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SHORT COMMUNICATION

Mosaic stunting in bareroot *Pinus banksiana* seedlings is unrelated to colonization by mycorrhizal fungi

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Abstract Mosaic stunting, the occurrence of random patches of chlorotic seedlings with reduced shoot and diameter growth amidst more robust cohorts within bareroot nurseries, is classically associated with poor colonization by mycorrhizal fungi. We examined possible relationships among soil fertility, mycorrhizas, and random patches of mosaic stunting in bareroot *Pinus banksiana* Lamb. and suggest this paradigm is not universal. Stunted seedlings were distributed among healthy seedlings, occupied field space for 2–3 years, and used nursery resources (i.e. irrigation, fertilization); consequently high rates of culling at harvest resulted in an economic stress for the nursery. Thus, an understanding of the cause(s) of stunting was necessary. Stunted 1 + 0 seedlings had significantly lower levels of nitrogen, phosphorus, potassium, and zinc than their healthy cohorts, despite similar soil nutrient levels. The numbers of mycorrhizal root tips on stunted and healthy seedlings were similar, and the taxa of mycorrhizal fungi, determined by isolations and DNA sequencing, were not consistently associated with stunted or healthy seedlings. We conclude that differences in *Pinus banksiana* mycorrhizas are not responsible for mosaic stunting, but

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may be caused by localized low soil N availability due to uneven distribution of added organic matter amendments.

Keywords Mycorrhizas · Organic amendment · Nursery · Chlorosis · Seedling nutrition

Introduction

Determining the cause of poor seedling growth can be challenging because of the potential influence of multiple factors: edaphic conditions, abiotic and biotic diseases, and nursery practices (e.g. fertilization, irrigation, pesticide use). One example of this poor growth is mosaic stunting, characterized by random patches of chlorotic seedlings with reduced shoot and diameter growth adjacent to normally-growing seedlings. Mosaic stunting has been observed in a variety of tree species at many bareroot nurseries (Landis 1998). Common nursery diseases have been ruled out as causes of mosaic stunting because seedlings lack classic foliar symptoms, root decay, or high soil populations of root-rot pathogens (Dumroese and James 2005; Linderman et al. 2007).

Needle discoloration (yellowing or purpling) and reduced shoot growth in mosaic-stunted seedlings indicate nutrient deficiencies. Ectomycorrhizal fungi (EMF) are important in conifer nutrient uptake, especially Phosphorus (P) (Smith and Read 1997). Because fumigation depletes soils of EMF (Trappe et al. 1984), many hypothesized that a lack of mycorrhizas on young seedlings caused nutrient deficiencies resulting in stunting (Trappe and Strand 1969; Henderson and Stone 1970; Croghan et al. 1987). This idea was supported when fertilization or application of EMF inoculum improved seedling growth (Campagna and White 1969; Henderson and Stone 1970; Ridge and Theodorou 1972; Marx et al. 1978; Hung et al. 1982). Consequently, investigations of mosaic stunting in conifers have focused on EMF in the Midwestern USA (Croghan et al. 1987) and Western USA (Linderman et al. 2007). These studies compared differences in colonization of stunted and non-stunted seedling roots as measured by counting mycorrhizal root tips. Different species of EMF have, however, varying effects on soil nutrient uptake efficiency, which depends on edaphic factors (pH, nutrient levels, texture) as well as physiology of the fungus (Ho and Zak 1979; Taylor and Peterson 2005). Therefore, the species of EMF colonizing seedling roots may be a factor in the incidence of mosaic stunting.

Mosaic stunting, characterized by asymptomatic root systems, in which root mass was decreased proportionately to the shoot mass, was observed in bareroot *Pinus banksiana* seedlings growing at the USDA Forest Service J.W. Toumey Nursery in Michigan, USA. We established a study to test three hypotheses: (1) stunted seedlings have significantly lower EMF colonization compared to healthy seedlings; (2) stunted seedlings have different taxa of EMF colonizing the roots; and (3) stunted seedlings have significantly lower foliar nutrient concentrations.

Materials and methods

Study area and general nursery production

The J.W. Toumey Nursery is located in Watersmeet, Michigan, USA (46.45° N; 89.28° W). This 24-ha nursery is situated on an outwash plain, with a fine sandy loam soil of the Pence-Vilas complex (NRCS 2007). Mean annual precipitation is 77 cm and mean annual

air temperature is 4 °C (Michigan Climatological Resources Program 2009). The principal stocktypes produced are 2 + 0 (years in seedbed + years in transplant bed) *Pinus banksiana* and 3 + 0 *Pinus resinosa* Aiton and *Pinus strobus* L. In the fall before seeds are sown, fields are fumigated with either dazomet (370–393 kg ha^{-1}; sealed with irrigation) or 67:33 methyl bromide:chloropicrin (392 kg ha^{-1}, sealed with tarps). All seedlings in this study were operationally produced.

Seedling and soil sampling

A randomized block design was used in field sampling (n = 10); briefly twenty seedlings were collected (ten stunted and ten healthy growing adjacent to one another) at ten plots, for each given stocktype (1 + 0 and 2 + 0). Seedlings were carefully extracted from the soil to preserve root systems, bagged, and placed on ice. In Year 1 and Year 2 (hereafter Y1 and Y2), we collected seedlings from each stocktype. In Y2 we re-sampled the Y1 1 + 0 plots, and we also extracted eight 15 cm deep, 2 cm diameter soil cores from each plot (four beneath stunted and four beneath healthy seedlings), which were pooled by plot for a total of 20 soil samples. All samples were processed at Michigan Technological University, Houghton, Michigan, USA.

Seedling, mycorrhiza, and soil measurements

Year 1

We measured height of both stocktypes and root-collar diameters (RCD) of 2 + 0 seedlings. After carefully washing roots, a 3-cm section from the third and sixth lateral roots (basipetal from the root collar) of each seedling was selected for quantification of mycorrhizas [similar to Amaranthus and Perry (1987) and Menkis et al. (2005)]. We counted mycorrhizal root tips on each root section using a dissecting microscope (10–40× power) and categorized tips by branching pattern (Grand and Harvey 1982).

Fungi were isolated from root tips of two representative seedlings from each group within a plot (stunted and healthy) within 24 h of initial sampling (Molina and Trappe 1982). We exicised three, 3-cm sections from the lateral roots of each seedling, pooled (stunted or healthy), surface sterilized for 30 s with agitation using a 1:10 (v v^{-1}) Clorox® solution (5.25 % sodium hypochlorite): water solution, and rinsed three times with sterile water (Zak and Bryan 1963). Two mycorrhizal root tips were removed from each 3-cm root section for a total of 480 individual root tips each sampling, and individually plated on 35 × 10 mm Petri dishes with a 2 % malt (2 M) agar medium with additions of antibacterials (100 ppm streptomycin [S] and 100 ppm tetracycline [T]) and 10 ppm of the fungicide benomyl (B) (2MSTB).

Plates were observed weekly and putative EMF were characterized by slow hyphal growth, presence of clamp connections on hyphae, and distinctive colony characteristics (Zak and Bryan 1963); cultures were transferred to fresh modified Melin-Norkrans agar (Marx 1969). After 3 weeks, we sorted cultures into mycorrhizal types by growth rate, mycelial mat color and texture, hyphal shape, pigmentation of agar, clamp connection presence and shape, and morphological differentiation of hyphae (Hutchison 1991).

Isolates from the 1 + 0 and 2 + 0 stock were identified to species level. The CTAB mini-prep method was used for DNA extraction of the putative EMF Types 1–3 (Gardes and Bruns 1993). The fungal specific primers ITS1F and ITS4 were used for the PCR amplification of the internal transcribed spacer region (White et al. 1990; Gardes and Bruns

1993) and RFLP methods followed those of Lilleskov et al. (2002). We prepared samples of unique RFLP types for sequencing using the QIAquick PCR Purification Kit with the primers ITS1F and ITS4, sent to the Nevada Genomics Center (Reno, Nevada, USA), and DNA-sequenced using ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1. The reactions were run using an ABI Prism 3730 DNA Analyzer. Sequence results were matched with highly similar sequences using the National Center for Biotechnology Information nucleotide database BLAST (Altschul et al. 1997). We used a sequence similarity across 500 bp of ≥97 % to identify to species level and ≥95 % to identify genus (Landeweert et al. 2003). Type 1 fungus was identified as *Sistotrema brinkmannii* (Bres.) J. Erikss., Type 2 as *Thelephora terrestris* Ehrh., and Type 3 as *Suillus luteus* (L.) Roussel. DNA results matched the morphology of the respective fungus in culture.

Year 2

After measuring height and RCD, we carefully washed and sampled roots as described above. Because of a lack of consistent differences for the Y1 sample, Y2 mycorrhizas were not quantified and categorized as in Y1, but root tips were plated and fungi isolated as described above. We compared cultures of Y2 isolates with the DNA-identified plate cultures from Y1.

After sampling roots for mycorrhizas, seedlings were separated into roots and shoots and dried for 48 h at 65 °C before determining mass for shoot-to-root ratio (S:R). Shoots (needles and stems) and roots of 1 + 0 seedlings and needles of 2 + 0 seedlings were ground with a Wiley Mill to pass a 40 mesh screen and analyzed at the Penn State Agricultural Analytical Resources Laboratory (University Park, Pennsylvania, USA) for nutrient concentration. Total N was determined on a Carlo Erba NA1500 Elemental Analyzer (Horneck and Miller 1998). Phosphorus, K, Ca, Mg, Mn, Fe, Cu, B, and Zn were analyzed after dry-ashing at 500 °C (Miller 1998).

Soil samples were passed through a 2-mm sieve, dried at 105 °C, and sent to the USDA Forest Service Rocky Mountain Research Station (Moscow, Idaho, USA) where pH, total C and N, and extractable K, Ca, and Mg were analyzed. The cations were extracted with pH neutral ammonium acetate and processed on a Perkin Elmer Atomic Absorption Spectrometer (Model 5100PC). Soil pH was measured on a 1:2 (v v^{-1}) soil:deionized water slurry; available P was estimated using the Bray 1 method and analyzed on an O·I. Analytical Flow Solution 3000 (College Station, Texas, USA); C and N were determined using a LECO CN2000 (St. Joseph, Michigan, USA).

Statistical analysis

A two-tailed paired-sample *t* test was used to test for differences among stunted and healthy seedlings for each stocktype for both years. The seedling and mycorrhiza variables used for Y1 samples were: seedling height (n = 50), RCD (n = 50), mean numbers of monopodial, bifurcate, dichotomous, and pinnate root tips morphologies (n = 100), and total mycorrhizal root tip counts (n = 100). Differences in mycorrhizal fungal types isolated from root tips of stunted and healthy seedlings were analyzed by the Pearson's Chi square test. The Y2 seedling and soil variables were: seedling height (n = 50), RCD (n = 50), shoot and root dry weight and S:R (n = 10 for 1 + 0 s; n = 50 for 2 + 0 s), mean foliar nutrient concentrations (n = 10), and mean soil nutrient concentrations from soils beneath sampled seedlings (n = 10). We considered tests significant when $p < 0.05$.

Results

In Y1, heights of healthy 1 + 0 and 2 + 0 seedlings were 265 and 322 % greater, respectively, and significantly different (all $p \leq 0.0001$) than stunted seedlings in those same stocktypes. Similarly, healthy 2 + 0 seedlings had significantly ($p \leq 0.0001$) greater RCD (235 %) than stunted seedlings. In Y2, heights of healthy 1 + 0 and 2 + 0 seedlings were 673 and 332 % greater, respectively, than their stunted cohorts (Table 1). Similarly, healthy 1 + 0 and 2 + 0 seedlings had significantly greater RCD (167 and 213 %), and shoot biomass (367 and 381 %) than the stunted seedlings. Root biomass was similar for the 1 + 0 crop, but healthy 2 + 0 seedlings had 289 % more root mass. Stunted seedlings had lower S:R (Table 1).

Of the mycorrhiza morphotypes, monopodial and bifurcate were the most common across stocktypes (Table 2). Morphotype representation between healthy and stunted 1 + 0 seedlings was not significantly different, but healthy 2 + 0 seedlings had significantly more of the monopodial morphotype than stunted seedlings ($p = 0.01$). The total numbers of mycorrhizal root tips on healthy and stunted 1 + 0s were similar, but healthy 2 + 0s had significantly more (about 20 %) mycorrhizal root tips than their stunted cohorts. The 2 + 0 seedlings had nearly twice the mycorrhizal root tips per cm of root than the 1 + 0 seedlings (Table 2).

In Y1, approximately 60 % of the 2 + 0 root tips and 27 % of the 1 + 0 root tips yielded presumptive EMF isolates. *Sistotrema brinkmannii* was isolated only from the root tips of 2 + 0 stunted seedlings, while *T. terrestris* and *S. luteus* were isolated from stunted and healthy 2 + 0 and 1 + 0 seedlings (Table 3). No significant differences were detected in the number of *T. terrestris* and *S. luteus* isolates obtained from stunted and healthy seedlings. Due to the late sampling date and slow development of Y1 seedlings caused by drought conditions, overall success of isolating EMF from root tips of 1 + 0 *P. banksiana* seedlings was much lower than for 2 + 0 seedlings.

In Y2, 1 + 0 seedlings yielded the most fungal isolates, with presumptive EMF growing on 47 % of the root tips plated, while 2 + 0 seedlings yielded EMF isolates from just 24 % of root tips. Most fungi isolated had similar growth characteristics in culture to *T. terrestris*, *S. luteus,* and *S. brinkmannii* isolated in Y1. However, a different EMF (Type 4) isolated from healthy and stunted 1 + 0 and 2 + 0 roots was identified as a *Laccaria* spp. after comparisons with known pure cultures of *Laccaria laccata* from fruit body tissue isolations.

In Y2, significantly more *S. luteus* and *T. terrestris* was isolated from healthy 2 + 0 seedlings, but no significant difference in *Laccaria* was observed between seedling groups (Table 3). In Y2 1 + 0 seedlings, significantly more *Laccaria* and *T. terrestris* were isolated from healthy seedlings than from stunted seedlings, while *S. luteus* was isolated at significantly higher rates in stunted seedlings; *S. brinkmannii* was isolated only from 1 + 0 healthy seedlings. Across both years in healthy seedlings, *S. luteus* was detected most, followed by *Laccaria* and *T. terrestris,* with *S. brinkmannii* occurring at the lowest (<3.3 %) frequency. In stunted seedlings across both years, *T. terrestris* was the most frequently isolated fungus from stunted seedling roots, followed by *S. luteus* and *Laccaria,* with *S. brinkmannii* occurring infrequently (<12.8 %).

No significant differences were observed in pH, total C and N, exchangeable Ca, K, and Mg concentrations, or extractable P between soils sampled below healthy and stunted 1 + 0 and 2 + 0 seedlings (Table 4). However, concentrations of N, P, K, Mn, and Zn were significantly lower in shoots and roots of stunted 1 + 0 seedlings than in healthy 1 + 0 seedlings (Table 5). When concentrations of these nutrients were converted to

Table 1 *Pinus banksiana* morphological traits (mean ± standard errors) of Year 2 stunted and healthy seedlings

Stocktype	Status	Height (cm)	RCD (mm)	Shoot biomass (g)	Root biomass (g)	Shoot:root
1 + 0	Healthy	7.40 ± 0.23	1.30 ± 0.04	1.10 ± 0.20	0.20 ± 0.04	5.5
	Stunted	1.10 ± 0.03	0.61 ± 0.01	0.30 ± 0.03	0.20 ± 0.02	1.5
	p	<0.0001	<0.0001	0.001	0.43	<0.0001
2 + 0	Healthy	17.25 ± 0.42	3.35 ± 0.06	12.0 ± 0.70	2.75 ± 0.22	4.4
	Stunted	5.20 ± 0.17	2.00 ± 0.04	3.15 ± 0.26	0.95 ± 0.08	3.3
	p	<0.0001	<0.0001	<0.0001	<0.0001	<0.01

Values are significantly different at $p < 0.05$

Table 2 Mycorrhizal root tip counts and morphology in healthy and stunted *Pinus banksiana* seedlings

Mycorrhizae morphotype	1 + 0 Seedlings			2 + 0 Seedlings		
	Healthy	Stunted	*p*	Healthy	Stunted	*p*
Monopodial	992	905	0.22	1,894	1,567	0.01
Bifurcate	21	40	0.32	231	196	0.54
Dichotomous	0	10	0.08	46	41	0.84
Pinnate	0	0		1	1	1.00
Total count*	1,013	955	0.44	2,172	1,805	0.005
Average number of mycorrhizae cm^{-1}	3.38	3.18	0.44	7.24	6.02	0.005

Values are significantly different at $p < 0.05$

* 600 cm of roots within each group of seedlings (healthy or stunted) were examined to obtain mycorrhizal root tip counts

Table 3 Mycorrhizal fungal types isolated (%) from Year 1 and Year 2 root tip isolations

Year	Stock type	Status	*Thelephora terrestris* (%)	*Suillus luteus* (%)	*Sistotrema brinkmannii* (%)	*Laccaria* spp. (%)
1	1 + 0	Healthy	19a	2a	0	0
		Stunted	25a	7a	0	0
	2 + 0	Healthy	39a	13a	0a	0
		Stunted	47a	8a	13b	0
2	1 + 0	Healthy	8a	1a	3a	58a
		Stunted	2b	13b	0b	9b
	2 + 0	Healthy	4a	36a	0	7a
		Stunted	2b	15b	0	6a

120 root tips isolated for each stock type/date/seedling condition. Values followed by different letter within each EMF, year, and stock type combination indicate significant difference ($p < 0.05$)

content using seedling biomass, healthy 1 + 0 seedlings contained 8–10 times more of these nutrients than stunted seedlings. In contrast, concentrations of Ca, Mg, Fe (shoots only), and B were significantly higher in stunted than in healthy 1 + 0 seedlings but healthy seedlings had significantly higher contents than stunted seedlings. Similarly for the

Table 4 Nutrients levels and pH of soils beneath Year 2 stunted and healthy *Pinus banksiana* seedlings

Stocktype	Status	pH	Extractable Ca (mg/kg)	Extractable Mg (mg/kg)	Extractable K (mg/kg)	C (%)	N (%)	C:N	Ortho-P (mg/L)
1 + 0	Healthy	4.79	461 (283–611)	41 (23–57)	69 (44–112)	2.00	0.08	23.80	138
1 + 0	Stunted	4.83	508 (334–774)	43 (28–57)	72 (38–131)	2.32	0.09	26.23	136
2 + 0	Healthy	4.82	444 (270–743)	33 (18–74)	62 (34–94)	1.64	0.07	23.94	NA
2 + 0	Stunted	4.83	474 (267–696)	37 (19–62)	54 (16–83)	1.48	0.06	24.05	NA

No significant differences were detected between stunted and healthy seedlings for any soil nutrients or pH ($p < 0.05$). The range of values for Ca, Mg, and K are listed following the mean

Table 5 Year 2 nutrient concentration of *Pinus banksiana* seedling tissues

Stocktype and tissue	Status	Macronutrients					Micronutrients				
		N (%)	P (%)	Ca (%)	Mg (%)	K (%)	Mn (ppm)	Fe (ppm)	Cu (ppm)	B (ppm)	Zn (ppm)
1 + 0 Shoots	Healthy	2.59a	0.28a	0.26a	0.14a	1.16a	490a	121a	9.4a	19.9a	106a
	Stunted	1.00b	0.12b	0.70b	0.19b	0.60b	385b	257b	9.3a	27.7b	57b
1 + 0 Roots	Healthy	1.46a	0.27a	0.42a	0.14a	1.24a	386a	1472a	83.9a	18.1a	149a
	Stunted	0.62b	0.19b	0.68b	0.14a	0.90b	111b	1164a	130.7a	28.9b	53b
2 + 0 Needles	Healthy	1.23a	0.12a	0.28a	0.09a	0.50a	141a	86a	4.0a	11.2a	48a
	Stunted	1.18a	0.13a	0.32b	0.11b	0.58b	148a	100a	6.1b	13.5b	59b

Values followed by different letter within each nutrient, year, and stock type combination indicate significant difference ($p < 0.05$)

2 + 0 s, the concentrations of macronutrients (Ca, Mg, K) and Cu, B, and Zn were significantly reduced in needles of healthy seedlings (Table 5) but healthy seedlings had significantly greater macro- and micronutrient contents.

Discussion

Mycorrhizal root tips

The stunting of *P. banksiana* seedlings at Toumey Nursery does not appear to be caused by a lack of mycorrhizas. Although we detected higher numbers of mycorrhizal root tips on healthy 2 + 0 seedlings, no differences were evident between healthy and stunted 1 + 0 seedlings. Also, the significant difference between 6 and 7 mycorrhizas per cm of root length on stunted and healthy 2 + 0 seedlings may not be biologically important. Therefore, we reject our first hypothesis that stunted seedlings have lower numbers of mycorrhizas than healthy seedlings. Our results are consistent with the findings of Linderman et al. (2007), who failed to find differences in the number of EMF on healthy and stunted 1 + 0 *Pseudotsuga menziesii* (Mirb.) Franco seedlings. This contrasts, however, with Croghan et al. (1987), who found that 79 % of healthy 1 + 0 *Picea glauca* (Moench) Voss seedlings had ectomycorrhizal root tips, while only 2 % of stunted seedlings were colonized.

Mycorrhizal fungi

Similar to mycorrhizal root tips, differences in EMF communities do not seem to be causing *P. banksiana* stunting. We observed a shift in the relative frequency of fungi colonizing roots from Y1 to Y2. The community of EMF is dependent on a number of factors including soil moisture (Theodorou 1978), nutrient availability (Lilleskov et al. 2002), and temperature (Domisch et al. 2002), and EMF communities on *P. resinosa* roots have been shown to vary during a 13-month period (Koide et al. 2007). Of the four fungal taxa isolated from *P. banksiana* seedlings, all, except *S. brinkmannii*, are known to form mycorrhizas on conifer nursery seedlings (Richter and Bruhn 1993; Menkis et al. 2005). *Suillus luteus* colonizes pine seedlings in the first few months after germination (Dahlberg and Finlay 1999) and was frequently isolated from healthy and stunted seedlings. *Thelephora terrestris* is an aggressive colonizer of pine seedling roots in nurseries (Colpaert 1999) and was the dominant EMF isolated from Y1 seedlings. *Laccaria* spp. also showed a strong temporal variance, as it was not isolated from any Y1 seedlings, but was commonly found in Y2. *Laccaria* is beneficial to the growth of pine nursery stock, often increasing P uptake and increasing outplanting performance (Tyminska et al. 1986; Richter and Bruhn 1989; Browning and Whitney 1992). *Sistotrema brinkmannii* was rarely isolated from Y1 and Y2 seedlings, and is typically classified as a wood decay fungus (Eriksson et al. 1984). However, recent work indicates that some species of *Sistotrema* may form mycorrhizal associations (Nilsson et al. 2006; Bubner et al. 2014), and in a laboratory pure culture synthesis we found no negative effects of *S. brinkmannii* on *P. banksiana* seedling growth (Potvin et al. 2012). Although there were inconsistencies across seedling age groups and sampling years, no major differences in mycorrhizal fungal taxa were evident between stunted and healthy seedlings. Therefore, we reject our second hypothesis that stunted seedlings would have different EMF communities than healthy seedlings.

Nutrients

In our study, levels of soil N (total), P (ortho), K, Ca, and Mg were similar beneath stunted and healthy 1 + 0 seedlings sampled in Y2. Despite these similar levels, we observed significantly lower concentrations of shoot N, P, and K in stunted 1 + 0 *P. banksiana* seedling shoots, but significantly greater levels of Ca and Mg. A similar trend was apparent in the roots of stunted 1 + 0s. In general, levels of 1 + 0 shoot N, P, and K were higher than the critical concentration in healthy seedlings, N and P were lower than adequate in stunted seedlings, and Ca and Mg were higher than adequate in stunted seedlings (Landis 1985; Stewart and Swan 1970). Except for Fe, which exceeded the adequate range regardless of seedling health, all 1 + 0 stunted seedlings had micronutrient shoot concentrations within the adequate range (Landis 1985; Stewart and Swan 1970). Stunted 2 + 0 seedlings, however, had concentrations of N and P similar to healthy 2 + 0s; these concentrations were in the adequate range for conifers in general (Landis 1985) but slightly below the recommendation for *P. banksiana* (1.5–2.5 and 0.18–0.35 % for N and P, respectively) (Stewart and Swan 1970). Healthy 1 + 0 seedlings had much higher N than healthy 2 + 0 seedlings. This decline in N concentration of healthy seedlings as they grow from 1 + 0 to 2 + 0s is likely due to a dilution effect discussed by Timmer et al. (1991), where larger seedlings, because of growth, have lower concentrations despite greater nutrient content.

Nitrogen is usually the most limiting nutrient in nurseries (Landis and van Steenis 2003). Croghan and LaMadeleine (1982) found lower concentrations of N and P (73 and

29 % respectively) in stunted 1 + 0 *P. glauca*. Trappe and Strand (1969) reported lower P concentrations in foliage of 2 + 0 stunted *P. menziesii* seedlings despite similar soil P concentrations under stunted and healthy seedlings. While deficiencies of Mn and Zn have commonly been reported in agricultural crops (Berger 1962), we could not find any studies linking low K, Mn, or Zn levels to stunting. Nitrogen appears to be the main factor in the stunting of our *P. banksiana*, as shoot N concentration of 1 + 0 stunted seedlings (1.0 %) was below the generally accepted range (1.2–2.0 %) for bareroot conifer seedlings (Landis 1985) and below the adequate range (1.5–2.5 %) reported for *P. banksiana* seedlings (Stewart and Swan 1970).

Because we found relatively little difference in mycorrhizal root tips and fungal taxa between stunted and healthy seedlings, it seems unlikely that mycorrhizas are causing the mosaic stunting of *P. banksiana* in this study. However, the low levels of nutrients, especially N, in 1 + 0 stunted seedlings indicate that a nutrient deficiency may be the probable cause. Recent work at the nursery suggests that unequal distributions of hardwood sawdust applied as an organic amendment prior to planting could reduce soil N availability in small, localized areas of the nursery beds (Koll et al. 2010). Although standard nursery practice includes additional N applications to off-set the high C:N of the sawdust, deposits of excess sawdust would lower available soil N for many months following application and cause pockets of seedlings with leaf chlorosis and stunting. As these high amounts of sawdust slowly decompose, N would be released and eventually be available late in the 1 + 0 growing season or early next year. These 1 + 0 seedlings would, however, likely show stunting the following year, as shoot growth of the 2 + 0 *P. banksiana* seedlings was determined by bud set conditions during the previous year (Kozlowski et al. 1973; Colombo 1986; Islam et al. 2009).

Conclusions and implications

Traditionally mosaic stunting has been attributed to a lack of ECM formation in nursery seedlings. We present an alternative to that paradigm, and support a more comprehensive approach in determining causal factors in growth deficiencies. In our study, healthy and stunted seedling roots were colonized by ECM without detection of definitive differences in ECM community across the seedling groups. Our stunted *Pinus banksiana* seedlings were the apparent result of poor soil preparation that caused random patches of high carbon-to-nitrogen ratios leading to localized low soil N. Thus, "mosaic stunting" is a general, non-specific designation of leaf chlorosis and poor growth in bareroot nursery seedlings, and the cause needs to be determined by experienced nursery personnel or through nursery-specific studies.

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