SURVIVAL AND GROWTH OF CHESTNUT BACKCROSS SEEDS AND SEEDLINGS ON SURFACE MINES[1]

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Abstract: The development of Chestnut backcrosses by The American Chestnut Foundation has resulted in seeds and seedlings that are now being planted on coal surface mines in the Appalachian Region. In West Virginia, two studies were established. The first study, initiated in 2008, involved planting seeds of two parental species of chestnut and three breeding generations (100% American, 100% Chinese, and B_1F_3, B_2F_3, and B_3F_2 backcrosses) into loosely-graded mine soils at the Glory surface mine in West Virginia. First year survival was Chinese 81%, American 66%, and backcrosses between 69 and 74%. After the 4th year, survival had declined further for all chestnut stock types except for Chinese: Chinese 80%, American 40%, B_1F_3 55%, B_2F_3 40%, and B_3F_2 44%. Average height after the 4th season was not significantly different among seed stock types: Chinese 45 cm, American 49 cm, B_1F_3 48 cm, B_2F_3 53 cm, and B_3F_2 48 cm. Significantly lower survival was found when seeds were planted with peat (44%) compared to seeds without peat (60%), and lower survival resulted when seeds were not protected with tree shelters. The second study, initiated in 2009, involved planting seeds and seedlings of these same five chestnut parental species and breeding generations into two substrates (brown sandstone (pH 4.5) and gray sandstone (pH 6.6)). Only six out of 250 seeds germinated, which was surprising with the good germination results on the Glory study the year before. Planted chestnut seedlings, however, showed much better establishment. After the 3rd year on brown sandstone and gray sandstone, respectively, seedling survival was American 77 and 80%, Chinese 84 and 100%, B_1F_3 88 and 72%, B_2F_3 70 and 68%, and B_3F_2 68 and 48%. Average height after the 3rd season for brown and gray sandstone substrates, respectively, was American 92 and 71 cm, Chinese 112 and 79 cm, B_1F_3 77 and 55 cm, B_2F_3 68 and 52 cm, and B_3F_2 82 and 39 cm.

Additional Keywords: chestnut backcrosses, Forestry Reclamation Approach, mine soils, tree seedlings, tree seeds

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Introduction

About 78% of the West Virginia landscape is forested and, with the prevailing climate, almost all land in this region will naturally revert to forestland eventually after disturbed by fire, farming, or mining. The climate and soil/geology of the central Appalachians is conducive to very productive hardwood forest growth.

Surface coal mining has been conducted on about 2.5 million ha (6 million ac) since 1930 in the USA (Paone et al., 1978; Plass, 2000). In Appalachia, the vast majority of surface mined land was originally covered by eastern deciduous forest. The earliest laws governing reclamation of surface mines were passed in Ohio, Pennsylvania, and West Virginia during the 1940s, and these early reclamation laws prescribed soil, subsoil, and overburden (the geologic material overlying the coal) be used to refill the excavated area. Leveling the land was often specified, and conifers and some hardwood trees species were planted to replace the forest that had been removed (Ashby, 2006; Brown, 1962; Limstrom, 1960; Plass, 2000). Reforestation was chosen because the land had been originally forested and reforested sites provided long-term site stabilization, wildlife habitat, and future economic value when trees are harvested (Torbert and Burger, 2000).

Since the late 1970s with the passage of a national surface mining law, a large amount of surface mined land in Appalachia has been reclaimed to pasture and hay land or wildlife habitat post-mining land uses (Plass, 1982; 2000), rather than forestland. The reasons for this land use change are related to: 1) quick economic returns to landowners by grazing and haying systems, 2) predictable bond release because the consistent ground cover gave good erosion control and water quality, and 3) better land stability compared to pre-law mined landforms where no grading was performed (Boyce, 1999). When maintained with fertilizer and lime, these pasture and hay land uses provide landowners with consistent income. When neglected, these lands collapse to weedy plant communities that gradually can return to a woody forest community, but long time periods are necessary for valuable hardwood species to invade and mature (Zipper et al., 2011).

Forestry post-mining land uses have gradually emerged during the early 2000s as a preferred post-mining land use option in the Appalachian Region, and especially in West Virginia. To encourage forest re-establishment on mined lands and to optimize the success of tree plantings, the Forestry Reclamation Approach (FRA) has been adopted by the Appalachian Regional Reforestation Initiative (ARRI). ARRI encourages use of the FRA's five-step process to reclaim coal mined land to forestland:

1. Create a suitable rooting medium for good tree growth that is no less than 1.2 m (4 ft) deep and comprised of topsoil, weathered sandstone, and/or the best available material;
2. Loosely grade the topsoil or topsoil substitutes established in step one to create a non-compacted growth medium;
3. Use ground covers that are compatible with growing trees;
4. Plant two types of trees – 1) early succession species for wildlife and soil stability, and 2) commercially valuable crop trees;
5. Use proper tree planting techniques (Burger et al., 2005).

Recent research has demonstrated the usefulness of the FRA by showing the successful establishment of native hardwood trees when applying this five-step process (Angel et al., 2008; Emerson et al., 2009). Coal operators and regulators are gradually seeing an increase of acreage being reclaimed to forestry post-mining land uses (Angel et al., 2009).

Brown and gray sandstone substrates have been studied as plant growth media for forestry land uses. Survival of hardwood trees was shown to be very good (>80%) during the first several years after planting in both brown and gray sandstone (Burger et al., 2007; Emerson et al., 2009). However, trees planted into brown sandstone have shown much greater growth than that in gray sandstone (Emerson et al., 2009; Thomas and Skousen, 2011). As reviewed by Skousen et al. (2011a and 2011b), studies have found that weathered rocks, especially sandstones produce excellent soil materials compared to unweathered materials. Working in Kentucky, Angel et al. (2008) found that weathered sandstone mine soils supported faster tree growth and more rapid colonization by native plants than either unweathered gray sandstone or a mixture of the two materials.

Prior to the 1900s, the eastern hardwood forests of the United States were comprised of an assemblage of 30 or 40 hardwood species. One of the most important species was the American chestnut (*Castanea dentata* (Marsh.) Borkh.), and past research estimated that this species occupied up to 25% of the forest (Russell, 1987). American chestnut trees were up to 50 m in height and produced tremendous volumes of timber because it grew straight, fast, and often produced three or four 4-m logs before the first branch was reached. Chestnut trees are also valued for their consistent annual nut production, which is eaten by humans and animals, and natural tannin for tanning leather (Freinkel, 2009).

Chestnut blight, discovered in 1904 in New York, is caused by a fungus (*Cryphonectria parasitica* (Murr.)Barr.), which quickly spread through the eastern US forests (Jacobs, 2007). By 1950, about 4 billion trees had perished, nearly one-fourth of the canopy cover of the eastern deciduous forest was gone, and an important wildlife and timber tree was lost. Many scientists consider the loss of the American chestnut to be the greatest ecological disaster of the 20th century. The blight fungus infects American chestnut through cracks or wounds in the bark, creating a canker which effectively cuts off circulation to the branches above the canker. The roots, however, remain alive. The ability to sprout has enabled American chestnut to persist in eastern forests, but only as an occasional understory shrub. For a broader description of the blight fungus and other diseases of the chestnut, please see Anagnostakis (1995) and Brewer (1995).

The American Chestnut Foundation (TACF), formed in 1983, is breeding surviving American chestnut with blight-resistant Chinese chestnuts (*Castanea mollissima)* (Burnham et al., 1986). Highly blight-resistant progeny of the backcrosses are then selected for further backcrossing to American chestnut (Hebard, 2005). It is hoped through careful selection that these backcrosses incorporate Chinese chestnut's blight resistance while retaining the desirable timber and nut-producing characteristics of the American chestnut. In 2009, TACF produced tree seedlings that are approximately 7/8 American chestnut and 1/8 Chinese chestnut (the B_3F_2 backcross is the third backcross to American chestnut and the second generation hybrid). TACF has begun testing of this final product in many eastern USA states.

The use of reclaimed surface mines for chestnut reestablishment has recently gained momentum (French et al., 2007b). The same factors that affect survival and growth of native hardwoods on surface mines will probably affect survival and growth of American chestnuts. Since the Appalachian coal region overlays the former range of the American chestnut, the establishment of blight-resistant backcrosses would coincide with TACF's goal of restoring the chestnut into eastern USA hardwood forests. Since 2002, TACF has been planting American chestnut and backcross chestnut on mined lands in various Appalachian states. In cooperation with the University of Kentucky, chestnut seeds were planted in 2005 on end-dumped spoil in eastern Kentucky composed of gray sandstone, brown sandstone, and run-of-mill spoil materials. Better growth was found in brown sandstone (Adank et al., 2008; French et al., 2007a). Researchers in Ohio have been examining chestnut direct seeding versus planted seedlings, mycorrhizal inoculation treatments, and protection of seedlings on surface mine lands (McCarthy et al., 2008 and 2010). A breeding orchard of backcross chestnut seedlings on mined land was established in Jefferson County, PA, and harvesting of nuts was performed in 2010 (Phelps, 2002).

As part of this effort, researchers at West Virginia University were provided with parental stock and backcross chestnut materials which were planted on surface mined lands in West Virginia. The objectives were to evaluate the survival and growth of chestnuts on surface mined lands using seeds and seedlings. The first experiment (Glory Study) evaluated seed establishment and growth of five chestnut parental species and breeding generations (hereafter called 'stock types' of American, Chinese, B_1F_3, B_2F_3, and B_3F_2; see Hebard (2005) for descriptions of backcross chestnuts) planted into a mixed brown/gray sandstone substrate over four years since planting. The second experiment (Nicholas Study), with three years of data, compared the establishment and growth of both seeds and seedlings of the same five chestnut stock types in a loosely-dumped brown sandstone material and in a compacted gray sandstone material.

Materials and Methods

Glory Study - 2008

The Glory surface mine is located Boone County, West Virginia. Overburden from the 5-Block and Clarion coal seams was used to construct a 1-ha plot for this experiment, which was comprised of 75% brown sandstone and 25% gray sandstone. The material was end dumped by trucks and a large bulldozer flattened the tops of the piles to create a roughly level surface (Fig. 1). Precipitation is about 112 cm with 60% falling between April and September, the recognized growing season (Wolf, 1994). The average annual temperature during the growing season is 20 degrees C.

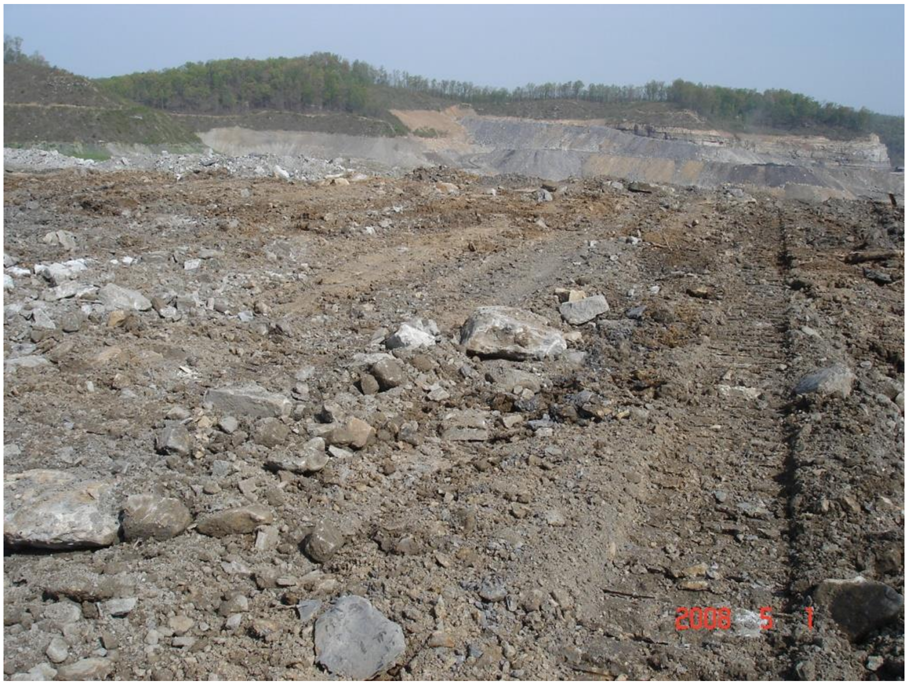

Figure 1. The 1-ha experimental site was constructed with mixed sandstone substrate, end-dumped with trucks, and the piles were flattened by one or two passes of a bulldozer.

On this 1-ha site, two areas were selected with one of the areas having tree shelters placed over planted seeds and the other area had no shelters. Each area (shelters or no shelters) was composed of four blocks. One half of each block was randomly assigned a peat or no peat treatment. Peat treatment was selected as an amendment because these mine soil materials were coarse-textured and generally had low nutrient and water-holding capacity (Haering et al., 2004). The application of organic matter, and in this case peat, helps to improve these capacities in mine soils. In each half block, five subplots were randomly assigned one of the five chestnut stock types (Fig. 2). Five seeds of the assigned seed stock type were planted in each subplot at a 2.4 x 2.4 m spacing. The chestnut seeds were produced from open-pollinated seed orchards of The American Chestnut Foundation in Meadowview, VA. Wooden stakes were driven into the soil at each seed location. In total, 80 seeds of each seed stock type were planted for a total of 400 seeds (2 areas x 5 seed stock types x 4 blocks x 2 peat treatments x 5 replications = 400 seeds).

Chestnut seeds were planted by digging a small 5-cm hole about 5 cm from the base of the wooden stake. Each seed was inoculated with mycorrhizal fungi provided by The American Chestnut Foundation before planting. In peat treatments, about 5 cm^3 of commercial peat was placed in the hole, and the seed was placed on the peat and covered with soil. In the no peat treatment, only soil was used to cover the seed. After planting, 45-cm tall, plastic tree shelters were placed over each planted seed in one area (blocks 1-4 with 200 seeds), and no tree shelters were placed on planted seeds in the second area (blocks 5-8 with 200 seeds). The tree shelters were secured to the stakes with twine. Shelters were removed during the second growing season in June 2009 because some chestnut trees were observed to be wilting and crowded in the shelters. Survival was noted and height of each live chestnut seedling was measured in late August 2008 to 2011.

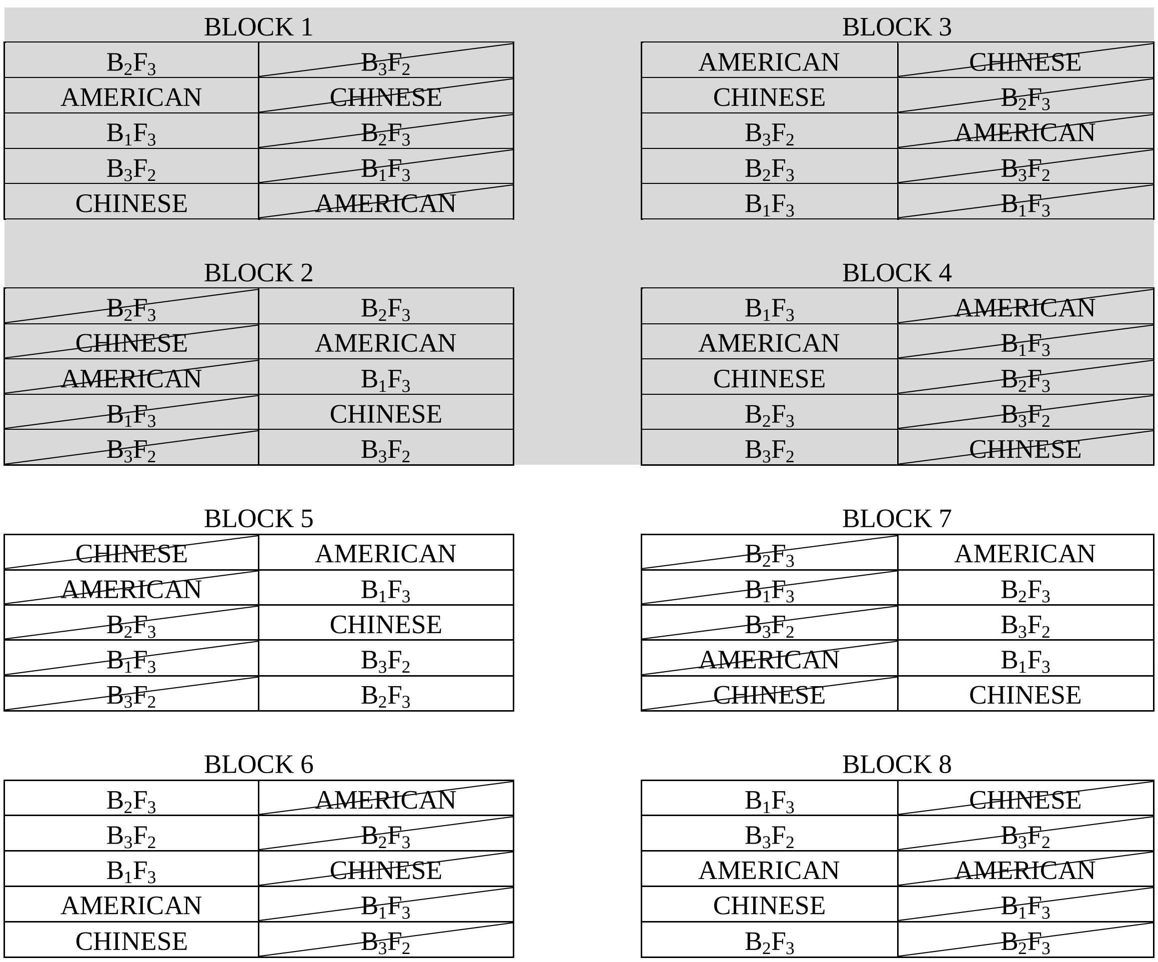

Figure 2. Two areas, one with tree shelters (blocks 1-4, shaded) and the other with no shelters (blocks 5-8, un-shaded), were established. Within blocks at each area, seeds of five Chestnut stock types (American, Chinese, B_1F_3, B_2F_3, and B_3F_2) were randomly assigned to subplots with and without a peat treatment (no peat = no cross-hatch, peat = cross-hatch).

Statistical analyses were performed using SAS 9.1 software (SAS Institute, 2005). Means were calculated for tree survival and height between chestnut stock types with and without shelters but statistical analysis were not performed for shelter treatment. Within areas (shelter or no shelter), ANOVA was used to evaluate differences in survival and height among seed stock types for each year across peat treatments. An alpha level of 0.05 was considered significant.

Nicholas Study – 2009

A second study was established a year later at the Nicholas Energy site about 15 km west of Summersville, WV. Nicholas Energy produces about 2.4 million metric tons of high quality coal per year using large shovels, trucks, and dozers to extract coal from the 5-Block, Clarion, Stockton and Coalburg seams. Two overburden types (brown and gray) were available as planting substrate at this site. The brown sandstone materials came from the surface which overlies the 5-Block coal seam, which was end dumped by trucks with no striking off or flattening of the piles (Fig. 3). The gray sandstone topsoil substitute material came from the overburden above the Clarion coal seam. This gray sandstone was placed and compacted for typical pasture reclamation and hydro seeding of forages, and was composed of unweathered, coarse-textured materials and rocks (Fig. 4). Precipitation at the site is about 118 cm with 55% falling between April and September, the recognized growing season (Carpenter, 1992).

Chestnut seedlings and seeds were provided by The American Chestnut Foundation from Meadowview, VA. Seedlings and seeds of the same five chestnut stock types used in the previous experiment were randomly planted in subplots of five blocks in both substrates (Fig. 5). In each block, 10 subplots were planted with all treatment combinations (stock type x seed/seedling) with five replications of trees planted in each subplot. A total of 125 seeds and 125 seedlings were planted in each substrate (5 chestnut stock types x 2 (seed or seedling) x 5 blocks x 5 replications = 250) for a total of 500 seeds and seedlings in both substrate types. Wooden stakes were driven in at the point where seedlings or seeds were planted on a 2.4 by 2.4 m spacing and labeled. The planting procedure involved digging holes large enough to place the roots of the seedlings into, while the seeds were planted approximately 3-4 cm deep in the soil and covered. No peat treatments or tree shelters were used in this study. Survival was noted and height of each live chestnut seedling was measured in August 2009 to 2011.

Figure 3. The 0.3-ha experimental area of brown sandstone and topsoil at Nicholas was constructed in 2009 and simply end-dumped with trucks. No leveling was done.

Figure 4. The 0.3-ha experimental area of gray sandstone at Nicholas was constructed in 2009 with primarily gray sandstone and some brown material, and graded by bulldozers.

Un-compacted Brown Sandstone

BLOCK 1

B_2F_3 - Seed	B_3F_2 - Seedling
AMER - Seed	CHIN - Seedling
B_1F_3 - Seedling	B_2F_3 - Seedling
B_3F_2 - Seed	B_1F_3 - Seed
CHIN - Seed	AMER - Seedling

BLOCK 2

B_2F_3 - Seed	B_2F_3 - Seedling
CHIN - Seedling	AMER - Seedling
AMER - Seed	B_1F_3 - Seedling
B_1F_3 - Seed	CHIN - Seed
B_3F_2 - Seedling	B_3F_2 - Seed

BLOCK 3

CHIN - Seed	AMER - Seedling
AMER - Seed	B_1F_3 - Seedling
B_2F_3 - Seed	CHIN - Seedling
B_1F_3 - Seed	B_3F_2 - Seedling
B_3F_2 - Seedling	B_2F_3 - Seed

BLOCK 4

B_2F_3 - Seedling	AMER - Seedling
B_3F_2 - Seedling	B_2F_3 - Seed
B_1F_3 - Seed	CHIN - Seed
AMER - Seedling	B_1F_3 - Seedling
CHIN - Seedling	B_3F_2 - Seed

BLOCK 5

B_1F_3 - Seedling	CHIN - Seed
B_2F_3 - Seed	B_1F_3 - Seed
AMER - Seed	B_3F_2 - Seed
B_3F_2 - Seedling	B_2F_3 - Seedling
CHIN - Seedling	AMER - Seedling

Compacted Gray Sandstone

BLOCK 1

AMER - Seed	CHIN - Seed
CHIN - Seedling	B_2F_3 - Seed
B_3F_2 - Seedling	AMER - Seedling
B_2F_3 - Seedling	B_3F_2 - Seed
B_1F_3 - Seed	B_1F_3 - Seedling

BLOCK 2

B_1F_3 - Seed	AMER - Seed
AMER - Seedling	B_1F_3 - Seedling
CHIN - Seed	B_2F_3 - Seed
B_2F_3 - Seedling	B_3F_2 - Seed
B_3F_2 - Seedling	CHIN - Seedling

BLOCK 3

B_2F_3 - Seed	AMER - Seedling
B_1F_3 - Seed	B_2F_3 - Seedling
B_3F_2 - Seedling	B_3F_2 - Seed
AMER - Seed	B_1F_3 - Seedling
CHIN - Seedling	CHIN - Seed

BLOCK 4

B_1F_3 - Seed	CHIN - Seed
B_3F_2 - Seed	B_3F_2 - Seedling
AMER - Seed	AMER - Seedling
CHIN - Seedling	B_1F_3 - Seedling
B_2F_3 - Seed	B_2F_3 - Seedling

BLOCK 5

AMER - Seedling	B_2F_3 - Seed
CHIN - Seed	AMER - Seed
B_3F_2 - Seedling	B_3F_2 - Seed
B_1F_3 - Seed	CHIN - Seedling
B_2F_3 - Seedling	B_1F_3 - Seedling

Figure 5. Completely randomized block design for seeds and seedlings in two substrates at the Nicholas Energy study site. Each chestnut stock type (American, Chinese, B_1F_3, B_2F_3, and B_3F_2) of seeds and seedlings were randomly planted in each of five blocks in both substrates.

Statistical analyses were performed using SAS 9.1 software (SAS Institute, 2005). Means were calculated for tree survival and height among chestnut stock types on both substrates, but they were not compared across substrates (no statistical analysis performed). Using ANOVA, significant differences for seedling survival and growth among chestnut stock types within substrate types were evaluated at an alpha level of 0.05.

Soil Sampling

For the Glory study site, soil samples were extracted at five locations in each block (at the four corners and center) to a depth of 15 cm to evaluate chemical properties. At the Nicholas site, soil samples were extracted from five locations across each substrate type to a depth of 15 cm. Samples were analyzed for pH (1:1 soil:water) with a Beckman 43 pH meter and elemental content by the West Virginia University Soil Testing Laboratory with a Mehlich 1 extract, which is composed of approximately 0.05*N* HCl and 0.025*N* H_2SO_4. The leachate from the extraction was analyzed with a Perkin Elmer Plasma 400 emission spectrometer for H, Al, P, K, Ca, and Mg. Cation exchange capacity was calculated by summing the above elements and base saturation was calculated as the sum of base cations divided by total cations. At Glory, means for soil properties on each site (shelters and no shelters) were calculated and significant differences between sites were evaluated by t-tests (SAS, 2005). At Nicholas, t-tests were used to determine significant differences between substrates for soil parameters.

Results and Discussion

Glory Study

At Glory, soil analysis revealed a pH range of 5.5 to 5.8 at both sites (Table 1). The shelter site had significantly more Ca and higher base saturation than the site where no shelters were placed on seeds. No other soil parameters we measured seemed different between sites. We expected some variation in soils between sites and these values are within anticipated ranges of soil chemical values.

Table 1. Chemical properties of soils in 2008 where five chestnut seed stock types were planted at the Glory surface mine in West Virginia. Seeds were planted on two sites with tree shelters and no tree shelters.

Block	pH	P	K	Ca	Mg	CEC	BS
		mg kg^{-1}	--------------	$cmol^+ kg^{-1}$	----------------		%
Shelters Ave	5.8	30	0.15	3.36 a[1]	1.93	10	55 a
No shelters Ave	5.5	33	0.14	2.10 b	1.96	10	44 b

[1] Values within columns with different letters are significantly different with an LSD test at $p<0.05$. If no letters, the values are not significantly different.

During the first year, chestnut seeds established and survived at a higher rate in the plots where tree shelters were placed on top of the seed compared to those in the plot that did not have tree shelters (81 vs 64%), which effect persisted through 2011 (Table 2). The tree shelters may have originally protected the seed from predators but there was no evidence that small mammals or deer had visited the plot. Tree shelters may have also slightly changed the climate and environment within the shelter during the first year. We noticed some heat stress and burning of leaves in shelters at the end of the first growing season, plus many of the seedlings were crowded in the shelters. Therefore, in June of 2009, in the middle of the second growing season, we removed the tree shelters, which may have eliminated some of the moisture stress and high temperature within the shelter tube (Bergez and Dupraz, 1997). More trees were lost between the 2nd and 3rd years when the shelters were removed, but essentially no more trees were lost from the 2nd to 4th years where no shelters had been placed. Tree height was consistently greater on the plot with shelters versus no shelters (Table 2).

Table 2. Chestnut seed germination/survival and height with and without tree shelters across all five seed stock types and peat treatments in the Glory Study from 2008 to 2011.

Treatment		**Survival**		
	2008	2009	2010	2011
		---------------------- % ----------------------		
Shelters	81	74	61	59
No Shelters	64	48	53	45
Ave	72	61	57	52
Treatment		**Height**		
	2008	2009	2010	2011
		--------------------- cm ----------------------		
Shelters	10	37	51	53
No Shelters	6	27	37	44
Ave	8	32	44	49

Seeds planted with peat showed significantly lower seed germination and establishment than seeds without peat (64% vs 81%) during the first year (Table 3). Survival was reduced an additional 10% regardless of peat treatment by the end of the 2nd growing season, with another 8 to 10% decline in survival by the 4th year. We do not know the reason(s) why peat treatment negatively affected seed germination during the first year. However, it is possible that the peat retained moisture making it unavailable for seed germination, and the peat may have reduced the pH surrounding the seed which may have negatively influenced germination (Skousen et al., 2009). Peat treatment had little effect on height (Table 3).

Table 3. Chestnut seed germination/survival and height with and without peat treatment across all five seed stock types and tree shelter treatments in the Glory Study from 2008 to 2011.

Treatment	**Survival**			
	2008	2009	2010	2011
	----------------------	%	----------------------	
Peat	64 b*	52 b	50 b	44 b
No Peat	81 a	70 a	64 a	60 a
Ave	72	61	57	52
Treatment	**Height**			
	2008	2009	2010	2011
	----------------------	cm	----------------------	
Peat	8	29	41	47
No Peat	9	35	48	51
Ave	8	32	44	49

*Peat treatment values within years with different letters are significantly different with an LSD test at $p<0.05$. If no letters, the values are not significantly different.

In the area where shelters covered the seeds, Chinese seeds showed significantly higher establishment than the other seed stock types (Table 4). While the other four seed stock types had a 20 to 30% reduction in survival from the first to the fourth years, Chinese remained about the same through four years.

Table 4. Seed germination and survival of five chestnut stock types **with tree shelters** across peat treatments in the Glory Study from 2008 to 2011.

Seed Type	**Survival with Tree Shelters**			
	2008	2009	2010	2011
	-----------------------	**%**	**------------------------**	
American	75 b*	68 b	48 c	48 c
Chinese	93 a	93 a	93 a	85 a
B_1F_3	83 b	70 b	68 b	65 b
B_2F_3	78 b	63 b	48 c	45 c
B_3F_2 - p	75 b	68 b	53 c	48 c
Ave	81	74	61	59

*Values within years with different letters are significantly different with an LSD test at $p<0.05$

In plots where no tree shelters were placed over seeds, all seeds germinated at about the same rate from 58 to 70% (Table 5). Chinese seeds did not decline from the original survival rate during the next four years, but all others did, declining at a rate of 20 to 25%. This rate of decline was similar to the decline in the area with tree shelters.

Table 5. Seed germination and survival of five chestnut stock types **without tree shelters** across peat treatments in the Glory Study from 2008 to 2011.

Seed Type	**Survival without Tree Shelters**			
	2008	2009	2010	2011
	----------------------	**%**	-----------------------	
American	58 c*	40 d	40 d	33 c
Chinese	70 b	68 b	75 b	73 b
B_1F_3	65 c	48 c	55 c	45 d
B_2F_3 - p	60 d	43 d	40 c	35 c
B_3F_2 - p	68 c	53 c	53 c	40 c
Ave	64	50	53	45

*Values within years with different letters are significantly different with an LSD test at $p<0.05$.

During the first year with tree shelters, seedlings of Chinese seeds showed a trend of higher growth than the other seeds, which may demonstrate the larger seed size and greater reserves available for growth with Chinese seeds (Table 6). By the fourth year, any height differences among seed stock types had disappeared, and in fact Chinese had the lowest average height of the five seed stock types. This will be an interesting trend to follow: to see if the backcrosses and American continue to outpace Chinese chestnut in height. Similarly, in the area with no tree shelters, seedlings from seeds showed a trend of shorter height, which trend continued through to the fourth year (Table 7).

Nicholas Study

Soil chemical properties were significantly different for most parameters between brown and gray substrates (Table 8). Soil pH was much lower at 4.5 for the brown compared to pH 6.6 for the gray. In many cases with gray sandstone soil substitutes in West Virginia, the pH is generally higher, sometimes nearly 8.0 (Emerson et al., 2009). The almost 10-fold greater P in the gray versus the brown materials has also been documented in studies of West Virginia mine soils (Emerson et al., 2009; Thomas and Skousen, 2011). Significantly greater quantities of Ca and Mg were found in gray versus brown sandstone, which then gave much higher base saturation values.

Table 6. Height of five chestnut stock types **with tree shelters** with peat and no peat treatments in the Glory Study from 2008 to 2011.

Seed Type	Height with Tree Shelters			
	2008	2009	2010	2011
	---------------------- cm	----------------------		
American	9	31	51	57
Chinese	13	44	56	49
B_1F_3	11	39	52	51
B_2F_3	9	37	47	59
B_3F_2	10	37	51	51
Ave	10	37	51	54

*Values within years with different letters are significantly different with an LSD test at p<0.05

Table 7. Height of five chestnut stock types **without tree shelters** across peat treatments in the Glory Study.

Seed Type	Height without Tree Shelters			
	2008	2009	2010	2011
	---------------------- cm	----------------------		
American	5	20	32	40
Chinese	8	29	35	41
B_1F_3	7	28	39	45
B_2F_3	6	30	39	47
B_3F_2	6	27	40	45
Ave	6	27	37	44

*Values within years with different letters are significantly different with an LSD test at p<0.05

Table 8. Chemical properties in 2009 of the two substrate types in the Nicholas Study, where five chestnut seed and seedling stock types were planted into brown and gray sandstone plots in West Virginia.

Substrate	pH	EC	P	K	Ca	Mg	CEC	BS
		dS m^{-1}	mg kg^{-1}	---------------	cmol$^+$ kg^{-1}	----------------		%
Brown	4.5 b	0.16	6.0 b	0.33 a	2.90 b	3.60 b	13 b	28 b
Gray	6.6 a	0.23	56.1 a	0.40 a	9.50 a	6.20 a	8 a	100 a

*Values with different letters for each parameter are significantly different with an LSD test at p<0.05

A surprising finding during this study of seeds and seedlings during this first year was that only a handful of the 250 seeds planted on either substrate germinated and established. Only six seeds germinated, with four being in gray sandstone and the other two in brown. These six germinated seeds were also not just one seed type; two were B_1F_3, two were Chinese, and one was B_2F_3, and the other B_3F_2. Therefore, no other information could be gathered about seed germination and establishment during this first year in this study.

For seedlings, after the first year, 100% survival was found in the brown sandstone for all stock types except for B_3F_2 with 89% (Table 9). These survival rates declined to between 68 and 88% after the third growing season. On the gray, Chinese had 100% survival through three years, while the others declined to between 48 and 80%. The B_3F_2 survived particularly poorly on the gray sandstone to 50%. We found hardwood tree survival to be between 60 to 70% on brown and gray substrate materials in other studies in West Virginia mine soils (Emerson et al., 2009; DeLong and Skousen, 2009), so this result is not surprising.

Chinese seedlings were significantly greater in height than the other chestnut seedling stock types but these height differences were partly due to the initial differences in the size of the seedlings when planted (Table 10). During the second growing season and subsequently during later years of measurement, height differences will be increasingly due to growing conditions and growth media differences. We have seen in some of our other studies that tree growth is much greater on brown substrates than on gray substrates (Thomas and Skousen, 2011) and we are beginning to see these differences in this study. Average height of all chestnut seedlings in the brown substrate was 86 cm compared to 59 cm in the gray substrate.

Table 9. Chestnut seedling survival for five stock types in the Nicholas Study in brown and gray substrates in 2009 to 2011.

Seed Type	**Substrate**					
	Brown			Gray		
	2009	2010	2011	2009	2010	2011
	---------------		**%**	---------------		
American	100 a*	100 a	77 ab	100 a	72 b	80 b
Chinese	100 a	96 a	84 a	100 a	100 a	100 a
B_1F_3	100 a	72 b	88 a	80 b	72 b	72 b
B_2F_3	100 a	70 b	70 b	87 b	66 b	68 b
B_3F_2	89 b	68 b	68 b	100 a	44 c	48 c
Ave	98	81	77	93	61	74

*Values within columns (seed stock and year) with different letters are significantly different at an alpha level of 0.05.

Table 10. Chestnut seedling height for five stock types in brown and gray substrates at the Nicholas Study in 2009 to 2011.

Seed Type	Substrate: Brown 2009	Brown 2010	Brown 2011	Gray 2009	Gray 2010	Gray 2011
	cm					
American	62 b*	80 b	92 b	59 b	61 b	71 a
Chinese	100 a	101 a	112 a	84 a	90 a	79 a
B_1F_3	36 c	56 c	77 c	37 c	42 c	55 b
B_2F_3	33 c	48 c	68 c	31 c	24 d	52 b
B_3F_2	26 c	52 c	82 bc	22 c	24 d	39 c
Ave	51	68	86	47	48	59

* Values within columns (seed stock type and year) with different letters are significantly different at an alpha level of 0.05.

Summary and Conclusions

In the Glory study, seeds of five chestnut parental species and breeding generations (stock types: American, Chinese, B_1F_3, B_2F_3, and B_3F_2 backcrosses) were planted into a mixed brown-gray sandstone substrate material in eight blocks with and without peat, and with and without tree shelters on a surface mine in southern West Virginia. The mixed sandstone soil material had a pH that varied across the blocks from pH 5.3 to 6.7, with the tree-sheltered blocks being slightly more acidic than the non-sheltered blocks. Germination and survival after the first year was 72% across all treatments and survival dropped to 52% after the fourth year. Seeds in the area without shelters and those in peat treatment had the lowest germination and survival. After the second year, Chinese seeds had significantly higher germination and survival at 81% compared to around 51 to 62% survival for American and backcross seeds, and this trend persisted through the fourth year. Average height of trees was between 45 and 53 cm for all chestnut stock types.

In the Nicholas Study, soil pH on the brown substrate material was 4.5 compared to 6.6 on the gray sandstone material. Only six seeds of the 250 planted seeds germinated, which was quite surprising compared to the good success we had with chestnut seeds the previous year at the Glory site. After the first year, planted seedling survival was >80% with all chestnut stock types on both brown and gray substrates. All declined to lower levels except for Chinese. Overall, we have seen good establishment success on mined lands with chestnut seeds in the

Glory Study, but poor establishment with seeds at the Nicholas Study. However, good survival and establishment was found for chestnut seedlings in the Nicholas Study. The results of this study demonstrate that chestnut survival values are similar to survival of hardwood species on reclaimed mine land. Growth is also comparatively similar to hardwood tree growth on these types of mine soils in other studies in the Appalachian Region.

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References

Adank, K.M., C.D. Barton, M.E. French, and P.B. de Sa. 2008. Occurrence of Phytophthora on reforested loose-graded spoils in eastern Kentucky. Proceedings America Society of Mining and Reclamation, 2008 pp 1-13. http://dx.doi.org/10.21000/JASMR08010001.

Anagnostakis, S.L. 1995. The pathogens and pests of chestnuts. Advances in Botanical Research 21: 125-145. http://dx.doi.org/10.1016/S0065-2296(08)60011-7.

Angel, P., C. Barton, R. Warner, C. Agouridis, T. Taylor, and S. Hall. 2008. Forest establishment and water quality characteristics as influenced by spoil type on a loose-graded surface mine in eastern Kentucky, Proceedings America Society of Mining and Reclamation, 2008 pp 28-65 http://dx.doi.org/10.21000/JASMR08010028

Angel, P., J. Burger, V. Davis, C. Barton, M. Bower, S. Eggerud, and P. Rothman. 2009. The forestry reclamation approach and the measure of its success in Appalachia. Proceedings America Society of Mining and Reclamation, 2009 pp. 18-36. . http://dx.doi.org/10.21000/JASMR09010018.

Ashby, W.C. 2006. Reflections of a botanist on reclamation to trees. Reclamation Matters, Volume 3, Issue 2, Fall 2006. American Society of Mining and Reclamation, Lexington, KY.

Bergez, J.E., and Z.C. Dupraz. 1997. Transpiration rate of Prunum avium L. seedlings inside an unventilated tree shelter. For. Ecol. Manage. 97: 255-264. http://dx.doi.org/10.1016/S0378-1127(97)00071-6

Boyce, S. 1999. Office of Surface Mining (OSM) revegetation team survey results. p. 31-35. *In*: K.C. Vories and D. Throgmorton (eds.), Proceedings of the Enhancement of Reforestation at Surface Coal Mines: Technical Interactive Forum. March 23-24, 1999, USDI, OSM, Coal Research Center, Southern Illinois University, Carbondale, IL.

Brewer, L.G. 1995. Ecology of survival and recovery from blight in American chestnut trees [Castanea dentata (Marsh.) Borkh.] in Michigan. Bull. Torrey Bot. Club 122: 40-57. https://doi.org/10.2307/2996402

Brown, J.H. 1962. Success of tree planting on strip-mined areas in West Virginia. West Virginia Agriculture and Forestry Experiment Station Bulletin 473. West Virginia University, Morgantown, WV.

Burger, J., D. Graves, P. Angel, V. Davis, and C. Zipper. 2005. The forestry reclamation approach. US Office of Surface Mining, ARRI, Forest Reclamation Advisory No. 2.

Burger, J., D. Mitchem, and W.L. Daniels. 2007. Red oak seedling response to different topsoil substitutes after five years. Proceedings America Society of Mining and Reclamation, 2007 pp 132-142. http://dx.doi.org/10.21000/JASMR07010132.

Burnham, C.R., P.A. Rutter, and D.W. French. 1986. Breeding blight-resistant chestnuts. Plant Breeding Reviews 4: 347-397. http://dx.doi.org/10.1002/9781118061015.ch11.

Carpenter, S. 1992. Soil survey of Nicholas County, West Virginia. US Dept. of Agriculture and the WVU Agricultural Experiment Station, Morgantown, WV. 138 p.

DeLong, C., and J. Skousen. 2009. Amendments for reforestation on a reclaimed West Virginia surface mine. In: Abstracts, American Society of Agronomy, Nov. 1-5, 2009. Pittsburgh, PA.

Emerson, P., J. Skousen, and P. Ziemkiewicz. 2009. Survival and growth of hardwoods in brown versus gray sandstone on a surface mine in West Virginia. J. Environ. Qual. 38: 1821-1829. http://dx.doi.org/10.2134/jeq2008.0479.

Freinkel, S. 2009. American chestnut: the life, death, and rebirth of a perfect tree. University of California Press. 304 p.

French, M.E., C.D. Barton, D. Graves, P.N. Angel, and F.V. Hebard. 2007a. Evaluation of mine spoil suitability for the introduction of American chestnut hybrids in the Cumberland Plateau. Proceedings America Society of Mining and Reclamation, 2007 pp 249-258. http://dx.doi.org/10.21000/JASMR07010249.

French, M.E., C.D. Barton, F.V. Hebard, D. Graves, S. Fei, and K. Adank. 2007b. Can surface mine reclamation help to restore American chestnut? p. 24-28. *In*: Reclamation Matters, Issue 2, Fall 2007. American Society of Mining and Reclamation, Lexington, KY.

Haering, K.C., W.L. Daniels, and J.M. Galbraith. 2004. Appalachian mine soil morphology and properties: effects of weathering and mining method. Soil Sci. Soc. Am. J. 68: 1315-1325. http://dx.doi.org/10.2136/sssaj2004.13155.

Hebard, F. 2005. The backcross breeding program of The American Chestnut Foundation. Journal of The American Chestnut Foundation 19(2): 55-77.

Jacobs, D.F. 2007. Toward development of silvical strategies for forest restoration of American chestnut (Castanea dentata) using blight-resistant hybrids. Biological Conservation 137: 497-506. http://dx.doi.org/10.1016/j.biocon.2007.03.013.

Limstrom, G.A. 1960. Forestation of strip-mined land in the Central States. USDA Forest Service Central States Forest Experiment Station, Agric. Hdbk. No. 166, Columbus, OH.

McCarthy, B.C., J.M. Bauman, and C.H. Keiffer. 2008. Mine land reclamation strategies for the restoration of American chestnut. Ecological Rest. 26: 292-294. http://dx.doi.org/10.3368/er.26.4.292.

McCarthy, B.C., K.E. Gilland, J.M. Bauman, and C.H. Keiffer. 2010. Factors affecting the performance of artificially regenerated American chestnut on reclaimed mine sites. Proceedings America Society of Mining and Reclamation, 2010 pp. 582-597. http://dx.doi.org/10.21000/JASMR10010582.

Paone, J., P. Struthers, and W. Johnson. 1978. Extent of disturbed lands and major reclamation problems in the United States. p. 11-22. In: F. Schaller and P. Sutton (Eds.), Reclamation of Drastically Disturbed Lands, 1st Edition, American Society of Agronomy, Madison, WI.

Phelps, T. 2002. Fifth generation orchard initiated at Penn State Arboretum. *In*: Chestnut Tree, Newsletter of the Pennsylvania Chapter of The American Chestnut Foundation. http://www.patacf.org/

Plass, W.T. 1982. The impact of surface mining on the commercial forests of the United States. p. 1-7. *In*: C.A. Kolar and W.C. Ashby (eds.), Post-mining Productivity with Trees. March 31-April 2, 1982, Southern Illinois University, Carbondale, IL.

Plass, W.T. 2000. History of surface mining reclamation and associated legislation. p. 1-20. *In*: R. Barnhisel et al. (Eds.), Reclamation of Drastically Disturbed Lands. 2nd Edition, Agron. Monogr. 41, ASA, SSSA, CSSA, Madison, WI.

Russell, E.W.B. 1987. Pre-blight distribution of Castanea dentata (Marsh.) Borkh. Bulletin of the Torrey Botanical Club 114(2): 183-190. http://dx.doi.org/10.2307/2996129

SAS Institute. 2005. SAS user's guide. Cary, NC.

Skousen, J., T. Keene, C. DeLong, E. Pena-Yewtukhiw, and T. Cook. 2009. Survival and growth of five chestnut seed stock types on mountaintop surface mines in West Virginia. Proceedings America Society of Mining and Reclamation, 2009 pp. 1276-1291. .
https://doi.org/10.21000/JASMR0901
Skousen, J., C. Zipper, J. Burger, P. Angel, and C. Barton. 2011a. Selecting topsoil substitutes for forestry mine soils. Proceedings America Society of Mining and Reclamation, 2011 pp 591-609. . http://dx.doi.org/10.21000/JASMR11010591. .

Skousen, J., C. Zipper, J. Burger, C. Barton, and P. Angel. 2011b. Selecting materials for mine soil construction when establishing forests on Appalachian mine sites. US Office of Surface Mining, ARRI, Forest Reclamation Advisory No. 8.

Thomas, C., and J. Skousen. 2011. Hardwood tree performance on amended brown and gray mine spoils after four years. Proceedings America Society of Mining and Reclamation, 2011 pp 655-675. http://dx.doi.org/10.21000/JASMR11010655

Torbert, J.L., and J.A. Burger. 2000. Forest land reclamation. p. 371-398. *In*: Reclamation of Drastically Disturbed Lands, 2nd Edition. American Society of Agronomy, Madison, WI.

Wolf, B. 1994. Soil survey of Boone County, West Virginia. US Dept. of Agriculture and the WVU Agricultural Experiment Station, Morgantown, WV. 115 p.

Zipper, C., J. Burger, J. Skousen, P. Angel, C. Barton, V. Davis, and J. Franklin. 2011. Restoring forests and associated ecosystem services on Appalachian coal surface mines. Environ. Management 47: 751-765. http://dx.doi.org/10.1007/s00267-011-9670-z.