Optimizing Select Fraser Fir (*Abies fraseri*) Reforestation Methods within an Artificial Bald at Mount Buckley, Great Smoky Mountains National Park

Benjamin Smith[1,†], Shawn P. Brown[2,†,*], John Frampton[3], AnneMargaret Braham[3], C. Elizabeth Stokes[4], and Richard Baird[5]

Abstract - *Abies fraseri* (Fraser Fir) has a range that is restricted to high-elevation stands in the southern Appalachian Mountains with few suitable locations. *Adelgid piceae* (Balsam Wooly Adelgid) depredation that induced high mortality and chronic losses, and other anthropogenic causes, have led to Fraser Fir being designated as an endangered species by the IUCN Red List. There is great interest in restoring Fraser Fir through managed reforestation efforts, especially in protected areas such as Great Smoky Mountains National Park. To optimize Fraser Fir reestablishment in formerly forested sites, we designed a study to examine reforestation success by comparing survival and growth using treatments including mycorrhizal seedling transplants, local seedling transplants, local soil inocula, and mulching conditions to suppress surrounding vegetation. We found that while some treatments increased seedling survival and growth, any benefit was slight compared to local seedling transplants from adjacent mature Fraser Fir stands and/or local soil inocula. Greenhouse manipulations were more costly and time intensive for very little added benefit. We conclude that transplanting local seedlings with native soil to restore damaged sites provides the maximum return on investment and is a viable option for reforestation of Fraser Fir, but a sufficient local seedling bank may not always be available.

Introduction

Abies fraseri (Pursh) Poir (Fraser Fir) is the only true fir endemic to the southern Appalachian Mountains and has a disjunct distribution that is restricted to high elevations in the southern Appalachian Mountains of southwestern Virginia, western North Carolina, and eastern Tennessee in the United States. At its lower elevational limits (~1300 m asl), Fraser Fir is a minor stand component, but increases in frequency with elevation, and pure stands occur at ~1900 m asl and above (Dull et al. 1988). At these higher elevations, *Sorbus americana* Marshall (Mountain-ash) is often the only canopy associate (Williams 1958). Fraser Fir's ecosystem services are numerous and include watershed protection (Cai et al. 2012), scenic attraction and education (Farmer et al. 2010), soil erosion control

---

[1]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695. [2]Department of Biological Sciences, The University of Memphis, Memphis, TN 38152. [3]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695. [4]FWRC-Sustainable Bioproducts Department, Mississippi State University, Starkville, MS, 39762. [5]BCH-EPP, Box 9655, Mississippi State University, Mississippi State, MS 39762. [†]Contirbuted equally. [*]Corresponding author - spbrown2@memphis.edu.

Manuscript Editor: Robert Carter

(White et al. 2012), and as habitat for several species of mosses and other biota including the endangered *Microhexura montivaga* Crosby & Bishop (Spruce–fir Moss Spider) and *Glaucomys sabrinus coloratus* Handley (Northern Flying Squirrel) (Hackett and Pagels 2003).

The Great Smoky Mountains National Park (GRSM) has ~74% of the remaining southern Appalachian spruce–fir forest types. This area of the park is considered to possess boreal climatic conditions (Potter et al. 2010) that vary between temperate and boreal zones as designated by the Köppen–Trewartha system originally defined by Köppen (1936). The insect pest *Adelges piceae* Ratz. (Balsam Woolly Adelgid [BWA] ; Hemiptera: Adelgidae), originally introduced into Maine on imported nursery stock, spread and led to widespread damage to Fraser Firs, which resulted in high mortality in the southern Appalachians (Smith and Nicholas 2000, Witter and Ragenovich 1986). In GRSM and nearby Pisgah National Forest, mature Fraser Fir had over 90% mortality due to this pest (Dull et al. 1988). Since these losses, some natural reforestation has occurred and excellent stand reestablishment has been seen, but for many sites, re-establishment has been poor (Baird et al. 2014). It is uncertain why some sites vary in natural regeneration potential.

The climate of the southern Appalachian spruce–fir forest is cool and humid and classified as temperate rain forest (Shanks 1954). Moisture is an important requisite for initial Fraser Fir survival and seedling establishment (Crandall 1958). In the 1950s (pre-BWA), annual mean precipitation for Clingmans Dome (where this study took place) in GRSM was 250 cm (Oosting and Billings 1951), and at nearby Newfound Gap and Mt. LeConte, average annual precipitation from 1991 to 2020 (post-BWA) was 187 and 214 cm, respectively, which suggests a trend of declining annual precipitation. Precipitation is likely critical for Fraser Fir regeneration in the higher elevations, but to our knowledge, no studies are available to validate this assumption. Southern spruce–fir soils can be extremely acidic with a root-zone pH of 3.6–4.6 across several soil subgroups, which further impacts forest composition (Kelly and Mays 1989).

On north-facing slopes within Fraser Fir stands, ground cover includes high levels of moss, *Oxalis* spp. (wood sorrel), and *Dryopteris* spp. (wood fern) coverage at 90%, 50% and 40%, respectively (Whittaker 1956). Areas of greater moss coverage may be important for seedling establishment due to moisture retention (Baird et al. 2014), although thick layers of moss may inhibit root establishment (Pauley and Clebsch 1990). Methods to enable greater moisture retention for seedling survival in drier sites are critical. Ecologically safe methods to support moisture retention while suppressing vegetation could be important in reestablishing Fraser Fir stands in anthropogenically damaged areas that can form balds containing grassy heath layers within GRSM.

Following the initial BWA infestation, Fraser Fir regeneration continued to decrease and remains erratic (Smith and Nicholas 2000). A study over 30 years after the initial BWA wave at GRSM compared stem density for trees 5–60 cm dbh in pure and mixed Fraser Fir stands. These results were compared with similar data from a 1946 pre-BWA GRSM investigation, with the new data indicating a decrease in overall fir densities. Mortality was noted to be selective for larger trees (Oosting and Billings 1951, Smith and Nicholas 2000). In GRSM between 1986 and 1990, Fraser Fir seed production and seedling germination increased with elevation, but production and germination were erratic over the 5-year period (Nicholas et al. 1992). While seed production occurred every year across most elevations of spruce–fir forest, the number of germinal seedlings/ha was often 0 following poor production years and rarely reached >1000 seedlings/ha following the best years of production at the highest elevations (1830–1980 m; Smith and Nicholas 2000). This erratic germination was likely due in part to the high percentage of empty seed (88–100%) they observed over the 5-year period, but site factors such as moisture and competing vegetation also likely played a role.

Mycorrhizal associations between rootlets of vascular plants and beneficial fungi are vital for successful establishment of many plants (Gould 2004). The loss of Fraser Fir significantly impacted soil rhizosphere biota, especially the ectomycorrhizal (EcM) fungi associated with this tree species (Baird et al. 2014). Conversely, changes in species dominance have led to changes in soil microbial community structure that may interfere with reestablishment of previous plant communities (Ingham 1992). Absence or reduced densities of certain EcM taxa may limit fir reestablishment. Baird et al. (2014) confirmed that the fungal fruiting bodies formed by EcM in our Fraser Fir were significantly lower in canopy gaps or where trees were sparse. However, EcM taxa such as *Laccaria* spp. and several other symbiotic genera were abundant under saplings and mature Fraser Fir and reported previously from similar forest types (Bills et al. 1986, Bird and McCleneghan 2005).

Artificial or natural mycorrhizae inocula have been shown to support improved seedling survival and growth of forest tree species in reforestation efforts (Brown et al. 2022, Policelli et al. 2020). Artificial inoculum, if produced commercially, has the potential to introduce exotic fungal genotypes into a location, which is prohibited and disallowed in National Park land (National Park Service 2006). Furthermore, these genotypes may not be locally adapted, limiting any potential benefits and may increase the chances of non-native plant establishment (Claridge et al. 2009). Laboratory-produced EcM inoculum of fungi originally isolated from the same location will ensure community genetic ecosystem stability (Policelli et al. 2020). An alternative approach would be to use locally native soil, containing natural fungal EcM inoculum, from adjacent stands (Sýkorová et al. 2016). Furthermore, seedlings that can be transplanted from the adjacent stands would have a natural mycorrhizal community already established on their roots, providing a stronger reservoir of symbiotic fungi across forest tree age classes.

Understory Fraser Fir seedlings have a high vigor response following overstory release via transplantation (Oosting and Billings 1951) and may provide an economic and logistical alternative to labor-intensive and costly multiple-year production of nursery seedling and laboratory-prepared EcM inoculum. Establishment of Fraser Fir in previous habitats that no longer support tree seedling establishment due to either vegetation competition or poorer soil structure might benefit from enhanced planting methods using EcM for enhancing seedling growth.

Currently, most high-elevation canopy gaps within GRSM that once contained Fraser Fir remain relatively open (Baird et al. 2014), even after 30 years since fir stand loss. Canopy gaps forming balds in the Clingmans Dome area of GRSM and adjacent mountains such as Mount Buckley and Mount Love are the direct result of Fraser Fir mortality from BWA between 1962 and 1970 (Busing et al. 1988, Dull et al. 1988). Vegetation in these balds can choke out Fraser Fir seedlings during early establishment stages, limiting their survival. Reforestation methods to maximize seedling growth and reestablish Fraser Fir seedlings onto these sites, with particular focus on cost effectiveness and reduced labor intensity, would allow managers to re-establish this tree species on highly eroded, vegetation-dense, and disturbed sites. Therefore, we hypothesize that (1) survival and growth of field-collected seedlings will differ from greenhouse-grown seedlings comprising 7 Fraser Fir open-pollinated families originating from Mt. Buckley, GRSM, and (2) 2 *Laccaria* spp. used as inoculum (Baird et al. 2014) will enhance Fraser Fir seedling survival and growth compared with natural field soils.

Field-Site Description

The site, located on Mt. Buckley adjacent to the Clingmans Dome area of GRSM, comprised of 9 plant species (understory vegetation; Baird et al. 2014) with no mature Fraser Fir within 10 m of the planting area. The site is on a steep slope (mean of ~42%) previously devastated by wildfire that burned down to the mineral soil in 1925 (Lix 1958). Exposed soils are highly eroded and shallow (<20 cm deep), and as of 2010, only understory-type vegetation was established in these soils (Baird et al. 2014). Plantings conducted in August 2012 and June 2013 were centered around the location 35.56154°N, 83.50906°W, and the May 2014 planting was located adjacently at 35.56183°N, 83.50850°W. All sites had an average elevation of 1950 m asl.

Methods

Seeds used in this study were collected from Fraser Fir cones collected in 1994 from the Clingmans Dome and Mt. Buckley area varying in elevations from 1667 to 1993 m asl (McKeand et al. 1995); these seeds had been stored at -20 to -25 ºC since collection (Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC). The open-pollinated families (i.e., seed from individual trees, at least half-siblings) we included in this study were NCSU 208, 211, 212 (seed was limited), 213, 219, 224, and 225. We initiated seed germination in May 2011, and our seedling-culture techniques in the greenhouse followed established protocols (Frampton and Benson 2012).

Per US National Park Service regulations (National Park Service 2006), employed personnel attempt to maintain Park Service lands free from exotic organisms and/or genotypes, either introduced naturally or through anthropogenic causes. Consequently, Fraser Fir seed selected for the current study were required to be from parent trees located near the planting site. Similarly, the isolates of mycorrhizae inocula (see below) used were prepared from laboratory cultures of basidiocarps that were collected by R. Baird from the Clingmans Dome area including Mount Buckley and Mount Love.

This study consists of 2 distinct planting experiments. During 2012 and 2013 (project 1), we established 2- or 3-seedling microplots within a semi-circular main plot area of 0.404 ha (1.0 ac), allowing for a minimum of 1-m spacing between microplots. We chose at random which seedlings were planted in each microplot. Initially, this project was designed as a multi-year planting experiment. After the first planting year (August 2012), we observed higher mortality, presumably due to frost heave and limited time for root establishment. Consequently, we planted the second-year plantings earlier in the season (June 2013). We established a total of 464 microplots, and site preparation for microplots included removing the thick shrubs, grasses, small rocks, and roots layers down to the mineral soil in circular areas ~50 cm in diameter at each planting location. We used similar site preparation for the planting done in 2014 (project 2; 180 microplots), but the 2014 plot was smaller, with an area of ~0.15 ha. (0.37 ac).

Planting years 2012–2013 (project 1)

In addition to testing plant-family effects, we also tested the different effects of EcM inoculum and/or mulching (cover) treatment on growth and survival. Mycorrhizal inoculation treatments included the following: (1) bare-soil plantings (no added EcM; control); (2) *Laccaria laccata* (Scop.) Cooke (REB # 205-MSU) and (3) *Laccaria nobiles* A.H. Smith (REB # 162-262 mix-MSU), all using a cornmeal–sand inoculum procedure (Baird et al. 1992); and (4) a natural soil inoculum which consisted of soil transferred from under nearby mature Fraser Fir trees. Coverage treatments included (1) no soil cover (bare exposed soil with no additional coverage; control) or (2) an artificial cover (34 cm- CocoDisc®; Timm Enterprises Ltd., Milton, ON, Canada). These CocoDiscs simulate the way bryophyte cover increases water-retention potential and provides weed suppression. All treatments and control microplot combinations were replicated 4 times using each family for a total of 464 plots. In plots covered with a CocoDiscs, we used three 15-cm long GreenStake® biodegradable staples (VivaGreen Group, Dublin, Ireland) to secure the CocoDiscs to the ground to prevent seedling damage or loss of CocoDiscs from heavy winds. We planted an average of 1200 seedlings from 2 age classes (1-year-old and 2-year-old greenhouse-grown seedlings) for both years using 3–4 replicates of a single half-sib family placed within each microplot. We planted seedlings from the greenhouse in August 2012 and June 2013.

In all cases, for EcM treatments, we added 15 cm^3 of either artificial prepared inoculum (cornmeal–sand) or natural soil (15 ml) into each dibble hole throughout the root zone of the seedling. For the first 2 years of the study, each established microplot consisted of 3 seedlings with 4 replicates/treatment except for family 212, for which we planted only 2 seedlings per microplot due to low viability of the seed during greenhouse establishment.

Planting year 2014 (project 2)

During June 2014, we planted 3-year-old greenhouse-grown seedlings and locally collected Fraser Fir seedlings in microplots adjacent to the project 1 site. The local seedlings were from natural stands growing near (within 1 km) the planting site and were lifted then planted within 2 hours into the study microplots. The planting area included 180 plots (4 seedlings/treatment/replication) that we established using 720 greenhouse-grown containerized seedlings, and 20 plots using 80 naturally occurring seedlings (same approximate age class as nursery seedlings) we collected from surrounding Fraser Fir sites. Due to unbalanced numbers of container-grown seedlings per family remaining at 3 years, we no longer separated families by microplots and randomly planted individual seedlings from the different families within the microplots across all the treatments. However, we recorded specific family information on seedlings for each microplot.

For project 2, we were testing if seeding type (greenhouse grown or wild transplanted), coverage (no coverage and artificial coverage [CocoDiscs]), and inoculum source (bare soil planted or natural soil inoculum [15 cm^3] collected from underneath nearby mature Fraser Fir) influenced seedling growth and survivability. We collected natural soil and seedlings (200) from an adjacent understory of Fraser Fir stand.

After seedling establishment, we repeatedly surveyed each individual plant for survivorship and height (cm). Data collection dates were 23 July 2013 (project 1), 23 September 2013 (project 1), 24 October 2014 (projects 1 and 2), and 18 November 2018 (projects 1 and 2).

Analyses

Project 1. Visually, seedlings planted in August 2012 appeared to have significant frost-heave, increased mortality, and reduced growth overtime, compared to June 2013 plantings. Because of the heaving damage, the 2012 and 2013 plantings had apparent differential seedling success. To confirm, regression analyses were conducted of heights over time, and these results indicated that seedling heights were significantly impacted by age of seedlings ($t = 29.71$, $P < 0.001$), planting year ($t = -9.06$, $P < 0.001$), and their interaction ($t = -2.52$, $P = 0.012$). Survivorship was different across planting events based on likelihood tests ($\chi^2 = 119.425$, $P < 0.0001$). Consequently, we analyzed the 2012 and 2013 planting events separately to compare how the planting time of year impacted treatment effects on growth and survivability.

To investigate if treatments affected growth and height of seedlings for each year analyzed, we used a repeated-measures ANOVA approach with Kenward–Roger first-order approximations and Kacker–Harville corrections, as different families likely have different growth distributions, and Kenward–Roger approximations are robust given these differences (Arnau et al. 2014). We nested replicates within microplots and used as the repeated measures variable. The models consisted of examining plant height against cover treatment, mycorrhizal treatment, plant family, and months since planting. In addition, to determine effect size of any significant factors, we calculated partial eta-squared (η^2_p) effect sizes (Cohen 1965). Also, where significant, we conducted Tukey HSD post-hoc tests to examine which treatments differed.

Further, to examine the impacts of our treatments on seedling survivability, we generated survivorship curves by tabulating average proportion of seedlings that remained alive for each treatment at each timepoint. We used a k-sample Anderson–Darling framework (Scholz and Stephens 1987) using the R (v 4.1.1; R Development Foundation, Austria, Vienna) package ‘k-Samples’ (v.1.2-9; Scholz and Zhu 2019) to test if survivorship differed across treatments; we did this for each treatment and across each year’s plantings separately. We used the exact *P*-value calculation method and a Šidák correction (αSID) for multiple comparisons. These assays test if observed distributions differ across categories (treatments) or belong to a common unspecified distribution. Where treatment effects were significant, we conducted post hoc AD tests to examine which treatments differed.

Project 2. To investigate if reforestation treatments impact growth of seedlings, we used a repeated measures ANOVA approach (same as project 1). The model consisted of plant height against seedling type (greenhouse grown vs. natural seedlings), coverage treatment (CocoDisc or not covered), natural mycorrhizal inoculation (yes, no), and time since planting (months). Where significant, we calculated partial eta-squared (η^2_p) effect sizes (Cohen 1965) and conducted Tukey HSD post-hoc tests to examine which treatments differed. To assess if treatments impacted seedling survivability, we used a similar k-sample Anderson–Darling framework as we did for project 1 for each treatment to test if different treatments resulted in differential survivorship.

To visualize effects of seedling planting treatments on seedling height over time (for both projects), we fit kernel-smoothing (loess curve) local-fit lines ($\lambda = 2$, $\alpha = 0.1$, $\Delta = 0$; Cleveland 1979) using the tri-cube weight function with 4 iterations in JMP Pro (v15; SAS Institute, Cary NC). To visualize survivability, we generated discrete survivorship curves across all sampling events, across treatments. All statistical analyses were conducted using a combination of JMP Pro (v15) and R (v 4.1.1).

Results

Project 1: comparison between planting years

Due to observed differences between seedling growth (Table 1) and survival between the 2012 and 2013 planted seedlings, these data were analyzed separately.

Table 1. Multiple regression analysis comparing seedling survival or heights over months and treatments for August 2012 and June 2013 planting dates.

Variable	*t*-ratio	*P*
Intercept	-2.67	0.0078
Months since planting (age)	29.71	<0.0001
Planting year (year)	-9.60	<0.0001
Age x year	-2.52	0.0120

The August 2012 planting event had stunted growth over time (months since planting) relative to the June 2013 plantings (mean ± SE = 12.09 cm ± 1.34 cm, 95% CI = 10.26–15.54 cm reduction in height compared to similar-aged seedlings from 2013). Further, plantings in 2012 (August) had significantly higher mortality rates than those planted in 2013 (June) based on likelihood tests ($\chi^2 = 119.425$, $P < 0.0001$). While individual mortality was high for both planting years (2012: 87.16%, 2013: 73.87%), mortality was much higher when seedlings were planted in August 2012. For those surviving seedlings, the 2012 seedlings grown in the greenhouse had survival rates at 75 months after planting varying from 5.37% to 12.50% for the different seed sources (families), compared to local transplanted seedlings at 11.45%. But for 2013, at 65 months after planting, greenhouse-growth seedling had survival varying from 19.79% to 40.40% (by families), while local transplanted seedlings had 32.29% survival rates. Thus, of the surviving seedlings, those planted in 2013 were ~18% more likely to survive compared to 2012, which perhaps suggests that planting earlier in the growing season is a cost-effective way to increase efficacy of replanting success.

Project 1: seedling survival

We saw differential effects on seedling survivorship with treatments depending on which year they were planted (August 2012 compared to June 2013; Table 2, Fig. 1). For both 2012 and 2013 planting events, seedling survivability was not impacted by inclusion of CocoDisc ground coverings. While non-significant, the presence of CocoDiscs did increase plant average survival in 2012 (15.36% survival at the terminal sampling when CocoDiscs were present compared to 6.89% without CocoDiscs; see Supplemental File 1, available online at https://www.eaglehill.us/SENAonline/suppl-files/s22-3-S2826-Brown-s1, and for BioOne subscribers, at https://www.doi.org/10.1656/S2826.s1), and also in 2013, but to a much lesser extent. Seedlings did have differential survivorship depending on seedling source (plant family) for both years, but in different ways across the 2 years. While survivability was much lower in the 2012 plantings, plant families 224 and 225 had significantly higher survivability, whereas in the 2013 plantings, plant family 212 had much higher survivorship (47.5% higher) than the next best

Table 2. Results of k-sample-based Anderson–Darling (AD) tests of seedling survivability across planting years. Presented are tests (with Šidák corrected α_{SID} for each test presented parenthetically), the AD test statistic (T.AD) and *P*-value. Significant results are indicated with "*".

Test	AD Test Statistic	*P*-value
2012 plantings (August)		
Cover treatment (α_{SID} = 0.025)	2.484	0.1000
Mycorrhizal treatment (α_{SID} = 0.013)	3.765*	0.0023
Plant family (α_{SID} = 0.0073)	5.654*	<0.0001
2013 plantings (June)		
Cover treatment (α_{SID} = 0.025)	-0.867	0.9052
Mycorrhizal treatment (α_{SID} =0 .013)	1.525	0.0972
Plant family (α_{SID} = 0.0073)	3.880*	0.0004

performing families (families 208 and 213). Mycorrhizal treatments improved survivorship of seedlings in 2012 (T.AD = 3.765, $P = 0.002$) but not in 2013. For the 2012 planting, natural inoculum and *L. laccata* had increased survivorship at our final sampling event (2018) compared to the control treatment (3.7% and 27.7% more surviving seedlings than controls, respectively), but *L. nobilis*-inoculated seedlings had decreased survivability.

Project 1: seedling height comparisons

Heights of reforested firs showed differential patterns depending on which year they were planted. We see, unsurprisingly, that months since planting impacts seedling total height. Overall, for 2012 and 2013, neither cover treatment nor plant family influenced seedling height (Table 3), but mycorrhizal treatment did affect seedling height in 2013 planting only ($F_{3,207.4}$=10.867, $P < 0.001$), albeit with a small effect size. Post-hoc tests indicated that mycorrhizal treatments led to greater plant heights over time compared to local transfer, but our controls were more ambiguous (*Laccaria laccata*^{A}: 41.043, *Laccaria nobilis*^{A,B}: 36.571, control^{B,C}: 33.824, local transfer^{C}: 27.584; least square means are reported and treatments with different superscript letters are significantly different per Tukey HSD test results). This result is also seen in Figure 2, which visualizes seedling height over time with

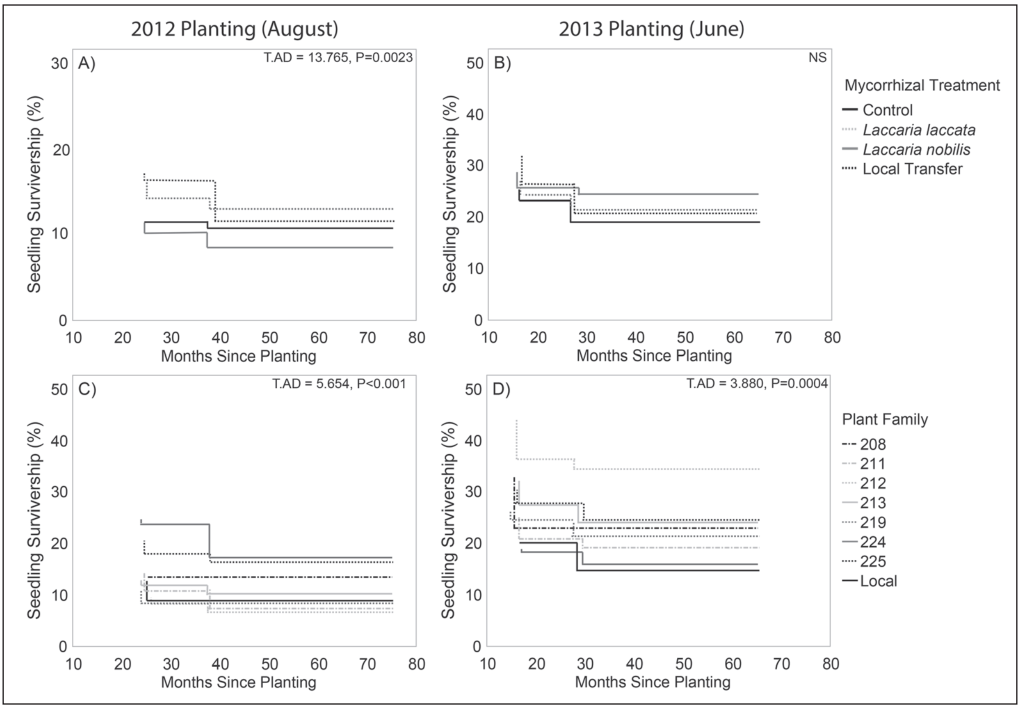

Figure 1. Suvivorship curves of Fraser Fir seedlings for 2012 plantings (A, C), and 2013 plantings (B, D) for mycorrhizal treatments (A, B) and across seedling families (C, D) at Clingmans Dome in Great Smoky Mountains National Park. Suvivor curves for mulch coverage treatments are not shown as this factor did not impact seedling suvivorship for either 2012 or 2013 (Table 2).

mycorrhizal treatment using kernel smoothing (Cleveland 1979). In the 2012 planting, all mycorrhizal treatments resulted in similar heights.

We examined if seedling survivability was affected by treatments using a k-sample Anderson–Darling framework (as above). While none of the treatments demonstrated significant differences in seedling survivability (Table 4), some treatments trended toward increased survivability (see Supplemental File 1), seedlings grown in containers had marginally better survivability at our terminal sampling date than wild plantings (63.19% compared to 47.61%), and the presence of Coco-Discs slightly reduced seedling survivability compared to no coverage (52.27% compared to 63.39%).

Project 2: seedling height comparisons

For all treatment categories, seedling height increased with time, but only seedling type (replanted local seedlings or greenhouse grown) had differential seedling heights (Table 5), albeit with relatively small effect sizes; inoculations

Table 3. Results of repeated-measures ANOVA on seedling heights for reforestation treatments. Presented are *F* statistics (degrees of freedom are based on Kenward–Roger first-order approximations with Kacker–Harville corrections), *P*-values, and where significant (*) partial η^2 effect sizes are presented parenthetically.

	Year	
Treatment	2012 (August)	2013 (June)
Cover treatment	$F_{1,101.1} = 0.328$, $P = 0.568$	$F_{1,210.3} = 3.507$, $P = 0.063$
Mycorrhizal treatment	$F_{3,97.06} = 1.217$, $P = 0.308$	$F_{3,207.4} = 10.867$, $P < 0.001$ (0.057)*
Plant family	$F_{7,98.98} = 0.740$, $P = 0.639$	$F_{7,209.1} = 0.608$, $P = 0.749$
Months since planting	$F_{1,182} = 420.677$, $P < 0.001$ (0.623)*	$F_{1,383} = 1104.856$, $P < 0.001$ (0.674)*

Table 4. Results of k-Sample-based Anderson-Darling (AD) tests of seedling survivability across study years. Presented are tests (with Šidák corrected α_{SID} for each test presented parenthetically), the AD test statistic (T.AD) and *P*-value.

Test	AD test statistic	*P*-value
Seeding type (α_{SID}=0.025)	0.504	0.2076
Inoculation treatment (α_{SID}=0.025)	-0.756	0.8155
Coverage treatment (α_{SID}=0.025)	-0.809	0.8630

Table 5. Results of repeated-measures ANOVA on seedling heights with treatments. Presented are *F* statistics (degrees of freedom are based on Kenward–Roger first order approximations with Kacker-Harville corrections), *P*-values, and where significant (*), partial η^2 effects size are presented parenthetically.

	F statistic	*P*-value (η^2_p)
Seedling type	$F_{1,949.1} = 21.935$	<0.001 (0.007)*
Inoculation treatment	$F_{1,888.1} = 1.225$	0.268
Coverage treatment	$F_{1,908.3} = 1.170$	0.279
Time since planting	$F_{1,3096} = 6221.752$	<0.001 (0.636)*

and coverage treatments did not affect seedling height. At our terminal sampling event (65 months post planting), wild seedlings were shorter than container-grown plants (mean ± SE = 59.325 cm ± 3.810 cm and 64.554 cm ± 1.433 cm, respectively; Fig. 3).

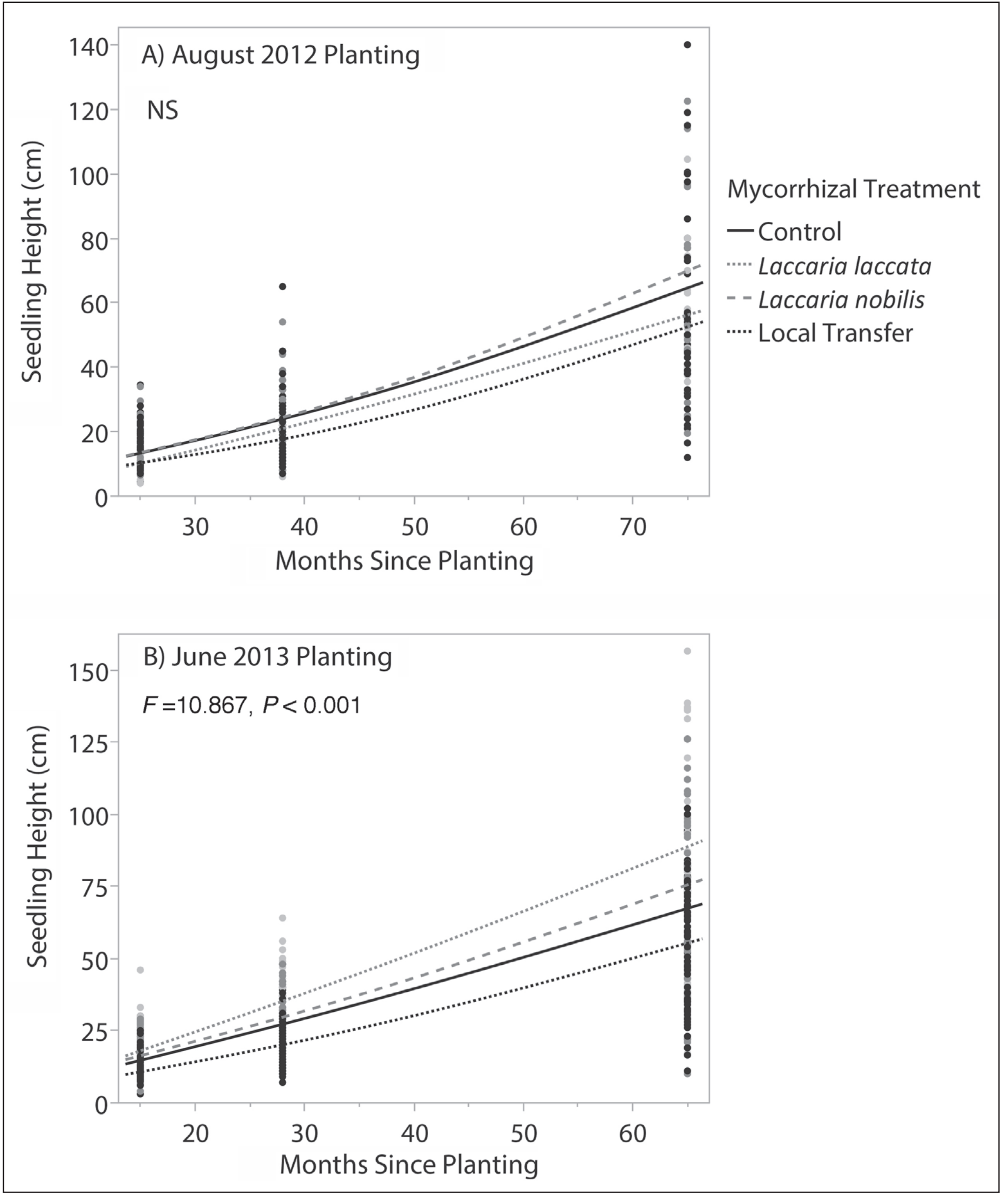

Figure 2. Growth of seedlings over time as related to mycorrhizal treatments for (A) the 2012 planting and (B) the 2013 planting. Mycorrhizal treatments did not affect seedling height for 2012 but did for the 2013 plantings, where *Laccaria* inoculation improved growth.

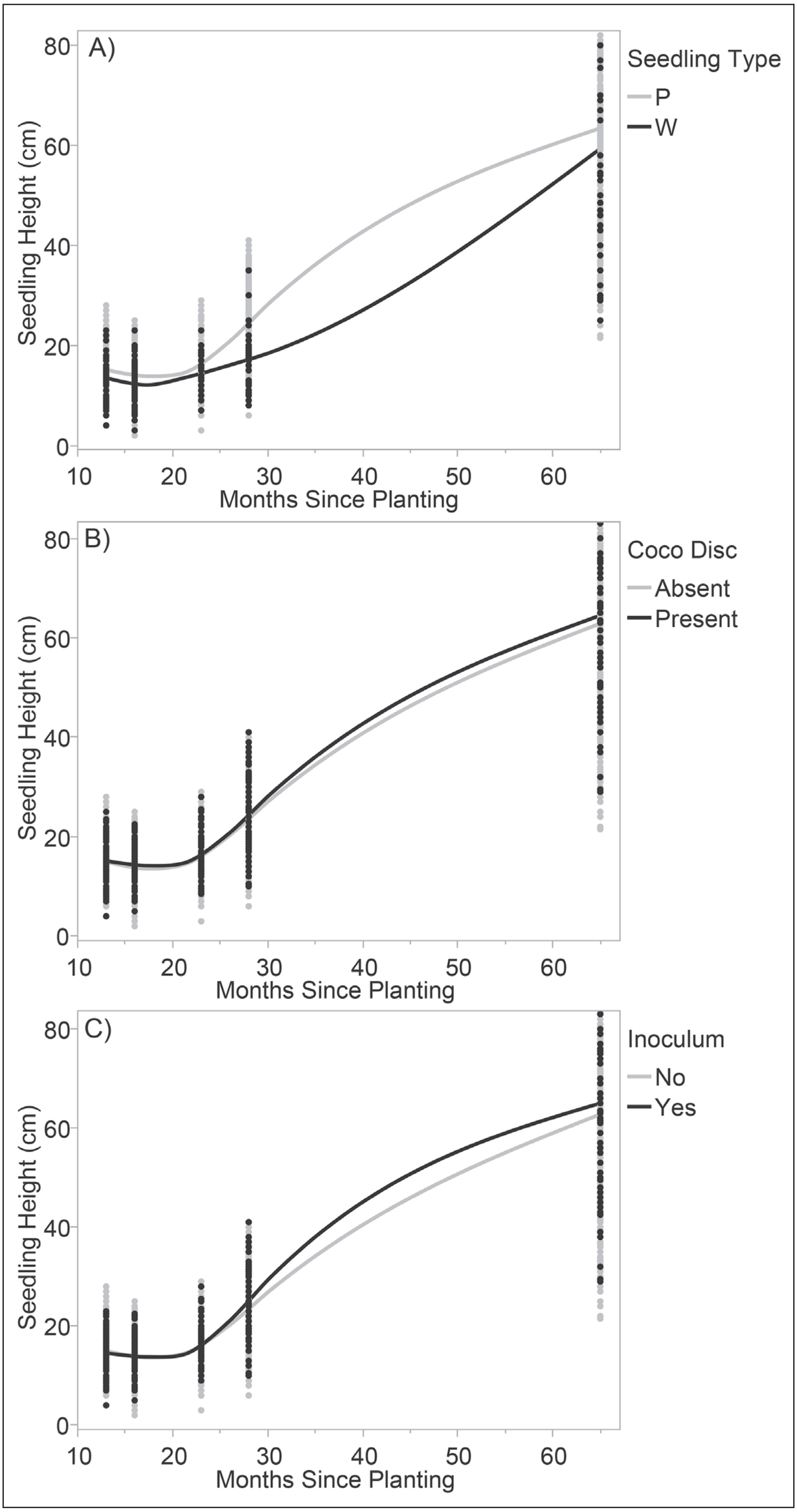

Figure 3. Growth of seedlings over time as related to (A) seedling type, (B) coverage treatment, and (C) inoculation treatment presented as kernel-smoothed local best-fit lines. Only seedling type differentially impacted seedling height with greenhouse-started seedlings having increased height compared to wild seedlings. P = greenhouse grown seedlings, W = wild transplanted seedlings.

Discussion

In the present investigation, we hypothesized that Fraser Fir survivorship and plant height would be impacted by site-preparation methods and mycorrhizal inoculants for reforestation success at the high-elevation bald located at Mt. Buckley in GRSM. The site was very dense in vegetation, including *Rubus ulimifolius* Schott (Thornless Blackberry), wood sorrel, and wood fern, on a steep slope (42%) with a rock layer close to the surface often making seedling planting challenging. Treatments were similar for the 2012 and 2013 planting events aside from timing of planting, but since there were significant age x planting year interactions (Table 1), we analyzed these planting events separately. The fall planting conducted in 2012 (August) had significantly greater mortality compared to the 2013 planting event (June). This result may be due to several reasons, which are not mutually exclusive. The seedlings used for fall 2012 planting date were ~12 months old (1-0) at planting, whereas the seedlings in the greenhouse continued their growth and were ~21 months old (2-0) by spring 2013 planting and thus had better-developed root systems and more aboveground growth, which may improve establishment. It has previously been reported that root size and substrate moisture were important factors for increased survival of subalpine and alpine *Abies* forests especially on warmer, drier sites with increased levels of solar radiation (Johnson and Yeakley 2016). At our planting site, which occurred on the southern-facing slope of Clingmans Dome, there was no overstory, resulting in increased initial radiation, temperatures, and vegetation crowding that may have affected survival. Here, soils were also poorly developed and there was much exposed bedrock. Therefore, when considering reforestation of high-elevation sites, seedling size becomes important due to environmental stressors especially with global warming becoming increasingly a factor in reforestation of boreal or alpine forests.

Another consideration affecting seedling survival and growth here is that onset of winter conditions occurred soon after the 2012 planting, which may have prevented adequate root establishment. A previous study determined that snow packing duration and freeze–thawing cycles are more important to survivorship of *Picea glauca* (Moench) Voss (White Spruce) seedlings compared to summer temperature and rainfall, due to the effects of frost and desiccation (Renard et al. 2016). During the first sampling date, the following spring, it was noted that many of the planted seedlings (~30%), regardless of treatments, had some level of frost-heaving and root exposure resulting in seedling mortality. In a previous high-elevation reforestation study, snow depth acted as insulation against frost damage (Goulet 2000). Furthermore, extent and method of ground preparation and soil type greatly influence the percent of frost heaving, conditions that were not matched between our 2 studies.

At our Mount Buckley 2012 planting site, grassy vegetation initially invaded all plots following the 2013 growing season. The neighboring shrub, fern, and herbaceous plant growth was suppressive to seedling survival, which might explain our relative low survival rates seen here. However, vegetation residue can protect seedlings from frost and snow damage by insulating the seedling (Fajardo and McIntire 2011), and protecting them from wind desiccation (Cavieres et al. 2006), and drought stress (Renard et al. 2016).

Establishment of seedlings in boreal forest ecotones depends on planting-bed composition (Wheeler et al. 2011). The microhabitat of a planting site can be influenced by physical composition (e.g., rocky, shallow, or deep soils), available soil nutrients, and existing local vegetation. Competition of seedlings with some plant species can limit survivorship, while moss layers suppresses other plant colonizers, facilitates soil stabilization, and enhances water retention (Simard et al. 1998, Wheeler et al. 2011). Because of these previous observations, we used biodegradable CocoDiscs as a substitute for moss cover. However, CocoDiscs did not impact seedling survivorship or height for either project 1 or project 2. With lack of substantial benefits to using CocoDiscs during initial planting, their omission at such sites will make these reforestation efforts more cost effective.

The Fraser Fir seedlings planted in June 2013 survived better and grew faster than those planted in 2012. All these seedlings were greenhouse grown, and we believe that these 2-0 seedlings benefited from an additional year of root and shoot growth and a full growing season before winter. Similarly, the seedlings planted in 2014 had the largest sizes at planting (both greenhouse grown and transplanted seedlings), plus a full growing season enabling them to compete with the ground cover.

Field seedling collections and mycorrhizae inocula preparation followed by pre-study screening are labor intensive and require greenhouse utilization for 3 years and laboratory inoculum preparations. To evaluate the effect of a reduced cost and time effort for Fraser Fir reforestation, natural local seedlings were lifted from Fraser Fir understories along with naturally inoculated soil, containing a complex native mycorrhizal population, and transplanted on our site. These were used for comparison to the greenhouse-grown seedlings and laboratory-produced mycorrhizal inoculum. The greenhouse-grown seedlings planted during the first 2 years of the study had similar mean percent survivorship to the natural local transplanted seedlings. However, at the time of the planting, average seedling height was greater for natural seedling transfers compared to those grown in the greenhouse. Seedlings that have the greatest heights may be critical for reducing light competition from dense vegetation. A previous study using seed-sowing and seedling transfers of several hardwood and conifer tree species in the Alaskan boreal forest resulted in seedling transfers having more variable survivorship, especially in the presence of different understory vegetation species (Cater and Chapin 2000). When vegetation was cleared prior to transplantation of seedlings, survivorship for White Spruce *Betula papyrifera* Marshall (Paper Birch) seedlings overcame dominant and established species (Connell and Slatyer 1977). From these results, it was assumed that vegetation removal within planting zones might be important for Fraser Fir seedling establishment.

Mycorrhizal inoculum is thought to be an important component of Fraser Fir seedling transplantation success by providing the plant an advantage in scavenging limited nutrients and water, especially in open areas or sites (Baird et al. 2014). Obtaining and preparing compatible mycorrhizal inoculum for specific tree seedlings can be labor intensive, time consuming, and expensive, especially if local genetic sources must be identified and utilized. In this study *L. lacccata* inoculum-treated seedlings had minimally significantly higher survivorship in 2012 compared to the natural soil inoculum and untreated plots, but they were similar to the control in 2013. Even though there may be a slight advantage conferred by prepared mycorrhizal inoculum on Fraser Fir seedling height growth for the 2013 planting, this treatment had a low effect size suggesting it may be minor biologically ($\eta^2 = 0057$; Table 3). Preparation time, need for endemic isolates, costs for preparation, technical expertise, and in-field applications may not be practical for all land managers. Furthermore, common mycorrhizal fungi such as *L. laccata* and *Cenococcum graniforme* may be inhibited by phenolic compounds formed by *Vaccinium, Athyrium,* and *Picea* spp. in spruce forests (Pellissier 1993). These concerns further lend support to the hypothesis that endemic natural seedlings are most likely associated with compatible mycorrhizal components for seedling establishment in the presence of competing vegetation. The introduced inoculum was not cost effective compared to collection of native soils from adjacent Fraser Fir stands and had only marginal benefits.

Developing a feasible approach to reestablish Fraser Fir stands in GRSM is possible. The results of this research using select site preparations and seedling families from endemic parent trees did not justify these additional measures that are labor intensive and costly. Further, implementation on US National Park lands required adherence to specific regulations that our design had to comply with, as outlined below along with our suggestions and recommendations for reforestation of Frasier fir on national park land:

1.Seeds from multiple parent trees from the same geographical area must be collected and seed vigor must be tested in advance.
2. To maintain genetic purity, any EcM inoculum cultures used must be obtained from endemic basidiocarps. Using natural adjacent soils can also provide EcM complexes locally adapted to forming symbiotic associations with the seedlings.
3. Greenhouse-grown seedlings require seed from the area where planting would be done to maintain genetic purity. Seedlings used for planting require at least 2 years of greenhouse growth as shown by survival rates of this study. However, keeping the seedling container media clean from contaminants is difficult during the required 2–3-year window and may be economically prohibitive. Natural Fraser Fir seedlings strategically collected from stands adjacent to the planting site would be the most economical and logistically feasible alternative while also reducing the possibility of the introduction of unwanted genetic microbial sources or pathogens affecting survival.
4. Clearing planting sites, including removing root masses of lesser vegetation (e.g., ferns) around the planted seedlings, gives seedlings time to establish for at least a year, which improves early survival outcomes. The soil clearing and planting is labor intensive, but GRSM park volunteers and other organizations such as AmeriCorps can provide labor at almost no cost,

and there are other volunteer operations active at other suitable reforestation sites. Also, using lifted local wild seedlings eliminates the need for ectomycorrhizal inoculum added to each planting hole. This approach may also be preferable as it simplifies compliance with the portions of the US Department of Interior policies (§ 4.4.1.2 and § 4.4.2.2) that the National Park Services has used to guide this type of reforestation effort (National Park Service 2006). These policies specifically require closely related genetic and ecological resources for restoration efforts.

5. Using cover such as CocoDiscs can be used but are required to be biodegradable per NPS regulations. However, this additional effort may not improve survivorship of the Fraser Fir seedlings.

Results of this study showed minimal or no benefits for outside introduction of endemic nursery-produced seedlings, EcM inocula, and artificial surface soil covers. Instead, we demonstrated that reforestation of Fraser Fir using natural seedling sources within the planting vicinity provides the seedlings' own complex of EcM that yielded similar or negligibly different reforestation potential with minimal costs and time of preparations. Based on these findings, the natural seedling approach could enable reforestation of Fraser Fir to be done more expediently, potentially resulting in reestablishment of Frasier fir within its natural range. We suggest that this is the best approach due to both regulatory issues and economic considerations.

Acknowledgments

The authors acknowledge AmeriCorps and Friends of the Smokies volunteers who participated in plot establishment, data collection, and plot maintenance across 3 planting dates. We thank personnel of the Park Service, Great Smoky Mountains National Park, especially Paul Super, Kristine Johnson, and Glenn Taylor for locating the reforestation site to conduct the research, guidance to appropriately establish the seedlings using accepted methodology, and field support to conduct the study. This work was partially supported by the Friends of the Great Smoky Mountains National Park (R. Baird), Highlands Biological Station, Grant-In-Aide program from 2010-2015 (R. Baird) and the North Carolina State University Christmas Tree Genetics Program.

Literature Cited

Arnau, J., R. Bendayan, M.J. Blanca, and R. Bono. 2014. Should we rely on the Kenward–Roger approximation when using linear mixed models if the groups have different distributions? British Journal of Mathematical and Statistical Psychology 67:408–429.

Baird, R.E., J.P. Wilson, and D.R. Sumner. 1992. Identity and pathogenicity of two *Marasmius* species from the sterile white Basidiomycete complex. Plant Disease 76:244–247.

Baird, R., C.E. Stokes, J. Frampton, B. Smith, C. Watson, C. Pilgrim, and M. Scruggs. 2014. Diversity and density of the EM fungal community present in high-elevation Fraser Fir forests of Great Smoky Mountains National Park. North American Fungi 9:1–21.

Bills, G.F., G.I. Holtzman, and O.K. Miller Jr. 1986. Comparison of ectomycorrhizal–basidiomycete communities in Red Spruce versus northern hardwood forests of West Virginia. Canadian Journal of Botany 64:760–768.

Bird, C., and C. McCleneghan. 2005. Morphological and functional diversity of ectomycorrhizal fungi on Roan Mountain (NC/TN). Southeastern Naturalist 4:121–132.

Brown, S.P., S.L. Clark, E. Ford, A. Jumpponen, A.M. Saxton, S.E. Schlarbaum, and R. Baird. 2022. Comparisons of interspecies field performance of Fagaceae (*Castanea* and *Quercus*) planted in the southeastern United States with attention to soil fungal impacts on plant performance. Forest Ecology and Management 525:120569.

Busing, R.T., E.E.C. Clebsch, C.C. Eagar, and E.F. Pauley. 1988. Two decades of change in a Great Smoky Mountains spruce–fir forest. Bulletin of the Torrey Botanical Club 115:25–31.

Cai, M., A.M. Johnson, J.S. Schwartz, S.E. Moore, and M.A. Kulp. 2012. Soil acid–base chemistry of a high-elevation forest watershed in the Great Smoky Mountains National Park: Influence of acidic deposition. Water, Air, and Soil Pollution 223:289–303.

Cater, T.C., and F.S. Chapin III. 2000. Differential effects of competition or microenvironment on boreal tree seedling establishment after fire. Ecology 81:1086–1099.

Cavieres, L.A., E.I. Badano, A. Sierra-Almeida, S. Gómez-González, and M.A. Molina-Montenegro. 2006. Positive interactions between alpine plant species and the Nurse Cushion Plant, *Laretia acaulis*, do not increase with elevation in the Andes of central Chile. New Phytologist 169:59–69.

Claridge, A.W., J.M. Trappe, and K. Hansen. 2009. Do fungi have a role as soil stabilizers and remediators after forest fire? Forest Ecology and Management 257:1063–1069.

Cleveland, W.S. 1979. Robust locally weighted regression and smoothing scatterplots. Journal of the American Statistical Association 74:829–836.

Cohen, J. 1965. Some statistical issues in psychological research. Pp. 95–121, *In* B. Wolman (Ed.). Handbook of Clinical Psycology. McGraw-Hill, New York, NY. 159 pp.

Connell, J.H., and R.O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. American Naturalist 111:1119–1144.

Crandall, D.L. 1958. Ground vegetation patterns of the spruce–fir area of the Great Smoky Mountains National Park. Ecological Monographs 28:337–360.

Dull, C.W., J.D. Ward, H.D. Brown, G.W. Ryan, W.H. Clerke, and R.J. Uhler. 1988. Evaluation of spruce and fir mortality in the southern Appalachian mountains. USDA/FS Protection Report R8-PR13. US Forest Service, Southern Region, Atlanta, GA. 92 pp.

Fajardo, A., and E.J.B. McIntire. 2011. Under strong niche overlap, conspecifics do not compete but help each other to survive: Facilitation at the intraspecific level. Journal of Ecology 99:642–650.

Farmer, J., D. Knapp, and G.M. Benton. 2010. An elementary school environmental education field trip: Long-term effects on ecological and environmental knowledge and attitude development. Journal of Environmental Education 38:33–42.

Frampton, J., and D.M. Benson. 2012. Seedling resistance to *Phytophthora cinnamomi* in the genus *Abies*. Annals of Forest Science 69:805–812.

Gould, A. 2004. Plant pathogenic fungi. Pp. 75–89, *In* R. Trigiano, M. Windham, and A. Windham (Eds.). Plant Pathology: Concepts and Laboratory Exercises. 1st Edition. CRC Press, Boca Raton, FL. 432 pp.

Goulet, F. 2000. Frost heaving of plant tree seedlings in the boreal forest of Northern Sweden. M.Sc. Thesis. SLU, Umea, Sweden. 16 pp.

Hackett, H.M., and J.F. Pagels. 2003. Nest-site characteristics of the endangered Northern Flying Squirrel (*Glaucomys sabrinus coloratus*) in Southwest Virginia. American Midland Naturalist 150:321–331.

Ingham, E.R. 1992. Interactions between invertebrates and fungi: Effects on nutrient availability. Pp. 669–690, *In* G. Carroll and D. Wicklow (Eds.). The Fungal Community: Its Organization and Role in the Ecosystem. 2nd Edition. Marcel Dekker, Inc., New York, NY. 952 pp.

Johnson, A.C., and J.A. Yeakley. 2016. Seedling regeneration in the alpine treeline ecotone: Comparison of wood microsites and adjacent soil substrates. Mountain Research and Development 36:443–451.

Kelly, J.M., and P.A. Mays. 1989. Root-zone physical and chemical characteristics in southeastern spruce–fir stands. Soil Science Society of America Journal 53:1248–1255.

Köppen, W. 1936. C.R. Köppen (Ed.). Das Geographische System der Klimate. Vol. 1. Handbuch der Klimatologie. Borntraeger, Berlin, Germany. 44 pp.

Lix, H.W. 1958. Short History of the Great Smoky Mountains National Park. Great Smoky Mountian National Park, Gatlinburg, TN, USA. 126 pp.

McKeand, S., F. Bridgwater, C. McKinley, J. Jett, and R. Arnold. 1995. 1994 seed collection from natural stands of Fraser Fir and plans for breeding and genetics research at NCSU. Limbs and Needles 22:4–7.

National Park Service. 2006. Management policies 2006. US Government Printing Office, Washington, DC. 168 pp.

Nicholas, N.S., S.M. Zedaker, C. Eagar, and F.T. Bonner. 1992. Seedling recruitment and stand regeneration in spruce–fir forests of the Great Smoky Mountains. Bulletin of the Torrey Botanical Club 119:289–299.

Oosting, H.J., and W.D. Billings. 1951. A comparison of virgin spruce–fir rorest in the northern and southern Appalachian system. Ecology 32:84–103.

Pauley, E.F., and E.E.C. Clebsch. 1990. Patterns of *Abies fraseri* regeneration in a Great Smoky Mountains spruce–fir forest. Bulletin of the Torrey Botanical Club 117:375–381.

Pellissier, F. 1993. Allelopathic inhibition of spruce germination. Acta Oecologica 14:211–218.

Policelli, N., T.R. Horton, A.T. Hudon, T.R. Patterson, and J.M. Bhatnagar. 2020. Back to roots: The role of ectomycorrhizal fungi in boreal and temperate forest restoration. Frontiers in Forests and Global Change 3:97.

Potter, K.M., J. Frampton, S.A. Josserand, and C. Dana Nelson. 2010. Evolutionary history of two endemic Appalachian conifers revealed using microsatellite markers. Conservation Genetics 11:1499–1513.

Renard, S.M., E.J.B. McIntire, and A. Fajardo. 2016. Winter conditions—not summer temperature—influence establishment of seedlings at White Spruce alpine treeline in Eastern Quebec. Journal of Vegetation Science 27:29–39.

Scholz, F.W., and M.A. Stephens. 1987. K-sample Anderson–Darling tests. Journal of the American Statistical Association 82:918–924

Scholz, F., and A. Zhu. 2019. kSamples: K-Sample rank tests and their combination. Version 1.2-9. Available online at https://cran.r-project.org/web/packages/kSamples/kSamples.pdf.

Shanks, R.E. 1954. Climates of the Great Smoky Mountains. Ecology 35:354–361.

Simard, M.-J., Y. Bergeron, and L. Sirois. 1998. Conifer seedling recruitment in a southeastern canadian boreal forest: The importance of substrate. Journal of Vegetation Science 9:575–582.

Smith, G.F., and N.S. Nicholas. 2000. Size- and age-class distributions of Fraser Fir following Balsam Woolly Adelgid infestation. Canadian Journal of Forest Research 30:948–957.

Sýkorová, Z., J. Rydlová, R. Slavíková, T. Ness, P. Kohout, and D. Püschel. 2016. Forest reclamation of fly ash deposit: A field study on appraisal of mycorrhizal inoculation. Restoration Ecology 24:184–193.

Wheeler, J.A., L. Hermanutz, and P.M. Marino. 2011. Feathermoss seedbeds facilitate Black Spruce seedling recruitment in the forest–tundra ecotone (Labrador, Canada). Oikos 120:1263–1271.

White, P.B., G.B. van de Gevel, and P.T. Soulé. 2012. Succession and disturbance in an endangered Red Spruce–Fraser Fir forest in the southern Appalachian Mountains, North Carolina, USA. Endangered Species Research 18:17–25.

Whittaker, R.H. 1956. Vegetation of the Great Smoky Mountains. Ecological Monographs 26:2–80.

Williams, W. 1958. Fraser Fir as a Christmas tree. US Department of Agriculture Forest Service/Extension Service, Washington, DC. 9 pp.

Witter, J.A., and I.R. Ragenovich. 1986. Regeneration of Fraser Fir at Mt. Mitchell, North Carolina, after depredations by the Balsam Woolly Adelgid. Forest Science 32:585–594.