Final report - year 3
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Title: Defining methods for reintroducing American chestnut to oak-hickory forests of the Allegheny Plateau

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Study summary:
This study examined the establishment success of BC_3F_3 hybrid American chestnut seed and seedlings planted on the Allegheny National Forest following each of three cuts of a three-stage shelterwood sequence commonly used to regenerate oak on the Allegheny Plateau of western Pennsylvania. The shelterwood sequence involves a preparatory cut (prep-cut), which reduces a fully-stocked stand to approximately 70 percent relative density in order to improve light conditions for establishment of new oak seedlings. Once the oak regeneration on site has developed an average root collar diameter of one-quarter inch, a shelterwood cut is implemented, which will leave about 50 percent relative density. Finally, once oak seedlings are three feet in height on average, an overstory removal cut will occur, removing most of the residual trees and releasing the developed regeneration to full sunlight. The results of this study will help refine our understanding of the growth, survival, and competitive ability of chestnut planted across a gradient of light and competition intensities. This information will help managers incorporate chestnut reintroduction into oak regeneration management activities.

- **Short term goals (3 years):** compare the early survival and growth of BC_3F_3 chestnuts planted as seeds and seedlings across three silvicultural treatments.
- **Long-term goals (10 years):** evaluate the effects of biotic (competing vegetation, seedling quality) and abiotic (available light) factors on long-term chestnut survival, growth, and competitive ability. Evaluate success of chestnuts planted as seeds vs. seedlings. Develop practical guidelines for reintroducing American chestnut to oak-hickory stands in the Allegheny Plateau.

Methods:

This study is located on the Allegheny National Forest in northwestern Pennsylvania. In the fall of 2016, 2500 hybrid American chestnut seeds from seven families were hand planted in the Vallonia nursery in Vallonia, Indiana. Five of the chestnut families planted are BC_3F_3 hybrids from The American Chestnut Foundation (TACF) and two are BC_2F_3 hybrids from the Connecticut Agricultural Experiment Station. The seedlings were lifted in March, 2017. In April, 2017, 757 of the largest chestnut seedlings and 617 chestnut seeds (from five TACF BC_3F_3 families, two in common with the seedling families, Table 1) were planted in a 3m x 3m grid, with 84 seedlings and 68 seeds planted in each of three replicates of the three silvicultural treatments for a total of 1,368 chestnuts (Table 1). All chestnuts are protected from browsing using five foot-tall tree shelters. In three to five years, depending on density of competing stems, chestnuts planted in the final removal cut sites will be manually released using cutting and/or herbicide treatments.

Table 1. Number of chestnuts planted for each family, separated by chestnut type; seed- or seedling-planted. Ł indicates families that were used in the seed- and seedling analysis. Also listed is the family source; The American Chestnut Foundation (TACF) or The Connecticut Agricultural Experiment Station (CAES).

Families	**Seedling-planted**	**Seed-planted**	**Family source**
D3-27-46	107		TACF
D5-28-88ł	104	108	TACF
D7-27-90		140	TACF
W1-12-141		140	TACF
W2-21-29	107		TACF
W4-32-87ł	107	149	TACF
W5-12-148	107		TACF
W7-21-11		75	TACF
W3-42	106		CAES
W4-31	106		CAES
TOTAL	**756**	**612**	

Seedling height and basal stem diameter were measured at the time of planting (for seedling-planted chestnuts) and near the end of the first two growing seasons (late summer 2017 and 2018). Height and species of the tallest competing seedling growing in a 1.3 m radius competition plot surrounding each chestnut was recorded during the first two growing seasons. The number of stems taller than the chestnut seedling in each competition plot was recorded during the 2018 growing season. Hemispherical photos were taken adjacent to a randomly selected subset of the chestnut seedlings at each site in 2018 (N=135) to evaluate canopy openness at the seedling strata across the silvicultural treatments. Basal area for the planting grids, including a 15 m buffer, was recorded in 2018 and a stand basal area/acre calculated to compare site conditions across the silvicultural treatments.

Analysis:

This study was analyzed as a completely randomized block design (CRD) for silvicultural treatment in the main plot, with each site representing a block. Family and chestnut type (seed/seedling), represented the subplot treatment factors, and were analyzed as complete block designs, with two blocks per site to account for intra-site variation. The seedlings were planted in an incomplete block design (IBD) within the two blocks per site, with 4-10 reps of each chestnut type by family combination per block within site, however enough seedlings survived in each chestnut type by family combination to allow the use of a CRD rather than IBD analysis.

We ran models analyzing the effect of treatments (silvicultural treatment, family, and year) on dependent variables with three different subsets of the data: 1. *Seedling-planted chestnuts:* Only those chestnuts planted as seedlings (7 families), 2. *Seed-planted chestnuts*: only chestnuts planted as seeds (5 families), and 3. *Seedling and seed-planted chestnuts*: Chestnuts with the same families planted as both seed and seedlings (2 families with the additional treatment effect of chestnut type; seed or seedling, only for 2018, Table 1). While this necessitated running multiple models for each dependent variable, it allowed us to ask slightly different questions and made use of all families, not just those represented in both the seedling- and seed- planted chestnuts. For each analysis, seedling response was analyzed using a mixed model analysis of variance to determine significant effects of the treatment factors and their interactions on height, basal diameter, and survival. Proc Mixed was used when a normal distribution fit the data, and Proc Glimmix with gamma or log normal distribution was used when the residuals were not normally distributed. Data were checked for homogeneity of variance and normality. Tukey's LSD procedure was used to compare specific means where significant differences were found.

Results:

Site conditions

Basal area averaged 95, 99, and 10 ft^2/acre for prepcut, shelterwood, and removal sites, respectively (Table 2). Similarly, canopy openness was comparable for prepcut and shelterwood sites; 24 and 25 percent, respectively, and markedly different in the removal sites; 65 percent (Table 2).

Table 2. Average basal area, canopy openness, and the number of taller stems and height of the tallest stems in competition plots for each

Silvicultural treatment	Basal area ft2/acre	No. taller stems	Canopy openness (percent)	Height of tallest competitor (cm)
Prepcut	**95**	**2**±0.3	**24**	**86**±3
338002	96	2±0.3	24	95±6
338004	80	2±0.3	23	80±5
338009	109	2±0.4	24	81±4
Shelterwood seed cut	**99**	**6**±0.8	**25**	**133**±5
321023	111	2±0.4	26	103±7
339007	82	5±1.0	26	74±4
289016	104	12±1.0	24	208±6
Removal cut	**10**	**6**±0.6	**65**	**278**±9
321006	17	6±0.5	81	244±8
351017	6	7±0.6	57	348±19
351018	7	5±0.6	57	249±17

During the second growing season (summer, 2018), the height of the tallest understory stem in competition plots was 86 cm in prepcut sites, 133 cm in shelterwood sites, and 278 cm in removal sites (Table 2). Birch spp. and red maple were the two most frequent tallest understory stems in competition plots, followed by American beech in prepcut and shelterwood sites, and black cherry in removal sites (data not shown).

Chestnut survival

Chestnut survival averaged 73 percent after two growing seasons across the nine sites. The seedling-planted chestnut analysis demonstrated a small but statistically significant drop in survival from 96 to 92 percent from 2017 to 2018 (see Table 3 for P-values for all seedling response analyses), and no other significant differences for treatments or their interactions.

The seed-planted chestnut analysis found differences among silvicultural treatments, families, years, and the interaction between silvicultural treatment and year was significant (Table 3). Survival was lowest for family D4-28-88 (35 percent ± 5). There were no differences in survival among the remaining families, which averaged between 48 and 60 percent survival (± 5 for each) over the course of the study. Survival remained highest in the prepcut sites in both years, averaging 62 percent (± 4) in 2018. The only difference in years was found in the removal sites, where survival dropped from 46 to 40 percent (± 4, Figure 1).

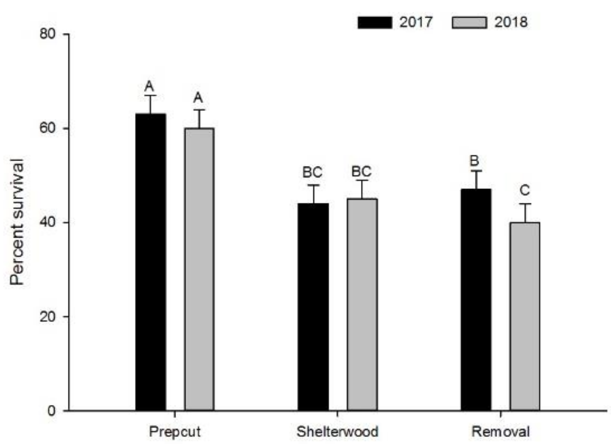

Figure 1. Seed-planted chestnut survival among the three silvicultural treatments for 2017 and 2018. Error bars indicate standard error. Letters indicate statistically significant differences.

The seedling- and seed-planted chestnut analysis demonstrated differences in survival between chestnut types (46 percent ± 3 for seed- and 94 percent ± 3 for seedling-planted chestnuts), and families, with a positive type by family interaction (Table 4) and silvicultural treatment by family by type interaction (results not shown).

Table 3. P-values for mixed model analyses of response variables height, basal diameter (BD), and survival for each of three analyses: seedling-planted chestnut, seed-planted chestnut, and seed- and seedling-planted chestnut. α= 0.05.

Fixed effect	**Seedling chestnut analysis**			**Seed-chestnut analysis**			**Seed and seedling analysis**		
Treatment effect	**Height**	**BD**	**Survival**	**Height**	**BD**	**Survival**	**Height**	**BD**	**Survival**
Silvicultural treatment (silv)	<0.0001	0.67	0.64	0.16	0.36	0.002	0.001	0.07	0.13
Type (seed or seedling)	NA	NA	NA	NA	NA	NA	<0.0001	<0.0001	<0.0001
Family	0.02	0.05	0.18	<0.0001	0.005	0.01	0.04	0.86	0.001
Year	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.02	NA	NA	NA
Silv*type	NA	NA	NA	NA	NA	NA	0.002	0.03	0.07
Silv*family	0.02	0.38	0.54	0.56	0.91	0.18	0.15	0.53	0.11
Silv*year	<0.0001	<0.0001	0.08	0.02	0.81	0.02	NA	NA	NA
Family*year	0.07	0.29	0.38	0.37	0.38	0.05	NA	NA	NA
Silv*family*year	0.002	0.36	0.29	0.27	0.98	0.97	NA	NA	NA
Family*type							0.77	0.02	0.001
Silv*family*type							0.15	0.31	0.04

Table 4. 2018 percent survival for the seed- and seedling-planted chestnut families. Letters indicate differences among treatments.

Family	Chestnut type	Second year survival
D5-28-88	Seed	34 ±4 C
	Seedling	95 ±4 A
W4-32-87	Seed	58 ±4 B
	Seedling	92 ±4 A

Chestnut height:

The seedling-planted chestnut analysis found significant differences for each main effect, as well as significant interactions for silvicultural treatment by family, silvicultural treatment by year, and silvicultural treatment by family and year (Table 3). Seedlings averaged 100 cm (±2) in year one (2017), compared with 128 cm (±2) in year two (2018). By 2018, the seedlings in the removal treatments were taller than seedlings in either the shelterwood or prepcut treatments for all families except W5-12-148 or W3-42; seedlings in these families were similar in height across the silvicultural treatments. Seedlings for all families were similar in height between the prepcut and shelterwood treatments in 2018. Initial seedling height was a significant covariate in the final model ($P < 0.0001$).

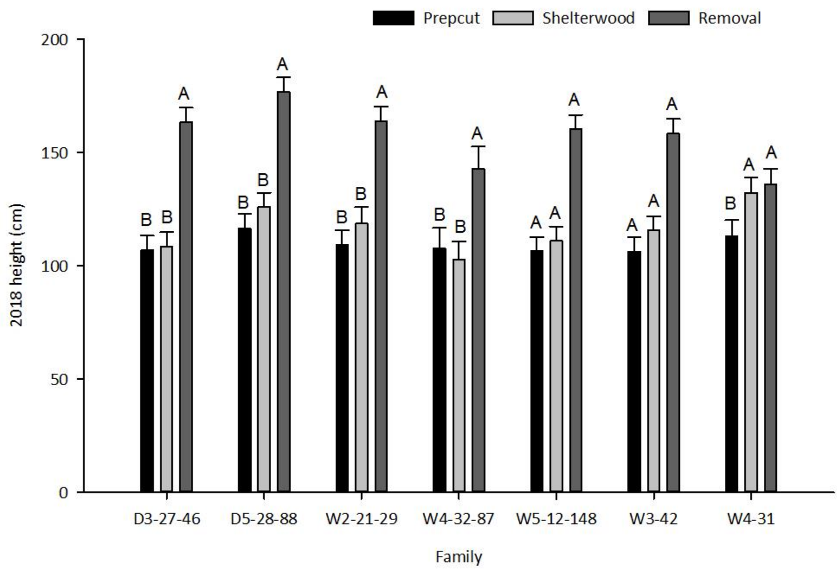

Figure 2. Mean height (cm) in 2018 (the second growing season) among families for each silvicultural treatment. Error bars indicate standard error. Letters indicate statistically significant differences among treatments.

The seed-planted chestnut analysis found differences in family and year, and the interaction between silvicultural treatment and year was significant (Table 2). Family W1-12-141 had the lowest height after two years, 15 cm (±1), compared to an average height of 21 cm (±1), for the remaining four families. Height after the first growing season averaged 19 cm (±1), compared to 23 cm (±1) in 2018. By 2018, chestnuts were taller in the removal sites than the shelterwood, and similar between the prepcut sites and each of the other two silvicultural treatments (Figure 3).

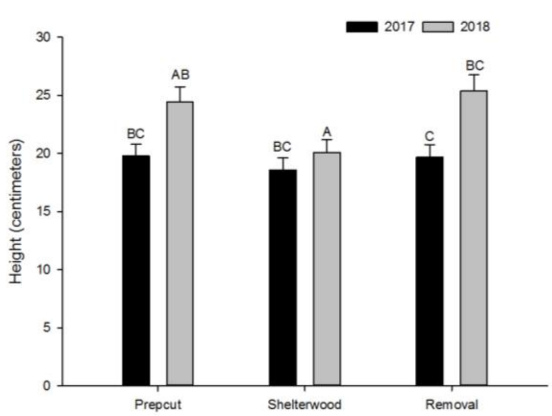

Figure 3. Mean height in 2017 and 2018 for seed-planted chestnuts among silvicultural treatments. Error bars represent standard error. Letters indicate statistically significant differences among treatments.

The seedling- and seed-planted chestnut analysis found differences in height among silvicultural treatments, chestnut type, and families (Table 3). Chestnuts from family D5-28-88 were nine centimeters taller on average after two growing seasons than those from W4-32-87 (86 cm ± 2, 77 cm ±2, respectively). The interaction between silvicultural treatment and chestnut type was significant; seedling-planted chestnuts were taller in removal sites than shelterwood or prepcut sites, while seed-planted chestnuts grew better in removal and prepcut sites than shelterwood sites (Figure 4).

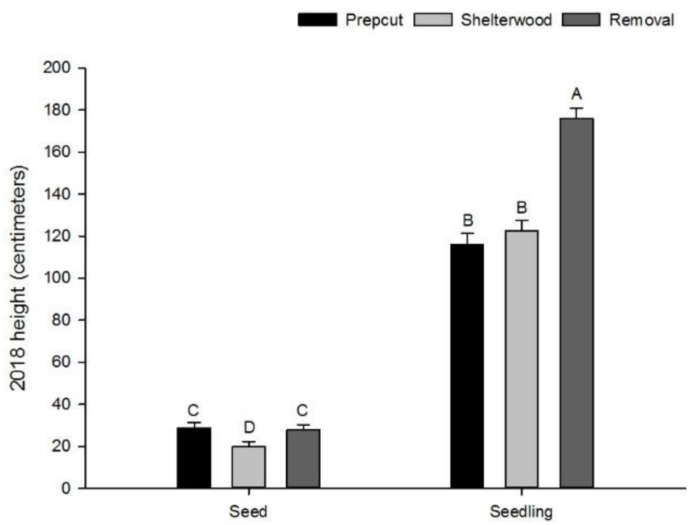

Figure 4. Mean height (cm) in 2018 for seed- and seedling-planted chestnuts among the silvicultural treatments. Error bars represent standard error. Letters indicate statistically significant differences among treatments.

Chestnut basal diameter:

The seedling-planted chestnut analysis found differences in basal diameter between the two years, and the interaction between silvicultural treatment and year was significant (Table 3). By 2018, the seedling-planted chestnut seedlings were larger in diameter in the removal sites than the other two silvicultural treatments (8.2 ± 0.2, 8.7 ± 0.2, 9.4 ± 0.2 mm in the prepcut, shelterwood, and removal sites, respectively). Families neared statistical differences ($P = 0.05$), with a slightly lower (not statistically) mean diameter for family W3-42 than the others.

The seed-planted chestnut analysis found differences in years and families (Table 4), but not silvicultural treatments and no interactions were significant (Table 3). Chestnuts averaged 1.9 mm (± 0.6) in diameter in 2017, compared with 2.6 mm (± 0.8) in 2018.

Table 4. Mean basal diameter (mm) over the two year study and for 2018 among seed-planted families. Letters indicate statistically significant differences in family means.

Family	Mean basal diameter (mm)	2018 basal diameter
D5-28-88	2.1 ±0.1 B	2.4. ±0.1
D7-27-90	2.5 ±0.1 A	3.0 ±0.1
W1-12-141	2.1 ±0.1 B	2.3 ±0.1
W4-32-87	2.2 ±0.1 AB	2.6 ±0.1
W7-21-11	2.4 ±0.1 AB	2.8 ±0.1

The seedling- and seed-planted chestnut analysis found differences in chestnut type, and family, and the interactions between family and type and among silvicultural treatment, family and type were significant (Table 3). Seedling-planted chestnuts were largest in diameter in the removal sites (10.5 ±0.03 mm) compared with the prepcut and shelterwood sites (8.6 ± 0.03 mm, 8.5 0.03 mm, respectively), while there were no differences in the seed-planted chestnut basal area among the silvicultural treatments. Seedling-planted chestnut from the DS5-28-88 family were larger in basal diameter than those in the W4-32-87 family (9.6 ±0.03 mm, 8.7 6 ±0.03 mm, respectively), while there were no differences in diameter between families for the seed-planted chestnuts.

Discussion:

Survival was high across treatments for seedling-planted chestnuts, likely due in part to the high quality of the seedlings used in this study and adequate soil moisture at the planting sites (Figure 5). Survival among seed-planted chestnuts, however was much lower, averaging 46 percent in 2018. Chestnut seeds are highly susceptible to predation by wildlife (Wang et al. 2013), and the large number that died in the first year in this study (49 percent) may have been eaten by small mammals. The tree shelters used in this study were designed to protect chestnut seedlings from browsing of leaves and stems, and may not sufficiently protect the nuts from predation from small mammals.

Height was greatest for most and basal diameter greatest for all seedling-planted chestnuts in the removal sites, compared with the shelterwood or prepcut sites. Removal sites had substantially lower basal area and higher canopy openness than the other treatments, indicating increased light availability. Several other studies have found increased early growth of planted chestnut seedlings in silvicultural treatments with high light availability (Clark et al. 2012, McCament and McCarthy 2005, Pinchot et al. 2017). Light available to the planted chestnuts will likely change over time as co-occurring vegetation continues to respond to the increased light from the harvest treatments, particularly in the removal treatment sites. The height of the tallest competitor is already greatest in this treatment, and over time it will only become more important that the chestnut seedlings can grow quickly to remain in the co-dominant or dominant strata. Pinchot et al. 2017 found that planted chestnuts growing in shelterwood with reserve treatments (high light availability) became less competitive relative to co-occurring vegetation than those growing in treatments with lower light levels. Competition control will likely be necessary for chestnuts planted in high light treatments.

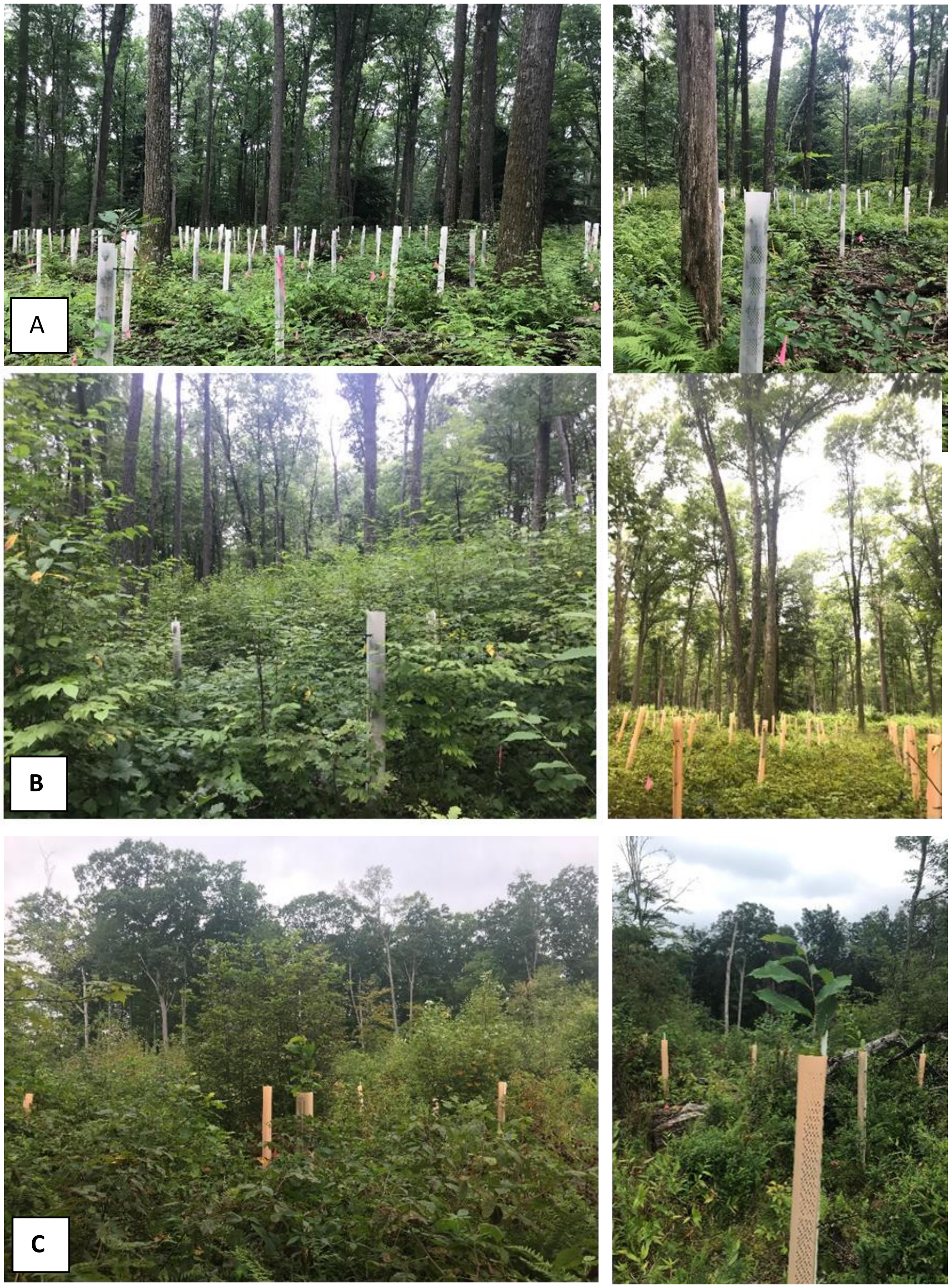

Figure 5. Chestnuts growing in A. prepcut, B. shelterwood, and C. removal sites, two years after planting.

Seed-planted chestnuts had the greatest height in the removal and prepcut treatments, with no statistically significant difference in height between prepcut and shelterwood treatments. The analysis comparing the same families grown from seed and seedlings found that height of seedling-planted chestnuts is more responsive to differences in the silvicultural treatments than the seed-planted chestnuts. The greater height of the seedling-planted chestnuts likely gave them a competitive advantage compared with the seed-planted chestnuts, which would have been particularly important in the removal sites, where height of competition was substantially greater than the other sites. In the removal sites, seed-planted chestnuts may simply not be able to keep up with competition, as is suggested by the lower survival for seed-planted chestnuts in these sites compared to the other two silvicultural treatments.

The relatively lower survival and growth of seed-planted chestnuts doesn't necessarily support planting only seedling chestnuts as part of reintroduction efforts. Planting seeds is generally logistically easier than planting seedlings; they are easier to store and transport, and are less laborious to plant compared with bareroot or containerized seedlings. Protection from predation is necessary to reduce mortality of seeds, and greater mortality for seed-planted chestnuts should be taken into consideration when determining the number of chestnuts to plant at a restoration site.

Longer term results are necessary to draw firm conclusions from this study about the use of oak silvicuilture for planted chestnut establishment. Over time vegetation dynamics will likely change dramatically across the three treatments The prepcut and shelterwood treatments have additional overstory manipulations planned, per the standard prescriptions for each of these treatments. The goal of reintroduction plantings is to establish populations of chestnut that will eventually reproduce and spread beyond the boundaries of the planting. For this to occur, it will be crucial for the chestnut seedlings to remain a part of the co-dominant and dominant strata over time. Longer term results will determine how successful the chestnuts are across the treatments.

Literature cited:

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