two of the common soil-borne pathogenic fungi in bare-root nurseries, *Pythium* spp. and *Fusarium* spp. were enumerated.

Pythium. Non-rhizosphere soil samples were collected from the selected plots in each nursery field in June 1994 or 1995. Ten cores per plot were collected midway between seedling rows with an 18-mm diameter soil sampling tube (Allmaras et al., 1988b) to a depth of 42 cm. Each core was sectioned sequentially into 6-cm increments and cores composited. Similar depth increments from all cores taken from a plot were combined into composite samples and stored for less than three days at 5 °C in a polyethylene bag before processing.

Soils were assayed using serial dilution plating with 0.1% water agar. Four subsamples, each 3 g, were removed from each composite. One subsample was oven-dried (105 °C) for 72 h to determine gravimetric water content, and the other subsamples were added to appropriate amounts of water agar for 1:10, 1:30 and 1:50 dilutions. Each dilution flask was vigorously agitated (200 rpm on a Lab-Line Orbital shaker, Melrose Park, IL) for 60 s, before 0.5-mL aliquots were removed and plated on a *Pythium* selective medium, P_5ARP (Jeffers and Martin, 1986). Petri dishes were incubated for 96 h in the dark at 20 °C. Colonies with *Pythium*-like growth (rapid, translucent and fibrous growth) were marked at 48, 72 and 96 h and subcultured on cornmeal agar (Difco). Pure isolates were then grown for seven days on cornmeal agar at 20 °C in the dark. Each isolate was examined microscopically (200×) and *Pythium* was identified based on:

1). Presence or absence of sporangia, oogonia, antheridia, and coenocytic mycelium;
2). Type and shape of sporangia, and
3). Vegetative growth rate.

The number of colony-forming units (cfu g^{-1} soil) was determined as:

$$\text{cfu g}^{-1}\text{ soil} = (2\ \text{CFU DF}) / \text{OD} \qquad (1)$$

where CFU = number of *Pythium* colonies counted for the dilution rate that had 10–60 CFU/petri dish; DF = dilution factor for plates read to obtain CFU; and OD is oven dry weight of soil (g).

Fusarium. Non-rhizosphere soil samples were collected from selected plots in each nursery field between 15 July and 15 August 1994 or 1995. Seven composited depth samples were obtained from each plot as previously described for *Pythium* samples. Soil samples were air-dried (48 h) before storage if processing was postponed for > 5 d after collection. Air-dried samples were then stored at 5 °C in polyethylene bags until processed (maximum of 3 wks).

Soils were assayed using serial dilution plating with 0.1% water agar. Two 10-g subsamples were removed from each thoroughly mixed sample. One subsample was used for determination of gravimetric

water content and a second subsample was added to 90 ml of 0.1% water agar. The mixture was vigorously agitated (200 rpm for 60 s on a Lab-Line Orbital shaker, Melrose Park, IL), and 10 ml of the resulting suspension was pipetted into the next flask to make the 10^{-2} dilution. The procedure was repeated to obtain two further ten-fold dilutions. Three 0.5-ml aliquots from each dilution were spread on each of three petri dishes containing *Fusarium*-selective modified PCNB agar (Papavizas, 1967). Petri dishes were incubated for 7 to 10 d at 22 °C in the dark. Colonies with *Fusarium*-like colony appearance were marked and subsamples of representative colonies were transferred to 2% water agar containing a small piece of sterilized carnation leaf (carnation leaf agar, CLA) and to potato dextrose agar (Dhingra and Sinclair, 1985). All isolates were incubated for 12 to 25 d at 22 °C under fluorescent lamps (three GE or Sylvania cool white tubes) supplemented with UV light (one Sylvania 40W tube, BLB series) with a 12-h photoperiod. Each isolate was examined microscopically (400×) and *Fusarium* were identified on the basis of characteristic macroconidia, microconidia and chlamydospores when present (Nelson et al., 1983). Number of cfu g^{-1} soil were determined as:

$$\text{cfu } g^{-1} \text{ soil} = [(100 / 1.5) (\text{CFU DF})] / \text{OD} \qquad (2)$$

where CFU = number of *Fusarium* colonies from the dilution rate with 10 to 60 cfu/petri dish, DF = dilution factor for the plates read to obtain CFU, and OD is oven dry soil. Random error was determined by the agreement among randomly selected plots in a field.

Soil sampling and characterization

Soil characterizations were made at times and places to describe root environment of the seedlings. Some characterizations were made *in situ* and others on soil samples taken to the laboratory.

Bulk density. Soil samples were collected from the seedling plots in each field between 5 July to 15 August 1994 or 1995. Ten cores were taken to a 60-cm depth from each plot using the previously described soil sampling procedure, sectioned into 2-cm increments, pooled by depth, and stored for drying in paper bags. After bulk density was determined the dry soil samples were pooled from 2-cm into 6-cm increments for pH and organic carbon measurement. Random error was determined from agreement among plots within a field.

Organic carbon. A small sample of dry soil from each depth, plot, and nursery was ball milled in a Spex 5300 Mixer Mill (Spex Industries, Edison, NJ), and a subsample processed for organic carbon content using a Fisons Instruments NA 1500 NC Elemental Analyzer (Carlo Erba Strumentazione, Milan, Italy).

pH. Soils used for measuring pH were the same as those used for organic carbon determination. Soil pH was measured at each depth in each plot of each nursery using a 3:1 mix of soil and 0.01 m $CaCl_2$ on a 43 Beckman pH meter (Irving, CA).

Texture. Subsamples of soil from composites of the 6-cm depth increments to originally measure bulk density were combined to create topsoil (0–30 cm) and subsoil (30–42 cm) samples. Sand, silt and clay were determined using a LaMotte Soil Texture Kit (Forestry Suppliers, Inc., Jackson, MS).

Soil penetrometer resistance. A Rimik CP10 static load cone pentrometer (Sydney, Australia) was used to measure soil resistance to penetration or CI. The penetrometer had semi-included cone angle of 30°, a cone base diameter of 12.8 mm, and a shaft diameter of 9.5 mm. Cone penetrometer measurements were made on 6 and 13 July and 15 August 1994 in the Minnesota nursery, 23 August 1995 in the Wisconsin nursery, and 6 June 1995 in the Michigan nursery. Nursery fields were irrigated for 4 h to approach saturation and penetrometer measurements were completed within 2 h after irrigation ceased. Three insertions were made at each of six locations based on a pre-determined grid in each plot. Measurements to a depth of 450 mm were recorded every 15 mm (30 individual readings per insertion). The 18 CI values for each depth were averaged within each plot, and a measure of error also determined on the agreement among plots.

Saturated hydraulic conductivity and associated bulk density. Soil samples for K_{sat} determination were taken on 5–6 Sept 1994 in the Minnesota nursery, 6 Sept 1995 in the Wisconsin nursery and 12 Sept 1995 in the Michigan nursery. After soil excavation to two pre-selected depths (8 and 21 cm for Minnesota fields, 10 and 31 cm for Wisconsin fields and 10 and 24 cm for Michigan fields), duplicate undisturbed cores of soil (5 cm diameter and 5 cm long) were taken at each depth. The ring and undisturbed soil core was wrapped in a polyethylene bag, covered on both ends with wooden blocks to stabilize and protect the core

during transport and stored at 5 °C until K_{sat} was measured using the falling head method (Klute and Dirksen, 1986).

To more closely evaluate poor seedling growth response to suspected compaction, K_{sat}, bulk density, and CI were measured in selected plots of the Minnesota and Wisconsin nurseries in 1996. Profiles of CI vs. depth were taken in plots with poor and better growth of white pine seedlings followed by undisturbed cores from 5 depth increments above 28 and 45 cm, respectively, in the Minnesota and Wisconsin nurseries. Undisturbed cores were taken from duplicate plots of each of two suspected compaction levels, low and high. Three and eight cores, respectively per depth were taken in each selected plot in the Minnesota and Wisconsin nurseries. K_{sat} and bulk density of these cores were measured in direct sequence.

Soil–water retention characteristic. Soil water content at various extraction pressures was determined using the same undisturbed soil cores taken for K_{sat}. Saturated soil cores were taken directly from the hydraulic conductivity apparatus and placed into Tempe cells. A soil–water characteristic was determined (weighable cells; Klute, 1986) at applied pressures of 1, 2, 3, 4, 10, 20, 40 and 60 kPa. After equilibrium at 60 kPa, soil cores were removed from the Tempe cells and oven dried for bulk density determination.

Seedling assessment

Pine seedlings were sampled 1 – 12 Sept at each nursery in 1994 or 1995. Within each sampling plot at each of the three nurseries, four 1-m^2 subplots were placed according to a stratified random design to obtain two subplots within each bed row in a plot. After the number of living (healthy or symptomatic) and dead seedlings in each subplot were recorded, a stratified random approach was used to select six seedlings for assessing root disease. Six seedlings were visually assessed for disease per subplot, three that appeared healthy and three that appeared diseased based on shoot appearance. These were then pooled together for assessments. Seedlings were stored in polyethylene bags at 5 °C until processed.

Root systems were washed in the laboratory to remove soil and examined for extent and location of any necrosis, water-soaked or grey-streaked tissue, or reddish-brown cortical discoloration. Each plant was rated for root rot severity on a 1 to 5 scale: where 1 = no evidence of symptomatic tissue; 2 = one lateral root with > 50% of the lateral root symptomatic; 3 = two or more laterals with > 50% of the roots symptomatic, or lower one-third of primary root affected; 4 = necrotic lesion or extensive symptomatic tissue in middle one-third of primary root; and 5 = necrotic lesion or extensive symptomatic tissue in upper third of primary root, or entire root system affected.

Fungi were isolated from necrotic lesions on affected roots by excising a root segment containing the affected tissue. Each lesion or extensive area on a segment was cut into two parts. One-half was immersed in 0.5% NaOCl for 1 to 2 min followed by two 1-min sterile distilled water rinses, pieces of the excised segment from the margin of the healthy necrotic tissue interface were plated on the *Fusarium*-selective medium described previously and petri dishes incubated in the dark at 20 °C for 7 to 10 d. *Fusarium*-like colonies were transferred to carnation leaf agar and to potato dextrose agar. *Fusarium* spp. were identified as described previously. The second one-half of the excised tissue was rinsed in tap water for 8 to 10 min and tissue pieces were aseptically removed and plated on 2% water agar. Colonies exhibiting *Pythium*-like growth were transferred to corn meal agar. Pure isolates were examined microscopically to identify *Pythium* spp., as previously described. No isolations were made from healthy-appearing root systems.

Data analyses and summary

Parametric analyses of variance (ANOVA) were performed on penetration resistance, bulk density, organic carbon, pH, K_{sat}, $\log_e$ *Fusarium*, and $\log_e$ *Pythium* data, and treatment comparisons were made with standard t-tests (Fisher's LSD) when the F-statistic was significant (Steel and Torrie, 1980; Wilkinson, 1992). *Pythium* and *Fusarium* cfu values, bulk density, organic carbon, CI, K_{sat} and soil water retention characteristic data were averaged by depth across the field before statistical analysis. For pH measurements, values from increments within topsoil and subsoil increments (0 to 30 cm and 30 to 42 cm, respectively) were combined and averaged prior to analysis, because no differences were found within these two zones.

Table 1. Some physical and chemical properties of soils in pine fields at three surveyed bare-root nurseries

Nursery location	Soil depth	pH[a]	Soil texture			
			Sand	Silt	Clay	Classification
	(cm)		g kg^{-1}			
Minnesota	0 – 30	4.60	790	135	75	loamy sand
	30 – 42	5.00	805	90	105	sandy loam
Wisconsin	0 – 30	4.66	770	160	70	sandy loam
	30 – 42	4.83	815	85	100	sandy loam
Michigan	0 – 30	4.19	950	50	0	sand
	30 – 42	4.44	1000	0	0	sand

[a] Determined using a 3:1 mixture of soil and 0.01 m Ca Cl_2.

Results

Cultural practices and soil properties

The nurseries selected after interview with the nursery managers all had soil textures ranging from sand to loamy sand with clay content ranging from 0 to 75 g kg^{-1} in the 0–30-cm depth (Table 1), while the Minnesota and Wisconsin nurseries had a sandy loam texture in the near-subsoil. Soil pH were suitable for the *Pinus* species and reflected some acidification in the 0–30-cm layer due to intensive use of incorporated biomass (Table 1). The water-retention characteristic (Figure 1) was not different between red vs. white pine fields in the Minnesota and Wisconsin nurseries so that each plotted point is a mean obtained from four cores. In the Michigan nursery the plotted point is a mean from two cores. These water-retention characteristic reflect the need for intensive frequent irrigation, because the water content ranges from 0.45 to 0.50 m^3 m^{-3} at saturation to 0.15 m^3 m^{-3} or less at −10 kPa water potential.

Cultural practices detailed by the nursery manager during the period after lifting the conifer seedlings and final seedbed preparation for the next seedling crop were distinctly different among nurseries (Table 2). Both the Minnesota and Wisconsin nurseries used a moldboard plow to incorporate biomass from the cover crops, but a disc was used in the Michigan nursery. The working depths (Table 2) suggested by the managers indicate a deeper moldboard tillage in the Wisconsin than Minnesota nursery. A rotary tiller was used for bed tillage just before sowing the *Pinus* seeds in the Minnesota and Wisconsin nurseries, but in the Michigan nursery the same disc was used as a pre-

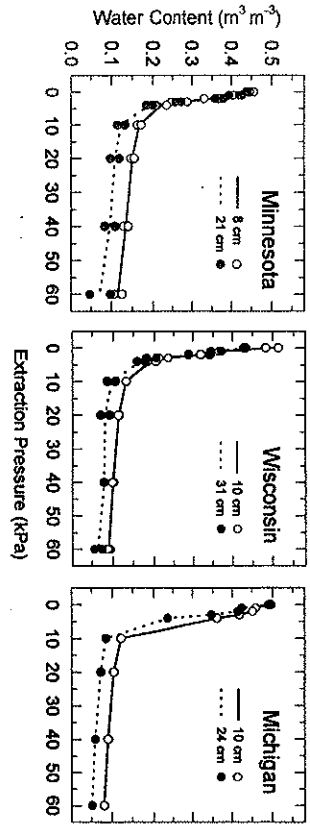

Figure 1. Soil water characteristic curves for both red and white pine fields at selected depths in each of three surveyed bare-root nurseries.

sowing tillage tool and a tool to incorporate biomass crops. The Michigan nursery did not use fumigation as was done in the other two nurseries. There were differences in number of cover crops and time lapse between harvest and sowing of *Pinus* seeds for the next woody crop. These cultural practices (Table 2) have been used for at least 20 years. Later discussions show consistent soil-profile-layer effects differing among nurseries even though the physical properties (Table 1, Figure 1) are not greatly different among nurseries.

Soil resistance to cone penetration

Cone index was used to locate depths of tillage and the maximum depth of rooting for both the *Pinus* species and the Graminaceae used for cover crop and incorporated biomass. Each CI curve in Figures 2, 3, and 4 with a maximum coefficient of variability (CV, expressed as a fraction) less than 0.14 was derived from

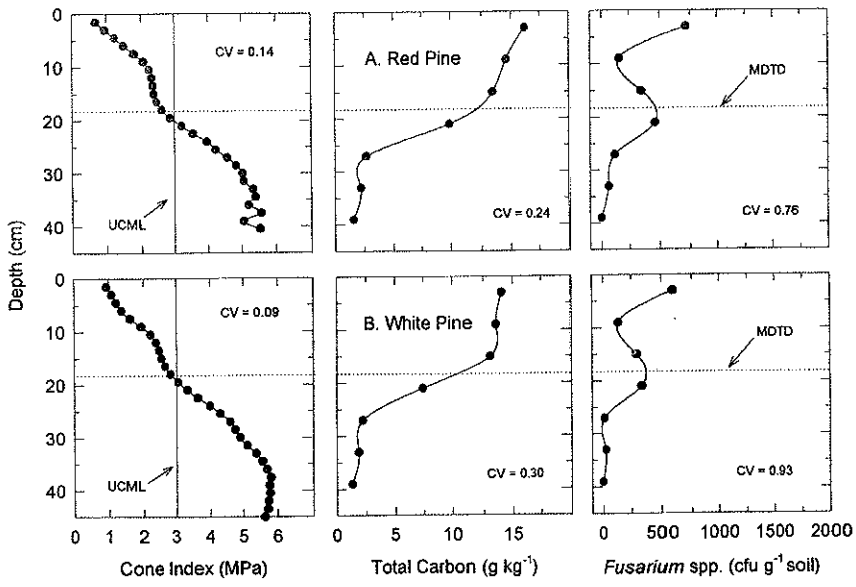

Figure 2. Cone index, total carbon, and *Fusarium* spp. levels in the soil profile of two pine fields (A. red pine, B. white pine) in the surveyed Minnesota, USA, bare-root nursery. (UCML is upper critical mechanical limit; MDTD is maximum depth of tillage disturbance; CV given as a decimal equivalent; 6 observations per plotted symbol).

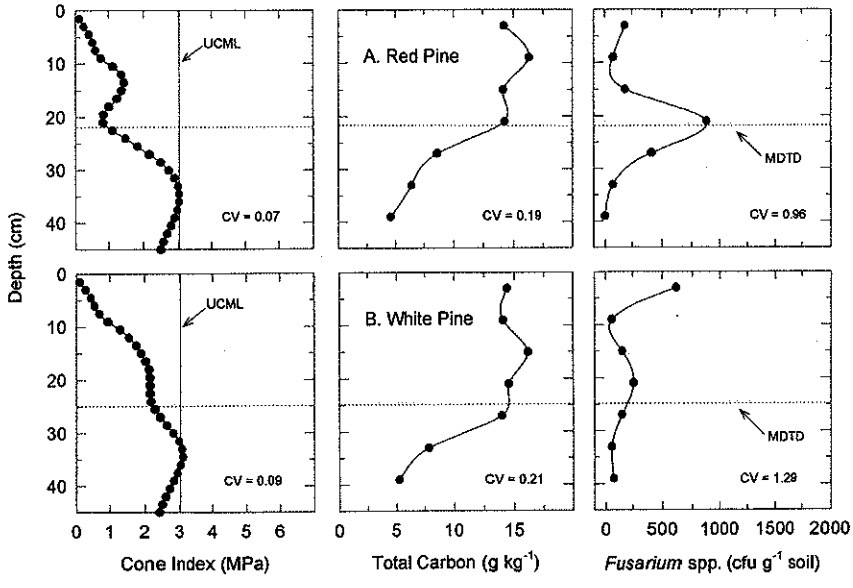

Figure 3. Cone index, total carbon, and *Fusarium* spp. levels in the soil profile of two pine fields (A. red pine, B. white pine) in the surveyed Wisconsin, USA, bare-root nursery. (UCML is upper critical mechanical limit; MDTD is maximum depth of tillage disturbance; CV given as a decimal equivalent; 4 observations per plotted symbol).

Table 2. Cultural practices used for pine seedling production in the three surveyed bare-root nurseries

Nursery location[a]	Cover cropping[b]			Fumigation			Pre-sowing tillage tool[c]
	Species[d] and lapsed time[e] (months)	Seeding rate	Incorporation tool[c]	Chemical	Rate	Effective depth (cm)	
Minnesota (18)	winter rye (4)	22 L ha^{-1}	mbd (20)	metam sodium	70 L ha^{-1}	20	rotary tiller
Wisconsin (18)	sorghum-sudan grass (1)	39 kg ha^{-1}	mbd (22)	methyl bromide-chloropicrin (67:33)	392 kg ha^{-1}	15	
	winter rye (4)	39 kg ha^{-1}	mbd (22)				
	sorghum-sudan grass (9)	39 kg ha^{-1}	mbd (22)				rotary tiller
Michigan (24)	oats (1)	29 L ha^{-1}	disc (16)	none	–	–	
	winter rye (5)	29 L ha^{-1}	disc (16)				
	buckwheat (10)	29 L ha^{-1}	disc (16)				
	oats (16)	29 L ha^{-1}	–				disc (16)

[a] Number in parenthesis is time lapse (months) from lifting conifer seedlings to sowing a new conifer crop; seedlings usually lifted in April and May.
[b] Cover cropping to provide cover and incorporated biomass.
[c] Mbd (xx) is moldboard plow and assumed working depth in (cm); disc (xx) is double disc, 36 cm diameter blades, (16) is assumed maximum working depth in cm.
[d] winter rye (*Secale cereale* L.), sorghum-sudan grass (*Sorghum bicolor* L.), oats (*Avena sativa* L.), and buckwheat (*Fagopyrum esculentum* L.).
[e] Lapsed time (months) is that between conifer seedling lift and cover crop planting.

18 separate penetrometer insertions. Cone index in these soil profiles ranged from 0.1 to 5.6 kPa. A sharp increase at the 10-cm depth in the white pine field of the Minnesota nursery (Figure 2) indicated a tillage pan created by rotary tillage of the seed bed (Table 2). The same rotary tillage was used in the red pine field, but such a tillage pan was not observed using the CI. A sharp increase in the Wisconsin nursery (Figure 3) related to rotary tillage occurred at 10–14 cm and 12–18 cm in the red pine and white pine fields, respectively. Bulk density profiles (not shown) confirmed the rotary tillage pans at 11 cm in both pine fields of the Minnesota nursery, and a series of small bulk density increases starting at 8 cm and extending to 15 cm confirmed the presence of a tillage pan in the Wisconsin nursery. Only one inflection of the CI curve occurred at 10 cm in the Michigan nursery (Figure 4), which coincided with the most recent disc pass when the *Pinus* bed was tilled before sowing (Table 2).

A maximum depth of tillage disturbance (MDTD) was observed at the depth where the CI no longer had a break in a monitonical increase due to soil overburden. These changes in CI were observed at 18 cm in both pine fields of the Minnesota nursery (Figure 2), and at 22 and 25 cm, respectively, in the red and white pine fields in the Wisconsin nursery (Figure 3). Another inflection of the CI at about 16 cm in the Michigan nursery (Figure 4) suggests an MDTD at 16 cm. The disc diameter (Table 2) suggests an MDTD > 10 cm. Bulk density curves (not shown) did not indicate a MDTD in the sandy soils of this study but did show a MDTD in a silt loam soil (Allmaras et al., 1988b).

Cone index was used not only to locate tillage pans and MDTD but also to indirectly identify maximum depth of plant rooting. The Upper Critical Mechanical Limit (UCML in Figures 2, 3 and 4) of 3 MPa was proposed and tested for corn (*Zea mays* L.) rooting in a homogeneous soil without macroporous structure (Boone et al., 1986). This is a conservative limit for cessation of root growth, because many consider a CI of 2 MPa as an indicator of serious rooting limitations and severe compaction (Hamblin, 1985). Laboski et al. (1998) have successfully used an UCML of 3 MPa to indicate corn rooting cessation in a sandy soil. Based on these studies, this same UCML (3 MPa) was used for studies reported here. The UCML was reached at

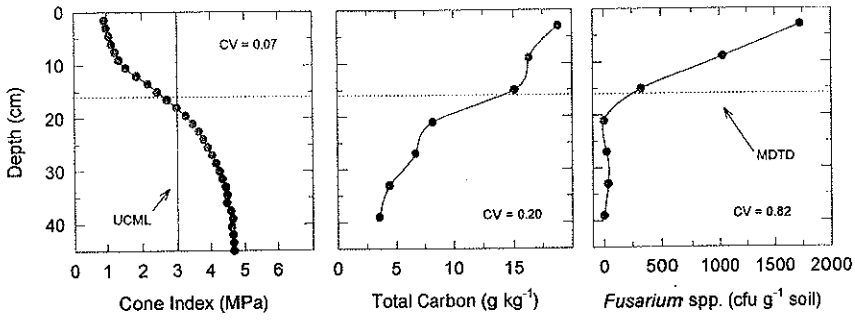

Figure 4. Cone index, total carbon, and *Fusarium* spp. levels in the soil profile of red pine field in the surveyed Michigan, USA, bare-root nursery. (UCML is upper critical mechanical limit; MDTD is maximum depth of tillage disturbance; CV given as a decimal equivalent; 6 observations per plotted symbol).

Table 3. Saturated hydraulic conductivity and bulk density in undisturbed soil cores taken from selected soil layers in the three surveyed bare-root nurseries

Nursery location	Soil depth (cm)	Bulk density[a] ($g\ cm^{-3}$)	K_{sat} [b] ($cm\ h^{-1}$)
Minnesota	5 – 10	1.46	14.7
	19 – 24	1.49	18.4
Wisconsin	7 – 12	1.36	15.2
	28 – 33	1.50	16.0
Michigan	7 – 12	1.28	17.3
	21 – 26	1.25	19.1

[a] Respective CV for bulk density in MN, WI and MI are 0.05, 0.03 and 0.04; respective number of observations for each mean are 6, 4, 6.
[b] Respective CV for K_{sat} in MN, WI and MI are 0.28, 0.35 and 0.35; respective number of observations for each mean are 6, 4, 6.

20 cm in the white and red pine fields of the Minnesota nursery (Figure 2). The UCML in the Wisconsin nursery was reached at 33 and 35 cm for the white and red pine fields, respectively (Figure 3). The MDTD and UCML nearly coincide in the Minnesota nursery, whereas the UCML occurred about 10 cm deeper than the MDTD in the Wisconsin nursery. The UCML occurred at 18 cm in the Michigan nursery (Figure 4); about 8 cm deeper than the MDTD.

Saturated hydraulic conductivity and associated bulk density

Saturated hydraulic conductivity (and bulk density) in the Minnesota and Wisconsin nurseries (Table 3) were first measured either within the depth of rotary tilling or below the MDTD; in the Michigan nursery these measurements were either within the zone of tillage or below the MDTD. All K_{sat} were greater below the MDTD which was somewhat unexpected due to the increasing CI below the MDTD. Bulk density also increased as CI increased in the Minnesota and Wisconsin nurseries. Conclusions about these K_{sat} changes are tentative because of large CV (Table 3).

Additional samplings of CI, K_{sat}, and bulk density in suspected low and high compaction plots in the same field reveal the sensitivity of K_{sat} and CI to compaction in these coarse textured soils. Peak maximum CI at 15 cm in the Wisconsin nursery were 1.5 and 3.6 MPa for the low and high suspected compaction; at 25 cm the respective values were 1.5 and 1.7 MPa. The shape and position of the peak CI were similar to that in Figure 3 for the white pine field. The CI profiles in the Minnesota nursery had only small inflections above 18 cm just as in Figure 2; the maximum values above 18 cm were 1.9 MPa, not different than in Figure 2. Above the MDTD however, K_{sat} were significantly ($P < 0.05$) lower in the high suspected compaction plot in both nurseries (Table 4). Below 26 cm, neither K_{sat} nor bulk density was changed due to suspected compaction in the Wisconsin nursery, but in the Minnesota nursery suspected high compaction reduced K_{sat} down to 25 cm, past the 18 cm MDTD; there was also significantly ($P < 0.05$) increased bulk density. This K_{sat} and bulk density change below the MDTD could be related to low soil strength and consolidation when irrigated. The K_{sat} values are indicative of drainage differences that may expose pine seedling roots to poor aeration.

Table 4. K_{sat} and bulk density (BD) profiles in plots of the Minnesota and Wisconsin surveyed nurseries with different suspected compaction levels

Nursery location	Soil depth	Suspected compaction[a]			
		Low		High	
		K_{sat}	BD	K_{sat}	BD
	(cm)	(cm h^{-1})	(g cm^{-3})	(cm h^{-1})	(g cm^{-3})
Minnesota	0–5	20.9	1.27	14.3	1.36
	5–10	19.9	1.32	14.8	1.42
	12–17	20.1	1.39	14.0	1.39
	18–23	18.8	1.34	11.7	1.42
	23–28	18.9	1.36	12.0	1.46
Wisconsin	4–9	20.2	1.39	18.5	1.39
	13–18	16.3	1.46	13.1	1.48
	21–26	16.8	1.50	14.4	1.45
	30–35	18.2	1.45	18.1	1.46
	40–45	17.7	1.43	18.1	1.45

[a] Suspected compaction (low or high) depending on growth of white pine seedlings and confirmed with CI above the maximum depth of tillage disturbance; above 18 cm in Minnesota nursery and 25 cm in Wisconsin nursery. LSD ($P < 0.05$) for comparing low and high means at the same depth are 4.75 cm h^{-1} for K_{sat} and 0.06 g cm^{-3} for BD in Minnesota nursery; those in Wisconsin nursery are 2.58 cm h^{-1} and 0.05 gm cm^{-3}.

Organic carbon

Organic carbon (OC) in all profiles (Figures 2, 3 and 4) represents a long term accumulation related to bare-root nursery culture (Table 2) imposed on soils that had values < 5 g kg^{-1} in the upper 20 cm before intense cultivation. All OC profiles may be characterized by a large amount near the surface, a negligible amount in the subsoil, and a transition zone. Concentrations of OC in the upper 18 cm (above the MDTD) ranged above 13 g kg^{-1}, and their mean OC was significantly ($P < 0.01$) larger than the mean OC in the 24–42-cm depth in the Minnesota nursery (Figure 2) and the Michigan nursery (Figure 4). Since the MDTD and UCML were estimated to occur between 18 and 20 cm, the high concentrations of OC above 18 cm were derived from pine seedling roots, rhizodeposition and incorporated biomass. The transition zone from 18 to 24 cm may have received OC from rooting past the UCML, an occasional past tillage disturbance deeper than 18 cm, and translocation of carbonaceous materials during percolation.

Profiles of OC in the Wisconsin nursery (Figure 3) were qualitatively similar to those in the Minnesota nursery (Figure 2). Profiles of OC differed between pine fields consistent with associated differences in MDTD and UCML. In both pine fields the OC concentrations decreased markedly at the MDTD but did not reach a distinct minimum as in the Minnesota nursery. However, the mean OC concentration above the MDTD was significantly ($P < 0.01$) larger than the concentration below 24 and 30 cm, respectively, in the red and white pine fields. The transition of OC concentration and the OC concentration below 30 cm in the Wisconsin nursery reflect somewhat the separation of the MDTD and UCML and the deeper UCML; this feature of the OC profile was not observed in the Minnesota nursery.

The OC concentration profile in the Michigan nursery (Figure 4) was markedly different from that in the other two nurseries. The largest inflections in the OC profile were near the MDTD and the UCML (16-cm depth).

Fungal populations in the soil

Inoculum levels of *Fusarium* spp. were highly dependent upon substrate OC levels, because most species in the *Fusarium* genus are facultative saprophytes. This dependency is shown by their similarity of depth functions (Figures 2, 3 and 4). Additional evidence for dependency is the increasing CV in the OC concentration and *Fusarium* populations at deeper depths, where OC was more sparse (not shown). Bulk density and CI did not exhibit such an increasing CV at deeper depths (not shown). *Fusarium* inoculum reached nearly 1800 cfu in the Michigan nursery (Figure 4), while maximum values were always < 1000 cfu in the other nurseries. This difference might be explained by the absence of fumigation in the Michigan nursery (Table 2). *Fusarium* inoculum in the Michigan and Wisconsin nurseries is non-existent below the UCML, but in the Minnesota nursery there is a significant unexplainable presence of inoculum in the 6 cm layer below the UCML.

Within the surface 26 cm of the Minnesota (Figure 2) and 33 cm of the Wisconsin (Figure 3) nurseries the profiles of *Fusarium* inoculum were qualitatively similar. A peak inoculum concentration occurred at or above the MDTD in the Wisconsin nursery and at or about 3 cm below the MDTD in the Minnesota nursery. These peak inoculum concentrations, and that at the surface of the Michigan nursery are large enough to produce root disease in *Pinus* species (Juzwik, pers. obsn.). In both the Minnesota and Wisconsin nurseries there is a minimum *Fusarium* inoculum concentra-

Table 5. Seedling mortality and root disease in pine fields in the three surveyed bare-root nurseries

Nursery location	Pine field type	Seedlings m^{-2}		Ave. root disease rating[a]
		live	dead	
Minnesota	red	225	< 1	1
	white	204	< 1	1.2
Wisconsin	red	419	21	1.4
	white	363	16	1.6
Michigan	red	220	7	1.5

[a] Root disease rating on 1–5, where 1 is no evidence of necrotic tissue and 5 is necrotic lesion or extensive necrosis in upper third of primary root, or entire root system is necrotic.

tion corresponding with the nominal depth of rotary tillage in the seed bed. When present, the inoculum concentration (cfu g^{-1} soil) of *Pythium* spp. [not shown] followed a profile similar to *Fusarium* spp. No *Pythium* spp. were detected in the red pine field of the Minnesota nursery but there were up to 20 cfu g^{-1} soil in the white pine field, and the profile distribution mimicked that of the *Fusarium* spp. (Figure 2). No *Pythium* spp. were observed in the Wisconsin nursery. In the Michigan nursery, inoculum of *Pythium* spp. were 10 cfu g^{-1} soil, and again the profile [not shown] mimicked the *Fusarium* spp. profile (Figure 4). Even though there were < 10 cfu g^{-1} soil, *Pythium* spp. were recovered from 29% of the diseased trees.

Root disease

Seedling mortality was negligible in the Minnesota nursery and moderate in the other two nurseries (Table 5). Although the seedling loss in the Minnesota nursery was small, *Fusarium* spp. were isolated from 33% of the white pine roots that had visual root rot. In the Wisconsin nursery the mortality ranged from 4 to 5% (Table 5) and *Fusarium* spp. were isolated from > 60% of the seedlings with necrotic root tissue. Mortality in the Michigan nursery was 3%, and *Fusarium* spp. were isolated from 94% of seedlings with visible root necrosis. *Pythium* spp. were rarely isolated (4%) from seedlings with visible root rot in the Wisconsin and Minnesota nurseries, but were obtained more frequently (29%) from necrotic roots of seedlings from the Michigan nursery.

Discussion

Resistance to soil penetration values, or CI, suggested presence of a rotary tillage-associated pan in the Minnesota and Wisconsin nurseries (Figures 2 and 3). Further measures made in the soil profile using K_{sat} (Table 4) revealed real reductions in water movement through the tiller-pans in portions of the Minnesota and Wisconsin fields known to have higher root disease incidence. Specifically, the two outer bed rows of white pine fields in the Wisconsin nursery exhibited higher levels of disease than the four bed rows in the center of the field (Juzwik and Rugg, 1996). Soil moisture levels were also higher for longer periods of time in these same outer bed rows compared to the inner ones (Juzwik et al., 1994) and this observation was undoubtedly due to decreased K_{sat} in the soil profile. Additionally, measures of K_{sat} in the white pine field of the Minnesota nursery revealed reduced water flow through the 28-cm depth in bed rows of the Minnesota nursery where disease incidence was highest compared to adjacent bed rows with much lower disease incidence. In both the Minnesota and Wisconsin nursery fields the disease-affected bed rows are those immediately adjacent to either overhead or on-the-ground irrigation pipes.

Cone indices in all surveyed fields also suggested that the arbitrary UCML of 3 MPa for rooting by red and white pine seedlings in the nursery soils surveyed is appropriate, although further evaluation of actual rooting occurrence is needed for validation. Published reports on UCMLs for woody species are lacking, particularly for the nursery situation. Soil strengths greater than 2.5 MPa have been attributed with impeding woody root growth on very dry soils in forested situations (Greacen and Sands, 1980). Differences were found in the ability of lateral vs. primary roots of *Eucalyptus* seedlings to penetrate soils of different strengths (Misra and Gibbons, 1996). In general, UCMLs for eucalypts, based on the values reported, ranged from 2.54 to 3.74 MPa.

Finally, cone indices were also used to detect MDTD in the surveyed fields where multiple passes of one to several different types of tillage tools were utilized. These depths of MDTD produced by moldboard tillage were near those suggested (Table 2) for the Minnesota and Wisconsin nurseries based upon size of the plow share. The MDTD produced by the disc (Table 2) in the Michigan nursery was suggested by disc diameter. Maximum penetration of the moldboard and disc were readily identified in finer textured

soils (Staricka et al., 1991; Allmaras et al., 1996). The maximum depth of rotary tiller operation was consistent with earlier tests (Juzwik et al., 1997), but did not form the MDTD where it was used (Table 2).

Despite the understanding gained from the CI, there is still a need for multiple measurements vs. depth to establish the MDTD, presence of tillage pans, and the UCML. Besides CI, BD, and carbon assessments, rooting observations of both the Graminaceae and the woody crops would be required.

Levels of soil organic matter, plant roots and soil microorganisms generally decrease with depth in the soil profile (Paul and Clark, 1996). Long-term soil management practices in the surveyed bare-root nurseries have obviously affected soil in the upper 30 cm. Distribution of cover crop residue in the soil profile is related to the type of tillage tool used for crop incorporation as has been shown in field crop production (Staricka et al., 1991). The OC profile within the zone of tillage (disc) disturbance is characteristic of residue incorporated by a disc as contrasted to that with a moldboard plow (Staricka et al., 1991). The resultant OC profile partially reflects this incorporation profile, although rooting of the woody crop is also an important contributor. The transition zone extending beyond 19 cm may be partially explained by rooting, but taxonomy of this soil indicates possible OC translocation associated with iron chemistry of the soil before intensive nursery management.

Furthermore, the distribution profile of *Fusarium* spp. in the soils is reflective of where the organic substrate from the recent cover crop was placed by an incorporating implement. This profile was modified somewhat in those nurseries in which biocides are used just prior to sowing of the woody species (Minnesota and Wisconsin nurseries; Table 2). Surface recolonization by *Fusarium* via blowing soil, surface water flow, and infested seed (Bloomberg, 1985; Ocamb and Juzwik, 1993; Vaartaja, 1964) occurred within 2 years of the fumigation events in the Minnesota and Wisconsin nurseries. The survival and build-up of the *Fusarium* between the 18–24-cm depth in these nursery soils suggest the inability of chemical treatments to reach these depths for reduction of inoculum concentration. Thus the occurrence of *Pinus* root disease in the second growing season of the woody crop in operationally fumigated fields can be explained. As the pine roots grow into the 18 to 24-cm zone, an abundant level of potential inoculum is waiting. Soil environmental factors such as water drainage and aeration then further determine whether the pathogenic fungi will successfully infect and thrive in host roots. We suspect that of the two pine species present in the surveyed fields, white pine is physiologically affected to a greater degree by unfavorable soil water and rooting conditions than is red pine. This would partly explain why disease incidence is lower in red pine seedlings in bed rows closest to irrigation lines than disease incidence in white pine growing in similar bed-row locations (Juzwik pers. obsn).

In summary, an understanding of the relationship between nursery cultural practices and distribution and level of potential soil-borne pathogens, as well as soil physical conditions that predispose seedlings to root disease, is necessary in an integrated pest management approach to control of soil-borne diseases. Specifically, nursery staff could use tillage to control depth placement of cover crop residue and subsequent build-up of fungal propagules, adjust tillage practices to prevent tillage pans within the seedling root zone (Allmaras et al., 1994; Larson et al., 1994), and maintain optimum soil moisture levels appropriate for the growth of woody species consistent with weather conditions (Juzwik et al., 1994; Rothrock, 1992). Consideration of tillage practices effects on residue placement can also be the basis for more effective use of fumigants (that are often not placed sufficiently deep) when a chemical control option is used.

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