Silviculture of the American chestnut in the Cumberland Uplands of Tennessee

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Narrative Summary:

American chestnut (*Castanea dentata* (Marshall) Borkhausen) historically was a rapidly growing tree that exploited canopy gaps in the eastern forests. These gaps are created naturally by windthrow or fire and make light available to chestnut seedlings on the forest floor (Ashe 1911). In forest environments, light is a limiting factor to chestnut growth and flowering (Paillet 2002). Chestnut blight, *Cryphonectria parasitica* (Murr.) Barr, and root rot, *Phytophthora cinnamomi* Rands, have devastated the species across its entire range, including the Cumberland Uplands of Tennessee (Frienkel 2007). A blight resistant hybrid is being developed by The American Chestnut Foundation (TACF) (Hebard 2012). Canopy gaps, both existing and artificial, could facilitate chestnut reintroduction and restoration (Dalgleish et al. 2015). Advanced hybrids are now ready for reintroduction trials; however, further research is needed on the ecological requirements of reintroducing chestnut to eastern forests, including its establishment in canopy gaps (Clark et al. 2011, Rhoades et al. 2009).

For the 2019 and 2020 season, we proposed an observational study on the survival and growth of over 700 seedlings from The American Chestnut Foundation (TACF) in 35 various sized light gaps in the Cumberland Uplands across 3 sites. Sites were previously established at the privately-owned Starr Farm (Eastern Highland Rim) and at the private conservation easement at Eagle Point Railroad (Cumberland Plateau). We established a new location near the Barker Pounds trailhead of the Cumberland Trail in the North Chickamauga Creek Gorge State Natural Area (Cumberland Plateau) with 9 light gaps: 3 small, 3 medium, and 3 large (open field) plantings. We compared canopy openness to the growth rate of the saplings. We measured height and root collar diameter (RCD). Canopy openness was measured hemispherical photography and calculated by Gap Light Analyzer 2.0 (Frazer et al. 1999).

Objectives:

The short-term goal was to evaluate the effects of canopy gap size on the growth rate of *Castanea dentata* hybrid seedlings on three sites in the Cumberland Uplands of Tennessee. The long-term goal is to determine the silvicultural requirements for *C. dentata* hybrids for reintroduction to canopy gaps in forest environments. We hope, through this project, to forge a lasting partnership between UT Chattanooga, TACF and the Cumberland Trail State Park, a major stakeholder in the region that manages much habitat suitable for American chestnut reintroductions.

Methods:

Study Sites. EPRR is a private conservation easement with hardwood forest on the rim of the Cumberland Plateau. A chestnut genotype x environment study was started in 2013 and 2014 with seedlings planted in various sized canopy gaps (Tom Saielli, personal communication). The Starr Farm is in Noah, Tennessee with hardwood forest on the Eastern Highland Rim. The landowner widened existing canopy gaps and began planting trees in 2014. We established hybrid chestnut seedlings in canopy gaps of a discontinued Loblolly pine plantation at Barker Pounds trailhead at North Chickamauga Creek Gorge State Natural Area near Soddy Daisy, Tennessee. It was created by generous partnership with Justin P. Wilson Cumberland Trail State Park.

Figure 1. (Left) Tall seedlings were measured with a telescopic measuring rod like this one at EPRR. (Right) Hemispherical photography is used to measure canopy openness at a small gap site at Barker Pounds.

Establishment of Barker Pounds: With the help of state park rangers, we selected naturally occurring canopy gaps within the Loblolly stands. There were 3 blocks, each including a small, medium, and large gap (open field) each with 25 trees. A dozer with a fire line plow prepared all nine plots by removing understory vegetation and turning the soil. The first planting took place on May 7th, 2019 with the help from Cumberland Trail State Park's rangers and students from Ivy Academy, Soddy Daisy, TN. We replaced seedlings that perished in the 2019 season on December 7, 2019 with help of UT Chattanooga student volunteers.

Seedlings: Not all trees were used for the final analysis for reasons such as death and deer browse. The families studied in 2019 season are listed below in Table 1.

Table 1. This table contains the chestnut families studied in the 2019 season.

EPRR					
Cross Name	Pedigree	Seed Type	Resistance Source	Year	n
C. dentata Harlan Co. KY	HarlanAC x OpAmerican	American		2013	1
				2014	29
C. dentata Haun	Haun x OpAmerican	American		2013	3
				2014	2
C. dentata MGC-12	MGC-12 x OpAmerican	American		2013	9
				2014	2
C. dentata Native	American x OpAmerican	Native		N/A	9
C. dentata Pryor 1-182 or US	Pryor 1-182 x OpAmerican	American		2013	14
				2014	38
C. dentata SA 319	SA 319 x OpAmerican	American		2013	1
C. dentata SA 408 x I-11	SA 408 x I-11	American		2011	1
C. dentata Tyler	Tyler 4-13 x OpAmerican	American		2013	7
				2014	42
C. mollissima Asheville CH	Asheville CH x OpChinese	Chinese		2014	42
				2015	1
C. mollissima CH-1	*C. mollissima* x OpChinese	Chinese		2013	5
				2014	1
C. mollissima "McInturff"	*C. mollissima* x OpChinese	Chinese		2013	1
D1-29-4	D1-29-4 x OpB3F2	B3F3	Clapper	2014	29
D2-20-153	D2-20-153 x OpB3F2	B3F3	Clapper	2014	34
D4-27-64	D4-27-64 x OpB3F2	B3F3	Clapper	2014	32
D5-17-89	D5-17-89 x OpB3F2	B3F3	Clapper	2015	1
D5-26-88	D5-26-88 x OpB3F2	B3F3	Clapper	2014	38
D7-28-145	D7-28-145 x OpB3F2	B3F3	Clapper	2014	34
Thoroughfare Gap F-1		F1		2014	1
TN Mon - 13 x GL158	TN Mon - 13 x GL158	B3	Clapper	2011	1
Unknown cross	Unknown	?		?	2
W1-15-133	W1-15-133 x OpB3F2	B3F3	Graves	2019	2
W1-24-31	W1-24-31 x OpB3F2	B3F3	Graves	2013	5
W1-29-8	W1-29-8 x OpB3F2	B3F3	Graves	2013	5
W1-30-6	W1-30-6 x OpB3F2	B3F3	Graves	2013	5
W1-31-7	W1-31-7 x OpB3F2	B3F3	Graves	2013	9
W1-32-69	W1-32-69 x OpB3F2	B3F3	Graves	2013	4

W2-31-33	W2-31-33 x OpB3F2	B3F3	Graves	2013	1
W3-31-140	W3-31-140 x OpB3F2	B3F3	Graves	2013	1
W3-31-86	W3-31-86 x OpB3F2	B3F3	Graves	2013	5
W5-31-13	W5-31-13 x OpB3F2	B3F3	Graves	2013	3
W5-32-61	W5-32-61 x Op B3F2	B3F3	Graves	2013	7
W6-31-33	W6-31-33 x OpB3F2	B3F3	Graves	2013	3
W7-31-74	W7-31-74 x OpB3F2	B3F3	Graves	2019	2
W8-13-80	W8-13-80 x OpB3F2	B3F3	Graves	2015	1
W8-32-15	W8-32-15 x OpB3F2	B3F3	Graves	2013	3

Starr Farm

Cross Name	Pedigree	Seed Type	Resistance Source	Year	n
American	American x OpAmerican	American		2014	8
D5-17-89	D5-17-89 x OpB3F2	B3F3	Clapper	2015	5
D8-10-19	D8-10-19 x OpB3F2	B3F3	Clapper	2015	3
TN-TTU Mix	(TNSUM1 x VA89) x OpB3	B3F2	Clapper	2018	2
TN-TTU-C9	(TNSUM1 x VA89) x OpB3	B3F2	Clapper	2016	1
TN-TTU-E6	(TNSUM1 x VA89) x OpB3	B3F2	Clapper	2016	2
TN-TTU-G22	(TNSUM1 x VA89) x OpB3	B3F2	Clapper	2016	1
TN-TTU-K2	(TNClay1 x GL28) x OpB3	B3F2	Clapper	2016	4
W1-32-69	W1-32-69 x OpB3F2	B3F3	Graves	2019	9
W2-32-108	W2-32-108 x OpB3F2	B3F3	Graves	2016	1
W3-8-119	W3-8-119 X opB3F2	B3F3	Graves	2015	7
W3-8-73	W3-8-73 x Op B3F2	B3F3	Graves	2017	1
W4-12-124	W4-12-124 x OpB3F2	B3F3	Graves	2015	3
W4-21-42	W4-21-42 x OpB3F2	B3F3	Graves	2018	2
W4-32-87	W4-32-87 x OpB3F2	B3F3	Graves	2016	1
W4-6-71	W4-6-71 x OpB3F2	B3F3	Graves	2017	2
W7-14-122	W7-14-122 x OpB3F2	B3F3	Graves	2018	2
W7-32-147	W7-32-147 x OpB3F2	B3F3	Graves	2016	3
W8-22-62	W8-22-62 x OpB3F2	B3F3	Graves	2017	1
W9-8-140	W9-8-140 x OpB3F2	B3F3	Graves	2015	4

Barker Pounds

Cross Name	Pedigree	Seed Type	Resistance Source	Year	n
TN-TTU-K2	(TNCLA2 x "AB238") x OpB3	B3F2	Clapper	2019	75
TN-TTU-L13	(TNCLA2 x "AB238") x OpB3	B3F2	Clapper	2019	62
TN-TTU-E6	(TNSUM1 x GL28) x OpB3	B3F2	Clapper	2019	75
TN-TTU-L13 x SA408	(TNCLA2 x "AB238") x SA408	B3 x B2F2	Clapper	2019	13

Barker Pounds Fall Replant					
Cross Name	Pedigree	Seed Type	Resistance Source	Year	n
TVA SE 4-12	(Myco4-6(American) x VA89) x OpB3	B3F2	Clapper	2019	42
TVA SE 4-5	(Myco4-6(American) x VA89) x OpB3	B3F2	Clapper	2019	72
TVA NE 4-29	(VA89 x T2(American)) x OpB3	B3F2	Clapper	2019	39
TN-TTU-F32	(TNSUM1 x VA89) x OpB3	B3F2	Clapper	2019	5
TN-TTU-B7	(TNSUM1 x VA89) x OpB3	B3F2	Clapper	2019	7

The families studied in the 2020 season are listed below in Table 2. Additional trees were monitored in the 2020 season; however, some seedlings were dead and never resprouted or the seedlings were planted after the initial measurements. Therefore, those seedlings were not included in this list to simplify.

Table 2. The chestnut families studied in 2020 season are described below.

EPRR					
Cross Name	Pedigree	Seed Type	Resistance Source	Year	n
Asheville CH	Asheville CH x Op	Chinese		2014	40
				2015	1
C. dentata Native	American x American	Native		native	9
C. mollissima (Chinese) "McInuiff"	*C. mollissima* x Op	Chinese		2013	1
CH-1		Chinese		2013	4
				2014	1
D1-29-4	D1-29-4 x Op	B3F3	Clapper	2013	1
				2014	26
D2-20-153	D2-20-153 x Op	B3F3	Clapper	2014	33
D4-27-64	D4-27-64 x Op	B3F3	Clapper	2014	31
D5-17-89	D5-17-89 x Op	B3F3	Clapper	2015	1
D5-26-88	D5-26-88 x Op	B3F3	Clapper	2014	35
D7-28-145	D7-28-145 x Op	B3F3	Clapper	2014	31
Harlan Co. KY or AC KY-1	HarlanAC x Op	American		2013	1
				2014	29
Haun	Haun x Op	American		2013	3
				2014	1
MGC-12	MGC-12 x Op	American		2013	9
				2014	2
Pryor 1-182 x OP or US	Pryor 1-182 x OP	American		2013	14
				2014	39
SA 408 x Ix11	SA 408 x Ix11	American		2011	1
Thoroughfare Gap F-1		F1		2014	1
TN Mon - 13 x GL158	TN Mon - 13 x GL158	B3	Clapper	2012	1
Tyler	Tyler 4-13 x Op	American		2013	6

				2014	39
W1-15-133	W1-15-133 x Op	B3F3	Graves	2019	1
W1-24-31	W1-24-31 x Op	B3F3	Graves	2013	5
W1-29-8	W1-29-8 x Op	B3F3	Graves	2013	4
W1-30-6	W1-30-6 x Op	B3F3	Graves	2013	5
W1-31-7	W1-31-7 x Op	B3F3	Graves	2013	9
W1-32-69	W1-32-69 x Op	B3F3	Graves	2013	4
W2-31-33	W2-31-33 x Op	B3F3	Graves	2013	1
W3-31-140	W3-31-140 x Op	B3F3	Graves	2013	1
W3-31-86	W3-31-86 x Op	B3F3	Graves	2013	3
W5-31-13	W5-31-13 x Op	B3F3	Graves	2013	3
W5-32-61	W5-32-61 x Op	B3F3	Graves	2013	7
W6-31-33	W6-31-33 x Op	B3F3	Graves	2013	3
W7-31-74	W7-31-74 x Op	B3F3	Graves	2019	2
W8-13-80	W8-13-80 x Op	B3F3	Graves	2015	1
W8-32-15	W8-32-15 x Op	B3F3	Graves	2013	3

Starr Farm					
Cross Name	Pedigree	Seed Type	Resistance Source	Year	n
D5-17-89	D5-17-89 x Op	B3F3	Clapper	2015	5
D6-13-122	D6-13-122 x Op	B3F3	Clapper	2019	2
D8-10-19	D8-10-19 x Op	B3F3	Clapper	2015	3
American	American x Op	American		2014	7
TT-Mix	TN-SM1-Nmix x OpB3	B3F3	Clapper	2018	2
TT-C9	TN-TTU-C9 x OpB3	B3F3	Clapper	2016	1
TT-E6	TN-TTU-E6 x OpB3	B3F3	Clapper	2016	2
TT-G22	TN-TTU-G22 x OpB3	B3F3	Clapper	2016	1
TT-K2	TNClay1 x GL28	B3F3	Clapper	2016	4
W1-32-69	W1-32-69 x Op	B3F3	Graves	2019	8
W2-32-108	W2-32-108 x Op	B3F3	Graves	2016	1
W3-8-119	W3-8-119 X opB3F2	B3F3	Graves	2015	6
W3-8-73	W3-8-73 x Op	B3F3	Graves	2017	1
W4-12-124	W4-12-124 x Op	B3F3	Graves	2015	3
W4-21-42	W4-21-42 x Op	B3F3	Graves	2018	2
W4-29-25	W4-29-25 x Op	B3F3	Graves	2019	2
W4-32-87	W4-32-87 x Op	B3F3	Graves	2016	1
W4-6-71	W4-6-71 x Op	B3F3	Graves	2017	2
W7-14-122	W7-14-122 x Op	B3F3	Graves	2018	2
W7-32-147	W7-32-147 x Op	B3F3	Graves	2016	3
W8-22-62	W8-22-62 x Op	B3F3	Graves	2017	1
W9-8-140	W9-8-140 x Op	B3F3	Graves	2015	4

Barker Pounds					
Cross Name	Pedigree	Seed Type	Resistance Source	Year	n
TN-TTU-B7	(TNSUM1 x VA89) x OpB3	B3F2	Clapper	2019	7
TN- TTU-E6	(TNSUM1 x GL28) x OpB3	B3F2	Clapper	2019	14
TN-TTU-F32	(TNSUM1 x VA89) x OpB3	B3F2	Clapper	2019	5
TN-TTU-K2	(TNCLA2 x "AB238") x OpB3	B3F2	Clapper	2019	10
TN-TTU-L13	(TNCLA2 x "AB238") x OpB3	B3F2	Clapper	2019	22
TN-TTU-L13 x SA408	(TNCLA2 x "AB238") x SA408	B3 x B2F2	Clapper	2019	13
TVA NE 4-29	(VA89 x T2(American)) x OpB3	B3F2	Clapper	2019	38
TVA SE 4-12	(Myco4-6(American) x VA89) x OpB3	B3F2	Clapper	2019	44
TVA SE 4-5	(Myco4-6(American) x VA89) x OpB3	B3F2	Clapper	2019	72

Measurements: For the 2019 season, preseason height and RCD were taken at the Starr Farm on 24 April 2019, Barker Pounds on 8 May 2019, and EPRR on 23, 26, and 27 April and 2, 3, and 8 May 2019. End-of-season growth measurements were taken at the Starr Farm on 3 October 2019, at Barker Pounds on 27 September 2019, and at EPRR on 22 and 24 September and 8 October 2019. For the 2020 season, preseason measurements were taken at the Starr Farm on 7 May 2020, at Barker Pounds on 4 May 2020, and at EPRR on 14, 17, 21, 24, and 28 April 2020. End-of-season measurements were taken at the Starr Farm on 13 January 2021, at Barker Pounds on 11 December 2020, and at EPRR on 18 December 2020 and 2, 4, 5, and 12 January 2021.

Hemispherical Photography and Gap Light Analyzer 2.0: Nikon D750 SLR camera and a Sigma 4.5 f/2.8 EX DC HSM Circular Fisheye lens, which have a 180 field-of-view and a large aperture for understory conditions, were used to take canopy photos (Jonckheere et al. 2017). To capture the gradient of light received by the understory seedlings based on their position in the canopy gap, a photo of the overhead canopy was taken for each tree. The camera was leveled one meter above the ground with the top of the photo was oriented north (Jonckheere et al. 2017). For the 2019 season, canopy photos were taken before senescence at Barker Pounds on 21 and 27 September 2019, at EPRR on 9, 22, and 25 September 2019, and at the Starr Farm on 1 October 2019. For the 2020 season, photos were only taken at EPRR on 10, 13, 17, and 20 August and 1 September 2020.

Gap Light Analyzer 2.0 software (Simon Fraser University, Burnaby, BC, Canada, and the Cary Institute of Ecosystem Studies, Millbrook, NY) calculated canopy openness, which is the percentage of open sky that is visible from the understory (Hall et al. 2017). Pixels of sky and foliage were separated by the blue color plane and a threshold of 160, except for several overexposed photos in which 180 was used (Jonckheere et al. 2017). Therefore, canopy openness was determined for each seedling for analysis of growth.

Soil Samples: In 2019, a composite soil sample was taken from each of the three sites and mailed to the University of Tennessee Extension office for a soil nutrient test. Additionally, we sent soil samples to Clemson University for testing because root rot was observed at Barker Pounds. Of the three composite samples from Barker Pounds, all three contained *Phytophthora cinnamomi*, a root rot causing fungus. This is typical of pine plantation soils (Steven Jeffers, personal communication).

Statistical Analysis: R and R Studio (R Core Team 2017) processed all analyses for this study. Mean vertical growth and RCD growth was tested with a one-way ANOVA. Seedling growth was modeled using step-wise multiple regression analysis, using a partial F-test to test each term's significance, including polynomial regression, in improving the model.

Results:

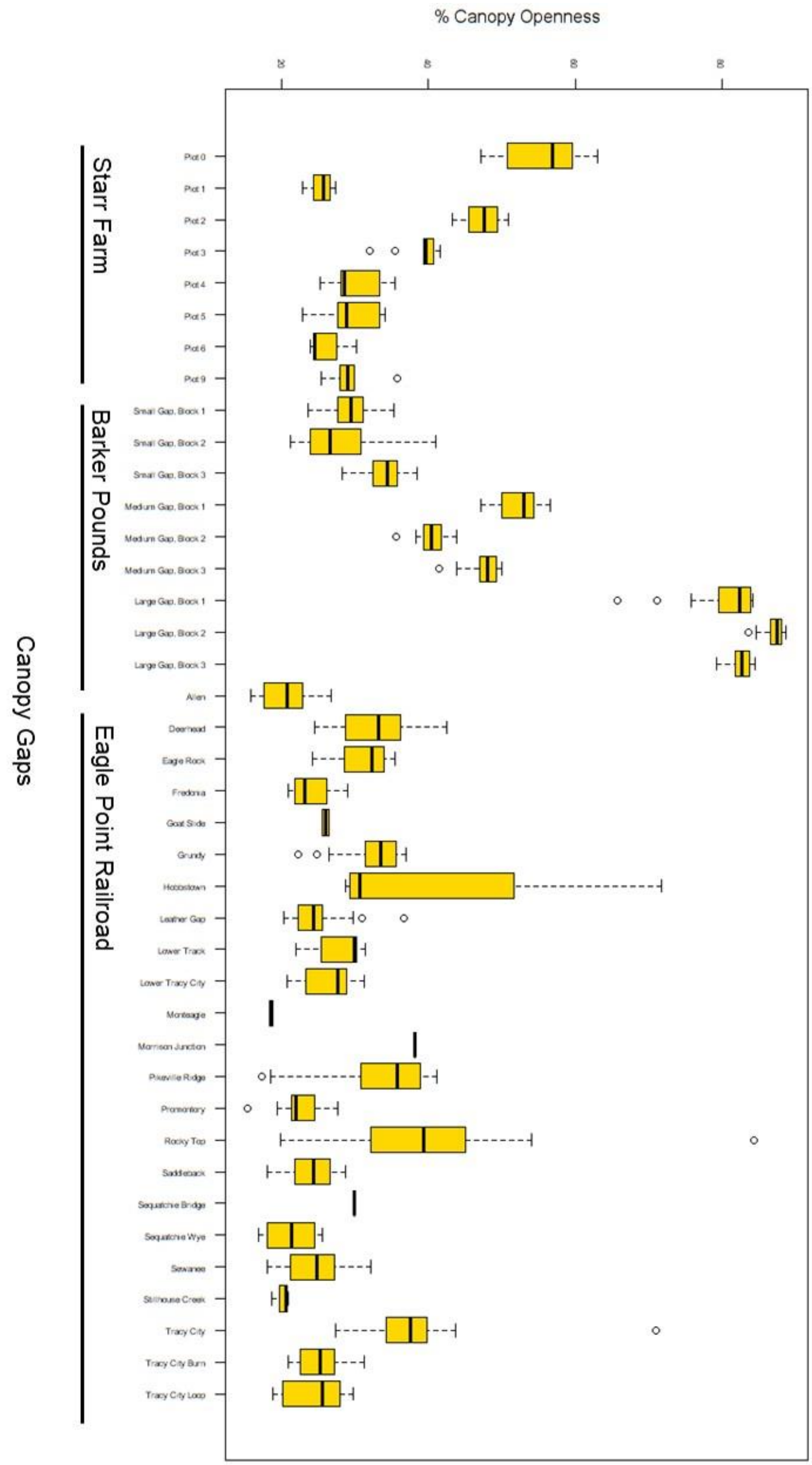
2019 Season

Detailed results of the 2019 season were reported in Hannah (Crawford) Nelms' undergraduate thesis, *Canopy openness as a predictor of growth for Castanea dentata seedlings in the Cumberland Uplands of Tennessee*. A citation is found below in the *Publications* section of this report.

Barker Pounds 2019 Season: There was a high mortality rate, 75.8%, in the first season at Barker Pounds with observed deer browse on 48.4%. Trees without browse that lived through the season grew between 0.0 cm (no observable growth) and 29.9 cm in vertical height and 0 mm (no observable growth) and 14 mm in RCD. High mortality prevented further statistical analysis based on growth.

Canopy Openness: Percent canopy openness was measured for individual seedlings at each site. Percent canopy openness at EPRR ranged from 15.31% to 84.43%. At Barker Pounds canopy openness ranged from 21.24% to 88.75%. At the Starr Farm, canopy openness ranged from 22.78% to 62.98%. There was a range of light conditions between seedlings within the same canopy gap (Figure 2).

Figure 2. Depending on the position of the seedlings within the gap and the distribution of understory vegetation, there was a range of light conditions experienced in the gap.



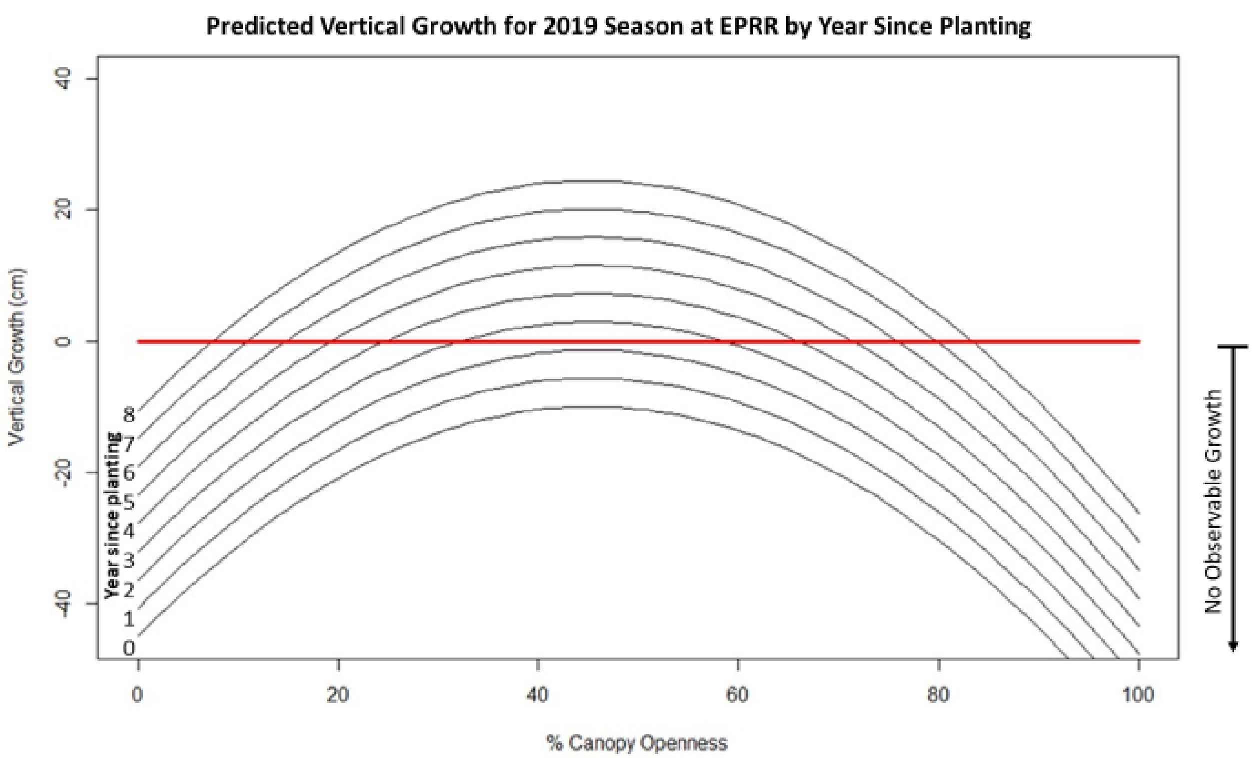
Vertical Growth: Seedlings that had observable deer browse or dead tops were not included in the analysis of vertical growth. In the 2019 season, seedlings at EPRR had a mean vertical growth of 8.32 cm and ranged from 0.0 cm (no observable growth) to 82.0 cm. The effect of seedling type on vertical growth was marginally significant ($p = .043$). Post-hoc analysis with Tukey's test revealed that Chinese seedlings grew marginally less than American seedlings and B3F3 seedlings ($p = .0244$ and $p = .0693$, respectively). There was no significant difference in growth between the B3F3 and American seedlings ($p = .876$).

Seedlings at Starr Farm in 2019 had a mean vertical growth of 62.7 cm and ranged between no observable growth and 178.0 cm. The effect of seedling type was not significant ($p = .362$).

2019 EPRR Growth Model: A linear regression was tested to predict vertical growth based on percent canopy openness and age of seedling. Vertical growth had a significant positive correlation with canopy openness and seedling age (respectively, $p < .001$ and $p < .001$). A step-wise regression of nested models was used to improve the simple linear model of vertical growth based on percent canopy openness. Using a partial F-test seedling age improved the model based on canopy openness ($p < .001$), but seedling type did not improve the model ($p = .102$). Adding the polynomial term, canopy openness^2, to the model based on canopy openness and seedling age improved the model using the partial F-test ($p = .003$).

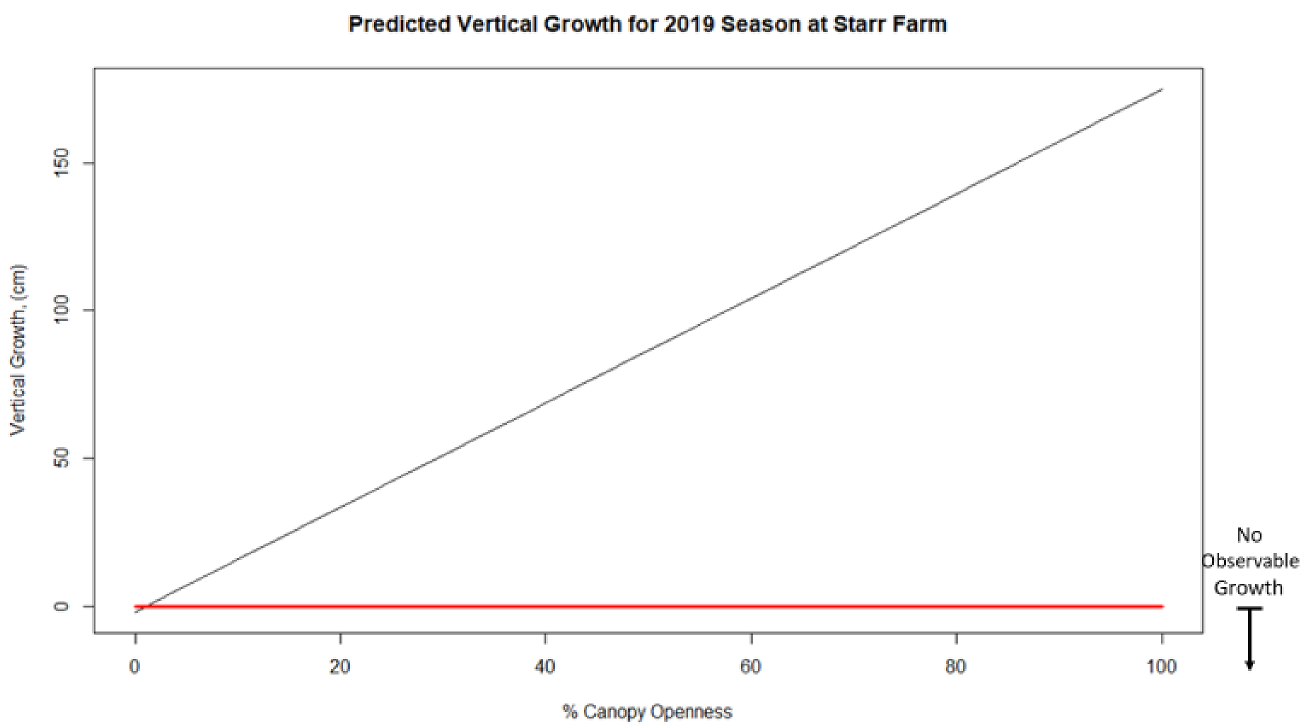
For the 2019 season at EPRR, the selected model predicted vertical growth based on seedling age, canopy openness, and canopy openness^2. The model has a significant regression ($p < .001$). Predicted vertical growth is equal to -45.0 + 4.30 (age) + 1.54 (% canopy openness) – 0.0175 (% canopy openness^2) cm, where age is years since planting and canopy openness is in percentages (Figure 3). All predictors in this model were significant.

Figure 3. Predicted 2019 growth for seedlings at EPRR based on year since planting.



2019 Starr Farm Growth Model: A linear regression was also tested to predict vertical growth based on percent canopy openness and age of seedlings at Starr Farm. Vertical growth had a significant positive correlation with canopy openness and seedling age (respectively, p = .00162 and p = .00714). A step-wise multiple regression of nested models improved the simple linear model that predicted vertical growth based on percent canopy openness. A partial F-test showed that neither seedling age, seedling type, or canopy openness^2 significantly improved the model (respectively, p = .283, p = .572, p = .334). Therefore, a simple linear regression for vertical growth based on canopy openness was selected for the 2019 season at Starr Farm. Vertical growth is equal to -1.77 + 1.77 (% canopy openness) cm, when canopy openness is measured in percentages (Figure 4). Vertical growth increased 1.77 cm for each percent canopy openness.

Figure 4. Predicted vertical growth for the 2019 season at Starr Farm. Seedling age sis not significantly improve the model.

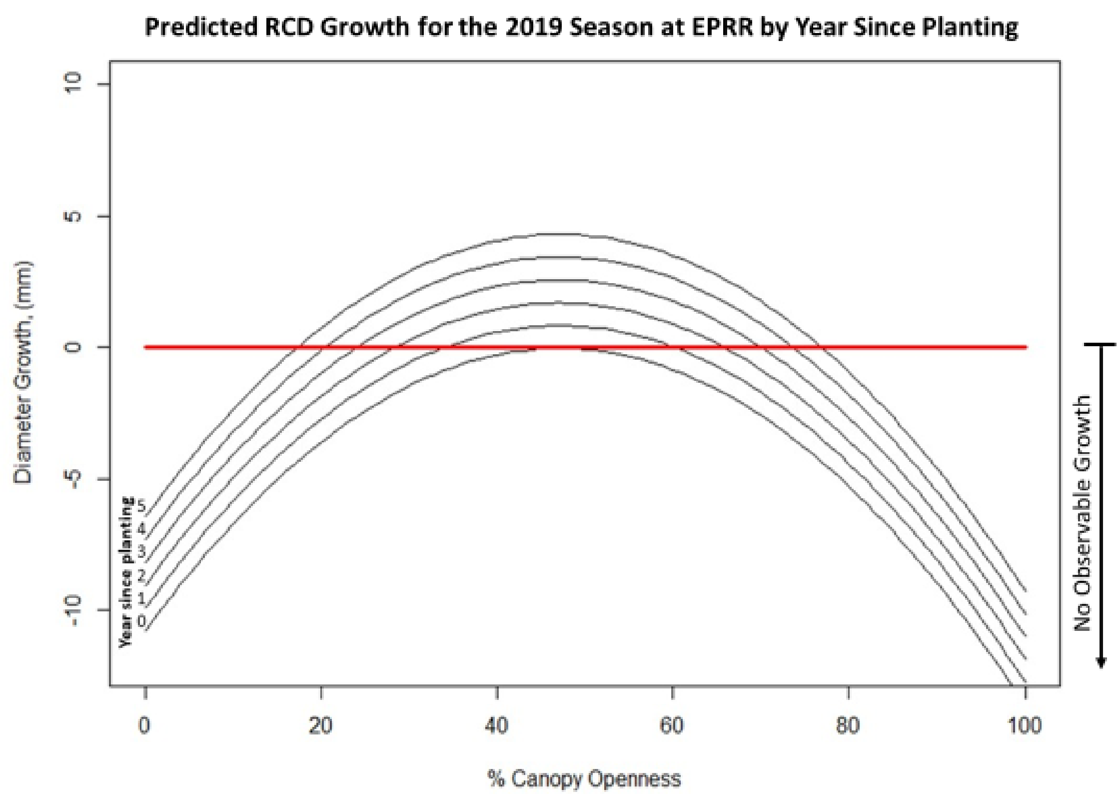


RCD Growth: In the 2019 season, seedlings from EPRR had a mean RCD increase of 3.25 mm and ranged from 0 mm (no observable growth) to 22 mm growth. The effect of seedling type on RCD growth was not significant (p = .488).

Seedlings at Starr Farm had a mean RCD increase of 20.0 mm and ranged between 1 and 92 mm growth. Seedling type had an effect on RCD (p < .001) according to a 1-way anova. Post-hoc analysis with Tukey's test revealed that American seedlings grew in RCD significantly more than B3F2 and B3F3 seedlings (p < .001 and p = .00152, respectively). However, there was no significant difference in RCD growth of the B3F2 and B3F3 seedlings (p = .199).

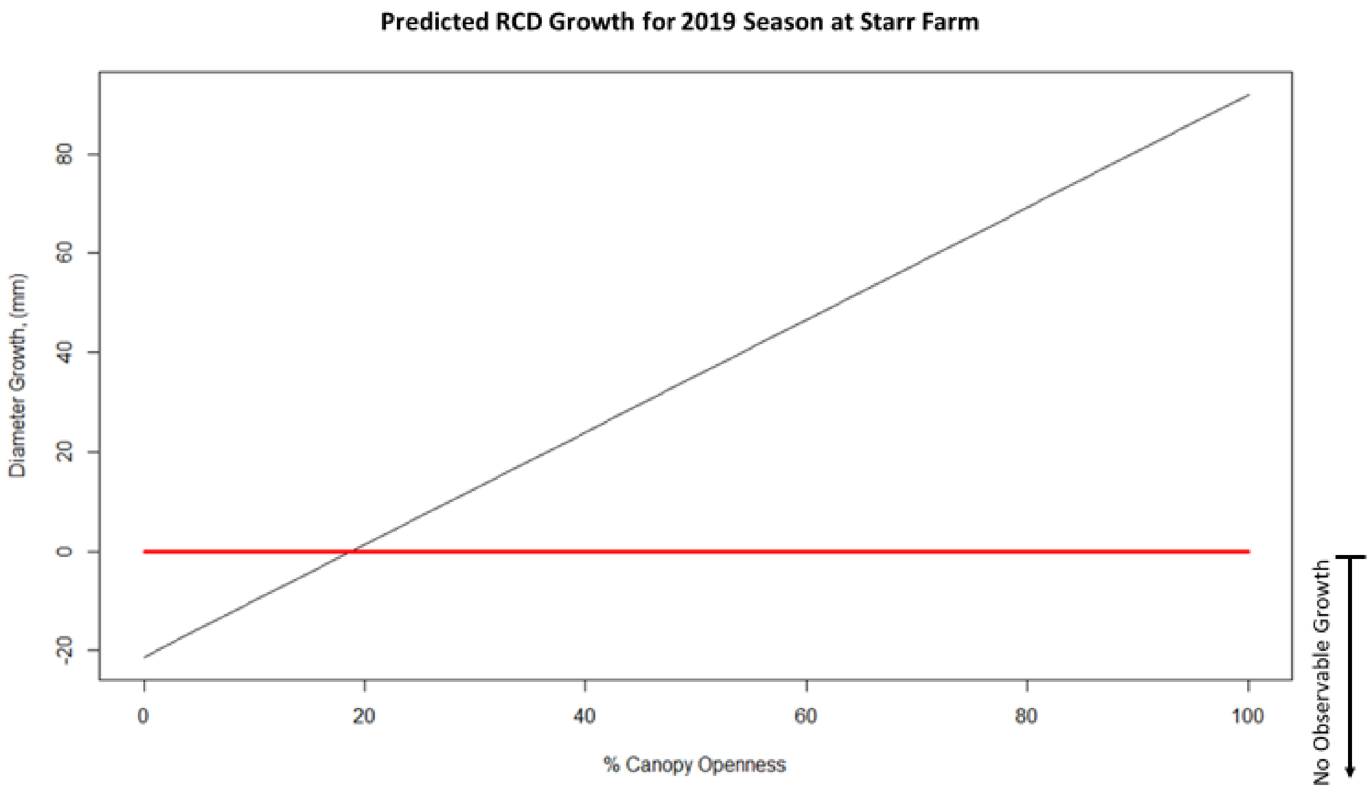
2019 EPRR RCD Model: A linear regression was tested to predict RCD growth based on percent canopy openness and seedling age. A significant positive linear correlation was found for canopy openness and seedling age ($p < .001$ and $p < .001$, respectively). A step-wise regression improved the simple linear model of RCD growth based on canopy openness. A partial F-test showed that adding seedling age as a predictor significantly improved the model ($p = .00129$), but seedling type did not significantly improve the model ($p = .733$). Adding the polynomial term, canopy openness^2, significantly improved the model based on canopy openness and seedling age also using the partial F-test ($p = .00378$). In the polynomial model, the predicted RCD growth is equal to -10.8 + 0.870 (seedling age) + 0.456 (% canopy openness) – 0.00485 (% canopy openness^2) cm, where age is years since planting and canopy openness is in percentages. All predictors in the polynomial model were significant (Figure 5).

Figure 5. Predicted RCD growth for the 2019 season at EPRR by seedling age, beginning at age = 0.



2019 Starr Farm RCD Model: A linear regression was tested to predict RCD growth based on percent canopy openness and seedling age at Starr Farm. A positive linear correlation was found between canopy openness and seedling age ($p < .001$ and $p < .001$, respectively). The simple linear regression of RCD growth based on canopy openness was analyzed using step-wise regression. Adding seedling age and seedling type as a predictor did not significantly improve the model, according to the partial F-test ($p = .123$ and $p = .775$, respectively). Adding a polynomial term, canopy openness^2, also did not improve the model according to the partial F-test ($p = .407$). A simple linear regression of RCD growth based on canopy openness was selected as the predictive model. RCD growth is predicted by percent canopy openness by -21.3 + 1.13 (% canopy openness) mm. RCD growth increased 1.13 mm for each percent canopy openness (Figure 6).

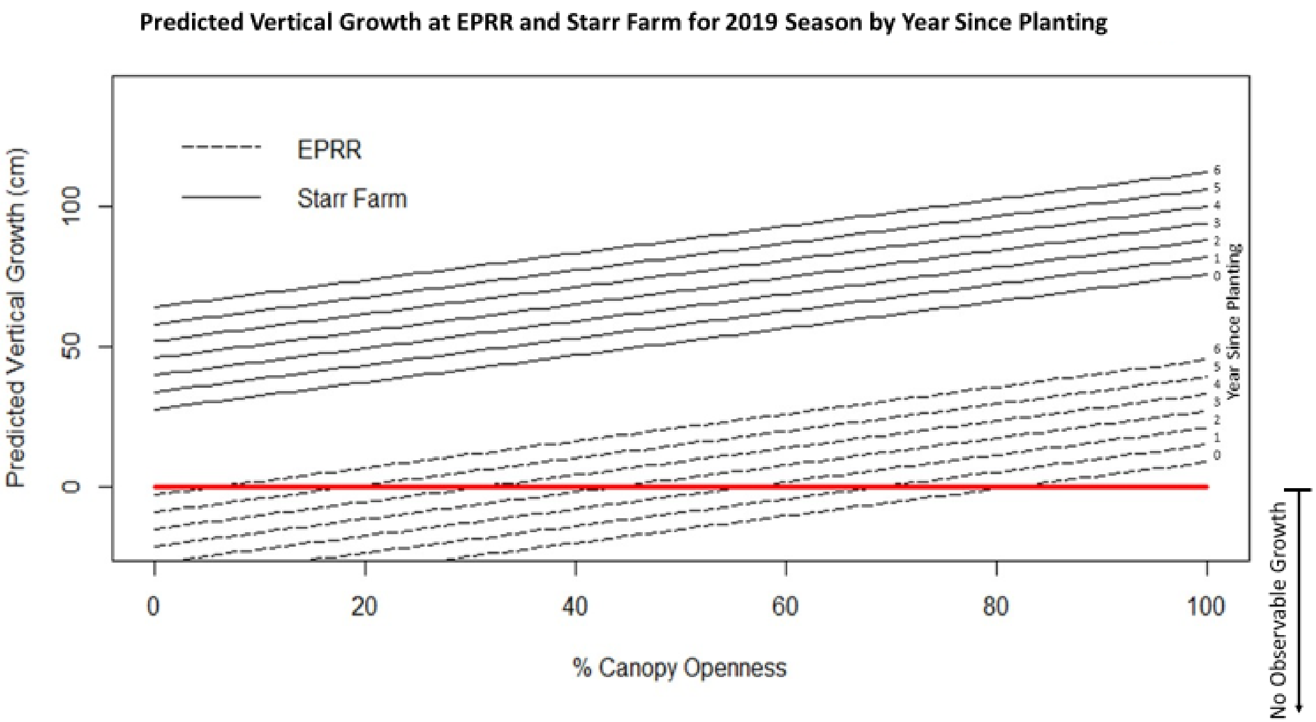
Figure 6. Predicted RCD growth for the 2019 season at Starr Farm. Seedling age did not significantly improve the model.



2019 EPRR and Starr Farm Combined Growth Model: Data from EPRR and Starr Farm was combined for analysis and comparison. A positive linear correlation of vertical growth based on canopy openness was significant ($p < .001$). Adding the site and seedling age as predictors significantly improved the model according to the partial F-test ($p < .001$ and $p < .001$, respectively). The canopy openness^2 term was not significant when added to the model ($p = .139$).

Therefore, the multiple regression model that predicted vertical growth based on the canopy openness, site (EPRR or Starr farm), and age of seedling was selected. It was significant ($p = .001$). Vertical growth is equal to -39.5 + 0.482 (% canopy openness) + 67.0 (1 if Starr Farm, 0 if EPRR) + 6.07 (seedling age) cm (Figure 7).

Figure 7. Predicted vertical growth at EPRR and Starr Farm. Beginning at year since planting = 0, vertical growth in a season increases with seedling age. Growth varies with the site and has a positive relationship with canopy openness.



2020 Season

Barker Pounds 2020 Season: High mortality (35.6%) was again observed at Barker Pounds. There is no further statistical analysis based on growth for the 2020 season.

Canopy Openness: Canopy openness was only remeasured for the 2020 season at EPRR. Time was a consideration so EPRR was chosen because canopy openness was most likely to change at this site because the plots were not mowed. Canopy openness in the 2020 season at EPRR ranged from 10.98% to 48.56% canopy openness.

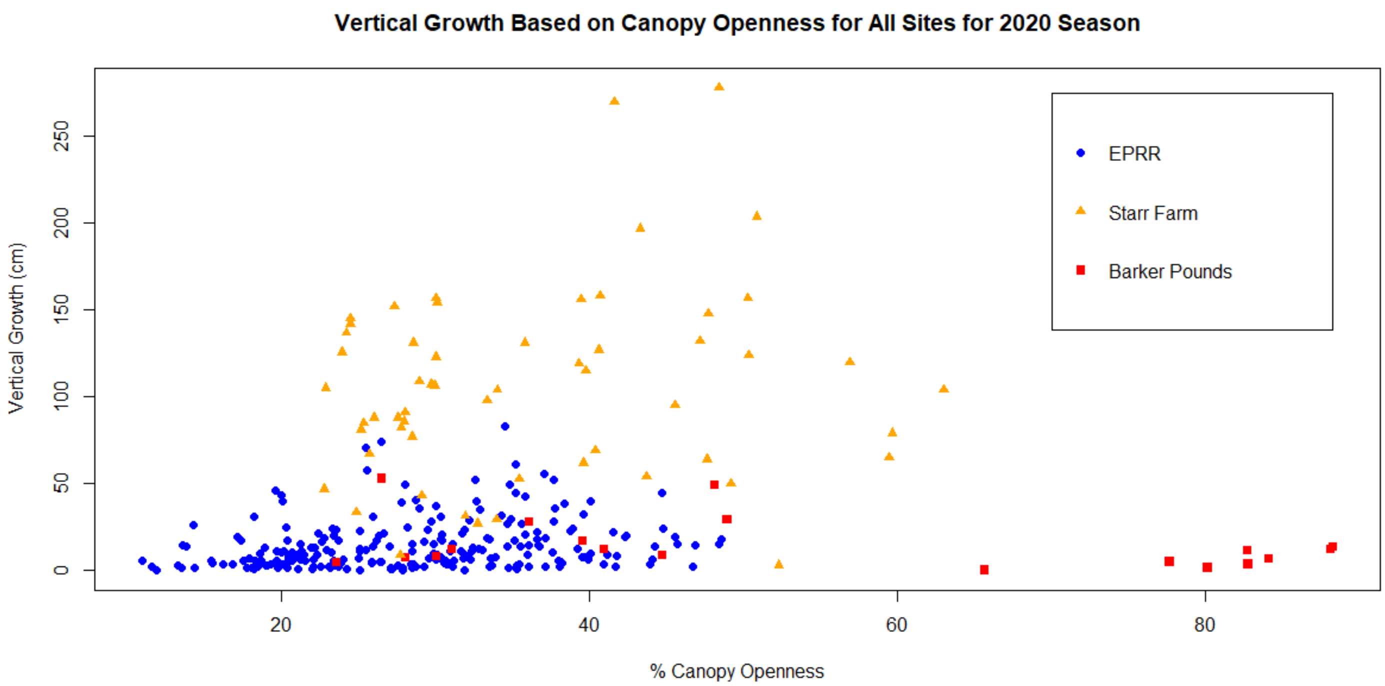
Vertical Growth: Many seedlings were measured but not used for analysis because they had observed deer browse or dead upper stems.

At EPRR, 232 seedlings were used for analysis. Seedlings from EPRR had a mean growth of 14.6 cm and grew between 0.0 cm (no observable growth) and 83.0 cm. Seedling type had no significant difference on growth (p = .194).

57 seedlings were used for analysis from Starr Farm. Mean vertical growth was 105.2 cm and ranged from 3.0 cm to 278.0 cm in growth. Seedling type had no significant difference on growth (p = .306).

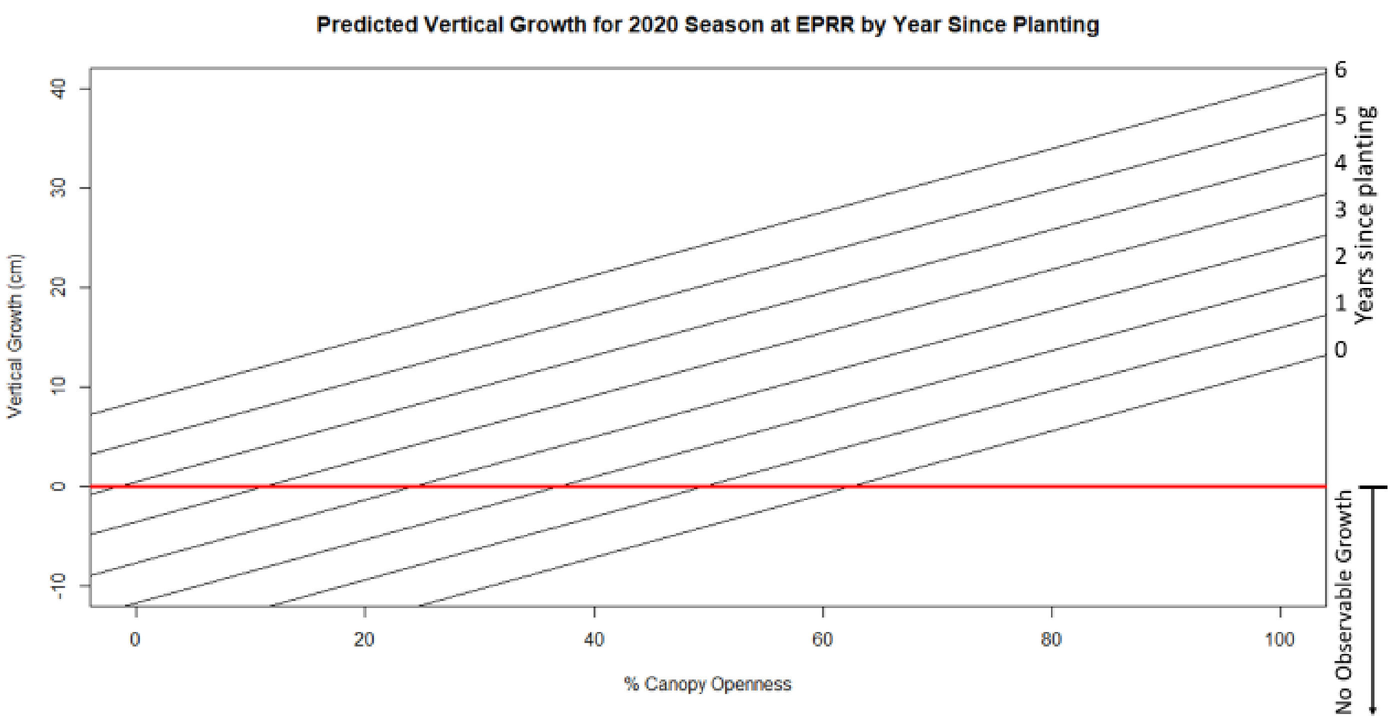
Barker Pounds only had 19 seedlings without observed browse or a dead upper stems. These seedlings had a mean vertical growth of 15.0 cm and ranged from 0.2 cm growth to 53.0 cm growth. This site was not used to create a predictive model.

Figure 8. The following graph shows the distribution of vertical growth based on canopy openness by site.



2020 EPRR Growth Model: Both percent canopy openness and age had a significant positive linear correlation (relatively, $p < .001$ and $p < .001$) to vertical growth. Canopy openness squared had no significant correlation to vertical growth ($p = .139$). A step-wise multiple linear regression was tested to improve the linear model of vertical growth based on percent canopy openness. A partial F-test supported that seedling age improved the simple linear model of vertical growth based on canopy openness ($p < .001$). Therefore, the model for to predict growth at EPRR used predictors seedling age and canopy openness. The model had a significant regression ($p < .001$). Predicted vertical growth is equal to -19.8 + 0.317 (% canopy openness) + 4.05 (age) cm, where age is in years since planting and canopy openness is in percentages (Figure 9). All predictors in this model were significant.

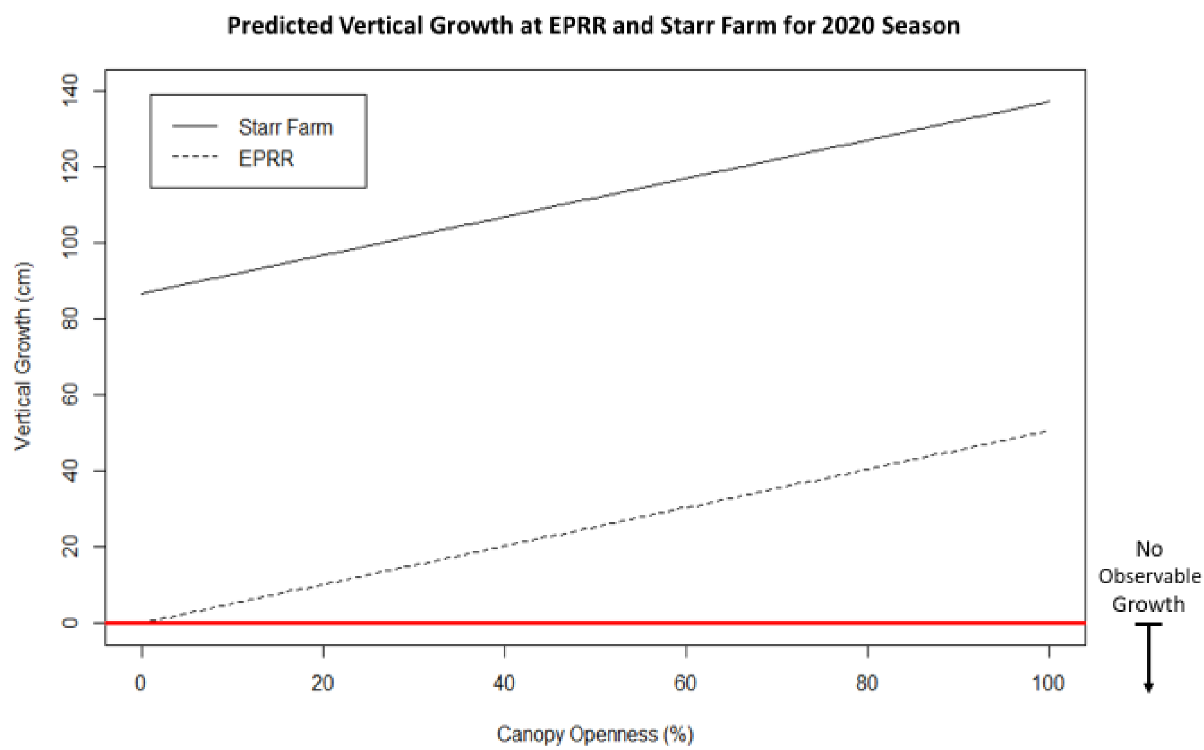
Figure 9. Predictive model of vertical growth at EPRR for 2020 is based on canopy openness and seedling age. Beginning at year since planting = 0, vertical growth in a season increases with seedling age. Growth has a positive relationship with canopy openness.



2020 Starr Farm Growth Model: A step-wise multiple regression was also applied to predict vertical growth at Starr Farm. However, neither canopy openness or age had a significant linear correlation (relatively, $p = .246$ and $p = .695$). Therefore, vertical growth had no significant predictors at Starr Farm for the 2020 season.

2020 EPRR and Starr Farm Combined Growth Model: A step-wise multiple regression was tested to predict vertical growth at the combined EPRR and Starr Farm. Barker Pounds was excluded because of the high mortality rate at the site. Canopy openness, age, and site were tested as predictors of canopy openness. Vertical growth based on canopy openness had a significant positive linear correlation ($p < .001$). Vertical growth based on age had a significant negative correlation ($p < .001$). A 1-way anova showed a significant difference in vertical growth based on site ($p < .001$). A partial F-test supported that seedling age improved the simple linear model of vertical growth based on canopy openness ($p < .001$). A partial F-test showed that site improved the model of vertical growth based on canopy openness and age ($p < .001$). However, age is not a significant predictor in the model of vertical growth based on canopy openness, age, and site. A partial F-test supported that age did not improved the model of vertical growth based on canopy openness and site ($p = .266$). Therefore, the model selected to predict vertical growth at Starr Farm and EPRR was based on the predictors of canopy openness and site. The model had a significant regression ($p < .001$). Predicted vertical growth is equal to 0.155 + 0.505 (% canopy openness) + 86.6 (1 if Starr Farm, 0 if EPRR) cm, where canopy openness is in percentages (Figure 10). Canopy openness and site were significant predictors in this model while the intercept was not significant.

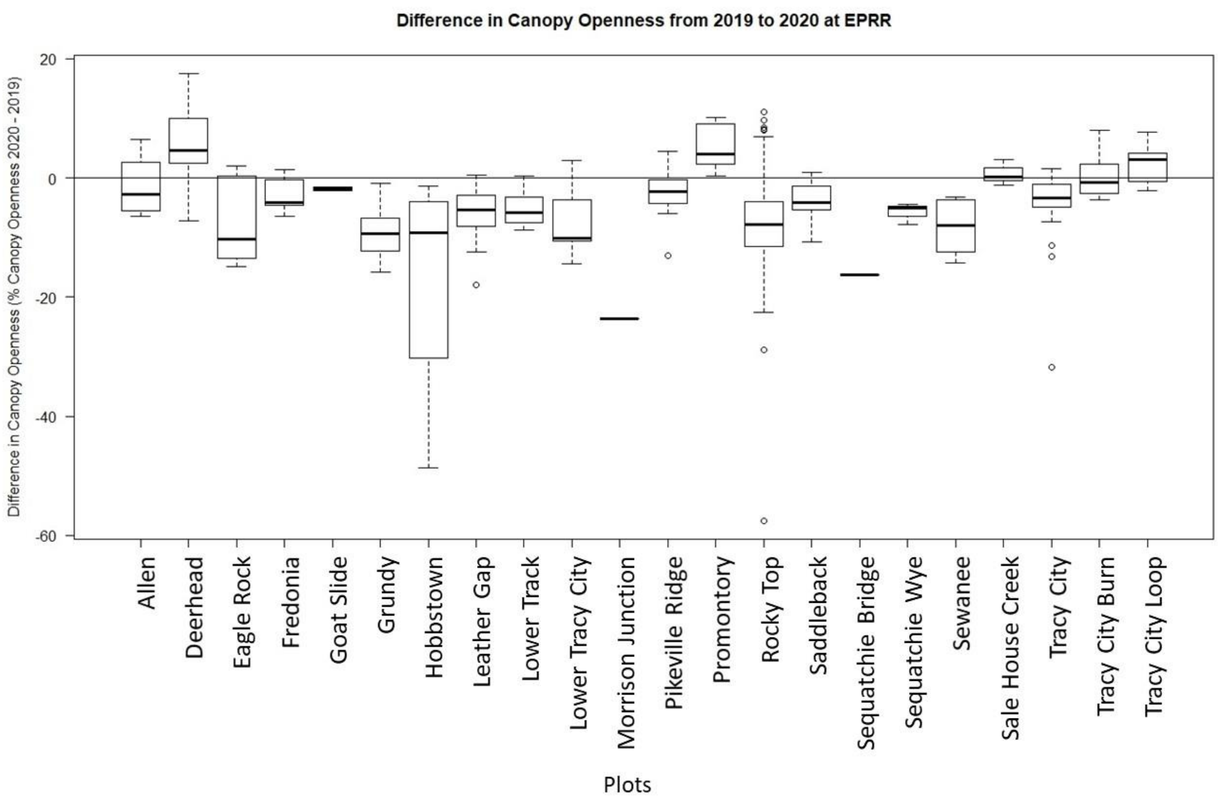
Figure 10. Predicted vertical growth at EPRR and Starr Farm for 2020. Growth varies with the site and has a positive relationship with canopy openness.



2019 and 2020 Season Comparisons

Comparing Canopy Openness from 2019 to 2020 at EPRR: Canopy openness was measured for both the 2019 and 2020 season at EPRR. In 2019, the mean canopy openness was 33.43%, and in 2020, the mean canopy openness was 28.66%. The difference between canopy openness in 2019 and 2020 was calculated (Canopy Openness 2020 – Canopy Openness 2019) for trees that had canopy openness measurements for both 2019 and 2020. The calculated mean difference was -4.56%. A Welch Two Sample T-test showed that the difference in mean canopy openness between 2019 and 2020 is not equal to zero ($T = 7.96$, $df = 841.4$, $p < .001$). Therefore, the canopy was less open an average in 2020 at EPRR. Canopy openness at EPRR appeared to either generally increase or decrease for each plot (Figure 11).

Figure 11. The change in canopy openness between 2019 and 2020 is depicted by the boxes deviation from the line at zero.



Comparing Vertical Growth from 2019 to 2020: Seedlings from EPRR and Starr Farm were selected that had record growth in both 2019 and 2020. At EPRR, these seedlings had an average of 4.39 cm more growth in 2020 than in 2019. At Starr Farm, these seedlings had an average of 41.3 cm more growth in 2020 than in 2019.

Discussion:

This study aimed to evaluate the effects of canopy gap size on the growth rate of *Castanea dentata* hybrid seedlings at three sites in the Cumberland Uplands of Tennessee. This was not determined for Barker Pounds because of high mortality likely due to root rot caused by *Phytophthora cinnamomi*. Therefore, data collected from Starr Farm and EPRR were used to create growth models. Growth generally increased with canopy openness. In 2019, canopy openness, site, and seedling age accounted for 51.5% of the variation in vertical growth in both sites. In 2020, site and canopy openness explained 63.7% of the variation in vertical growth in both sites. In both cases, canopy openness was a significant predictor. Individual site models of Starr Farm in 2019 and EPRR in 2020 also have a positive linear relationship with canopy openness. These results are consistent with other silvicultural studies that suggest that reintroduced American chestnut grows more in increased light (Pinchot et al. 2017, Rhoades et al. 2009, McCament and McCarthy 2005, Belair et al. 2014, Saielli et al. 2014). However, vertical growth had a parabolic relationship with canopy openness at EPRR in 2019 and had no trend with canopy openness for Starr Farm in 2020.

For the combined sites, canopy openness, with no other predictors, explained 7.42% of the variation of vertical growth in 2019 and 12.3% of the variation of vertical growth in 2020. Ecology deals with countless biotic and abiotic effects. Therefore, the percent of variance is expected to be small. However, small effects are exaggerated over generations (Moller and Jennions 2002).

A parabolic function of canopy openness best fit the model of vertical and RCD growth for EPRR in 2019. Since many studies have shown that chestnut growth increases with light (Pinchot et al. 2017, Rhoades et al. 2009, McCament and McCarthy 2005, Belair et al. 2014, Saielli et al. 2014), it is possible that there are other ecological conditions in the forest associated with increased light that decrease growth. Plots at EPRR were maintained by occasionally trimming of competition, whereas at Starr Farm, the plots were regularly mowed. At EPRR, increased competition was observed for chestnut seedlings in large gaps, which might reduce nutrient availability for seedlings in high light positions and decrease growth (Belair et al. 2014). Chestnut growth responds negatively to decreased soil moisture due to woody competition (Belair et al. 2014, Brown et al, 2014). The center of gaps, or positions with the greatest canopy openness, have a higher air temperature (Galhidy et al. 2006). Therefore, it is possible that the hot, dry 2019 summer likely furthered the effect of decreased soil moisture on chestnut seedlings (NOAA). Optimal canopy openness for seedlings EPRR in the 2019 season appeared to be between 40% and 50% canopy openness due to ecological factors making high light environments detrimental after a threshold. However, the same trend did not occur in the 2020 season at EPRR.

Site was a significant predictor of growth. We found through nutrient samples that Starr Farm's soils had more phosphorus, potassium, calcium, magnesium than the other sites. EPRR and Barker Pounds had deficient magnesium (UT Extension). Soil characteristics influence a species' natural distributions in mesophytic forests (Muller 1982). Therefore, soil characteristics will influence the growth of *C. dentata*. While chestnut can persist on poor soils, it increases growth in fertile soils (Wang et al. 2013, Rhoades et al. 2009). Site selections for chestnut reintroduction should be done thoughtfully.

Age was also a significant predictor of growth in many models. Older seedlings generally grew more. This trend is not expected for the lifespan of the tree. Chestnut growth is more rapid in the first decade and thereafter begins to decline (Ashe 1911).

It is apparent through the measure of canopy openness for each individual tree that not all seedlings receive the same amount of light even within the same canopy gap. This is due to position within the gap and shade of competing species. Pinchot et al. (2017) noted that the silvicultural treatment with the most available light also had the most understory woody competition. This is important because planted chestnuts are less likely to outcompete vegetation (Griffin et al. 1991, McNab 2003), and chestnut seedlings must maintain their dominance to reach the canopy (Loftis 1985, Belair et al. 2014). Even though chestnut has the potential to grow more in high light conditions, fast-growing shade intolerant species and stump sprouts in a cleared canopy gap challenge introduced chestnut seedlings in high light (Loftis 1985, Belair et al. 2014). Reduced competition in the first years after planting may be critical for successful restorations (Belair et al. 2014). Chestnut compared to other hardwood species has a very plastic response to light (Wang et al. 2006, Joesting et al. 2009, Belair et al. 2014). Therefore, moderate light conditions, or smaller canopy gaps, for initial planting might be favorable for reduced competition, and then, more light could be introduced (Rhoades et al. 2009, Belair et al. 2014, Wang et al. 2006). The canopy gaps could be culled after the seedlings are established to release the seedlings (Wang et al. 2006, Griscom and Griscom 2012). Large canopy gaps would likely need regular maintenance of competition.

We also aimed to determine the silvicultural requirements for *C. dentata* hybrids for introduction to canopy gaps in forest environments. The present study shows the early success of canopy gaps for reintroductions. To lessen establishment costs, canopy gaps are a possible alternative to high impact introductions (McNab et al. 2003). Chestnut hybrid founder stock is already limited and costly, and TACF is still working to improve blight resistant selections (Westbrook et al. 2020). Site quality will be important for successful reintroductions. Gaps should be selected based on canopy openness. Moderate canopy openness may be favorable for low maintenance sites. This should be considered for expansive plantings, where regular maintenance is not possible or too costly. Low maintenance sites might be culled once seedlings are better established (Wang et al. 2006, Griscom and Griscom 2012).

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