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Abstract

Five genotypes of chestnut were planted at surface mined sites in six states within ARRI area in order to test the utility of backcross American chestnut as a component of the FRA to surface mine reclamation, and to test the utility of surface mined sites to restoration of American chestnut to Appalachian forests. The genotypes were comprised of Chinese and American chestnut and three levels of backcrossing composing a gradient between the species. This report summarizes establishment and short-term (2 year) performance of the chestnut genotypes in the six states. Chestnuts were planted as seednuts. Germination and establishment averaged about 75% over all genotypes and states, ranging from less than 10% to about 90%. The instances of extremely poor germination and establishment may have been due to the absence of tree shelters, whose presence or absence was tested in some states. Shelters were used in all states except when being tested. It was concluded that shelters are critical to establishment of chestnut planted as seeds. Subsequent to establishment, survival of seedlings was good at most sites, averaging 66% with shelters in the first year and 55% in the second year. In one state, Maryland, mechanical damage by deer and four-wheelers, plus drenching rains on a site reclaimed with traditional methods, combined to reduce survival by the second year to 26%. At the Bernice site in Pennsylvania, traditional reclamation method combined with alkaline soils and high phosphorous levels to reduce survival the second year to 0. Other sites tested were acidic. There was not much tree growth on these nutrient poor sites (typical soil analyses indicated deficiencies for most elements tested). After 2 years, trees in shelters averaged 48 cm tall, ranging from 19 to 104 cm from state to state. After 4 years, trees on all sites surveyed exceeded 50 cm in average height. American chestnut had fewer, smaller trees than Chinese chestnut, overall, but there were exceptions at some sites. One of the exceptions may have been associated with a greater tolerance of Chinese than American chestnut to hypoxia. The backcross trees usually became more similar to American chestnut as the proportion of American parentage increased.

Introduction

The majority of the Appalachian coal field lies beneath mixed-species, hardwood forests that provide landowners with a profitable and very suitable land use. However, in the past, removal of coal by means of surface mining removed the option of landowners to once again return their

land to valuable forests. Site reclamation was accomplished through grading and compaction methods which suited grassland production, discouraging forest production, and, consequently, many times with little end-use value to the landowner. Recently, research has been conducted in the Appalachian area to examine reclamation methods that favor tree production. One such program experiencing success is the Forestry Reclamation Approach (FRA), where a species-mix of hardwood trees are planted under favorable growing conditions to provide value once again to the landowner.

These cleared areas of land provide desirable environments for tree establishment and growth due to the lack of competing vegetation and the requirement under the Surface Mining and Control and Restoration Act that they be restored, which includes revegetation. One species of particular interest to The American Chestnut Foundation (TACF) is the American backcross chestnut that has been developed in the breeding program at Meadowview, Virginia. These American chestnut materials possess resistance to the chestnut blight that destroyed approximately four billion American chestnut trees in the first part of the 20th century. The Appalachian coal fields are in the heart of the native range of American chestnut, and are a key region with regards to not only the growth, but also the restoration of the species. Therefore, testing American chestnut on surface mined areas is of extreme interest to TACF.

However, since few American chestnut have been available for planting using the FRA, little is known regarding the growth, performance, and survivability of chestnut on surface mined sites, nor is much known regarding the ideal substrate for maximum chestnut growth.

Goals and Objectives. The primary goal of the project was to determine whether American chestnut planted under FRA guidelines grow well on surface mined lands at locations across the entire ARRI region. This information may help us determine the range of adaptation of chestnuts originating in southwest Virginia, in combination with analysis of differences in site factors across this area. Secondarily, we hope to determine the blight resistance and growth of trees with varying fractions of Chinese and American chestnut parentage. We hope this research will lead to recommendations for establishing American chestnut and American chestnut backcross trees on surface mined areas being reclaimed, and to determine site conditions conducive to chestnut growth.

The objectives of the study are to determine: 1) performance and growth of chestnut on formerly surface mined lands, 2) performance and level of disease resistance of backcross chestnuts compared to American and Chinese chestnut parents, and 3) site effects on the performance and growth of chestnut.

Executive Summary

Five genotypes of chestnut were planted at surface mined sites in six states within the region of the Appalachian Regional Reforestation Initiative (ARRI), in order to test the utility of backcross American chestnut as a component of the Forestry Reclamation Approach (FRA) to surface mine reclamation, and to test the utility of surface mined sites to restoration of American chestnut to Appalachian forests. The genotypes were comprised of Chinese and American chestnut and three levels of backcrossing composing a gradient between the species. The backcross genotypes in the gradient were one-fourth, one-eighth and one-sixteenth Chinese, on average, with the remainder American chestnut. Chinese chestnut is resistant to the disease, chestnut blight, which has eliminated the susceptible American chestnut as a large forest tree throughout its range. The backcross trees were expected to have an average level of blight resistance either intermediate between the two parental species or similar to the resistance of Chinese chestnut. This report summarizes establishment and short-term (2 year) performance of the chestnut genotypes in the six states. The trees will have to be measured over longer periods (5, 10 and 20 years) to detect effects of their relative blight resistance and most effects of their varying proportions of Chinese and American heritage. It is expected that those factors will influence their growth, blight severity, survival and nut production and will interact with site characteristics. Chestnuts were planted as seednuts. Germination and establishment averaged about 75% over all genotypes and states, ranging from less than 10% to about 90%. The instances of extremely poor germination and establishment may have been due to the absence of tree shelters, whose presence or absence was tested in some states. Shelters were used in all states except when being tested. It was concluded that shelters are critical to establishment of chestnut planted as seeds. Subsequent to establishment, survival of seedlings was good at most sites, averaging 66% with shelters in the first year and 55% in the second year. In one state, Maryland, mechanical damage by deer and four-wheelers, plus drenching rains on a site reclaimed with traditional methods, combined to reduce survival by the second year to 26%. At the Bernice site in Pennsylvania, traditional reclamation method combined with alkaline soils and high phosphorous levels to reduce survival the second year to 0. Other sites tested were acidic. There was not much tree growth on these nutrient poor sites (typical soil analyses indicated deficiencies for most elements tested). After 2 years, trees in shelters averaged 48 cm tall, ranging from 19 to 104 cm from state to state. American chestnut had fewer, smaller trees than Chinese chestnut, overall, but there were exceptions at some sites. One of the exceptions may have been associated with a greater tolerance of Chinese than American chestnut to hypoxia. The backcross trees usually became more similar to American chestnut as the proportion of American parentage increased. It was concluded that:

- Chestnut can be established and persist on surface mined sites throughout the ARRI region.
- Tree shelters are an important factor in survival of seedlings from directly sown nuts.
- Better growth occurred at sites where FRA prescriptions for site preparation were followed.

- Differences in establishment and growth between Chinese and American chestnut and their backcross hybrids were observed, but all types could be established in all states.
- Strong differences between states in chestnut growth and establishment were not observed on the good sites. More precise experimentation might be required to detect those differences by measuring tree growth.

Materials and Methods

Plant Materials American, Chinese, and three levels of American-backcross chestnut were used in the study. The three backcross levels are denoted B_1-F_3, B_2-F_3 and B_3-F_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) or third (F_3) filial cross at the indicated level of backcrossing. It was expected that the B_3-F_2s would be intermediate in blight resistance, ranging from highly susceptible to highly resistant to chestnut blight, whereas the B_1-F_3s and B_2-F_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 by open pollination. The B_2-F_2 parents of the B_3-F_3 nuts also had been the product of open pollination, as well as the Chinese and American chestnut, whereas the parents of the B_1-F_3s and B_3-F_2s had been produced by controlled pollinations.

Measurements. It was not expected that blight would become evident in the plantations before 5 years, so only survival and growth parameters were assessed, as well as site factors.

Planting Method. Chestnut seednuts were planted at surface mined sites in KY, MD, OH, PA, TN & WV in the spring, in April or May of 2008. Approximately 125 seed of each of the five genotypes were made available to each state. The plantings at each site were designed, implemented and measured by personnel at the Morrill Act Land-Grant university for the state, except that in OH the personnel were from Ohio University.

Kentucky. At each planting location a ≈ 10-cm deep hole was prepared using a dibble bar or shovel. A teabag of fertilizer (Treessentials, Duluth, MN) was placed in the bottom of the hole and covered with 2-4 cm of planting mix (Scotts® general potting medium). Each chestnut was placed on the planting mix, roots down, and covered with an additional 2-4 cm of planting mix. Seeds in half the plots were protected with 60-cm Tubex® shelters that are anchored to the ground with white oak stakes, following the manufacturer's instructions. Shelters were not placed on the seeds in the other half of the plots.

Maryland. An approximately 10-cm deep hole was filled with potting mix, as specified below in experimental design, and planted nuts protected with 25-cm tall, 5 cm diameter aluminum cylinders.

Ohio. Nuts were planted using 30-cm-tall plastic tree shelters to protect them. Some shelters were anchored with stakes and others not, but there was no difference in persistence of the shelters depending on the presence of a stake.

Pennsylvania. Nuts were protected with 25-cm tall, 5 cm diameter aluminum cylinders.

Tennessee. No information currently available.

West Virginia. Seeds were planted by digging a small 5-cm-deep hole about 5 cm from the base of the wooden stake. Each seed was inoculated with mycorrhizae fungi before planting. In peat treatments, about 5 cm^3 of commercial peat from a local gardening store 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, we placed 45-cm-tall, plastic tree shelters on top of each planted seed in half the planted sites. No shelter was used in the other half.

Site Preparation. *Kentucky*. Brown weathered sandstone spoil was dumped out of the end of trucks ("loose-dumped") into piles that averaged about 3.5-m in height and placed in parallel rows so that they closely abutted one another across a 1.5-hectare site. The tops of the spoil piles were "struck-off" with one pass of a bulldozer (Caterpillar D9, straight blade) down the length of each parallel ridge of spoil, pushing it into the parallel valleys on both sides.

Maryland. The site was located in the Upper Georges Creek watershed in Western Allegany County, Maryland. It had been reclaimed for use as pasture/hayfield by the traditional method under the

Ohio. End dumping was used to prepare the final planting medium at a site in Jockey Hollow, OH.

Pennsylvania. Various methods were used on different sites. On the State Game Lands 100 site, preparation was back-dragging clay soil until hard. On the Fisher Mining Company site, end-dumped topsoil was applied per the Forest Reclamation Approach. The Bernice site was composed of glacial till amended with biosolids.

Tennessee. No information currently available.

West Viriginia. The Glory surface mine is located near Van, in Boone County, West Virginia. Overburden from the Number 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 rough level surface.

Experimental Design. There was considerable variation in experimental design since several investigators wished to test additional factors to the genotype of chestnut. This variation in approach was encouraged since not all aspects of chestnut planting on mine sites has been investigated. However, in hindsight, the implementation was more non-uniform than may have been desirable.

Kentucky. A completely randomized design was used to test three factors: with or without 36-cm-tall plastic tree shelters; five genotypes; and three replications, for thirty treatment-replicate combinations. Each cell consisted of 25-tree plots of the same genotype.

Maryland. The original design was a randomized complete block design located at one site. There were six blocks composed of 25-tree plots arranged in rows with one genotype per plot (equal to a row). Contingency led to splitting of the test, without randomization between splits, when one planting mix was substituted for another beginning at the second row of the fourth block. The planting mix used in the first three blocks and row one of the fourth was a mixture of one-third each ground, milled peat moss, horticultural perlite, and large horticultural vermiculite, with mycorhizzal inoculum (spores of *Pisolithus tinctorius*). The planting mix used beginning in the second row of the fourth plot through the sixth row was composed of dried, milled peat moss, hardwood bark mulch, mushroom compost, in a ratio of 8:7:8, respectively. No mycorhizzal inoculum was added to the second mix.

Ohio. A randomized, complete block design was used with single-tree plots. Each block was a single mound from end dumping used to prepare the site. Blocks were further grouped into three larger areas. The first larger area contained 265 nuts planted into 53 mounds. The second larger area contained 34 mounds, and the third area 44 mounds. The larger blocks reflected site heterogeneity and different sources of end-dumped surface soils.

Pennsylvania. Six-hundred, fifteen nuts were planted at three sites, using randomized complete block designs, with three blocks at each ste, and replication of single-tree plots in the blocks. There were 9 replicates per genotype within a block at the “State Game Lands 100” site and variable numbers of replicates per genotype and block at the “Fisher Mining” and “Bernice” sites, but a total of 240 nuts at the two sites, and equal numbers of each genotype between

blocks. At each of those two sites, there were 57 pure American nuts planted, 48 Chinese and B1-F3, 45 B3-F2 and 42 B2-F3. The same randomization was used at the Fisher and Bernice sites.

Tennessee. Six-hundred nuts were planted in 4 randomized complete blocks, about 35 seeds for each treatment-block combination, in single tree plots, with replication in the block.

West Virginia. Two-hundred, fifty nuts, 50 per genotype, were planted using a split-plot design, in four complete blocks with genotypes randomized within blocks and plots, and peat or no peat randomized between plots. All nuts were protected with 45-cm-tall, plastic tree shelters. Five-tree plots of the same genotype were the basic experimental unit. A second set of 250 nuts was planted with an identical design, but no tree shelters.

Results and Discussion

Overall Survival. Survival was good at all sites, when tree shelters were used, averaging over 60% of planted chestnuts in 2008. The Maryland site was devastated by deer and 4-wheelers over the winter of 2008-2009, and survival dropped to 26% in 2009, but survival in the other states was above or near 50% in 2009 (Table 1). Bizzari and McCarthy (unpublished) surveyed these same sites in 2011, except MD and WV. Scousen el al (in review), surveyed WV in 2010 and 2011. These investigators found that survival remained around above 50% at most sites. There was heterogeneity between sites within and between states, which will be discussed below, but, overall, these data indicate that chestnut can be established and persist on surface mined lands.

At some states, more than one site was planted, and this affected survival in some cases. In Pennsylvania, the Bernice site was wet and alkaline, and there was no survival in year two. At the other two sites in Pennsylvania, survival was excellent (80%) (data not shown). The Pennsylvania sites will be discussed more thoroughly below.

Table 1. Percent establishment in 2008 and survival over 2 years of chestnut at surface mined sites in six states, with and without tree shelters in instances where both treatments were used.*

State	Establishment		2008		2009	
	Shelter	None	Shelter	None	Shelter	None
KY	80a	7b	66a	6b	61a	4b
MD	64		53		26	

OH	84		80		80	
PA	67		61			
TN			55		45	
WV			81a	65b	74a	48b
Mean	**74**	**7**	**66**	**34**	**55**	**32**

*** Significant differences between shelter treatments within states and years are indicated by the percent survival not being followed by the same letter.**

Overall Height Growth. There was considerably more variation in height growth than survival (Table 2). The taller trees in Kentucky may reflect use of fertilizer in the planting hole. In the unpublished surveys by Bizzari and McCarthy and Skousen in 2011, referred to above, mean height at all sites exceeded 50 cm, but only exceeded 100 cm in Kentucky. In orchard settings, chestnut height after four growing seasons is usually in excess of 300 cm (Hebard, 2006), and trees on surface mined sites do not grow so fast, unsurprisingly. These results indicate that chestnut could be a component species in the Forest Reclamation Approach. The long-term growth of chestnut on these sites as soils build up will be a key determinant of the utility of the surface mined sites to restoring chestnut.

Tree shelters gave better survival and height in Kentucky and Tennessee, and clearly should be used when chestnut seednuts are planted on surface mined lands.

Table 2. Mean height (cm) over 2 years of chestnut established at surface mined sites in six states in 2008, with and without tree shelters in instances where both treatments were used.*

State	**2008**		**2009**	
	Shelter	**None**	**Shelter**	**None**
KY	43a	1b	104a	46b
MD	16		19	
OH	22		32	
PA	25			
TN				
WV	10a	6b	37a	27b

Mean	**23**	**4**	**48**	**36**
*** Significant differences between shelter treatments within states and years are indicated by the mean height not being followed by the same letter.**				

Effects of Genotype on Survival. Overall, American chestnut survived less than Chinese chestnut, and there was a gradient between the two reflective of the average proportion of parental species genotype in the backcrosses, with the B_1-F_2 seedlings having the highest proportion of Chinese parentage (averaging 25%) followed by the B_2-F_3 (averaging 12.5%) and the B_3-F_2 (averaging 6.25%)(Table 3). This may have reflected the larger nut size of the Chinese (averaging about 1.5 nuts/gram) than American chestnut (averaging about 3 nuts/gram). However, the relative viability of the nuts used in this study is unknown and a potentially confounding factor.

The lower survival of nuts with a higher fraction of American parentage was not always clear, depending on the state, such as Maryland, where the survival of Chinese chestnut was lower than that of American chestnut. In conjunction with the high degree of mechanical damage in Maryland, the better survival of American than Chinese chestnut may reflect the relatively sparse randomization at the Maryland site, where the basic experimental unit was 25-tree plots of the same species. This genotype by environment interaction alternatively could have been due to additional unidentified environmental factors, in whole or in part. A formal test of such an interaction across all states was not done due to the disparity in experimental designs.

Table 3. Survival over 2 years of chestnut genotypes at surface mined sites in six states, with tree shelters.*

State	**2008**					**2009**				
	Chin	**B_1-F_3**	**B_2-F_3**	**B_3-F_2**	**Amer**	**Chin**	**B_1-F_3**	**B_2-F_3**	**B_3-F_2**	**Amer**
KY	75a	69a	64a	63a	55b	68a	69a	59a	60ab	51b
MD	53	60	49	54	51	25	30	23	25	29
OH	84	86	84	79	75	84	86	84	79	75
PA	69	66	57	59	57					
TN	61	53	59	50	50	52	48	55	36	32
WV	92a	82ab	78b	75b	75b	92a	70b	62c	68bc	68bc

Mean	72	69	65	63	61	64	61	57	54	51

*** Significant differences between genotypes within states and years are indicated by the mean not being followed by the same letter. In many cases, the significant differences were based on least square means. These were generally close to the arithmetic means, which are the data reported.**

Effects of Genotype on Height. As well as having lessened survival, American chestnut seedlings often were shorter than trees in the other genotypes. Overall, height decreased with the proportion of Chinese genotype, but, again, not in all states (Table 4). As with survival, this decrease in height may reflect the smaller nut size of American chestnuts in comparison to Chinese, or may reflect differences in viability.

Table 4. Mean height (cm) over 2 years of chestnut genotypes at surface mined sites in six states, with tree shelters.*

State	**2008**					**2009**				
	Chin	**B_1-F_3**	**B_2-F_3**	**B_3-F_2**	**Amer**	**Chin**	**B_1-F_3**	**B_2-F_3**	**B_3-F_2**	**Amer**
KY	66ab	74a	60b	64b	61b	109a	108a	102a	105a	97a
MD	16a	17a	16a	17a	17a	21a	20a	18ab	20a	16b
OH	22	26	21	13	16	32	35	32	22	25
PA	27a	27a	26a	25ab	23b					
TN										
WV	13a	11a	9a	10a	9a	44a	39a	37a	37a	31a
Mean	29	31	26	26	25	52	51	47	46	42

*** Significant differences between genotypes within states and years are indicated by the mean not being followed by the same letter. In many cases, the significant differences were based on least square means. These were generally close to the arithmetic means, which are the data reported.**

Site Effects. Table 5 presents an analysis of variance for first year height growth in Pennsylvania. There were significant main effects for genotype and site, but block within site was not significant. (One would not expect a major block effect on these sites if the material dumped to form the seed bed was fairly uniform; the large block sizes in all states but Ohio also would reduce variability between blocks). The Bernice site supported significantly worse growth than the other sites, perhaps because the soils were wet and alkaline (pH 7.3), on which acid-loving chestnut trees grow poorly. Another factor in the poor growth at the Bernice site

may have been a high phosphorous level, which was reported at 444 kg/ha. The soils of the State Game Lands 100 had a pH of 4.5, while the Fisher site had a pH of 5.7, both within the preferred range for chestnut, with no mineral excesses, only deficiencies. The end dumping at the Fisher site would have produced a drier soil than the compacted Bernice site. The soil moisture at the State Game Lands is unclear but one would expect it to be high because the site was prepared by back-dragging clay until it was hard..

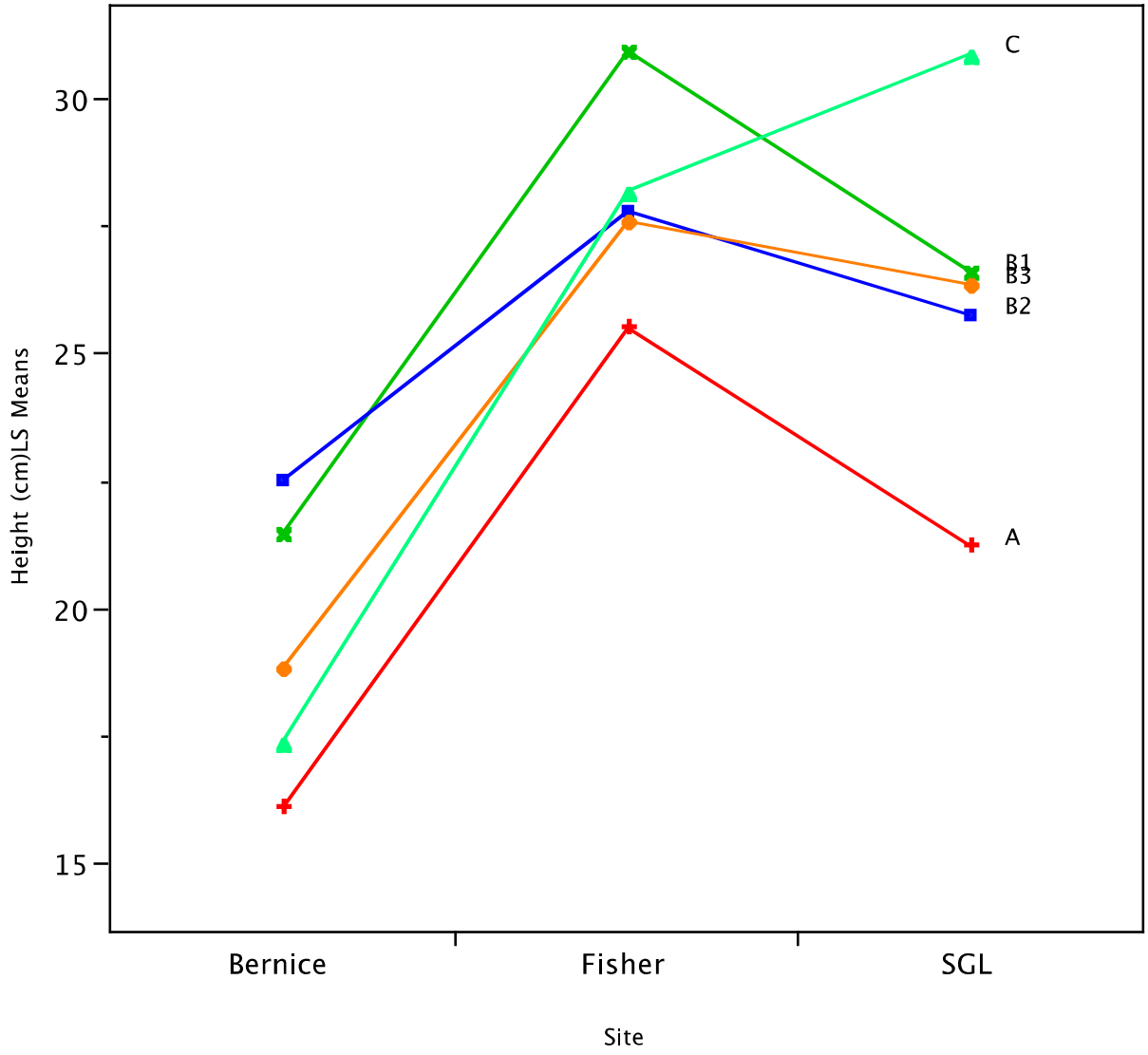
Interestingly, there was significant site by genotype interaction in Pennsylvania. The main contributor to the interaction was the markedly larger height of Chinese chestnut than the other genotypes at the State Game Lands 100 site; at the Bernice and Fisher sites, the height of Chinese chestnut was intermediate among that of the other genotypes (Figure 1). Perhaps Chinese chestnut is more tolerant than the other genotypes of tight soils such as would have occurred at the State Game Lands in contrast especially to the Fisher site. We have additional observational evidence for this conjecture from orchard locations.

Pennsylvania was the only state where there was separation of disparate sites combined with data availability to make this analysis possible and reasonably convincing. In Maryland, there was a significant block effect, but it was obscured by the 25-tree plots in which trees were planted plus a disparity in planting media coinciding with and confounding the block effect.

Table 5. Analysis of variance for height growth (cm) after one growing season of five chestnut genotypes planted as nuts on three surface-mined sites in Pennsylvania in 2008

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Prob > F**
Model	20	7350.320	367.516	7.2162	<.0001
Error	357	18181.866	50.930		
C. Total	377	25532.186			
Effect Tests					
Genotype	4	1216.4551		5.9713	0.0001
Site	2	4431.0837		43.5020	<.0001
Block[Site]	6	514.0399		1.6822	0.1243
Genotype*Site	8	899.6582		2.2081	0.0263

Figure 1. Least square mean heights after one growing season of five chestnut genotypes planted as nuts on three surface mined sites in Pennsylvania in 2008.



Conclusions

- Chestnut can be established and persist on surface mined sites throughout the ARRI region.
- Tree shelters are an important factor in survival of seedlings from directly sown nuts.
- Better growth was seen at sites where FRA prescriptions for site preparation were followed.
- Differences in establishment and growth between Chinese and American chestnut and their backcross hybrids were observed, but all types could be established in all states.

- Strong differences between states in chestnut growth and establishment were not observed on the good sites. More precise experimentation might be required to detect those differences by measuring tree growth.

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