10-2015

Evaluating the Use of Tree Shelters for Direct Seeding of *Castanea* on a Surface Mine in Appalachia

Christopher Barton
University of Kentucky, barton@uky.edu

Jarrod Miller
University of Maryland Extension

Kenton Sena
University of Kentucky, kenton.sena@uky.edu

Patrick Angel
Office of Surface Mining

Michael French
American Chestnut Foundation

Repository Citation

Barton, Christopher; Miller, Jarrod; Sena, Kenton; Angel, Patrick; and French, Michael, "Evaluating the Use of Tree Shelters for Direct Seeding of *Castanea* on a Surface Mine in Appalachia" (2015). *Forestry and Natural Resources Faculty Publications*. 6.
https://uknowledge.uky.edu/forestry_facpub/6

Evaluating the Use of Tree Shelters for Direct Seeding of *Castanea* on a Surface Mine in Appalachia

Notes/Citation Information
Published in *Forests*, v. 6, no. 10, p. 3514-3527.

Digital Object Identifier (DOI)
http://dx.doi.org/10.3390/f6103514

Forests **2015**, *6*, 3514-3527; doi:10.3390/f6103514

OPEN ACCESS

forests

ISSN 1999-4907

www.mdpi.com/journal/forests

Article

Evaluating the Use of Tree Shelters for Direct Seeding of *Castanea* on a Surface Mine in Appalachia

Christopher Barton [1,*], Jarrod Miller [2], Kenton Sena [1], Patrick Angel [3] and Michael French [4]

[1] Department of Forestry, University of Kentucky, Lexington, KY 40546, USA; E-Mail: kenton.sena@uky.edu

[2] College of Agriculture and Natural Resources, University of Maryland Extension, Princess Anne, MD 21853, USA; E-Mail: jarrod@umd.edu

[3] U.S. Office of Surface Mining, London, KY 40741, USA; E-Mail: pangel@osmre.gov

[4] The American Chestnut Foundation, Hope, IN 47246, USA; E-Mail: michael@acf.org

* Author to whom correspondence should be addressed; E-Mail: barton@uky.edu; Tel.: +859-257-2099; Fax: +859-323-1031.

Academic Editors: Phillip G. Comeau and Eric J. Jokela

Received: 4 August 2015 / Accepted: 25 September 2015 / Published: 7 October 2015

Abstract: American chestnut (*Castanea dentata*), once a primary constituent of the eastern hardwood forest ecosystem, was nearly extirpated from the forest canopy by the accidental introduction of chestnut blight (*Cryphonectria parasitica*). An intensive breeding program has sought to breed blight resistance from Chinese chestnut into American chestnuts, while maintaining as much of the desirable American chestnut phenotypes as possible. Previous studies suggest that these blight resistant American chestnuts, termed "restoration chestnuts", are capable of thriving on reclaimed surface mines. We direct seeded pure Chinese, pure American, and three backcross lines into brown sandstone minesoil on a mine site in Pike County, KY. To investigate the effects of tree sheltering on survival and growth, we installed tree shelters on half the plots, and left the rest of the plots unsheltered. Results indicated that shelters were highly effective at reducing initial mortality. In addition, while pure Chinese chestnut survival was highest, the three backcross lines have also survived well on this site. Our study demonstrates that American, Chinese, and backcrossed chestnuts can survive through five growing seasons on reclaimed surface mines with the use of tree shelters.

Keywords: American chestnut; mined land reforestation; tree shelters; herbivory

1. Introduction

The American chestnut (*Castanea dentata* (Marshall) Borkh.) was a major component of eastern hardwood forest, composing 25% to 35% of forests on Appalachian slopes in the early part of the 20th century [1]. Chestnut fruited reliably every year, providing a highly nutritious and consistent food source for a wide range of species. In addition, chestnut was an important timber species, producing strong, lightweight, rot-resistant wood used in fence posts, roofing, and other applications [2]. However, with the introduction of the invasive fungal pathogen *Cryphonectria parasitica* ((Murrill) Barr), the disease known as chestnut blight swept across the eastern U.S., all but eliminating chestnut from the forest canopy.

In response to this ecological devastation, a breeding program was initiated by the U.S. Forest Service and the Connecticut Agricultural Experimental Station and carried forward more recently by The American Chestnut Foundation (TACF). Chinese chestnuts (*Castanea mollissima* Blume), which coevolved with the pathogen and have high levels of resistance, were crossed with remnant mature American chestnuts (not resprouts) that escaped the blight or exhibited some level of resistance [3–5]. Subsequent generations demonstrating blight resistance were backcrossed with American chestnut to conserve as much of the original American chestnut phenotype as possible while still conferring blight resistance from Chinese chestnut [3–5].

More recently than the introduction of chestnut blight and ecological extirpation of chestnut from eastern forest, surface mining for coal has presented a major perturbation of native ecology in Appalachia. The surface mining process results in the removal of native forest systems, leading to direct habitat loss and forest fragmentation. In addition, native soil on Appalachian sites is typically not retained during the mining process; thus, post-mining soils tend to be composed of fragmented overburden characterized by high bulk density, unfavorable pH, and high salinity [6]. Due to these characteristics, reclaimed mine sites in Appalachia tend to be dominated by non-native grasses and legumes, habitat that is unnatural compared to pre-mining ecosystems [6].

To address these problems, researchers, reclamation practitioners, and regulators have developed the Forestry Reclamation Approach (FRA), a series of five guidelines for optimizing forest establishment on mined sites. These guidelines, in brief, are: (1) select the best available growth medium; (2) minimize compaction; (3) minimize vegetative competition; (4) plant both early and late-succession trees; and (5) use proper planting techniques [7]. A number of studies have confirmed that sites planted according to FRA are characterized by high tree survival and growth [8–10]. Because a majority of the land affected by surface mining lies in the center of the historical American chestnut range, Appalachian mined land has been targeted for blight-resistant chestnut restoration efforts [11].

A number of studies have investigated the potential for using the FRA to successfully establish chestnuts on mined land. In Ohio, McCarthy *et al.* [12] found that first-year survival of bareroot seedlings planted into mined land was heavily influenced by soil physical characteristics, particularly bulk density. Seedlings planted into compaction-mitigated treatments survived well (90%–95%).

A study in Pike County, KY, found high first-year survival (>75%) of container-grown pure American chestnut seedlings in end-dumped (minimal compaction) mine spoil [13]. In a West Virginia study, five varieties of chestnuts were planted as seeds directly into mine spoil. First-year survival was high (80%), declining to 60% after four years [14]. These studies suggest that American chestnuts, and their backcrosses with Chinese chestnuts, show short-term success when planted in minesoils that are placed according to FRA guidelines.

Increasingly, comparing pure American, pure Chinese, and various American/Chinese backcrosses has been a major research focus. Because these backcrosses are novel, very few studies have assessed their growth and survival characteristics to date. In a Virginia study, researchers concluded pure Chinese chestnuts grew taller than either pure American or any of the three hybrids tested (B_1F_3, B_3F_2, B_2F_3) after two years [15]. In West Virginia, Chinese chestnuts had the highest survival through four years (90%), while the four remaining lines (pure American, B_1F_3, B_2F_3, and B_3F_2) were statistically similar (50%–60% survival [14]). In an Ohio study, researchers found that three year old Chinese, B_1F_3, B_2F_3 and B_3F_1 lines had higher survival and height growth rates than pure American chestnut [16]. In a different study in Ohio, researchers found that, although initial height growth was higher in backcrossed chestnuts, American chestnut height growth surpassed backcrosses after five years [17]. Finally, a North Carolina study found that chestnut seedlings (pure American, pure Chinese, and B_1F_3, B_2F_3, and B_3F_3) vary in their photosynthetic efficiency, with pure American chestnut and backcrosses photosynthesizing more efficiently than Chinese under high light conditions [18]. Continued research into long-term growth and survival of blight-resistant restoration American chestnuts is critical.

A major threat associated with seed plantings of chestnuts is initial predation, as well as post-sprouting herbivory. To combat this, tree sheltering has been investigated for use in improving survival and growth of a number of other tree species. Shelters can improve microclimate conditions as well as reduce pressure from herbivory. However, it has been demonstrated that interior air temperature in solid-walled tree shelters can be greater than ambient conditions during the daytime and potentially high enough to negatively affect seedling performance [19–21]. Tree shelters also influence the quality and quantity of light reaching the seedlings, depending on the shelter material [21]. In addition, the humidity within a solid-walled tree shelter is typically greater than ambient conditions [20,22]. With chestnuts, higher temperatures and humidity are of particular concern, in that those conditions may eventually increase blight incidence. As such, it has been recommended to remove shelters after establishment [14] and utilize alternative exclosure methods if necessary to prevent large herbivore (e.g., white-tailed deer) damage [23].

Some studies have specifically investigated the use of tree shelters in American chestnut plantings, with conflicting results. One study found that tree shelters did not significantly influence growth or survival of chestnuts planted into reclaimed mine land after the first growing season [14]. In contrast, some research suggests that sheltering improves survival of direct-seeded chestnuts into reclaimed mine land both by reducing predation of seeds and reducing herbivory of seedlings [12]. Thus, strategies for using tree shelters to improve establishment and growth of American chestnut in restoration projects remain unclear.

This study was implemented to address the following three objectives: establishment of chestnut plantings on a surface mined site in Appalachia, comparison of growth and survival among species and backcrosses, and potential use of tree shelters in improving early growth and survival. Pure American, pure Chinese, and three restoration chestnut lines were planted into minesoil on a reclaimed surface mine and received a sheltering treatment (sheltered or unsheltered). Height and survival were recorded through the fifth growing season after planting.

2. Methods

2.1. Site Selection

This study was conducted on Bent Mountain, an active surface mine site in Pike County, KY (37°35′53.1″ N, 82°24′30.6″ W). This mine is located in Kentucky's eastern coalfield in the Cumberland Plateau physiographic region [24], which is predominately forested. Climate is temperate humid continental with average annual precipitation of 114 cm and an average monthly precipitation of 10 cm. Average temperature is 13 °C, with a mean daily maximum and minimum of 31° and 18° in July and 8° and −4° in January [25]. Ultisols are the predominant soil order in the area [26]. The soil series at the study site is Dekalb, which are typically on upper side slopes and ridges [27]. The geologic unit that is affected by surface mining in the Bent Mountain area is the Lower and Middle Pennsylvanian (Carboniferous, 318.1–306.5 Ma) Breathitt Formation. The formation consists of interbedded sandstone, siltstone, shale and coal. Sandstone, shale and siltstone, in that order, are the most abundant rock types. In general, the sandstone is light gray, massive, fine to medium grained, and weathers to a yellowish or reddish brown. The shale is dominantly medium gray, silty, and contains siderite nodules [28]. The formation contains more than seven coal seams that are being mined.

2.2. Plot Construction and Planting

Predominately brown, weathered sandstone was end-dumped into piles approximately 3.5 m high, closely abutted to one another, across a 1.5 ha site. After dumping, piles were "strike-off" graded by a single pass of a Caterpillar D-9 bulldozer, reducing topographic variation and resulting in a final spoil depth of approximately 2.5 m. Spoil placement was conducted in accordance with Forestry Reclamation Approach (FRA) recommendations [7]. Surface mines in Appalachia are frequently reclaimed using mine spoil (fragmented rock overburden) rather than native topsoil (permitted by soil substitution waivers under SMCRA); thus, this experimental design simulates actual soil conditions under FRA mine reclamation. Standard soil testing was conducted to ensure that minesoil chemical conditions were favorable for establishment of restoration chestnuts. Two species of chestnut (American and Chinese) and three backcross generations (B_1F_3, B_2F_3, and B_3F_2) were used across two shelter treatments (shelter or no shelter) and replicated three times, for a total of thirty treatment plots. The 1.5 ha study area was irregularly shaped and plots were established such that all were positioned on top of the spoil piles and not on the sides. Each plot was randomly assigned a chestnut type and shelter treatment. Each plot was 10 × 10 m in area and contained 25 chestnuts from only one species or backcross generation. Nuts were planted on 2 m centers, making a total of 750 chestnuts planted in the study. Plot layout was in a factorial completely randomized design, in which response variables (seed germination, seedling height, and seedling survival) were observed for all factor-level combinations of independent variables (chestnut lineage and shelter treatment).

2.3. Chestnut Lineages

The five types of chestnut used in this study were as follows: pure American, pure Chinese, and three individual backcross generations, all provided by The American Chestnut Foundation's breeding program. The three backcross lines are denoted B_1F_3, B_2F_3 and B_3F_2, and should average one-quarter (B_1), one-eighth (B_2), and one-sixteenth (B_3) Chinese chestnut, respectively, with the remainder American. These were either the second (F_2, product of $F_1 \times F_1$ cross) or third (F_3, product of $F_2 \times F_2$ cross) filial cross at the indicated level of backcrossing. It was expected that the B_3F_2s would be intermediate in blight resistance, ranging from highly susceptible to highly resistant to chestnut blight, whereas the B_1F_3s and B_2F_3s are expected to be resistant, ranging perhaps from intermediately to highly resistant. The American chestnut are expected to be highly susceptible to blight and the Chinese resistant to highly resistant. All nuts were produced at the Meadowview Research Farm in Virginia. The parents of the Chinese and American nuts were the product of open pollination, whereas the parents of the B_1F_3 (NB8), B_2F_3 (SA330), and B_3F_2 (BG323) had been produced by controlled pollinations.

At each planting location a 7.5 cm deep hole was prepared using a dibble or shovel. A teabag of fertilizer (Treessentials, Duluth, MN, USA) was placed in the bottom of the hole and covered with 5 cm of planting mix (Scotts® general potting medium). The teabag was a 10 g biodegradable planting packet containing a blend of: 16% total nitrogen, 6% available phosphoric acid, 8% soluble potash, 6.92% combined sulfur and trace elements consisting of 0.52% zinc, 0.54% iron, 0.54% magnesium, 0.26% copper, 0.05% boron, and 0.56% manganese. Fertilizer was used because mine soils tend to be less fertile than the native soil that occupied the site prior to mining. Chestnuts were stratified in bags of moist peat in a refrigerator (~2 °C) for approximately seven months prior to planting. The nuts were examined prior to planting and only those with an observed viable radicle were used. Each chestnut was placed on the planting mix, and covered with an additional 1.5 cm of planting mix and a thin layer (1 cm) of minesoil at the surface. Chestnuts on sheltered plots were protected with 60 cm Tubex® shelters that were anchored to the ground with white oak stakes, following the manufacturer's instructions. In order to more effectively reduce rodent predation, shelters extended into the ground, and rocks were piled around the base. Planting and shelter establishment occurred on 7 May 2008.

2.4. Data Collection and Analysis

Nut germination was evaluated one-month after planting and calculated as the percentage of nuts planted that sprouted. Due to the high mortality observed in the unsheltered plots, efforts were undertaken to dig up some of the planted nuts for observation. Even though the planting location was obvious due to the soil disturbance and discovery of the planting mix, ungerminated nuts were not found and failed germination was attributed to rodent predation. Annual tree height was recorded in August of each year. Notes were also taken in regards to observed plant health (discolored leaves, presence of basal phloem cankers, crown dieback, sign of herbivory such as chewed stems). Percent survival was calculated by comparing the number of trees alive in the following year with the number of nuts planted in 2008. Species and shelter effects on seedling height were determined using analysis of variance for a repeated measures factorial completely randomized design with PROC GLIMMIX (SAS 1999, Campus Drive, Cary, NC, USA). Significant pairwise differences were detected for significant ANOVAs by comparisons of LSMEANS (/pdiff statement, SAS). Survival data of the seedlings were analyzed with repeated measures logistic regression models (PROC GENMOD). The logistic regression models included all main effects (species and shelter) and the interaction (species × shelter), with survival as the dependent variable, and treatments as the independent variables. Probabilities of seedling survival were calculated by back transformation of the least-squares mean (LSM) from the logistical models $(e^{LSM})/(1 + e^{LSM})$. A Chi-square analysis ($\alpha = 0.05$) was used for survival given that data were not normally distributed due to the reduced sample size from high mortality in the unsheltered plots.

3. Results

Germination ranged from 77%–84% for all five chestnut types when sheltered and 1%–12% when not sheltered (Table 1). There was no interaction of shelter × chestnut type or main effects of chestnut type for germination percentage, but there was a significant shelter effect ($p < 0.0001$). The first year survival was lower for all chestnut types (18% and 16% lower for mean survival of sheltered and unsheltered chestnuts, respectively) when compared to germination. After the first growing season, there was a significant shelter effect ($p < 0.0001$), but no chestnut type effect. Among sheltered trees, the pure American chestnut had the lowest survival (54%), while all other types were similar (64% to 74%). Among plots where chestnuts were not sheltered, there were no differences among survival rates, which ranged from 1%–10% (Table 2).

Table 1. Mean percent germination (and standard deviation) of chestnut seeds planted into mine soil in eastern Kentucky, compared within chestnut types and between sheltering treatments. Different letters indicate a significant shelter effect ($p < 0.05$) within species.

Species	Shelter	No Shelter
American	82 a ± 10	12 b ± 13
B_1F_3	77 a ± 6	6 b ± 8
B_2F_3	77 a ± 15	1 b ± 2
B_3F_2	84 a ± 12	6 b ± 2
Chinese	78 a ± 9	6 b ± 11

Although there was no significant chestnut type main effect for survival each year through 2012, there was a significant effect within sheltered trees by chestnut type for each year ($p < 0.0001$ each year).

After five growing seasons, survival was 70% in pure Chinese chestnut when sheltered. Survival declined consistently across the three backcross lines through the 2012 growing season: B_1F_3 (60% ± 7%), B_2F_3 (54% ± 18%), and B_3F_2 (41% ± 8%). Pure American chestnut, which had the lowest first year survival (54% ± 8%), exhibited the most significant decline in survival through the end of 2012. However the three backcross lines were similar to both pure American and pure Chinese after the third growing season (2010–2012) (Table 3).

There was also no interaction of shelter × chestnut type or main effects of chestnut type for height for all years of the study. However, sheltering had a significant influence on seedling height growth ($p < 0.0001$) for each year of the study. Mean heights ranged from 60–73 cm among sheltered and 16–33 cm among unsheltered chestnuts (Table 3).

Table 2. Mean percent survival (and standard deviation) of chestnuts planted into mine soil in Eastern Kentucky, compared within chestnut types and between sheltering treatments. Different letters indicate a significant shelter effect ($p < 0.05$) within species. (SD of "x" indicates sample size is too low).

Species	2008	2009	2010	2011	2012
			Shelter		
American	54 b ± 8	50 b ± 10	42 b ± 20	28 b ± 17	26 b ± 15
B_1F_3	69 a ± 6	69 a ± 6	65 ab ± 5	59 ab ± 13	60 ab ± 7
B_2F_3	64 a ± 18	58 a ± 15	57 ab ± 16	52 ab ± 13	54 ab ± 18
B_3F_2	65 a ± 2	60 ab ± 4	57 ab ± 2	48 ab ± 7	41 ab ± 8
Chinese	74 a ± 10	68 a ± 13	72 a ± 10	71 a ± 20	70 a ± 14
			No Shelter		
American	10 ± 11	6 ± 4	6 ± 5	8 ± 7	5 ± 6
B_1F_3	5 ± 6	2 ± 2	0 ± 0	0 ± 0	1 ± x †
B_2F_3	1 ± 2	0 ± 0	0 ± 0	0 ± 0	1 ± x †
B_3F_2	4 ± 4	2 ± 4	1 ± x	1 ± x	0 ± 0
Chinese	6 ± 11	6 ± 11	8 ± 14	8 ± 14	7 ± 11

† In the 2012 survey two trees (one B1F3 and one B2F3) were observed in plots that were previously noted as having 100% mortality. In both instances the seedlings were growing within the canopy of a naturally colonized autumn olive (*Elaeagnus umbellata*) shrub and hidden in previous surveys.

Table 3. Mean height in cm (with standard deviation) of chestnuts planted into mine soil in eastern Kentucky, compared within chestnut types and between sheltering treatments. Different letters indicate a significant shelter effect ($p < 0.05$) within species. (SD of "x" indicates that sample size is too low).

Species	2008	2009	2010	2011	2012
			Shelter		
American	60 b ± 19	97 b ± 25	129 b ± 29	167 a ± 27	183 a ± 45
B_1F_3	73 a ± 18	107 a ± 24	137 ab ± 27	159 ab ± 33	174 a ± 46
B_2F_3	60 b ± 18	102 ab ± 24	129 b ± 32	144 b ± 36	150 a ± 46
B_3F_2	63 b ± 16	104 ab ± 20	135 ab ± 28	161 ab ± 37	178 a ± 58
Chinese	66 ab ± 19	108 a ± 25	148 a ± 44	174 a ± 48	196 a ± 63
			No Shelter		
American	24 ± 17	76 ± 34	93 ± 25	105 ± 35	106 ± 46
B_1F_3	27 ± 15	60 ± x	0 ± 0	0 ± 0	100.0 ± x †
B_2F_3	33 ± x	0 ± 0	0 ± 0	0 ± 0	130 ± x †
B_3F_2	16 ± 9	32 ± 12	59 ± x	59 ± x	0 ± 0
Chinese	26 ± 8	61 ± 23	75 ± 30	94 ± 45	115 ± 30

† In the 2012 survey two trees (one B1F3 and one B2F3) were observed in plots that were previously noted as having 100% mortality. In both instances the seedlings were growing within the canopy of a naturally colonized autumn olive (*Elaeagnus umbellate*) shrub and were likely hidden in previous surveys.

Within sheltered trees, the B_1F_3 line had significantly more height growth than the other backcrosses and pure American lines. Chinese chestnut heights were similar to all other lines examined. However, by the end of the second growing season, growth of Chinese chestnut was similar to all three backcross lines, and growth of both pure Chinese and the B_1F_3 line was significantly greater than pure American. By the end of the fourth growing season (2011), pure American and pure Chinese trees were similar in height, and both significantly taller than B_2F_3. After five growing seasons, all five lines were similar in height, ranging from 150 cm (B_2F_3) to 196 cm (Chinese).

4. Discussion

Average survival of our sheltered chestnuts after four growing seasons (2011) was comparable to survival of sheltered chestnuts after four growing seasons (60%) in West Virginia [14]. Other studies report first year survival of sheltered trees planted as seeds: 76% in Virginia [15] and >80% in Ohio [12]. These values are higher than first-year survival in our study (65%). Two of these studies also assessed survival of trees planted as unsheltered seeds. In Ohio, McCarthy *et al.* [12] found a significant sheltering effect; however, survival of unsheltered trees was around 55% in their study, which was much greater than survival of unsheltered trees found in our study. Similarly, unsheltered chestnuts in West Virginia survived well through four growing seasons (57%). Given very low survival of our unsheltered trees, it seems likely that conditions on our site were less suitable than these others. Small mammals (e.g., mice and voles) are known to utilize end-dumped spoil piles as habitat [29] and are suspected to be responsible for predating on unprotected chestnut seeds in this study. We note that average mortality in sheltered trees has increased only slightly since the end of the first growing season, suggesting that the critical time period for chestnut survival in our study was during germination and early establishment.

Tree sheltering has also been investigated for use in improving survival of a number of planted tree species. In Colorado, researchers working with Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) found that tree sheltering significantly improved survival (59%–78% in shelter treatments compared to 35% in unsheltered treatments), which they attributed to reduced herbivory as well as improved microclimate [30]. A study by Andrews *et al.* [31] did not find a tree shelter effect on survival of pin oak (*Quercus palustris* Munchh.), green ash (*Fraxinus pennsylvanica* Marshall) or American sycamore (*Platanus occidentalis* L.) in a riparian forest restoration site in Kentucky. In a study on a cork oak (*Quercus suber* L.) forest restoration site in Tunisia, tree sheltering improved second-year survival and growth, likely due to protection from browse [32]. Researchers in eastern Oregon found that willow (*Salix* spp.) and cottonwood (*Populus balsamifera* L. ssp. *trichocarpa* [Torr. & A. Gray ex Hook.] Brayshaw) survival was improved by tree sheltering, due to improved microclimate conditions (e.g., humidity) [33]. In Pennsylvania, restoration ecologists reported mixed success with tree shelters. They found that some species (e.g., black walnut, *Juglans nigra* L.) did not survive well in tree shelters. However, survival and growth of white ash (*Fraxinus americana* L.) was higher in sheltered treatments than unsheltered treatments. Due to heavy browsing and scraping pressure from white-tailed deer, the authors found that tree shelters were less useful for long-term protection against deer browsing. In addition, they identified unexpected issues with tree shelters including songbird mortality, vegetative species colonization (facilitated by utilization of shelters as a perch), and eventual girdling issues for fast-growing trees [23].

Considering only sheltered trees, we found that survival of pure Chinese chestnuts was significantly higher than survival of pure American chestnuts after five years, with the three backcross lines intermediate. In the West Virginia study, Chinese chestnut survival was significantly higher than pure American, and the three backcross lines (the same lines tested in this study) were similar to pure American and significantly lower than pure Chinese (ranging from 50% for B_3F_2 to 60% for B_1F_3).

In Ohio, Fields-Johnson *et al.* [15] reported similar survival among pure Chinese (89%), pure American (87%) and B_1F_3 (84%) after the first growing season, with B_2F_3 exhibiting significantly lower survival (66%) and B_3F_2 similar to both groups (73%). An apparent trend in the longer term studies is higher survival in Chinese chestnut, which is anticipated given that species' natural resistance to chestnut blight. Similarly, lower survival in American chestnut was anticipated given that species' historical susceptibility to the disease. Finally, we would expect American/Chinese backcrosses to fall somewhere in between their pure parents. However, chestnut blight was likely not a primary factor contributing to differences in survival in this study, given that only one tree showed the presence of basal phloem cankers associated with chestnut blight.

Another disease known to cause American chestnut mortality is inkstain disease or Phytophthora root rot, caused by the oomycete pathogen *Phytophthora cinnamomi*. This pathogen was historically introduced to the American Southeast and was found to contribute to chestnut mortality and stress long before the more devastating introduction of chestnut blight [2]. In 2011 we performed soil baiting followed by PCR amplification and sequencing of the ITS region to determine if *Phytophthora cinnamomi* was contributing to seedling mortality [34]. *P. cinnamomi* was not detected in the mine soil; however, in a nearby (approximately 1000 m away) American chestnut experiment, *P. cinnamomi* was diagnosed on dead seedlings and *P. cryptogea* was detected in both spoil and water infiltrated from brown sandstone soil reclaimed using the FRA [34]. Thus, while we are not confident that *P. cinnamomi* contributed to chestnut mortality in our study, it is likely that *P. cinnamomi* will colonize eventually and may contribute to mortality in the future. Initial soil analyses confirmed that soil conditions, particularly pH (4.87), were suitable for establishment of American chestnut; however, even mine sites with favorable soil chemical characteristics can be harsh sites for tree establishment. Mine soils are frequently characterized by low soil moisture and low organic matter content; these conditions are unfavorable for tree establishment, but can be ameliorated by continued vegetative growth.

In our study, sheltering also significantly influenced tree height. We found that trees protected by the 60 cm shelters were significantly higher than unsheltered trees after five growing seasons. Similarly, McCarthy *et al.* [12] found that unprotected trees were characterized by lower growth rates than trees sheltered with 120 cm tubes, a relationship they attributed to heavy herbivory on unprotected trees. In contrast, Skousen *et al.* [14] reported similar average heights of trees between sheltering treatments. While McCarthy *et al.* [12] reported evidence of herbivory on their site, Skousen *et al.* [14] observed that herbivory did not appear to be a major factor in growth and survival on their site. We only noted one instance of foliar herbivory of the 2 years and older seedlings on our site Andrews *et al.* [31] noted that growth of pin oak, green ash, and American sycamore was higher in sheltered trees than unsheltered controls. Height of California blue oak (*Quercus douglasii*) seedlings was significantly improved by sheltering primarily due to browse protection [35]. Growth of cork oak in Tunisia was also improved by sheltering [32]. Finally, willow and cottonwood in eastern Oregon experienced higher growth rates due to improved microclimate conditions (e.g., humidity) [33]. Comparing height growth solely among sheltered trees, we found that B_1F_3 outgrew nearly all four other lines after the first growing season. However, by the end of five growing seasons, height growth was similar across lines. In West Virginia, no differences were found in height growth among lines after four growing seasons [14]. Remarkably, first-year growth in our study was greater than fourth-year growth in West Virginia. The West Virginia site was constructed using a mixture of brown and gray spoils, while the plots in our study were constructed using predominately brown spoils [14]. A series of recent studies have indicated that brown spoils tend to provide a better growing medium for native hardwoods than gray, less weathered spoils [8,10,36,37]. Soil pH in West Virginia (5.8) was within the preferred range of the American chestnut (5.5–6.5 [38]). Given observations of heat stress during the second growing season [14], we suggest that soil moisture conditions were less favorable in the West Virginia study than in our study. Clay in our study was >17%, high compared to other studies in end-dumped spoil reclamation systems (e.g., 10% clays after nine years [37]). High clay fraction contributes to high moisture holding capacity, due to the high surface area to mass ratio of clay particles.

During the second and third growing seasons surveyed in the current study (2009 and 2010), we observed that Chinese chestnut significantly outgrew American chestnut. The reason for this difference in height growth is unclear, but similar results were observed by Skousen *et al.* [14] and Gilland and McCarthy [16] on minelands reclaimed using the FRA in West Virginia and Ohio, respectively. Gilland and McCarthy [16] suggested that increasing levels of leaf pubescence observed in Chinese (and those lines containing more Chinese characteristics) may aid in mitigating water or light stress that is incurred on the open reclaimed mine sites. Although chestnut trees prefer soils with pH from 5 to 6, they have been shown to grow on a wide range of soil pH levels found on reclaimed mines [13,14,39]. The pH of this site (4.87) was slightly lower than the preferred range. It is possible that subtle differences in soil chemistry may result in differing growth responses by the chestnut lines examined and may have contributed to the observed difference in initial height between Chinese and American. Conversely, the growth differences may be attributed to initial nutrition differences between the nut varieties at the time of planting. Although we did not measure nut size prior to planting, we observed that the Chinese nuts were larger than the American or any of the backcross lines. Studies have shown Chinese chestnut seedling height differ among visually distinguished nut size classes [40] and that initial seedling height in Chinese, American and other chestnut backcross lines is correlated to seed weight where higher weights result in higher height growth [41]. Bauman *et al.* [17] also observed higher initial height growth in backcross lines than in pure American chestnuts planted into reclaimed mine land in Ohio; however, consistent with our study, they observed that pure American chestnuts eventually surpassed backcross lines in height growth. We conclude, as they, that differences in nut size partially explain variation in initial seedling height growth.

5. Conclusions

American chestnut backcrosses have shown similar survival and height growth characteristics as the more blight resistant Chinese chestnut after direct planting into brown sandstone minesoil. This study supports growing evidence that surface mined land in Appalachia can serve as a “launching pad” for American chestnut restoration. Over time, as planted chestnuts mature and begin fruiting, we anticipate that they will slowly disperse out into the surrounding forest. Eventually, the American chestnut may reclaim its role as a dominant canopy tree in the mesic hardwood forests of the eastern U.S. With respect to planting techniques, it appears that shelters are an effective protection against predation of chestnut seeds by small mammals during early phases of seed germination and sprouting.

Acknowledgments

We would like to thank Fred Hebard with TACF for providing the chestnuts and for technical assistance. We would also like to acknowledge various students from Barton's lab who "volunteered" to help with the project establishment and monitoring. Thanks also to Bob Paris who provided assistance with establishment. Funding for this project was provided by the American Chestnut Foundation through a grant from the United States Department of Interior, Office of Surface Mining Reclamation and Enforcement.

Author Contributions

Christopher Barton and Jarrod Miller conceived and designed the study; Jarrod Miller, Patrick Angel, and Michael French collected data; Christopher Barton, Jarrod Miller, and Kenton Sena analyzed the data; Christopher Barton and Kenton Sena wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Russell, E.W.B. Pre-blight distribution of Castanea dentata (Marsh.) Borkh. *Bull. Torrey Bot. Club* **1987**, *114*, 183–190.
2. Wang, G.G.; Knapp, B.O.; Clark, S.L.; Mudder, B.T. *The Silvics of Castanea Dentata (Marsh.) Borkh., American Chestnut, Fagaceae (Beech Family)*; General Technical Report SRS-173; USDA Forest Service, Southern Research Station: Asheville, NC, USA, 2013.
3. Hebard, F. The backcross breeding program of The American Chestnut Foundation. *J. Am. Chestnut Found.* **2005**, *19*, 55–77.
4. Diskin, M.; Steiner, K.C.; Hebard, F.V. Recovery of American chestnut characteristics following hybridization and backcross breeding to restore blight-ravaged *Castanea dentata*. *For. Ecol. Manag.* **2006**, *223*, 439–447.
5. Jacobs, D.F.; Dalgleish, H.J.; Nelson, C.D. A conceptual framework for restoration of threatened plants: The effective model of American chestnut *(Castanea dentata)* reintroduction. *New Phytol*. **2013**, *197*, 378–393.
6. Zipper, C.E.; Burger, J.A.; Barton, C.D.; Skousen, J.G. Rebuilding soils on mined land for native forests in Appalachia. *Soil Sci. Soc. Am. J.* **2013**, *77*, 337–349.
7. Burger, J.A.; Graves, D.; Angel, P.; Davis, V.; Zipper, C. *The Forestry Reclamation Approach, Forest Reclamation Advisory No. 2*; USDOI Office of Surface Mining: Washington, DC, USA, 2005.
8. Agouridis, C.; Angel, P.; Taylor, T.; Barton, C.D.; Warner, R.; Yu, X.; Wood, C. Water quality characteristics of discharge from reforested loose-dumped mine spoil in eastern Kentucky. *J. Environ. Qual*. **2012**, *41*, 454–468.

9. Cotton, C.; Barton, C.D.; Lhotka, J.; Angel, P.; Graves, D. Evaluating Reforestation Success on a Surface Mine in Eastern Kentucky. In *National Proceedings: Forest and Conservation Nursery Associations—2011. Proc. RMRS-P-65, USDA Forest Service*; Riley, L.E., Haase, E.L., Pinto, J.R., Eds.; Rocky Mountain Research Station: Fort Collins, CO, USA, 2012; pp. 16–23.
10. Wilson-Kokes, L.; Emerson, P.; DeLong, C.; Thomas, C.; Skousen, J. Hardwood tree growth after eight years on brown and gray mine soils in West Virginia. *J. Environ. Qual*. **2013**, *42*, 1353–1362.
11. Barton, C.D.; French, M.E.; Fei, S.; Ward, K.A.; Paris, R.; Angel, P. Appalachian surface mines provide a unique setting for the return of the American chestnut. *Solutions* **2010**, *1*, 84–87.
12. McCarthy, B.C.; Gilland, K.E.; Bauman, J.M.; Keiffer, C.H. Factors affecting performance of artificially regenerated american chestnut on reclaimed mine sites. In *Bridging Reclamation, Science and the Community*, Proceedings of the American Society of Mining and Reclamation, Pittsburgh, PA, USA, 5–11 June 2010.
13. French, M.E.; Barton, C.D.; Graves, D.; Angel, P.N.; Hebard, F.V. Evaluation of mine spoil suitability for the introduction of American Chestnut hybrids in the Cumberland Plateau. In *30 Years of SMCRA and Beyond*, Proceedings of the American Society of Mining and Reclamation, Gillette, WY, USA, 2–7 June 2007.
14. Skousen, J.; Cook, T.; Wilson-Kokes, L.; and Pena-Yewtukhiw, E. Survival and growth of chestnut backcross seeds and seedlings on surface mines. *J. Environ. Qual*. **2013**, *42*, 690–695.
15. Fields-Johnson, C.W.; Burger, J.A.; Evans, D.M.; Zipper, C.E. American chestnut establishment techniques on reclaimed Appalachian surface mined lands. *Ecol. Restor.* **2012**, *30*, 99–101.
16. Gilland, K.E.; McCarthy, B.C. Performance and phenotypic variation of American chestnut (*Castanea dentata*) hybrids on newly reclaimed mine sites in eastern Ohio, USA. *Ecol. Restor.* **2014**, *32*, 379–387.
17. Bauman, J.M.; Keiffer, C.H.; McCarthy, B.C. Growth performance and chestnut blight incidence (*Cryphonectria parasitica*) of backcrossed chestnut seedlings in surface mine restoration. *New For.* **2014**, *45*, 813–828.
18. Knapp, B.O.; Wang, G.G.; Clark, S.L.; Pile, L.S.; Schlarbaum, S.E. Leaf physiology and morphology of *Castanea dentata* (Marsh.) Borkh., *Castanea mollissima* Blume, and three backcross breeding generations planted in the southern Appalachians, US. *New For.* **2014**, *45*, 283–293.
19. Ponder, F. Shoot and root growth of northern red oak planted in forest openings and protected by treeshelters. *North. J. Appl. For*. **1995**, *12*, 36–42.
20. Kjelgren, R.; Montague, D.T.; Rupp, L.A. Establishment in treeshelters II: Effect of shelter color on gas exchange and hardiness. *HortScience* **1997**, *32*, 1284–1287.
21. Bellot, J.; Ortiz de Urbina, J.M.; Bonet, A.; Sanchez, J.R. The effects of treeshelters on the growth of *Quercus coccifera* L. seedlings in a semiarid environment. *Forestry* **2002**, *75*, 89–106.
22. Minter, W.F.; Myers, R.K.; Fisher, B.C. Effects of tree shelters on northern red oak seedlings planted in harvested forest openings. *North. J. Appl. For*. **1992**, *9*, 58–63.
23. Robertson, D.J. Trees, deer, and non-native vines: Two decades of northern Piedmont forest restoration. *Ecol. Rest*. **2012**, *30*, 59–70.
24. Fenneman, N.M. *Physiography of Eastern United States*; McGraw-Hill: New York, NY, USA. 1938.

25. Hill, J.D. *Climate of Kentucky*; Progress Report 221; Kentucky Agricultural Experiment Station: Lexington, KY, USA, 1976.
26. *Natural Resources Conservation Service*; 1999 Soil Survey Centennial Planning Guide; U.S. Department of Agriculture: Washington, DC, USA, 1998.
27. Hayes, R.A. *Soil Survey of Pike County*, *Kentucky*; US Department of Agriculture, Soil Conservation Service and Forest Service: Washington, DC, USA, 1982.
28. Wolcott, D.E.; Jenkins, E.C. *Geologic Map of the META Quadrangle, Pike County, Kentucky, Map GQ-497*; Department of the Interior, United States Geological Survey: Washington, DC, USA, 1966.
29. Larkin, J.L.; Maehr, D.S.; Krupa, J.J.; Cox, J.J.; Alexy, K.; Unger, D.E.; Barton, C.D. Small mammal response to vegetation and spoil conditions on a reclaimed surface mine in eastern Kentucky. *Southeast Nat*. **2008**, *7*, 401–412.
30. Jacobs, D.F. Reforestation of a salvage-logged high-elevation clearcut: Engelmann spruce seedling response to tree shelters after 11 growing seasons. *West. J. Appl. For*. **2011**, *26*, 53–56.
31. Andrews, D.M.; Barton, C.D.; Czapka, S.J.; Kolka, R.K.; Sweeney, B.W. Influence of tree shelters on seedling success in an afforested riparian zone. *New For.* **2010**, *39*, 157–167.
32. Chaar, H.; Mechergui, T.; Khouaja, A.; Abid, H. Effects of treeshelters and polyethylene mulch sheets on survival and growth of cork oak (*Quercus suber* L.) seedlings planted in northwestern Tunisia. *For. Ecol. Manag*. **2008**, *256*, 722–731.
33. Hall, J.; Pollock, M.; Hoh, S. Methods for successful establishment of cottonwood and willow along an incised stream in semiarid eastern Oregon, USA. *Ecol. Restor*. **2011**, *29*, 261–269.
34. Adank, K.M.; Barton, C.D.; French, M.E.; DeSa, P. Occurrence of Phytophthora on reforested loose-graded spoils in Eastern Kentucky. In Proceedings of the American Society of Mining and Reclamation, Lexington, KY, USA, 14–19 June 2008.
35. McCreary, D.D.; Tietje, W.; Davy, J.; Larsen, R.; Flavell, D.; Doran, M.; Garcia, S. Tree shelters and weed control enhance growth and survival of natural blue oak seedlings. *Calif. Agric*. **2011**, *65*, 192–196.
36. Miller, J.; Barton, C.D.; Agouridis, C.; Fogle, A.; Dowdy, T.; Angel, P. Evaluating soil genesis and reforestation success on a surface coal mine in Appalachia. *Soil Sci. Soc. Am. J.* **2012**, *76*, 950–960.
37. Sena, K.; Barton, C.D.; Angel, P.; Agouridis, C.; Warner, R. Influence of spoil type on chemistry and hydrology of interflow on a surface coal mine in the eastern US coalfield. *Water Air Soil Poll*. **2014**, *225*, 2171, doi:10.1007/s11270-014-2171-y.
38. U.S. Department of Agriculture. *Natural Resources Conservation Service, The PLANTS Database*; National Plant Data Team: Greensboro, NC, USA, 2014. Available Online: http://plants.usda.gov (accessed on 2 June 2014).
39. McCarthy, B.C.; Bauman, J.M.; Keiffer, C.H. Mine land reclamation strategies for the restoration of American chestnut. *Ecol. Rest*. **2008**, *26*, 292–294.
40. Shepard, E.; Miller, D.D.; Miller, G. Effect of seed weight on emergence and seedling vigor of Chinese chestnut. *HortScience* **1989**, *24*, 516.

41. Clark, S.L.; Schlarbaum, S.E.; Saxton, A.M.; Hebard, F.V. Nursery performance of American and Chinese chestnuts and backcross generations in commercial tree nurseries. *Forestry* **2012**, *85*, 589–600.