Survival, Growth, and Blight Incidence of Pure and Hybrid American Chestnuts After Nine Growing Seasons on a FRA-Reclaimed Coal Mine

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Introduction

The American chestnut (*Castanea dentata*) was once the dominant hardwood species in the eastern United States, representing 20-25% of forest canopy trees in the mixed mesophytic forests of the Appalachians, and serving as an important resource for people and wildlife (Lutts 2004). In the early 1900s, a fungal blight (*Cryphonectria parasitica*) was introduced from imported Japanese nursey stock that caused topkill of American chestnuts. The disease spread rapidly through Appalachia, and by the 1950s, only a few seemingly blight resistant trees remained, as well as resprouts from the dead trees (McEwan et al. 2006, Dalgleish et al. 2016). The resprouts repeatedly die back such that they never reach mature height, rendering the American chestnut canopy tree, functionally extinct (Dalgleish et al. 2016).

Since 1983, the American Chestnut Foundation (ACF) has been conducting a backcross breeding program to produce a tree resistant to the blight (Figure 1). The process begins by crossing American and Chinese chestnuts (*Castanea mollissima*), then repeatedly backcrossing the progeny to American mother trees (hybrid x American), and subsequently intercrossing (hybrid x hybrid) to produce offspring that segregate for blight resistance. All offspring are inoculated with blight to assess their level of resistance; trees with the highest blight resistance are selected to produce the next generation. Nuts from ACF hybrid trees are planted in various landscapes to 'field-test' their resistance to blight.

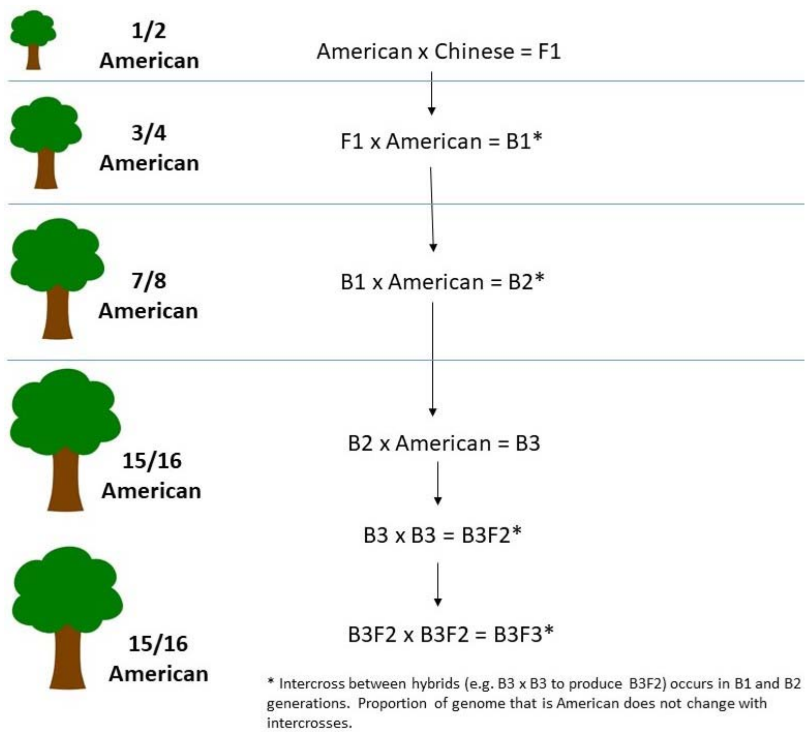

Figure 1. Schematic of the American chestnut backcross program (Clapper 1954, Jaynes and Graves 1963, Hebard 2001).

Coal surface mining has also impacted the forests of Appalachia. Over 400,000 ha have been disturbed to date in Appalachia, much of which is still need of reclamation. The extent of coal surface mining in the Appalachians, and thus land in need of reclamation, overlaps significantly with the historic range of the American chestnut (Figure 2). This overlap presents the opportunity to field-test American chestnut hybrids for blight resistance, growth, and survival on land already in need of reforestation. The use of the forestry reclamation approach (FRA) under the Surface Mining Control and Reclamation Act (SMCRA) has improved the success of coal mine reforestation in Appalachia (Burger et al. 2005) through better substrate selection and site preparation methods. The FRA consists of five steps: 1) Create a suitable growth medium at least 1.2 m (4 ft) thick of topsoil, weathered sandstone, or the best available material; 2) Loosely grade the growth medium to minimize compaction; 3) Seed with a tree-compatible groundcover mix; 4) Select a mix of early successional native trees and slower growing commercially valuable trees; 5) Plant trees properly to minimize mortality (Burger et al. 2005). With the implementation of FRA methods, chestnuts have the greatest opportunity for success.

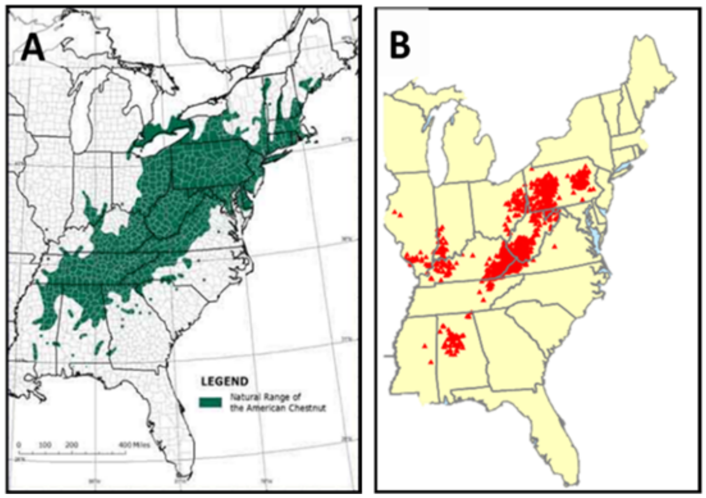

Figure 2. A) Map of the historic range of American chestnut in the United States (TACF 2014), B) Map of active coal surface mines in the eastern United States as of 2012 (CDC 2012).

However, many chestnuts do not grow well on reclaimed coal mines (Fields-Johnson 2011, Skousen 2016). In optimal conditions, they can grow over 50 cm per year, but several studies have shown growth closer to 10 cm per year on reclaimed coal mines (Clark et al. 2012, Gilland and McCarthy 2014). While poor survival and growth of chestnuts on coal mines is easily observable, the cause behind poor performance is less clear. Although chestnuts were historically most common on dry, south-facing ridge tops with sandy soils, one study observed that seedling survival decreased as percent sand and coarse fragments in the soil increased, and that canker incidence nearly doubled on xeric sites compared to mesic sites (Braun 1935, McEwan et al. 2005, Rhoades et al. 2009). Other studies have cited that chestnuts grow more slowly in poorly drained or extremely dry soils with high pH, but observed truncated growth on reclaimed mines with mesic, acidic soils (Gilland and McCarthy 2012).

In addition to responses to site physical characteristics, American chestnut survival, growth, and blight incidence are affected by competing vegetation. Griffin et al. (1991) observed higher chestnut survival and growth, and lower blight incidence when chestnuts were in the presence of competing hardwood vegetation compared to clear-cut landscapes. Clark et al. (2012) found that reducing the canopy cover of competing hardwood vegetation improved ground-line diameter and height growth, though mortality and blight incidence were both nominally higher. Conversely, studies testing the FRA methods consistently show decreased survival and growth of planted trees in the presence of competitive vegetation, suggesting that the type of vegetation competing with planted trees influences survival and growth (Burger et al. 2005, Burger et al. 2009, Fields-Johnson et al. 2012, Franklin et al. 2012).

In this study, we have revisited a chestnut experiment that was established in 2008 at the Powell River Project near Wise, VA to re-measure the trees and assess them for blight symptoms, as well as gather site data specific to each individual tree including slope position, aspect, and soil texture (Fields-Johnson 2011). Finally, as the experiment set-up in 2008, several of the tags identifying the trees were lost. We therefore collected tissue samples from the unknown trees and are using genomic methods to determine their ancestry. Samples are being analyzed by Jason Holliday's lab technician in the Department of Forest Resources and Environmental Conservation. Using these data, we hope to isolate some possible causes for stunted chestnut growth on this coal mine reclamation to better inform future reforestation efforts on former coal mines.

Goals

To investigate how site physical properties and vegetation competition have affected the survival, growth, and incidence of *Cryphonectoria parasitica* in American, Chinese, and hybrid chestnuts planted on a reclaimed coal mine after nine growing seasons.

Methods

Reclamation Methods

The study site was initiated in 2008 as part of a larger Forestry Reclamation Approach (FRA) experiment that assessed coal mine reforestation success within grading and seeding treatment combinations (Fields-Johnson 2011, Fields-Johnson et al. 2012, Zipper et al. 2016). Growth media in Block 1 was a mix of weathered sandstone and unweathered sandstones, and in Block 2, a mix of unweathered sandstones, siltstones, and some shale. Soil textures in Blocks 1 and 2 are similar, but soil chemistry differs due to the different parent materials (Table 1). During final grading, experimental blocks were either graded smooth or loose. Within each grading treatment, three seeding treatments were applied on 0.4 ha plots, for a total of six treatment combinations at each of the two experimental blocks at the Powell River Project (Figure 3). Block 1 was hydraulically seeded in the fall of 2007 and Block 2 was hydraulically seeded in the winter of 2007/2008. After seeding, a mix of native tree bare root seedlings was planted across the entire site at an overall rate of 1,845 trees/ha, and included an FRA mix of crop trees, such as chestnut and black oak, as well as wildlife trees such as redbud and white pine.

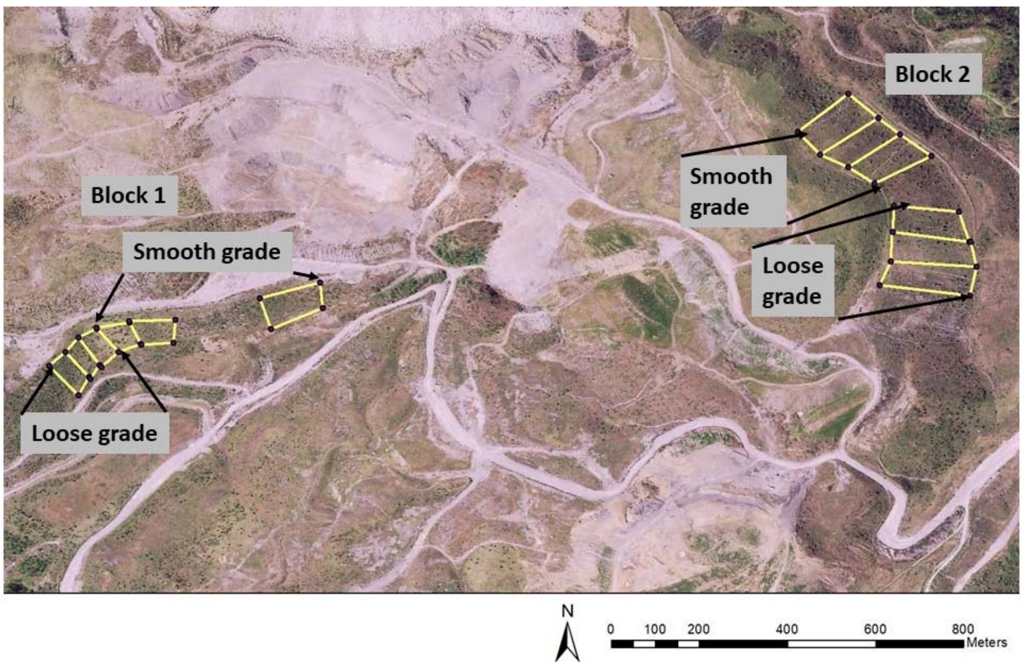

Figure 3. Map of the two experimental blocks within the grading x seeding experiment.

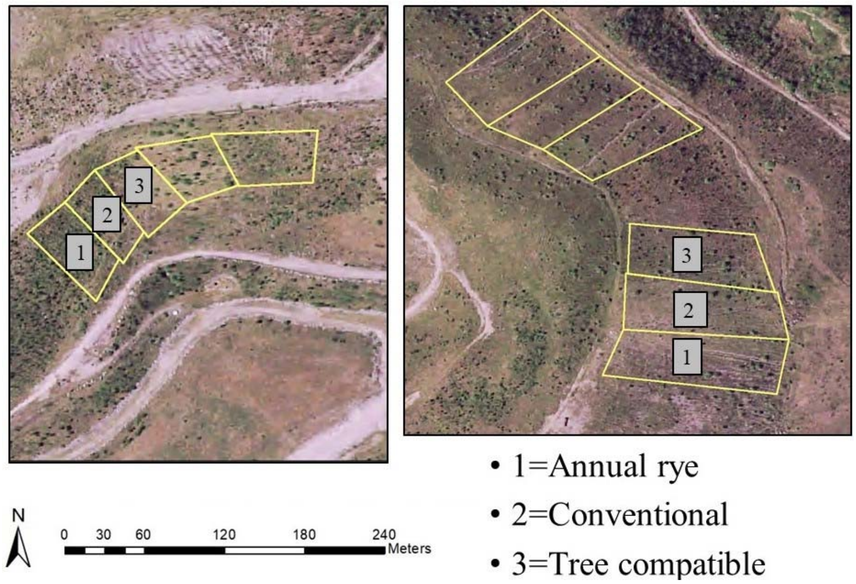

Figure 4. Maps of seeding treatments within the loose graded plots of blocks 1 and 2 (Esri ArcGIS 2016).

Table 1. Physical and chemical characteristics of the loose graded plots in the two experimental blocks in 2008 and 2016.

Block	Seeding treatment	Downhill aspect*	Texture*	2008 pH**	2008 EC**	2016 pH ± SE	2016 EC ± SE
1	**Annual rye**	SE	Sandy loam (SL)	5.96	401.5	6.98 ± 0.13 B,a†	360.4 ± 58.8 B
	Conventional	SE	Sandy clay loam (SCL)	5.52	898.5	6.52 ± 0.12 B,b	452.8 ± 54.5 B
	Tree compatible	SE	SCL	5.51	1356.7	6.78 ± 0.12 B,ab	532.1 ± 54.5
2	**Annual rye**	ESE	SCL	7.93	325.4	7.70 ± 0.10 A,a	779.2 ± 48.0 A
	Conventional	E	SCL	8.10	325.4	7.89 ± 0.10 A,a	675.7 ± 46.3 A
	Tree compatible	E	SCL	7.46	343.2	7.30 ± 0.10 A,b	627.6 ± 44.8

* Data from 2016 sampling.
**Data from Fields-Johnson 2010.
†Differences between groups determined by the Holm-Sidak method with α=0.05. Uppercase letters indicate differences between blocks within seeding treatments, lowercase letters indicate differences among seeding treatments within blocks.

Table 2. Seeding treatments and soil amendments applied to the experimental site in 2007.

Seeding treatment	Species	Application rate (kg/ha)
Annual rye	Annual ryegrass (*Lolium multiflorum*)	22
Tree compatible	Annual ryegrass (*Lolium multiflorum*)	22
	Perennial ryegrass (*Lolium perenne*)	11
	Timothy grass (*Phleum pratense*)	6
	Birdsfoot trefoil (*Lotus corniculatus*)	6
	Ladino clover (*Trifolium repens*)	3
	Weeping lovegrass (*Eragrostis curvula*)	2
Conventional	Cereal rye (*Secale cereale*)	34
	Orchardgrass (*Datylis glomerata*)	22
	Perennial rye (*Lolium perenne*)	11
	Korean lespedeza (*Kummerowia stipulacea*)	6
	Birdsfoot trefoil (*Lotus corniculatus*)	6
	Ladino clover (*Trifolium repens*)	6
	Redtop	3
	Weeping lovegrass (*Eragrostis curvula*)	2

Soil amendments	Material	Application rate (kg/ha)
	Nitrogen	22
	Phosphorous	68
	Potassium	18
	Wood fiber mulch	1680

Chestnuts were planted on the site in March of 2008 after the site had been graded and seeded among the aforementioned mix of native bare root tree seedlings. Chestnuts were planted within only the loose-graded plots to minimize compaction related mortality, as chestnuts are particularly sensitive to compaction. Within the loose-graded plots, chestnuts were planted in rows across all three different seeding mixes on Blocks 1 and 2 (Figure 4). Chestnuts were all planted as nuts that were produced by the American Chestnut Foundation (ACF), a mix of two lines of pure American chestnuts, one line of pure Chinese chestnuts, and one line each of B1F3, B2F3, and B3F2. After planting, a tree tube was installed for each nut and the genotype was recorded on the tree tube. Trees were labeled with permanent metal tags after germination. Data for the first two years of this study were published in 2012.

Field Sampling Methods

On the two experimental sites, we measured the ground-line diameter and height of all surviving chestnut trees. We assessed every tree for blight symptoms and recorded the severity of blight incidence. Overall health was scored to indicate the extent of blight damage to each tree as from Turchetti et al. (2008) (Table 3a). The trunk, lower crown, middle crown, upper crown, and overall tree were all given a score on the extent of blight symptoms as from Tizado et al. (2012) (Table 3b). At each tree, we recorded slope for the exact tree location using a meterstick and level and downhill aspect with a compass. At each tree, we also recorded landscape position as a proxy for degree of soil compaction (Table 4). Scaled percent cover of vegetation, height of vegetation relative to the associated chestnut, and dominant species were visually estimated and recorded at each tree (Table 5).

Table 3. Tree health (a) and blight symptom (b) indices.

A.

Overall tree health index	
0	Dead, other cause
1	Live and healthy
2	Live and blighted
3	Branch dead from blight
4	Top dead from blight
5	Resprout
6	Whole stem dead from blight

B.

Blight symptom index	
0	None
1	Visible symptoms, 10% of tree/tree part
2	Visible symptoms, 11-25% of tree/tree part
3	Visible symptoms, 26-50% of tree/tree part
4	Visible symptoms, 51-80% of tree/tree part
5	Visible symptoms, 81+% of tree/tree part

Table 4. Slope position index.

Slope position index	
0	Below slope (flat)
1	Bottom of slope
2	Middle of slope
3	Upper middle of slope
4	Top of slope

Table 5. Vegetation cover (A) and vegetation competition (B) indices based on methods from Evans et al. (2013).

A.

Vegetation cover index	
0	0% cover, no vegetation
1	1-25% vegetation cover
2	26-50% vegetation cover
3	50-75% vegetation cover
4	76-90% vegetation cover
5	91-100% vegetation cover

B.

Vegetation competition index	
1	No vegetation
2	Vegetation less than 1/2 tree height
3	Vegetation 1/2 to 3/4 tree height
4	Vegetation >3/4 tree height
5	Vegetation taller than tree

Soil Collection and Analysis

A soil sample was collected adjacent to each surviving chestnut tree, then air-dried and sieved to 2 mm on the Virginia Tech campus. Field-texturing methods were used to assess the soil texture on the sieved soil (Thien 1979). Soil pH and electrical conductivity (EC) were measured using the saturated paste method (Rhoades et al. 1989).

Genomic assignment of hybrid classes

As some of the tree tags were lost since planting in 2008, we are using genomics to determine the hybrid ancestry of these samples. Specifically, tissue samples were collected from the unknown trees and genomic DNA extracted using a Qiagen DNeasy kit. We are currently in the process of generating genotype-by-sequencing libraries for these samples, which involves isolating a random sample of the genome and sequencing on a high-throughput Illumina sequencer. Based on previous extensive experience with this method in chestnut, we expect to acquire approximately 70,000 genetic variants that will be used to infer hybrid class when combined with our >500 other sequenced samples from the ACF breeding program. We anticipate these results will be available before the end of 2017.

Statistical Analyses

We analyzed all measured health and growth characteristics (e.g. blight severity, height, survival) to determine potential influence by physical characteristics measured in the field (e.g. vegetation metrics, slope, or aspect), genetic background, and soil chemistry. For independent variables with a fixed number of categories (e.g. block, seeding treatment, or vegetation indices), we analyzed the data using analysis of variance (ANOVA) using SigmaPlot Version 12.5. Where independent variables did not have a fixed number of categories (e.g. pH or slope), we performed Spearman correlation analyses using SigmaPlot Version 12.5. For the purposes of this study, we have combined the two pure American chestnut genotypes for analysis, as they did not differ statistically from one another in any of the measured parameters. A summary of all results from statistical analyses can be found in Appendix 1.

Results

Survival

Chestnut survival was similar in Blocks 1 (44.6%) and 2 (44.0%). Chestnuts in the annual rye seeding treatment had higher survival (51.2 ± 2.2 %) than trees in the conventional seeding treatment (36.7 ± 2.2 %), though neither seeding treatment differed from the tree compatible seeding treatment ($p=0.005$) (Table 6). Survival also differed among the tree genotypes (Figure 5). Overall, Chinese chestnuts had higher survival (72.7 ± 2.8 %) than any of the other genotypes ($p<0.001$. The B2F3 trees had the lowest nominal survival (23.6 ± 2.8 %), however survival of the pure American chestnuts did not differ from any of the hybrids.

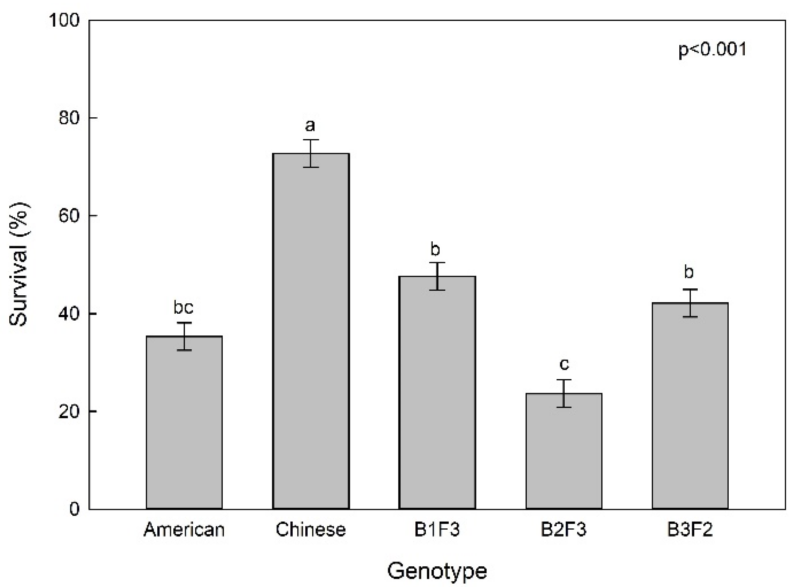

Figure 5. Survival among the five chestnut genotypes after nine growing seasons ± 1 SE. Differences between groups calculated with the Holm-Sidak methods with α=0.05. Different letters indicate significant differences among groups.

Table 6. Summary of survival, ground-line diameter, and height among blocks and main treatments.

Block	Survival (% ± SE)	Groundline diameter (cm ± SE)	Height (cm ± SE)
1	44.6 ± 1.8	5.08 ± 0.39 a	327.6 ± 14.9 a
2	44.0 ± 1.8	1.60 ± 0.30 b	87.2 ± 11.5 b

Seeding treatment	Survival (% ± SE)	Groundline diameter (cm ± SE)	Height (cm ± SE)
Annual rye	51.2 ± 2.2 a	3.18 ± 0.43	194.5 ± 16.5 b
Conventional	36.7 ± 2.2 b	2.85 ± 0.45	176.8 ± 17.2 b
Tree compatible	45.0 ± 2.2 ab	4.00 ± 0.40	250.9 ± 15.1 a

Genotype	Survival (% ± SE)	Groundline diameter (cm ± SE)	Height (cm ± SE)
American chestnut	35.3 ± 2.8 bc	1.94 ± 0.63 b	146.4 ± 24.1 b
Chinese	72.7 ± 2.8 a	5.05 ± 0.37 a	260.6 ± 14.1 a
B1F3	47.7 ± 2.8 b	3.40 ± 0.48 ab	203.0 ± 18.4 ab
B2F3	23.6 ± 2.8 c	3.47 ± 0.69 ab	230.8 ± 26.2 ab
B3F2	42.1 ± 2.8 b	2.84 ± 0.52 b	196.2 ± 20.0 ab

Differences between groups determined by the Holm-Sidak method with α=0.05.
Different letters indicate differences among groups.

Ground-line diameter

Ground-line diameter (GLD) for all trees across the experiment was variable, with a mean GLD ± standard error of 3.27 ± 0.27. Ground-line diameter did not differ among seeding treatments. Ground-line diameter was greater for Chinese chestnuts compared to American chestnuts and B3F2 chestnuts, however none of these genotypes differed from the B1F3 or B2F3 trees ($p<0.001$) (Figure 6).

Mean GLD was greater in Block 1 (5.08 ± 0.39) than Block 2 (1.60 ± 0.30) ($p<0.001$) (Table 7). Differences in measured parameters between the blocks are likely a reflection of overall physical differences between the sites. For example, trees in Block 1 primarily faced SE and SSE and trees in Block 2 faced primarily E and ENE, and GLD was significantly affected by directional aspect ($p<0.001$) (Figure 6A). Likewise, in the range of aspects within this study, there was a strong positive correlation between aspect degrees and GLD ($R=0.609$, $p=2.06E^{-16}$) (Figure 6B). Soil pH and GLD were weakly correlated, which may also be contributing somewhat to the observed block effect, as Block 2 had significantly higher pH than Block 1($R=-0.392$, $p=8.45E^{-7}$) (Table 1).

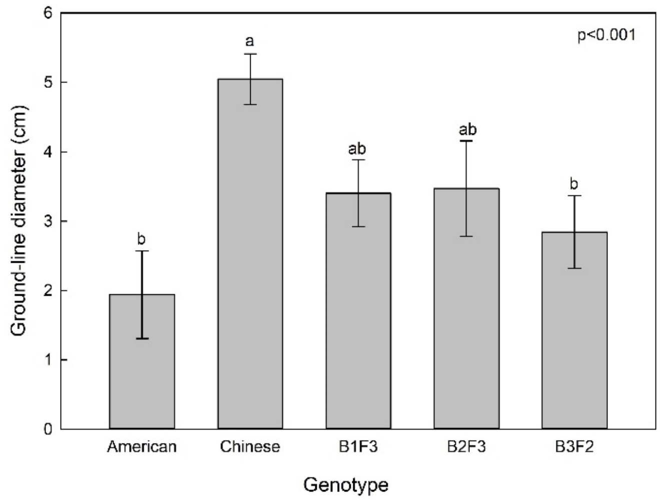

Figure 6. Ground-line diameter among the five chestnut genotypes after nine growing seasons ± 1 SE. Differences between groups calculated with the Holm-Sidak methods with α=0.05. Different letters indicate significant differences among groups.

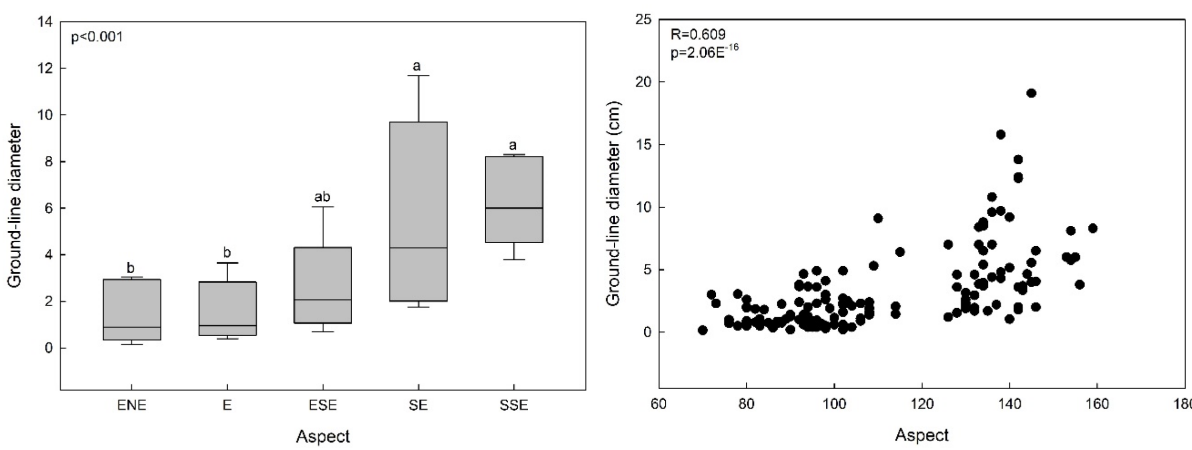

Figure 7. A) GLD of trees at all recorded directional aspects. Error bars represent 10% and 90% confidence intervals. Differences between groups calculated with Dunn's method with α=0.05. Different letters indicate significant differences among groups. B) GLD vs. aspect (□) for all live chestnut trees.

There were no differences in GLD related to vegetation cover, however there were differences in GLD associated with the vegetation competition index (p<0.001) (Figure 8). Generally, as vegetation competition increased, GLD diameter decreased, though differences were not significant until vegetation was taller than the tree (vegetation competition index=5). There were no trees where the vegetation competition index was 1 (no vegetation present), suggesting that some environmental factor was resulting in both poor tree survival and poor herbaceous vegetation establishment.

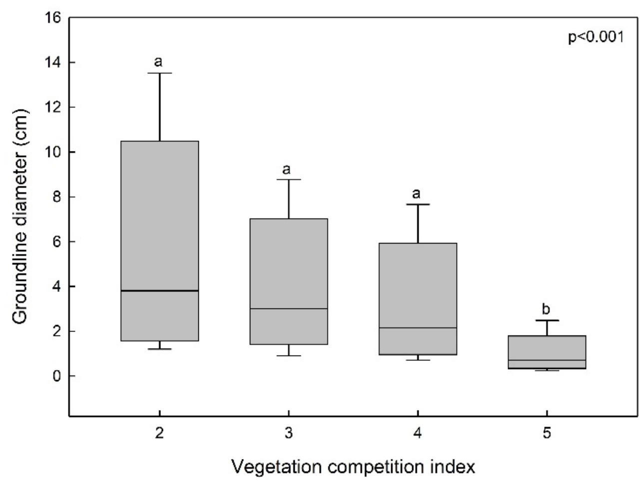

Figure 8. Ground-line diameter among the five vegetation competition classes after nine growing seasons. Error bars represent 10% and 90% confidence intervals. Differences between groups calculated with Dunn's method with α=0.05. Different letters indicate significant differences among groups.

There was a weak correlation between GLD and % slope (R=0.172, p=0.0368). There were no differences in ground-line diameter related to slope position or soil texture, nor was there a significant relationship between electrical conductivity (EC) and ground-line diameter.

Height

Mean height ± standard error of all trees in the experiment was 193.0 ± 13.6. Height was significantly higher in Block 1 (327.6 ± 14.9) than Block 2 (87.2 ± 11.5) (p<0.001) (Table 6). Height was higher in the tree compatible seeding treatment (250.9 ± 15.1) than both the conventional seeding treatment (176.8 ± 17.2) and annual rye seeding treatment (194.5 ± 16.5) (p=0.003) (Figure 9). Chinese chestnuts (260.6 ± 14.1) had greater height after 9 years than American chestnuts (146.4 ± 24.1), though height of the pure chestnuts did not differ from any of the hybrids (p<0.001) (Figure 10).

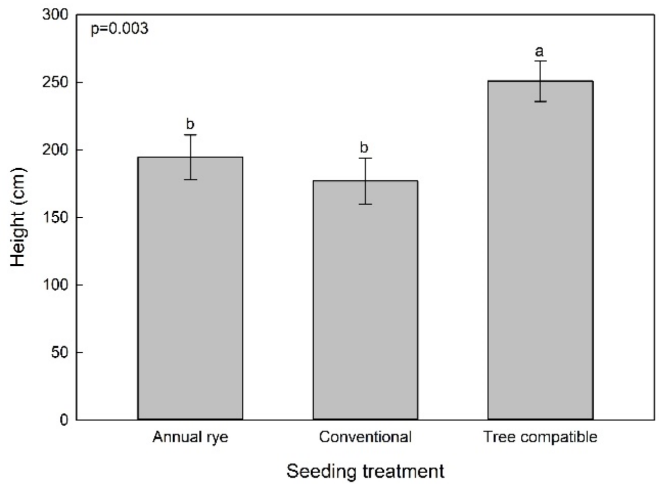

Figure 9. Chestnut height among the three seeding treatments after nine growing seasons ± 1 SE. Differences between groups calculated with the Holm-Sidak methods with α=0.05. Different letters indicate significant differences among groups.

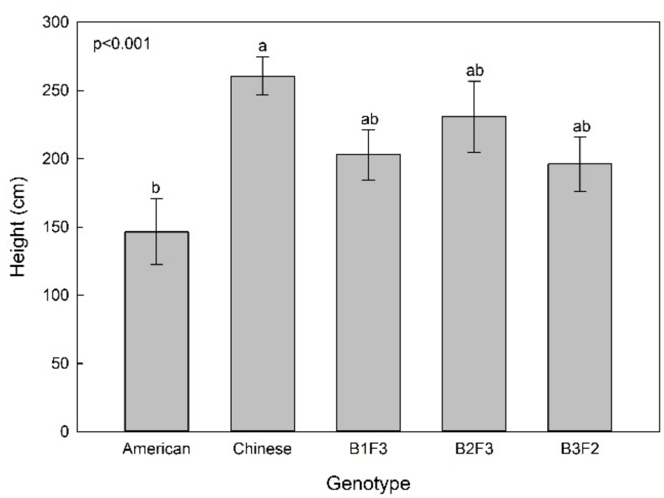

Figure 10. Height among the five chestnut genotypes after nine growing seasons ±1 SE. Differences between groups calculated with the Holm-Sidak methods with α=0.05. Different letters indicate significant differences among groups.

Trees located at slope position 4 (top of the slope) were taller than trees located at slope position 1 (bottom of slope) (p=0.021) (Figure 11). Height and % slope were weakly correlated (R=0.184, p=0.0263). Trees at SSE and SE aspects were taller than trees at E, ESE, and ENE aspects (p<0.001) (Figure 12a). Likewise, there was a positive correlation between degrees aspect and tree height (Figure 12b).

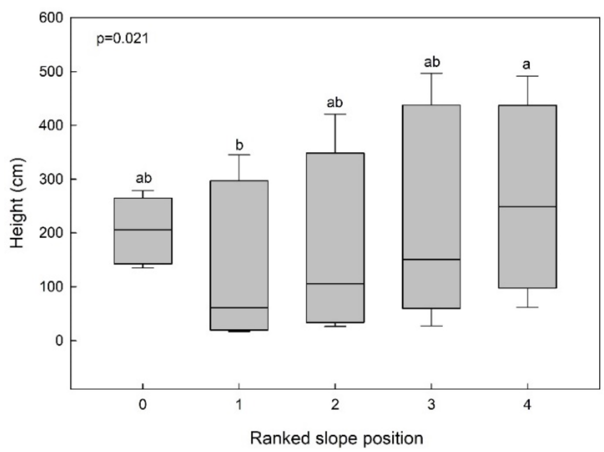

Figure 11. Mean tree height at ranked slope positions. Error bars represent 10% and 90% confidence intervals. Differences between groups calculated with Dunn's method with α=0.05. Different letters indicate significant differences among groups.

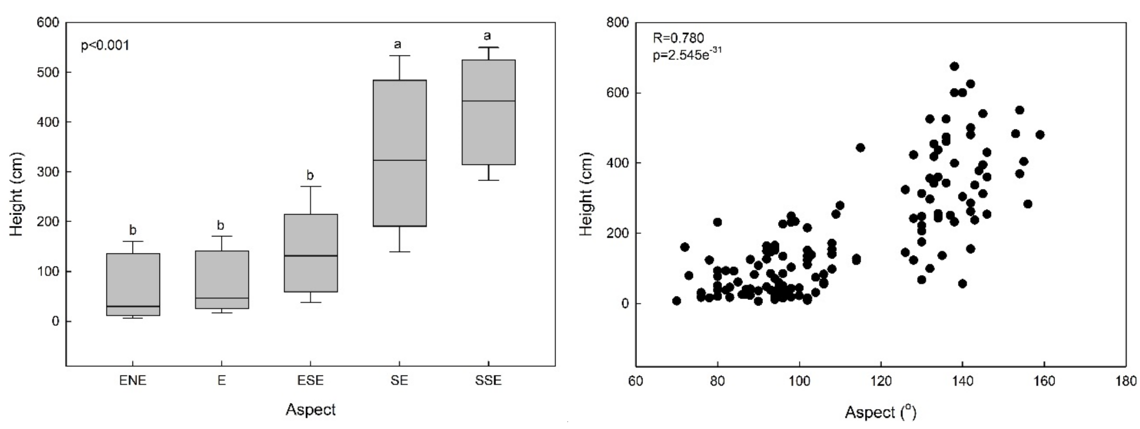

Figure 12. A) Mean tree height at aspect directions. Error bars represent 10% and 90% confidence intervals. Differences between groups calculated with Dunn's method with α=0.05. Different letters indicate significant differences among groups. B) Pearson's correlation between tree height and degrees aspect.

There was a negative correlation between height and soil pH ($R=-0.547$, $p=7.509E^{-13}$) (Figure 13). While height was variable, generally, trees were shorter as pH increased. Block 2 overall had higher pH, and trees in this block were much shorter after ten growing seasons than Block 1, which had lower and more variable pH. There was a weak negative correlation between height and soil electrical conductivity (EC) ($R=-0.234$, $p=0.00436$). There were no differences in tree height with soil texture.

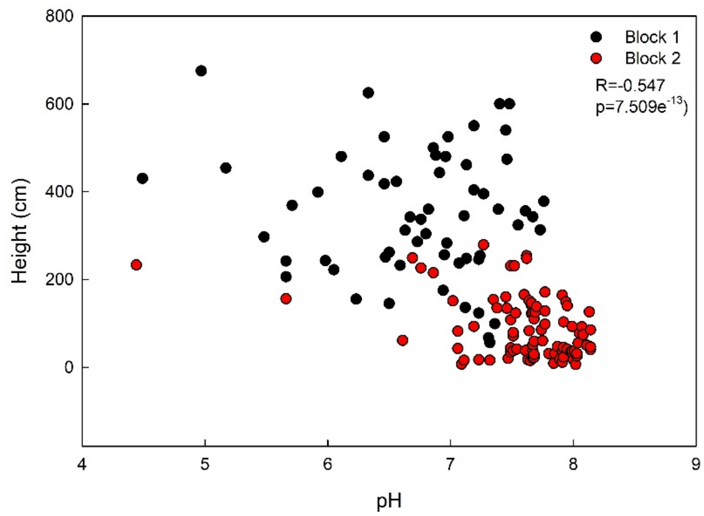

Figure 13. Pearson's correlation between tree height and soil pH in blocks 1 and 2.

There were no differences in tree height among the vegetation cover classes, however there were differences among the vegetation competition classes. Similar to the relationship with ground-line diameter, tree height in vegetation competition class 5 (vegetation taller than tree) was lower than in the other competition classes ($p<0.001$) (Figure 14).

<u>Tree health and blight incidence</u>

Overall, approximately half of the nuts that germinated in 2008 have died (Table 7). Only a few of the trees that have died were definitively killed by blight, however there may have been a number of trees killed by 'unknown' causes that actually succumbed to blight. Of the remaining live trees, about 60% are affected by blight symptoms, and approximately half of those are resprouts. Within Block 1, most of the remaining trees are either live and blighted (cankers present, but no dead branches or stems) or are resprouts. Within Block 2, most of the remaining trees are alive and healthy.

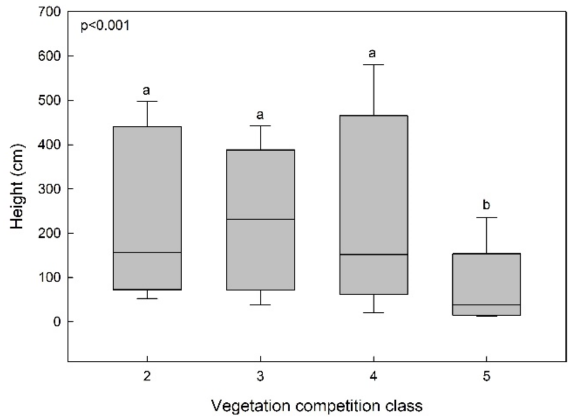

Figure 14. Height among the five vegetation competition classes after nine growing seasons. Error bars represent 10% and 90% confidence intervals. Differences between groups calculated with Dunn's method with α=0.05. Different letters indicate significant differences among groups.

Table 7. Summary of tree health indices for main treatment groups.

	Tree health index (% of trees)						
Block	**0 - Dead, other /unknown cause**	**1 - Live, healthy**	**2 - Live, blighted**	**3 - Branch dead from blight**	**4 - Top dead from blight**	**5 - Resprout**	**6 - Tree dead from blight**
1	52.4	11.9	14.3	4.0	3.2	14.3	0.0
2	51.8	24.1	4.7	0.5	6.3	11.0	1.6
Seeding treatment							
Annual rye	46.1	16.9	10.1	1.1	6.7	18.0	2.2
Conventional	57.9	20.7	7.4	0.8	6.6	6.6	0.0
Tree compatible	50.5	19.6	9.3	3.7	1.9	14.0	0.9
Genotype							
American	57.6	28.8	3.4	3.4	1.7	5.1	0.0
Chinese	27.1	31.4	17.1	1.4	5.7	15.7	1.4
B1F3	50.0	14.1	10.9	3.1	6.3	15.6	0.0
B2F3	76.2	3.2	4.8	0.0	4.8	9.5	1.6
B3F2	52.5	18.0	4.9	3.3	6.6	13.1	1.6
Overall	49.4	19.3	9.7	2.0	5.7	12.8	1.1

In the annual rye treatment and tree compatible seeding treatments most of the remaining live trees were alive and healthy or were resprouts and in the conventional seeding treatment they were primarily alive and healthy. Among most of the genotypes, the majority of the remaining trees were either alive and healthy or were resprouts. The B2F3 trees however, had high mortality, and only a few of the remaining trees were healthy.

Trunk blight, lower crown blight, and overall blight severity were all higher in Block 1 (1.4 ± 0.2) than Block 2 (0.4 ± 0.2) ($p<0.001$) (Table 8). Trunk blight ($p=0.010$) and lower crown blight ($p=0.024$) were lowest in the conventional seeding treatment, and did not differ between the annual rye and tree compatible seeding treatments. There were no differences in any of the blight severity indices among genotypes.

Table 8. Blight severity index in main treatments for all regions of tree anatomy.

Block	Trunk blight ± SE	LC blight ± SE	MC blight ± SE	UC blight ± SE	Total blight ± SE
1	1.4 ± 0.2 a	1.1 ± 0.2 a	0.6 ± 0.2	0.3 ± 0.2	1.1 ± 0.2 a
2	0.4 ± 0.2 b	0.5 ± 0.1 b	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1 b
Seeding treatment					
Annual rye	0.9 ± 0.2 ab	1.1 ± 0.2 a	0.7 ± 0.2	0.5 ± 0.2	1.1 ± 0.2
Conventional	0.4 ± 0.2 b	0.3 ± 0.2 b	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2
Tree compatible	1.4 ± 0.2 a	1.0 ± 0.2 a	0.7 ± 0.2	0.5 ± 0.2	1.0 ± 0.2
Genotype					
American	0.5 ± 0.3	0.2 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.3 ± 0.3
Chinese	1.2 ± 0.2	0.9 ± 0.2	0.6 ± 0.2	03 ± 0.1	1.0 ± 0.2
B1F3	1.1 ± 0.3	1.2 ± 0.2	1.0 ± 0.2	0.6 ± 0.2	1.1 ± 0.2
B2F3	1.2 ± 0.4	0.9 ± 0.3	0.6 ± 0.3	0.7 ± 0.3	1.1 ± 0.3
B3F2	0.5 ± 0.3	0.8 ± 0.2	0.7 ± 0.2	0.5 ± 0.2	0.8 ± 0.2

Differences between groups determined by Dunn's method with α=0.05. Different letters indicate significant differences among treatment groups.

Trunk blight was more severe at slope position 4 (top of the slope) compared to the bottom of the slope ($p=0.006$) (Figure 15). Slope position was not related to blight severity for any other region of the tree. There was a positive correlation between degrees aspect and trunk blight severity ($R=0.407$, $p=3.69E^{-7}$), lower crown blight severity ($R=0.339$, $p=3.08E^{-5}$), and overall blight severity ($R=0.342$, $p=2.6E^{-5}$), however none of the blight indices were significantly related to directional aspects.

Soil pH was weakly correlated with trunk blight severity (R=-0.300, p=2.49E^{-4}), lower crown blight severity (R=-0.224, p=6.81E^{-3}), and overall blight severity (R=-0.246, p=2.92E^{-3}). Soil EC and texture were not related to any of the blight severity indices.

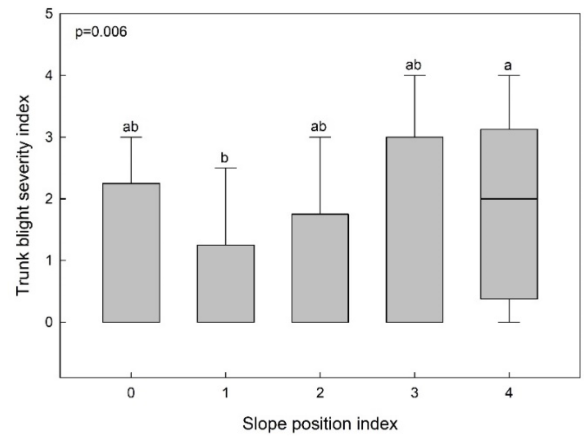

Figure 15. Trunk blight severity at the five recorded slope position categories. Error bars represent 10% and 90% confidence intervals. Differences between groups calculated with Dunn's method with α=0.05. Different letters indicate significant differences among groups.

The vegetation competition index was related to trunk blight severity (p<0.001), lower crown blight severity (p<0.001), and overall blight severity (p<0.001) (Figure 16). Overall, trees with less competitive vegetation had more severe blight symptoms than those with more competitive vegetation, though trees with extremely competitive vegetation were also much smaller and less likely to show blight symptoms.

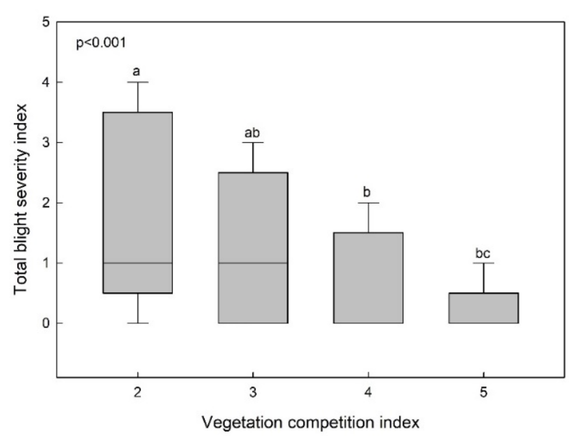

Figure 16. Total tree blight severity and vegetation competition index. Error bars represent 10% and 90% confidence intervals. Differences between groups calculated with Dunn's method with α=0.05. Different letters indicate significant differences among groups.

Discussion

Survival and growth

Survival was only tested among main treatment groups, as we did not record physical data for dead trees. Similar to the results after two growing seasons, survival did not differ between blocks, which suggests that chemical and physical properties that were found to differ between the blocks, such as pH and aspect, would not have affected survival (Fields-Johnson 2011). As in 2009, survival was higher in the annual rye seeding treatment than the conventional treatment, emphasizing that seeding treatments can have long-term effects on the success of a reclamation (Burger et al. 2008, Burger et al. 2009). In addition, differences in survival among genotypes were similar to those in 2009. Chinese chestnuts continue to have the highest survival, B2F3 chestnuts continue to have the lowest nominal survival, and the remainder of the hybrids and pure American chestnuts have intermediate survival. Chinese chestnuts are particularly tolerant of sandy or gravelly soils, so they may be ideally suited for growth on reclaimed coal mines with sandstone growth media (Strang 2012). Indeed, other studies with finer textured soils have observed lower survival of Chinese chestnuts that did not differ from similar hybrids (Gilland and McCarthy 2014).

Ground-line diameter and height differed between blocks, which appears to be largely driven by the marked differences in aspect, and to a lesser degree, soil pH. At relatively southerly aspects, trees were larger in terms of GLD and height, compared to trees at relatively easterly aspects. Block 1 has an almost exclusively SE and SSE aspect and Block 2 has aspects primarily ranging from ENE to ESE. Trees were also larger in lower pH soils, but most of the higher pH soils were in Block 2, so the strength of the correlation could be a product of the relationship between tree size and aspect. In Block 2, trees were only slightly larger in the few locations where soil pH was lower. In this study, it is likely that pH rarely was high enough to limit growth, so other environmental factors were the drivers in tree performance.

As with survival, height was also affected by seeding treatment, such that trees were taller where less competitive vegetation had been seeded. The tallest chestnuts were in the tree compatible seeding plots. Annual rye plots had nominally taller trees than those in the conventional seeding plots. Similarly, tree height and ground-line diameter were lower where vegetation competition was higher. Where the competing vegetation was taller than the chestnut tree, trees were particularly small. Anecdotally, many of the trees that were dead or missing seemed to be in locations where the vegetation was particularly thick and aggressive. It is well-established that vegetation competition can impede tree establishment, hence why one of the FRA steps specifies seeding a non-aggressive cover to maximize reforestation success.

Chinese chestnuts were taller and had larger GLD than pure American chestnuts, and larger GLD than B3F2 chestnuts. While there is variability in hybrid phenology, other studies have observed particularly poor growth of pure American chestnuts compared to pure Chinese chestnuts or hybrids on coal mine reclamations (Bauman et al. 2014, Gilland and McCarthy 2014).

Blight incidence

Although growth was better in Block 1 and in the annual rye and tree compatible seeding treatments, blight symptoms were also generally more severe. This is likely a function of the higher density of surviving trees (including blight carrier tree species such as oaks), and thus faster spread of disease because trees are closer together. Furthermore, chestnuts typically exhibit blight symptoms when they are larger, so because the trees are larger on Block 1, perhaps blight symptoms manifested in them sooner than in Block 2. In addition, Block 1 has a south-facing aspect, compared to the east-facing aspect of Block 2, and blight severity was significantly correlated with aspect. South aspects are hotter and drier, and Rhoades et al. (2009) found that blight incidence is higher on xeric sites compared to more mesic sites. There were weak correlations between pH and trunk, lower crown, and total tree blight severity, but as with growth, we think that aspect is a much stronger driver of blight severity.

We expected that blight incidence would vary among genotypes, but there were no differences in blight severity among any of the genotypes. Other studies have found that pure American chestnuts have more cankers than hybrids and we were surprised that genotypes did not differ in our study (Bauman et al. 2014, Gilland and McCarthy 2014). However, if a tree was dead, we were only able to attribute the death to blight if some part of the tree was still present, so it is possible that many of the dead American chestnut and hybrid trees died from blight infection and effects from blight went undetected.

We also expected that blight incidence would be higher where vegetation competition was greater, assuming that trees that had to expend more energy competing for resources would be less able to respond to pathogens, but instead found that trees with lower vegetation competition actually had higher incidence of blight than those with more competitive vegetation. We suspect that as with the seeding treatments, where all trees were performing better, disease spread was more rapid. More competitive vegetation limited chestnut growth such that blight symptoms have not yet manifested.

Conclusions

The purpose of the original 2008 study was to investigate the effects of seeding treatment and chestnut genotype on survival and growth. Its purpose was not to assess how site characteristics affect chestnut survival and growth. Therefore, there were marked differences between the two blocks in terms of their growth mediums, aspects, and soil chemistry. We attempted to compensate for this variability by recording physical measurements at every tree, but a fully replicated study focusing on site factors is needed, particularly in terms of growth mediums and the related soil chemistry and texture, and aspect.

Overall, better execution of the FRA method (i.e. higher quality growth medium and less aggressive herbaceous cover) resulted in better chestnut survival and growth, but also higher incidence of blight. However, it is unclear whether the higher blight incidence within plots with better application of the FRA was due to the treatments themselves or to some other factor. For example, it is possible that where the FRA was better implemented, larger sized trees were more likely to show blight symptoms or disease spread more quickly because there were overall more trees, including blight carriers such as oaks. We hope to study this site again in a few years to reassess survival, growth, and blight occurrence, when ostensibly a greater proportion of the trees will be larger and more likely to exhibit blight symptoms.

Finally, to date, American chestnuts have been planted early in coal mine restorations. However, American chestnuts are sensitive to particularly dry soils and particularly sandy soils, and due to the added drought stress, have more blight cankers than on mesic sites. They also perform well with some hardwood competition, slowly growing in the understory until the canopy opens. We hypothesize that allowing the non-competitive vegetation and native tree seedlings to establish for a few years before planting chestnuts on a mine reclamation site may improve survival, growth, and blight resistance of chestnuts. Waiting to plant chestnuts may allow the herbaceous community to develop soils with improved water holding capacity which would minimize drought stress on young chestnuts, and also may allow the native tree mix to function as nurse plants to minimize stress on young chestnuts.

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Appendix 1 – Summary results from statistical analyses

A. Summary of ANOVA statistical tests.

Independent variables	Survival	GLD	Height	Trunk blight	Lower crown blight	Middle crown blight	Upper crown blight	Total blight
	Dependent variables							
Block	n.s	p<0.001	p<0.001	p<0.001	p=0.021	n.s.	n.s.	p=0.014
Seeding treatment	p=0.005	n.s.	p=0.003	p=0.010	p=0.024	n.s.	n.s.	n.s.
Genotype	p<0.001	p<0.001	p<0.001	n.s.	n.s.	n.s.	n.s.	n.s.
Slope position	Not tested	n.s.	p=0.021	p=0.006	n.s.	n.s.	n.s.	n.s.
Aspect direction	Not tested	p<0.001	p<0.001	n.s.	p<0.001	n.s.	n.s.	n.s.
Texture	Not tested	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ranked veg cover	Not tested	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ranked veg comp	Not tested	p<0.001	p<0.001	p<0.001	p<0.001	n.s.	n.s.	p<0.001

B. Summary of correlation analyses

	Independent variables	Dependent variables: GLD	Height	Trunk blight	LC blight	MC blight	UC blight	Total blight
Spearman correlation	Vegetation cover index	n.s.	n.s.	n.s.	n.s.	R=-0.203, p=0.0145	n.s.	n.s.
	Veg competition index	R=-0.514, p=2.0E-7	R=-0.406, p=4.67E-7	R=-0.422, p=3.32E-8	R=-0.403 p=6.3E-7	R=-0.252, p=2.28E-3	R=-0.228, p=5.83E-3	R=-0.421, p=1.73E-7
Pearson correlation	% slope	R=0.172, p=0.0368	R=0.184, p=0.0263	n.s	n.s	n.s	n.s	n.s
	Degrees aspect	R=0.609, p=2.06E-16	R=0.78, p=2.545E-31	R=0.407, p=3.69E-7	R=0.339, p=3.08E-5	n.s	n.s	R=0.342, p=2.6E-5
	pH	R=-0.392, p=845E-7	R=-0.547, p=7.51E-13	R=-0.300, p=2.49E-4	R=-0.224, p=6.81E-3	n.s	n.s	R=-0.246, p=2.92E-3
	EC	n.s	R=-0.234, p=4.36E-3	n.s	n.s	n.s	n.s	n.s