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Growth Rate of Surrounding Vegetation after Exposure to Imazapyr at Wild Basin Wilderness Preserve

Abstract

The herbicide Imazapyr was injected into four ligustrum trees at Wild Basin Wilderness Preserve in 2014. While known as an effective measure for containing invasive species, it is also a persistent chemical known to have harmful effects on neighboring plants in some circumstances. In order to see if this was the case at Wild Basin, apical meristems of yaupon hollies and Lindheimer's silk tassels surrounding the injected trees were measured from February to April of 2015. The expected results included decreased meristem growth on plants downslope from or nearer to the injected specimen.

It was concluded that being downslope from or nearer to the injected tree did indeed decrease the growth rate of nearby vegetation. This could be due to the effects of herbicide spread or other environmental factors. This shows that even an herbicide with comparatively few negative chemical qualities can still have detrimental effects in a community. Vegetation close to and downslope from the injected trees are at particular risk and should be monitored when handling invasive species removal.

Introduction

Imazapyr is a non-selective herbicide that is absorbed by leaves and roots and eventually accumulates in the meristem (The Nature Conservancy, et al. 2004). It disrupts production of

acetolactate synthase, an enzyme that plays an important role in the synthesis of amino acids. Since this enzyme is only found in plants and microorganisms, it is nontoxic to humans and other animals (Wisconsin Department of Natural Resources 2012). It does not bioaccumulate but it can be persistent in the environment for over a year (Cox 1996).

The EZ-Ject system is used to isolate and selectively apply a dry form of this herbicide. According to the manufacturer's website, a lance injects an herbicide shell into the base of a tree, stump, or brush (EZ-Ject 2014). This information is pertinent to park managers and others interested in controlling invasive species. If these results show that Imazapyr and EZ-Ject can effectively manage invasive species, it can be widely promoted among park systems. Rather, if this study shows Imazapyr and EZ-Ject eradicate invasive species at the cost of healthy native populations, it should probably be used less frequently in wilderness preserves.

This study focused on non-target tree growth rates after Imazapyr, an herbicide, was applied to Japanese privet at Wild Basin Wilderness Preserve. At Wild Basin Wilderness Preserve, Imazapyr is used on invasive trees greater than 3 inches in diameter; this includes chinaberry, Chinese tallow trees, and Japanese privets (also known as ligustrum). By studying the growth rate of vegetation surrounding the targeted specimens we can learn how effectively EZ-Ject and Imazapyr can isolate and eradicate a particular individual as well as its potential effects on neighboring trees.



Similar studies have been undertaken considering the efficacy of Imazapyr, Imazapyr's movement in different soils, and a variety of other hypotheses. When injected as a capsule, it

kills over 90% of target plants (Vitelli and Maddigan 2011). However, a study by Lewis and McCarthy showed that targeted Imazapyr injections, much like EZ-Ject, not only killed all targeted plants, but also killed 17.5% of neighboring non-injected target species within 3 meters 62 weeks after treatment (Lewis and McCarthy 2008). Therefore it has already been established that this herbicide can move throughout a community, although the mechanisms are uncertain and are likely affected by methods of application. Soil type has an effect on sorption and mobility. Sandy clay is particularly vulnerable to herbicide spread and clay or clay loam has more resilience (Wethje, Glenn, et al. 1987). The areas of relevance to this study are all within the Brackett-Rock outcrop complex, which is primarily well-drained limestone. The topsoil is typically gravelly clay loam and the soil below that is clay loam until bedrock is reached (USDA and NRCS 2012). Imazapyr is known to dissipate faster in warmer temperatures, such as Austin's subtropical climate (McDowell, R.W., et al. 2008). Typically, Imazapyr is slowly degraded by microbial metabolism and has a half-life of one to five months in soil. It is rapidly degraded in water (The Nature Conservancy, et al. 2004). Distance from the injected tree and the pH of the soil are also known to affect Imazapyr's ability to spread (Vizantinopolous, S. and P. Lolos 1994).

Based on this research and the conditions at Wild Basin, effects on the growth of meristems on neighboring plants are to be expected. These effects will probably decrease with distance from the injected tree. It is likely that the leaching of Imazapyr will be reduced due to the time since injection, but since the herbicide is so effective and potent, it may be possible to find visible results of its spread despite these limiting circumstances.

Methods and Materials:



This study focused on four Japanese privet (*Ligustrum japonicum)* specimens at Wild Basin Wilderness Preserve in Austin, Texas. Each of these specimens had been injected with Imazapyr using EZ-Ject in either May or September of the previous year. These trees were marked with pink Teflon tape and their locations within the park can be found in Appendix A. Since measurements did not begin until February, this means they were taken nine months after injection for trees injected in May and five months after injection for trees injected in September. Once the trees were identified, the nearby area was examined for yaupon holly (*Ilex vomitoria*) and Lindheimer's silk tassel (*Garrya ovata lindheimeri*). These species were selected due to their abundance in the area and then marked with orange flags. In an effort to acquire a variety of samples, each area had at least one tree upslope from the injected ligustrum and a downslope counterpart. Each site also has a tree that is within one meter from the injected tree and another that is over five meters away. After selecting yaupon and silk tassels surrounding the injected privet, at least two apical meristems were identified per specimen. Details on these trees and their meristems can also be found in Appendices A and B. Using plastic twist ties, the apical meristems were marked and then measured using a tape measure. Then measurements were taken biweekly, until late April.

After data collection concluded, SPSS was used for statistical analysis. Growth rate data was first transformed by adding one to all values in order to compensate for zero growth rates automatically eliminated by SPSS. Data was then checked for normality, and it was discovered that growth rate followed a gamma distribution. Thus, a general linear model was used to determine the statistical differences between upslope and downslope and between close and far

trees. Up or downslope and near or far were used as main effects in the generalized linear model and the interaction between these was also included. The products of these calculations can be found in Appendix D.

Results

There is a statistically significant variation between the growth of trees depending on their proximity and slope from the injected ligustrum. Trees that were close and downslope grew at the slowest rate; followed by trees that were farther away and downslope. Upslope trees grew consistently faster but the trees that were furthest away had the highest growth rates. This correlation can be seen in the charts in Appendix E as well as the marginal means shown in Table 1. This statistical test concludes that the data found supports the original hypothesis; slope and proximity from the injected tree and were significant predictors of growth rate.

Table 1:	Up_DownSlope	Far_Close	Mean Growth (cm)	Std. Error
	0	0	7.130000	1.716590
	0	1	2.91818	.473672
	1	0	2.250000	.699333
	1	1	2.188888	1.419027
Upslope is indicated as 0 and downslope is identified as 1. Far, referring to trees over five meters from the injected specimen, can be identified as 0, and closer trees are indicated as 1.				

Discussion

The results of this study indicate that Imazapyr is moving throughout the ecosystem despite using an isolated application process. These results may also be linked to environmental conditions: for example, vegetation nearer to the injected tree may receive less light and this can

contribute to a disparity between the specimens. In addition, trees that are downslope are often more susceptible to disturbance after from storms and from urban runoff. However the visible decline in nearby plant growth is congruous with similar studies such as Lewis and McCarthy's "Nontarget Tree Mortality after Tree-of-Heaven (*Ailanthus altissima*) Injection with Imazapyr".

In future studies, it would be helpful to tag and measure a greater number of meristems. The fairly small number of meristems in this study was a limitation that can be easily remedied. Controlling for environmental factors is more difficult, but it would benefit the study by removing variables such as light levels or soil variances that can influence plant growth. Noting more specific chemical details of the soil would also prove illuminating and relevant to the results. Lastly, monitoring the growth of surrounding vegetation before and immediately after injection would add important knowledge to our understanding of the behavior of persistent chemicals in the environment. Fortunately, Wild Basin Wilderness Preserve has begun to record the growth of surrounding vegetation after a recent injection in mid-March.

If it is found that these herbicidal injections cause more damage than an invasive species, alternative ways to approach the issue should be explored. With this study's results in mind, it is important to encourage the consideration of the damage invasive species can do to the environment. They can overtake and displace native species, which has cascading effects throughout an ecosystem. Introduced species can be truly devastating to a community and their threat should be taken seriously or prevented if possible. Implications of this study suggest that even an herbicide with comparatively few negative chemical qualities can still have detrimental effects to a community. Vegetation close to and downslope from injected trees are at particular risk and should be monitored.

Literature Cited

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Appendix A: Locations within Wild Basin



Appendix B: Site Descriptions

Area 1:

After passing trail marker D on Warbler trail, take a right to find the site of ligustrum one. This tree was injected in May of 2014. This tree is on the bank of an ephemeral creek. Due to this, the area has a notably high insect population and diversity. The ligustrum itself is elevated and rests on slanted but moist, loamy soil covered in leaf litter. In addition, the area is filled with detritus and dead branches.



There are three yaupon hollies marked for study in this area. The first is a male about .3 m (about 1 ft) southeast from the injected tree. It is nearer to the ephemeral creek and surrounded by detritus while receiving indirect sunlight. It is 1.93 m (76 in) tall with noticeable budding on top. Two apical meristems were tagged and measured on this specimen.

The second yaupon is also male but it is in a shaded area. This area has a high density of leaf litter and is on thin but loamy soil. It is 1 m (3.3 ft) northwest from injected ligustrum 1 but due to the proximity of areas one and two, it is the equidistant from injected ligustrum #2. It is 2.18 m (7 ft 2in) tall with the top .6 m (2 ft) being bare. Meristem A is on a branch with 3 other stems while Meristem B in on a branch with 2 stems.

The third yaupon in this area is a female that was too tall to accurately measure. It is 10 m (32 ft 10 in) northeast from the injected ligustrum, separated by the ephemeral creek. It is surrounded on three sides by water and is shaded by a tall Cottonwood *(Populus deltoides)* tree to its east. Two meristems were tagged and measured on this specimen: meristem A is 1.3 m (53

in) from the ground with no other stems on its branch while meristem B is .9 m (38 in) from the ground with no other stems on its branch.

Area 2:

Only a 4.34 m (14ft 3in) behind ligustrum one, ligustrum two is also on Warbler trail, slightly past trail marker D. It is slightly farther away from the creek bank. This tree was also injected in May of 2014. It is on a slanted hill, southwest of ligustrum one. Its surroundings are dryer than ligustrum one, but it has the same loamy soil and a plethora of detritus. The branches of a large Ashe Juniper, which provides the area with shade, surround the ligustrum and most other relevant specimens.

Yaupon 1 is a male and about 1.04 m (41 in) tall. It is .61 m (2 ft) south from the injected ligustrum. There is only one meristem being studied on this plant; meristem A has no other major stems on its branch. It is only .96 m (38 in) from Yaupon 2.

Yaupon 2 is .68 m (2 ft 3 in) southeast from the injected ligustrum. It is a male about 2 m (78 in) tall but the top is mostly barren. It is in a mostly shady area. There is only one meristem being studied on this plant. Meristem A does not share its branch with any other stems.

Yaupon 3 is a female about 5.5 m northwest (18 ft) from the injected ligustrum. This area is just above a slight drop off but the soil is still moist and loamy. It is about 2.86 m (9 ft 4 in) tall. There are three tagged meristems on this tree and although they are in different area of the tree, they are all about .83 m (33 in) from the ground. Meristem A has 4 other stems in its branch, meristem B shares its branch with 2 other stems, and C shares its branch with 0 other stems.

Silk tassel 1 m (3.3 ft) east of injected ligustrum 2 and it is nearly surrounded by the tangled branches of an Ashe Juniper. It is 1.57 m (5 ft 2 in) tall but is sagging and the whole plant is very near to the ground. Meristem C is only .19m (7.5 in) from the ground. It is in a mostly shaded area. Many of the leaves on this plant specimen have brown speckles on their leaves.

Area 3:

The third ligustrum can be found on waterfall trail, below the scenic view. It is a few meters from waterfall pool and elevated from the creek bed. Getting to the ligustrum requires some climbing. The soil is thin and rocky but moist. It is especially notable for the concentration of greenbrier vines (*Smilax bonanox)*. This tree was injected with Imazapyr in May 2014.



Silk tassels 1 and 2 are on thin, rocky soil with lots of dead leaves. Silk tassel 1 is about 1 m (3.3 ft) northeast from the ligustrum and silk tassel 2 is about 1.5 m (5 ft) south of the ligustrum, however measuring this was difficult due to the steep, uneven setting.

Yaupon 1 is 11 m (36 ft 1 in) southwest from the injected ligustrum and across the creek bank. Its on hard and rocky soil, but it is only a short distance from the running creek. It is in direct sunlight, with little other plant density or leaf litter. There were three meristems tagged and measured on this tree. Meristem A is 1.8 m (74 in) from the ground, Meristem B is 1.6 m (66 in) from the ground, and Meristem C is 1.7 m (70 in) from the ground.

Area 4:

The fourth injected tree is across the creek bank, near trail marker G on creek trail. Unlike the others, this ligustrum was injected in September of 2014. It is about 1.5 m (5 ft) from the moving stream with soft soil close to the bank that grows harder and rockier with distance. There are some small woody plants and human litter in this area.

Yaupon 1 is about .9 m (3 ft) east and upstream from the injected site. It is about 1.5 m (5 ft) from the moving stream and is in an area surrounded by woody plants and some human litter, probably carried by the creek. The main trunk is a little over 5 m (16 ft) tall. Meristem A is 1.4 m (59 in) from the ground and Meristem B is 1.1 m (46 in) from the ground.

Yaupon 2 is about .4 m (1.5 ft) west and downstream from the injected ligustrum. Meristem A is .9 m (38.5 in) from the ground and Meristem B is 1.3 m (51 in) from the ground. It is about 3.5 m (11.48 ft) tall and grows slanting towards downstream.

Silk tassel 1 is 6.2 m (20 ft 4 in) southwest from the injected ligustrum. It is further away from the creek and is therefore in more rocky soil. It is in a very shady area but may receive direct sunlight in the early morning. There is plant debris and ample dead leaves in the area. There are three tagged meristems on this specimen: A is 1.8 m (74.2 in) from the ground, B is 1.2 m (48 in from the ground), and C is 1.2 m (49 in) from the ground.

Appendix C: Data

	19-Feb	Net Growth	6-Mar	Net Growth	12-Mar	Net Growth	27-Mar	Net Growth	11-Apr
Ligustrum 1									
Off Warbler Trail									
Yaupon 1									
about 1 ft (down) from Ligustrum									
Apical Meristem A			.3cm	0 cm	.3cm	0 cm	.3 cm	0 cm	.3 cm
Apical Meristem B			.2cm	0 cm	.2cm	0 cm	.2 cm	0 cm	.2 cm
Yaupon 2									
about 3 ft (up) from Ligustrum									
Apical Meristem A			.15 cm	0 cm	.15cm	.05 cm	.2 cm	6 cm	6.2 cm
Apical Meristem B			.1cm	.1 cm	.2cm	.3 cm	.5 cm	3.7 cm	4.2 cm
Yaupon 3									
About 10m (up) from Ligustrum									
Apical Meristem A			.3cm			.7 cm	1 cm	11.2 cm	12.2 cn
Apical Meristem B			.1cm			.5 cm	.6 cm	4.1 cm	4.7 cm
Ligustrum 2									
Off Warbler Trail, Slightly North of 1									
Lindheimer Silktassel 1									
about 3ft (down) from Ligustrum									
Apical Meristem A	.7cm			.5 cm	1.2cm	1.3 cm	2.5 cm	2 cm	4.5 cm
Apical Meristem B	.9cm			.1 cm	1cm	1 cm	2 cm	3.2 cm	5.2 cm
Apical Meristem C	.7cm			.2 cm	.9cm	1.6 cm	2.5 cm	2.9 cm	5.4 cm
Yaupon 1									
about 2ft (up) from Ligustrum									
Apical Meristem A	.3cm			.1 cm	.4cm	.6 cm	1 cm	8.7 cm	9.7 cm
Yaupon 2									
about 1.5ft (up) from Ligustrum									
Apical Meristem A	.2cm			0 cm	.2cm	0 cm	.2 cm	.1 cm	.3 cm
Yaupon 3									
About 5.5 m (up) from Ligustrum									
Apical Meristem A			.4cm			.3 cm	.7 cm	2.6 cm	3.3 cm
Apical Meristem B			.15cm			.15 cm	.3 cm	3.7 cm	4.0 cm
Apical Meristem C			.15cm			.55 cm	.7 cm	2.4 cm	3.1 cm

Ligustrum 3									
Near Waterfall Pool									
Lindheimer Silktassel 1									
About 3ft (up) from Ligustrum									
Apical Meristem A	1.25cm			.5 cm	1.75 cm	1.25 cm	3 cm	.7 cm	3.7 cm
Apical Meristem B	1.25cm			.25 cm	1.5 cm	1 cm	2.5 cm	1.75 cm	4.25 cn
Lindheimer Silktassel 2									
about 5ft (down) from Ligustrum									
Apical Meristem A	1cm			1 cm	2 cm	1 cm	3 cm	4.4 cm	7.4 cm
Apical Meristem B	.5cm			.75 cm	1.25 cm	2.75	4 cm	8.7 cm	12.7 cn
Yaupon 1									
11m from Ligustrum, Across Creek									
Apical Meristem A					.3 cm	1.2 cm	1.5 cm	2.3 cm	3.8 cm
Apical Meristem B					.2 cm	.3 cm	.5 cm	1.1 cm	1.6 cm
Apical Meristem C					.5 cm	.7 cm	1.2 cm	1.9 cm	3.1 cm
Ligustrum 4									
On Creek Trail, Across Creek Bank									
Yaupon 1									
About 3 ft (up) besides Ligustrum									
Apical Meristem A	.4cm	.1 cm	.5cm			0 cm	.5 cm	.1 cm	.6 cm
Apical Meristem B	.2cm	0 cm	.2cm			.1 cm	.3 cm	.1 cm	.4 cm
Yaupon 2									
About 1.5 ft (down) from Ligustrum									
Apical Meristem A	.3cm	0 cm	.3cm			0 cm	.3 cm	.2 cm	.5 cm
Apical Meristem B	0.5cm	0 cm	0.5 cm			.1 cm	.6 cm	.3 cm	.9 cm
Silktassel 1									
6m 28in SW from Ligustrum									
Apical Meristem A			1cm			5.5 cm	6.5 cm	10.5 cm	17 cm
Apical Meristem B			.5cm			2.6 cm	3.1 cm	7 cm	10.1 cn
Apical Meristem C			.9cm			.7 cm	1.6 cm	13.9 cm	15.5 cn

Appendix D: Statistical Analysis Results

Estimated Marginal Means 1: Grand Mean

Estimates

Mean	Std. Error	95% Wald Confidence Interval	
		Lower	Upper
3.621767677	.4882624528	2.664790854	4.578744499

Estimated Marginal Means 2: Up_DownSlope

Estimates

Up_DownSlope	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
0	5.024090909	.8903720610	3.278993737	6.769188081
1	2.219444444	.4010467360	1.433407286	3.005481603

Estimated Marginal Means 3: Far_Close

Estimates

Far_Close	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
0	4.690000000	.9267889207	2.873527094	6.506472906
1	2.553535354	.3076738335	1.950505721	3.156564986

Generalized Linear Models

[DataSet4]

Model Information

Dependent Variable	Ratetransformed
Probability Distribution	Gamma
Link Function	Identity

Case Processing Summary

	N	Percent
Included	28	100.0%
Excluded	0	0.0%
Total	28	100.0%

Categorical Variable Information

			N	Percent
Factor	Up_DownSlope	0	16	57.1%
		1	12	42.9%
		Total	28	100.0%
	Far_Close	0	8	28.6%
		1	20	71.4%
		Total	28	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean
Dependent Variable	Ratetransformed	28	1.000000000	10.40000000	3.364285714

Continuous Variable Information

		Std. Deviation
Dependent Variable	Ratetransformed	2.606633175

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	55.022	1	.000
Up_DownSlope	8.249	1	.004
Far_Close	4.787	1	.029
Up_DownSlope * Far_Close	4.517	1	.034

Dependent Variable: Ratetransformed
Model: (Intercept), Up_DownSlope, Far_Close, Up_DownSlope * Far_Close

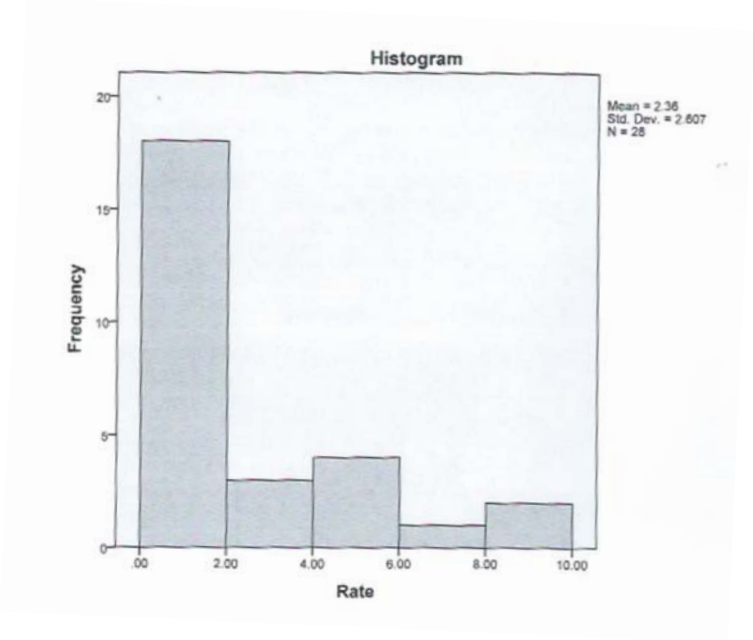
Descriptives

			Statistic	Std. Error
Rate	Mean		2.3643	.49261
	95% Confidence Interval for Mean	Lower Bound	1.3535	
		Upper Bound	3.3750	
	5% Trimmed Mean		2.1270	
	Median		1.4400	
	Variance		6.795	
	Std. Deviation		2.60663	
	Minimum		.00	
	Maximum		9.40	
	Range		9.40	
	Interquartile Range		3.52	
	Skewness		1.399	.441
	Kurtosis		1.155	.858

Extreme Values

			Case Number	Value
Rate	Highest	1	10	9.40
		2	26	8.00
		3	28	7.30
		4	5	5.95
		5	27	4.80
	Lowest	1	2	.00
		2	1	.00
		3	11	.03
		4	24	.07
		5	23	.07[a]

a. Only a partial list of cases with the value .07 are shown in the table of lower extremes.



Goodness of Fit[a]

	Value	df	Value/df
Deviance	8.504	24	.354
Scaled Deviance	29.342	24	
Pearson Chi-Square	11.560	24	.482
Scaled Pearson Chi-Square	39.889	24	
Log Likelihood[b]	-50.570		
Akaike's Information Criterion (AIC)	111.140		
Finite Sample Corrected AIC (AICC)	113.867		
Bayesian Information Criterion (BIC)	117.801		
Consistent AIC (CAIC)	122.801		

Dependent Variable: Ratetransformed
Model: (Intercept), Up_DownSlope, Far_Close, Up_DownSlope * Far_Close

a. Information criteria are in small-is-better form.

b. The full log likelihood function is displayed and used in computing information criteria.

Omnibus Test[a]

Likelihood Ratio Chi-Square	df	Sig.
15.392	3	.002

Dependent Variable: Ratetransformed
Model: (Intercept), Up_DownSlope, Far_Close, Up_DownSlope * Far_Close

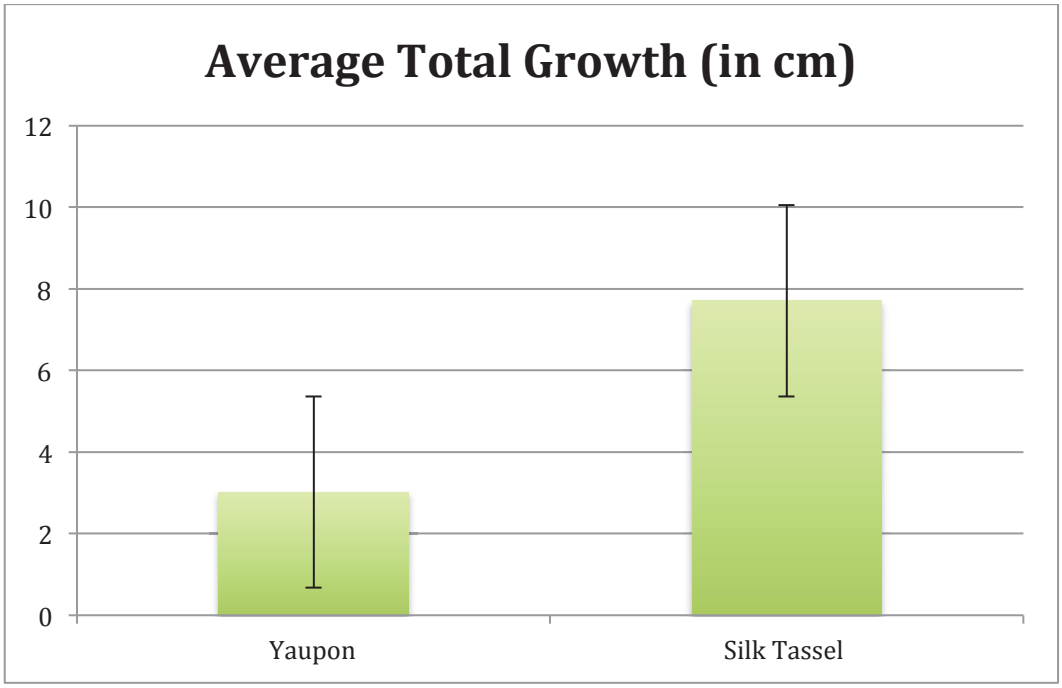
a. Compares the fitted model against the intercept-only model.

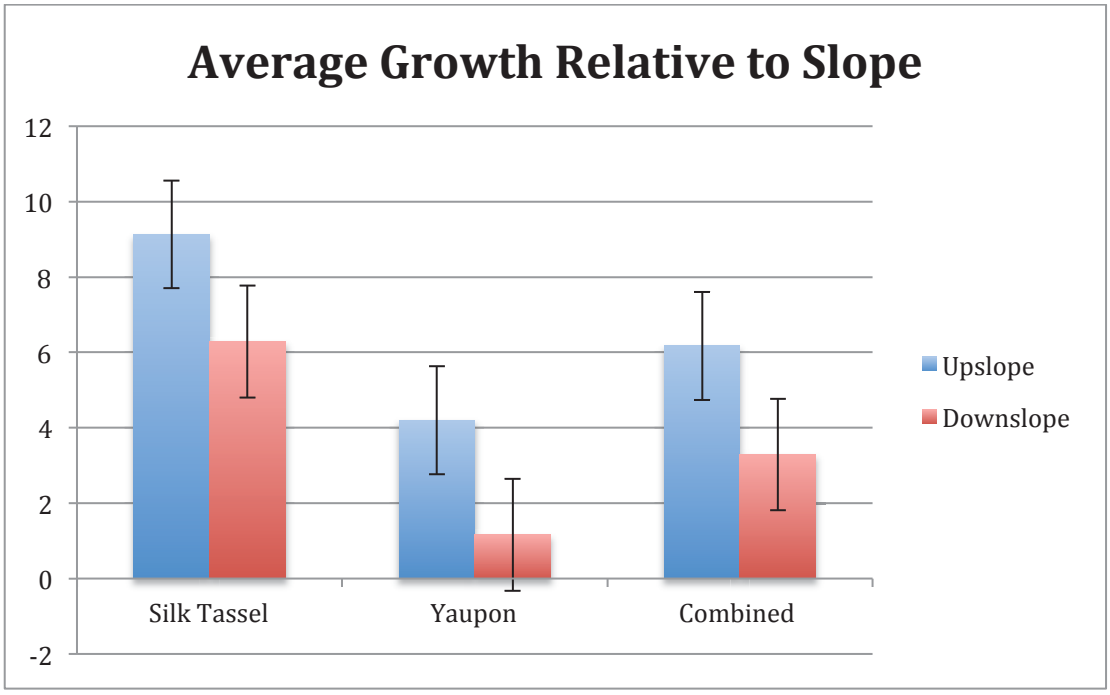
Estimated Marginal Means 4: Up_DownSlope* Far_Close

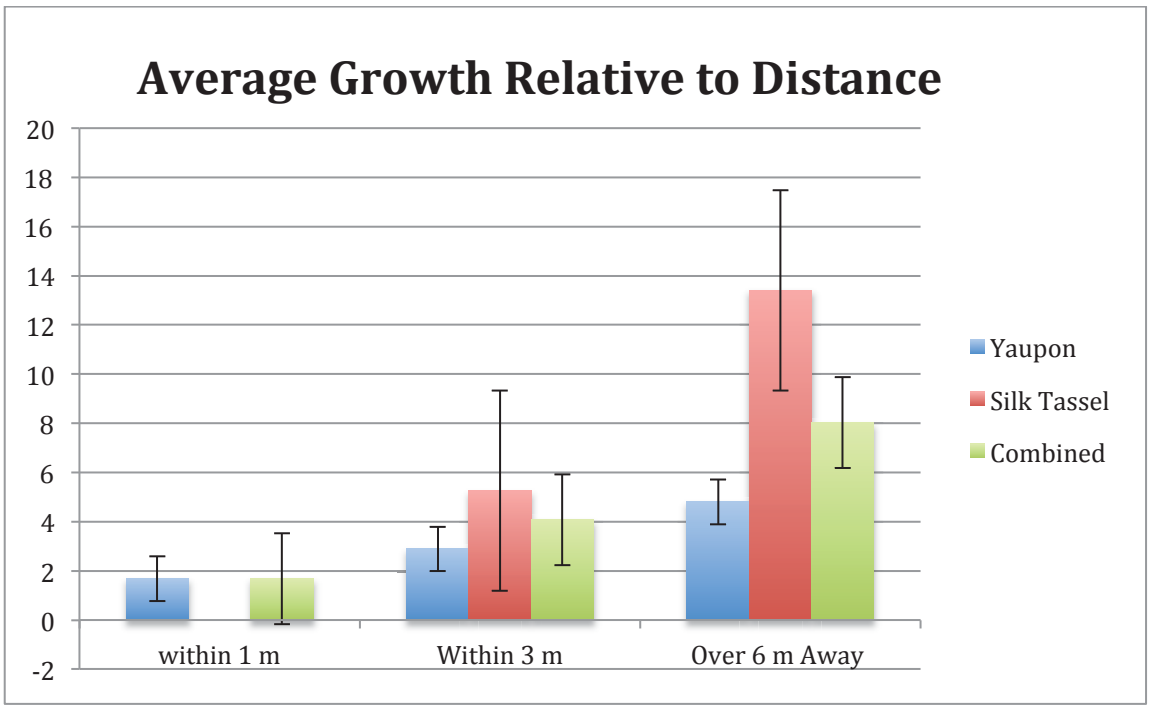
Estimates

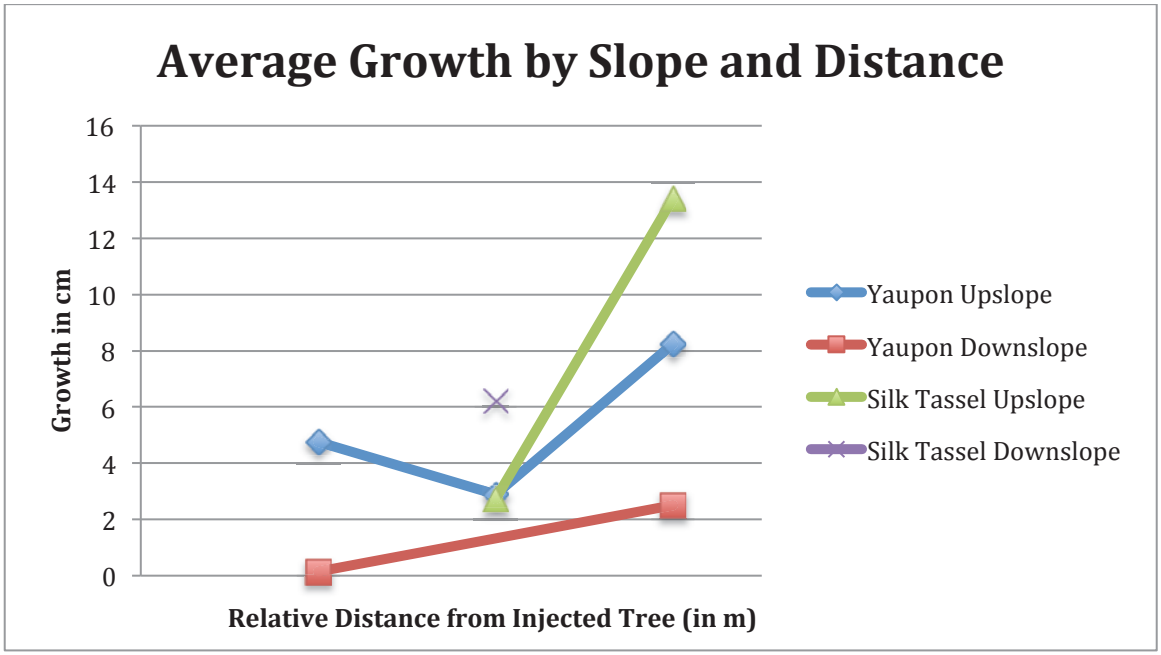
Up_DownSlope	Far_Close	Mean	Std. Error	95% Wald Confidence Interval	
				Lower	Upper
0	0	7.130000000	1.716590780	3.765543895	10.49445611

Appendix E: Additional Charts






Average Growth Relative to Distance
20
18
16
14
12
10
8
6
4
2
0
-2
within 1 m
Within 3 m
Over 6 m Away
Yaupon
Silk Tassel
Combined


Average Growth by Slope and Distance
16
14
12
10
8
6
4
2
0
Growth in cm
Relative Distance from Injected Tree (in m)
Yaupon Upslope
Yaupon Downslope
Silk Tassel Upslope
Silk Tassel Downslope