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IMPACTS OF GOAT BROWSING AND DISEASE ON *LILIUM GRAYI*, GRAY'S LILY, ON ROAN MOUNTAIN.

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Talent Expansion in Quantitative Biology

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Table of Contents

I.	Abstract.....	3
II.	Introduction.....	4-8
III.	Methods.....	9-13
IV.	Results.....	14-25
V.	Discussion.....	26-30
VI.	Literature Cited.....	31-32

Abstract

The flora of southern Appalachian high elevation balds has strong representation of northern disjuncts and regional endemics. Among the endemics, the showy *Lilium grayi* (Gray's Lily), is most noteworthy for its historical significance and for a high public profile. As bald vegetation changes in response to human and natural environmental shifts, active bald management has been implemented on public lands. Among managed balds, the Roan Mountain massif supports a large population of *L. grayi*. The purpose of this study was to describe the demography of adult plants, compare browsed and non-browsed plots, and determine the extent to which disease may impact survival and reproduction of *L. grayi* on Roan Mountain. There were no significant differences between browsed and control plots in measures of plant morphology, vigor, or reproductive output, but browsed plots had significantly more juvenile plants compared to controls. Along a transect, spatial analyses uncovered clusters of diseased and healthy plants and showed that plants in close proximity tended to be alike in disease status and those distant were more unlike. A pathogenic fungus, *Pseudocercospora inconspicua*, may be the disease pathogen.

Introduction

Roan Mountain is home to one of the rarest ecosystems in the world, temperate mountain balds. The Southern Appalachian Grassy Balds of Roan Mountain are classified as a G1 biome, which is the rarest possible ranking, assigned to biomes with only one to five occurrences throughout the world (<http://www.natureserve.org/explorer/ranking.htm>). The invasion of several woody plant species, specifically Canada blackberry (*Rubus canadensis*), have decreased the size of the balds immensely and threatened many of the noteworthy species on the balds (Donaldson 2009). With such severe rarity, the grassy balds should be protected before the ecosystem is lost altogether due to encroachment by woody plants and the shrinking of the balds.

A bald ecosystem is defined as an “area of naturally-occurring treeless vegetation located on a well-drained site below the climatic tree line in a predominantly forested region” (Mark 1958). The Roan Mountain massif is home to some of these balds. A current widespread opinion is that Roan Mountain balds are not sustainable because native woody species can outcompete the grasses, sedges, and other native herbaceous bald species. Two main schools of thought to bald origins and maintenance have emerged; origination via anthropogenic or natural factors (Weigl 1995). Anthropogenic factors are thought to have maintained the balds by human-induced interactions. Fires and clearing of the forests by Native Americans are some of the proposed anthropogenic factors that maintained the balds. The “Megaherbivore Theory” is the best example of bald maintenance by a natural factor, and is one of the more widely accepted theories for bald maintenance.

The “Megaherbivore Theory” of Southern Appalachian grassy balds hypothesizes a past naturally functioning ecosystem that was able to maintain the balds (Weigl 1995). Weigl proposes that megaherbivores, such as elk, bison, and woolly mammoths, once roamed the Grassy Balds but were eventually forced off, first, by the extinction of the mammoths after glaciation, then by European hunters and settlers who brought their own livestock (Weigl 1995). The disappearance of the megaherbivores left a herbivore void in the ecosystem. Now woody plants, specifically Canada blackberry, have no natural major herbivores and can spread freely on the grassy balds. The ecological question facing the balds is what is the appropriate human response to the invasion of the blackberry and other woody shrubs? Currently, the United States Forest Service uses mowers to push back the tree line, but others have suggested a more natural approach that more closely resembles the hypothesized historic natural processes. The objective of the Baatany Goat Project is to more closely mimic the activity of megaherbivores (Donaldson 2009).

Goats may serve to replace the now-absent megaherbivore(s) of the balds and function to reduce the shrubs naturally. Unlike sheep and cattle, who are grazers, goats are browsers. Compared to grazers, browsing animals feed more heavily on woody shrubs, invasion of which is one of the major causes of the decline of Appalachian grassy balds. Started in 2008 by Jamey Donaldson, the project uses a more natural process of bald management by introducing goats to the balds for three months during the summers. With renewed browsing, it is important to examine the impacts of browsing on the rare species of the balds.

Gray's Lily is one of the rare species thought to be effected by the decline of the balds. It is a globally-rare (G3 = 21-100 global populations), Federal Species of Concern listed as Threatened-Special Concern in North Carolina (Buchanan and Finnegan 2010) and Endangered in Tennessee (Crabtree 2008). It is identified by its bright red flowers and leaf whorls (leaf numbers vary per whorl) that run up the stem (Fig. 1). The lily is threatened by removal of habitat and with disease, the latter likely caused by a fungal pathogen. Mowing and goat browsing provide two different types of habitat modification for lilies. The Western Lily faces similar problems to Gray's Lily and the habitat modification choices reflect that (Imper 1998). A plant with moderate to high light requirements, Gray's Lily seems to need a prairie-like habitat with ground cover but enough space to reach the sunlight. Mowing removes everything in its path, leaving lilies exposed to the harsh environment. Furthermore, mowers mulch the cut plant material which often contributes to thatch buildup and may impede seed germination and seedling establishment. In contrast, browsing thins out the canopy cover allowing the lily to reach sunlight but still offers protection under shrubs. Thus, a working hypothesis is that browsing will provide more improved root zone habitat with canopy sunlight compared to what is currently being provided by the increased number of woody shrubs.



Figure 1. A photo of Gray's Lily with a whorl of leaves and four flowers (J. Donaldson 2008).

Many Gray's lilies are affected by some type of disease, most likely a fungal pathogen that causes plants to die early in the season. Besides Roan Mountain, there are only a few genetically pure Gray's Lily populations left. Most are threatened by hybridizing with the Canada Lily (*Lilium canadense*) (Skinner 2002). Therefore, preservation of one of the last remaining "pure" Gray's Lily populations should be considered a conservation priority.

Consequently, maintaining the health and size of the lily populations on Roan is just one way to help preserve the remaining genetically pure Gray's lilies in the Southern Appalachians. Maintaining the health and size of the lily populations on Roan is just one way to preserve the remaining Gray's lilies on the Roan Mountain massif. Gray's Lily seeds fall from the parent plant and do not use a dispersal vector for transportation other than wind. This causes lily

plants to cluster which makes it easier for a fungal pathogen to spread and infect other lilies around it. By identifying the pathogen, it will enable a comparison to be drawn between other scenarios of similar species, pathogens, and/or habitat and to look for the best possible solution to the current lily disease problem on Roan Mountain.

The purpose of this study was to re-survey the long-term plots established by Jamey Donaldson in 2008 and to use data from the three years to compare browsed to control for impacts on plant morphology, reproduction and health. Our hypothesis was that the goat browsed plots would have significantly younger and healthier lilies than the non-browsed because browsed areas should facilitate seedling recruitment and make it more likely for seedlings and juvenile plants to be able to reach the important resources and cover needed to survive.

A demography study was initiated to learn more about the spatial and temporal patterns associated with Gray's Lily; specifically, whether disease occurs in clusters and how disease patterns change throughout the season. Due to their non-specialized dispersal mechanisms, plant clustering is expected, and clustering should allow for easier dispersal of the pathogen from plant to plant. Finally, a pathology analysis was conducted to provide a start to identifying the pathogen(s) and combating the increasing health issues facing the lily.

Methods

Field Methods

A. Effects of Browsing

In 2008, the Baatany Goat project was started on Roan Mountain in an effort to maintain the balds. At that time, a study of the population biology of Gray's Lily was started. The lily monitoring study design was comprised of twelve plots, five in the goat browsing areas and seven control plots (Fig.2). The plots were selected to encompass the most abundant areas of Gray's Lilies. Three years of data have been compiled using these plots. Plots are five meter radius circular plots marked in the centers by metal stakes. At survey times, plot circumference was delineated by tying a string, 5 meters in length, to the middle stake and then walking along the outside to create a visibly distinct circumference. For the most recent data collection, 2010, the plots were divided and surveyed as four quadrants: NE, NW, SE, and SW by using a compass. Thorough surveys were conducted to identify Gray's Lily adults, juveniles, and seedlings.

The following measurements were recorded for each lily: height (in centimeters), number of leaf whorls, number of flowers, health, fungus coverage in relation to the number of whorls, canopy, and type of browse damage [invertebrate or vertebrate (usually mammal)]. Health was scored on a five point scale: 1, 1.5, 2, 2.5, 3, with one representing close to dead and three signifying excellent health. Whorls visibly infected by fungus or bacteria were recorded on a numerical scale using completely infected whorls as whole numbers and any infection that didn't cover the entire whorl was considered a half. For example, 2 plus 2-½

whorls, would signify infection was found on a total of four whorls. Browsing was recorded as invertebrate or vertebrate / mammal-mediated. The canopy category identified the woody species, excluding blackberry, because the majority of the Gray's Lily were associated with blackberry, and there appears, at least anecdotally, to be a correlation between Gray's Lily health and proximity to Green Alder (*Alnus viridis* ssp. *crispa*).

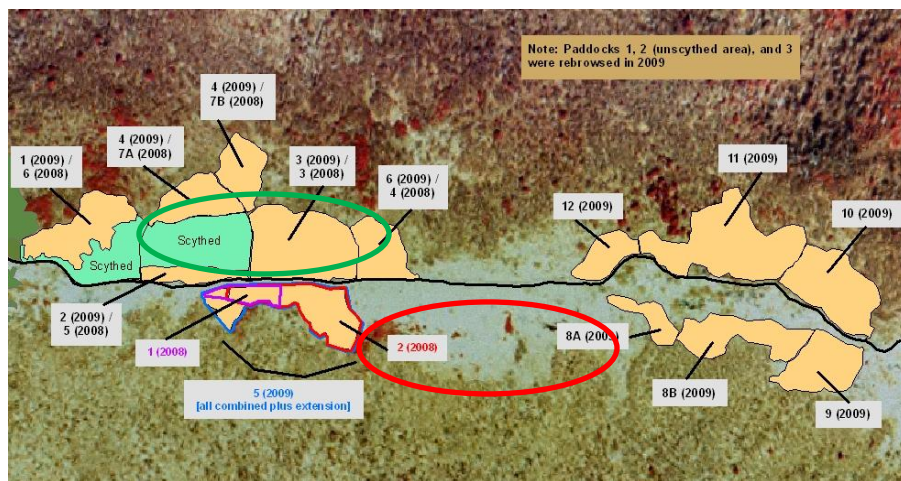


Figure 2. Goat- browsed (in green) and control (in red) plots established by Jamey Donaldson in 2008.

B. Pathology

The diminishing health of Gray's Lily seemed to be affected by an unknown pathogen(s) that needed to be identified to assess the proper management strategies. To identify potential pathogens, twelve plants were selected to be sent into North Carolina State University (NCSU) Plant and Insect Disease Clinic. Four plants were selected in each of three health categories: good, mediocre and poor health. Two plants were dug up and sent to the clinic for information on the roots, while the other ten plants did not include roots but were cut below their bottom leaf whorl.

C. Adult Demography, Temporal, and Spatial Patterns

A study of the demography of adult Gray's lilies was initiated in 2010. A line transect was established starting at the top of Jane Bald, located at the elevation sign, and followed the Appalachian Trail to the Memorial Rock on top of Grassy Ridge (Fig. 3).

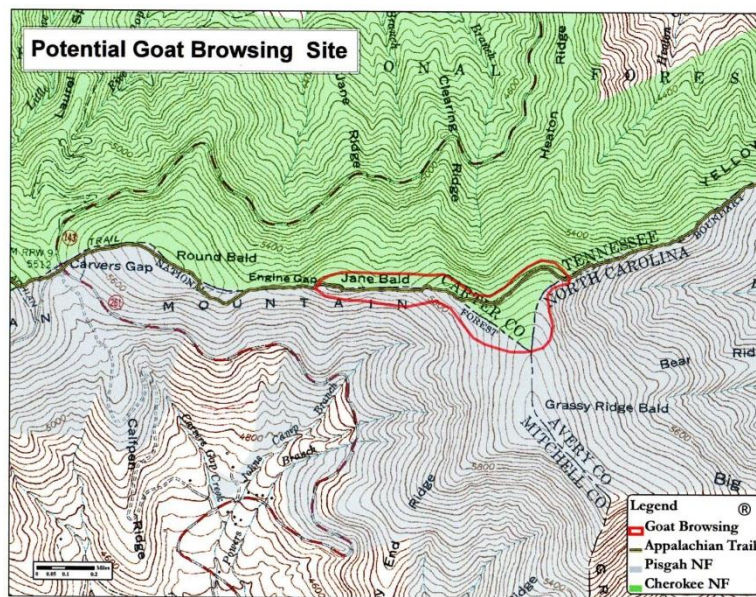


Figure 3: Location of the demography transect enclosed in the circle and following the Appalachian Trail and towards the east, the county dividing line.

The Appalachian Trail and Grassy Ridge trails were used as the x-axis. A meter tape was laid along the trail to record the x-axis coordinate. Reproductive plants were surveyed on either side of the trail, for a y-axis distance of 2-3 meters on either side of the trail. The North Carolina side was recorded as the southern side and the Tennessee side was recorded as northern side. Plants were tagged and the data was recorded using the same variables and

measurement scales as for the plot design described above. Every two weeks the plants were re-evaluated for; health, fungus, and browsing damage.

Statistical Methods

A. Effects of Browsing

To determine whether browsing influenced Gray's lilies, nested ANOVA was used to compare browsed and control plots. Plots were nested within the treatment main effect of browsed or control treatments. When significant plot effects were present, treatment effects were tested using the "plots within treatments" mean square as the denominator for F-ratios.

The PROC GLM procedure in SAS was used to compare the treatment effects and plots within treatments (SAS Institute 2002). A total of twelve ANOVA analyses were conducted, one for each variable (height, whorls, flowers and health) and for each of the three years, each followed by *a posteriori* Tukey and Student- Newman-Keuls comparisons of means. Pearson's correlations were calculated for variables among and within the plots and years.

B. Adult Demography, Temporal, and Spatial Patterns

Cluster analysis was used on transect data to uncover spatial patterns related to morphology, reproduction and health and to examine the temporal progression of health along the transect. SaTScan software was used to find statistically significant clusters (Kulldorf 2010). A circular scanning window was used with a scanning window of up to 50% of the population. Changing the window shape from circular to elliptical or changing the window size from 50% to 20% did not significantly influence the results. To estimate significance of clusters, 999 randomizations were used to generate P values. Data for each of the five two-week interval censuses were tested for clusters for the variables; height, number of whorls, number of flowers, and health status. For significant clusters, the data were then presented in a scatterplot to better illustrate where the clustered lilies were located on the transect. Spatial autocorrelation was used to further analyze the spatial patterns. The ability for the statistical

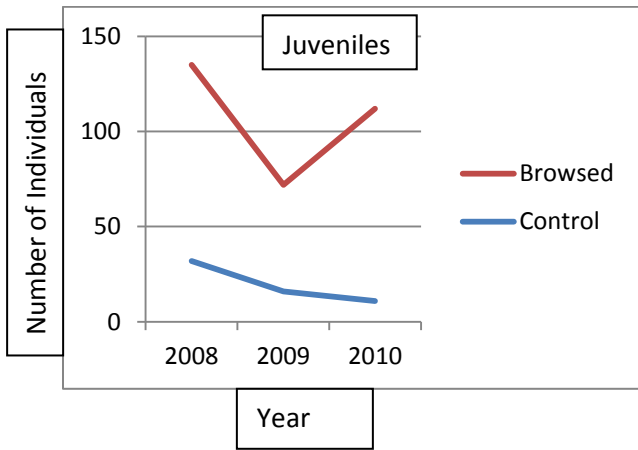
method to test individuals for a single variable made it invaluable in our research. SAM software was used to test for spatial autocorrelation using the Moran's I test for autocorrelations on the variables; height, whorls, flowers, and health. Spatial autocorrelation was used to distinguish whether plants close to each other are more alike, or unlike, than expected (Rangel, et al. 2010).

Results

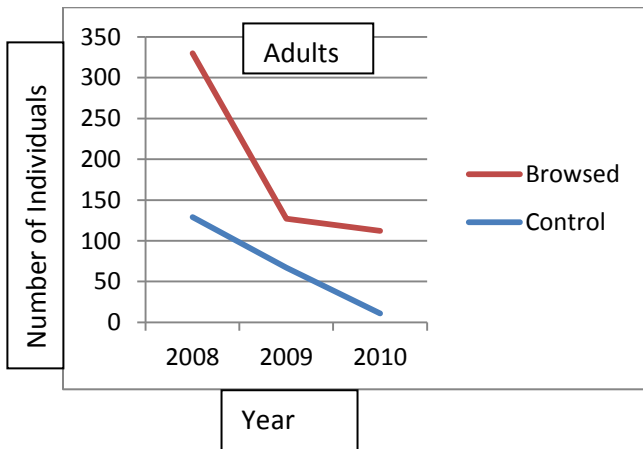
Effects of Browsing

Figure 4 shows numbers of Gray's Lily juveniles, adults, and total number of lilies in the area. The y-axis is the number of plants and the x-axis is the survey year. The data from 2008 was a baseline because the browsed plots were surveyed before the goats browsed. In the control treatment, there was a steady decline in juveniles, adults, and total plants. In the browsed treatment, juveniles showed an increase in 2010 from the previous year and the decrease in adults decreased in severity compared to 2009. The adult decline appears to be decreasing in severity. The total number of plants showed an increase from 2009 to 2010.

a)



b)



c)

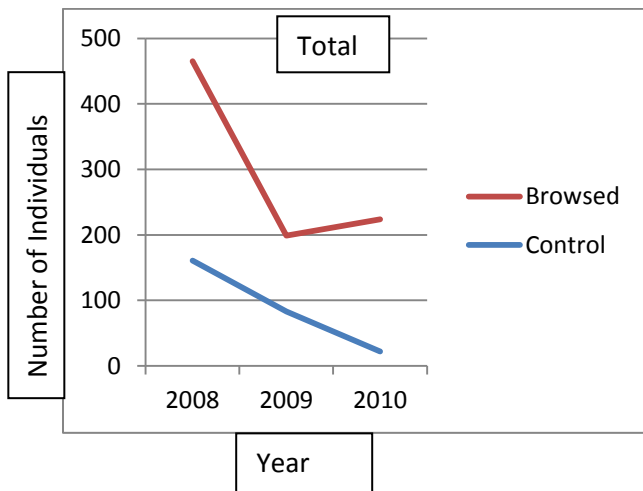


Figure 4. The number of Gray's Lily plants found in browsed and control plots for each year. a) juveniles, b) adults, and c) all plants.

The following table shows the correlation coefficients with plants from browsed and control plots combined within each year (Table 1). For all pairs of years, measures of morphology, vigor and reproduction were significantly correlated with positive correlation coefficients. In 2008, all the measurements were correlated. In 2009, health was marginally negatively correlated with height and positively correlated with flowers. Finally, in 2010, health was only significantly correlated with number of flowers.

2008	height	whorls	flowers	health
height	1.00000	0.88711 <.0001	0.62951 <.0001	0.13580 <.0001
whorls	0.88711 <.0001	1.00000	0.62213 <.0001	0.13797 <.0001
flowers	0.62951 <.0001	0.62213 <.0001	1.00000	0.18767 <.0001
health	0.13580 <.0001	0.13797 <.0001	0.18767 <.0001	1.00000
2009	height	whorls	flowers	health
height	1.00000	0.88255 <.0001	0.51419 <.0001	-0.13486 0.0516
whorls	0.88255 <.0001	1.00000	0.49265 <.0001	-0.06816 0.3268
flowers	0.51419 <.0001	0.49265 <.0001	1.00000	0.18231 0.0082
health	-0.13486 0.0516	-0.06816 0.3268	0.18231 0.0082	1.00000
2010	height	whorls	flowers	health
height	1.00000	0.88159 <.0001	0.56490 <.0001	0.07091 0.1758
whorls	0.88159 <.0001	1.00000	0.64319 <.0001	0.06747 0.1978
flowers	0.56490 <.0001	0.64319 <.0001	1.00000	0.15083 0.0038
health	0.07091 0.1758	0.06747 0.1978	0.15083 0.0038	1.00000

Table 1. Pearson Correlation coefficients (top value) and the associated P-value (bottom value) for Gray's Lily surveyed in plots from 2008 to 2010.

Table 2 shows the nested ANOVA results for comparison of browsed to control plots. There were significant treatment effects for; health and flowers in 2008, whorls in 2009, and height and whorls in 2010 and significant plot differences for all variables in all years.

Health 2008					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	10.26700311	10.26700311	25.34	<.0001
pLot(TRMNT)	10	19.42710728	1.94271073	4.80	<.0001
Error	453	183.5061838	0.405090		
Health 2009					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	0.03081986	0.03081986	0.06	0.8114
pLot(TRMNT)	10	26.38503954	2.63850395	4.89	<.0001
Error	197	106.3215789	0.5397034		
Health 2010					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	0.1323514	0.1323514	0.21	0.6502
pLot(TRMNT)	10	116.7608214	11.6760821	18.17	<.0001
Error	354	227.4502441	0.6425148		
Height 2008					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	188.56952	188.56952	0.44	0.5066
pLot(TRMNT)	10	22846.11932	2284.61193	5.35	<.0001
Error	453	193321.7425	426.7588		
Height 2009					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	960.26772	960.26772	5.40	0.0211
pLot(TRMNT)	10	17262.41030	1726.24103	9.71	<.0001
Error	197	35015.30531	177.74267		
Height 2010					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	5395.55698	5395.55698	21.18	<.0001
pLot(TRMNT)	10	14306.99159	1430.69916	5.62	<.0001
Error	354	90192.3609	254.7807		
Whorls 2008					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	1.5079217	1.5079217	0.42	0.5160
pLot(TRMNT)	10	210.9587937	21.0958794	5.91	<.0001
Error	453	1616.919516	3.569359		
Whorls 2009					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	18.1476868	18.1476868	11.58	0.0008
pLot(TRMNT)	10	152.8303353	15.2830335	9.75	<.0001
Error	197	308.6719821	1.5668629		
Whorls 2010					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	32.4588465	32.4588465	16.78	<.0001
pLot(TRMNT)	10	109.3506176	10.9350618	5.65	<.0001
Error	354	684.7928250	1.9344430		
Flowers 2008					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	1.80660801	1.80660801	5.94	0.0152
pLot(TRMNT)	10	3.56022438	0.35602244	1.17	0.3080
Error	453	137.6882228	0.3039475		
Flowers 2009					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	0.00311880	0.00311880	0.10	0.7472
pLot(TRMNT)	10	0.80047242	0.08004724	2.67	0.0043
Error	197	5.89622762	0.02993009		
Flowers 2010					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRMNT	1	0.26973181	0.26973181	2.83	0.0937
pLot(TRMNT)	10	4.60804299	0.46080430	4.83	<.0001
Error	354	33.79769472	0.09547371		

Table 2. Nested ANOVA results comparing browse and control plots for flowers, whorls, height and health for each year.

Pathogen

The result from the North Carolina Plant Disease and Insect Clinic analysis of 12 plants, reported the fungus, *Pseudocercospora inconspicua*, on all four plants with disease symptoms

but this fungus was absent from all other specimens (Table 3). It was concluded that *Pseudocercospora inconspicua* is a likely pathogen affecting Gray’s Lily on the Roan Mountain balds.

Health Status	<i>Pseudocercospora inconspicua</i> (# with P.i./total)	Other Pathogens
Healthy	No, 0/4	None.
Moderate	No, 0/4	<i>Geotrichum</i> (1/4), <i>Trichoderma</i> (1/4), and <i>Phomopsis</i> (2/4).
Poor	Yes, 4/4	<i>Fusarium</i> (1/4)

Table 3: NCSU Plant Disease and Insect Clinic’s analysis of twelve Gray’s Lily plants of different health categories.

Adult Demography, Temporal, and Spatial Patterns

Table 4 shows the descriptive statistics for adult lilies along the transect throughout the survey period from July 9 to September 15, 2010 (Table 4). Health and browsed means are for each survey period during the season, that is, each two-week measurement is shown, while height, flowers, and whorls measurements were only recorded on the initial survey.

	Mean	St.Dev	Range
Height	84.00	27.80	30-159
Flowers	1.77	1.64	0-7
Whorls	5.89	1.65	1-9
Health	1.80	0.70	1-3
Browsing	2.20	0.60	1-3

Table 4: Descriptive statistics for Gray's Lily along a transect used to study plant demography.

Date	Mean	St.Dev
7/9/2010- 7/22/2010	2.2	0.7
8/4/2010	2.0	0.7
8/18/2010	1.8	0.7
9/1/2010	1.6	0.7
9/15/2010	1.4	0.6

Table 5: Health of Gray's Lily at each survey period a transect used to study plant demography. Health was measured on a five-point scale with lower values indicating poorer health.

The mean health and standard deviation is shown for each survey period (Table 5). A steady decline of 0.2 units on the 1-3 scale was observed throughout the season.

Date	Cluster Size	Cluster Type	P
7/9/2010- 7/22/2010	12	healthy	0.007
7/9/2010- 7/22/2010	11	diseased	0.045
8/4/2010	16	diseased	0.002
8/4/2010	13	healthy	0.01
8/18/2010	16	diseased	0.001
9/1/2010	18	healthy	0.034
9/15/2010	18	healthy	0.003

Table 6: Characteristics of significant clusters at each survey period based on health status as identified using SaTScan software.

There were no significant clusters for height, fruit, whorls, or browsing. Table 6 shows significant health status clusters identified using SaTScan software. Two healthy and diseased clusters were found for each of the first two survey periods. The last three survey periods contained just one cluster, either healthy or diseased. Throughout the season, diseased and healthy clusters were each comprised of the same core group of plants, but clusters gained plants during subsequent weeks.

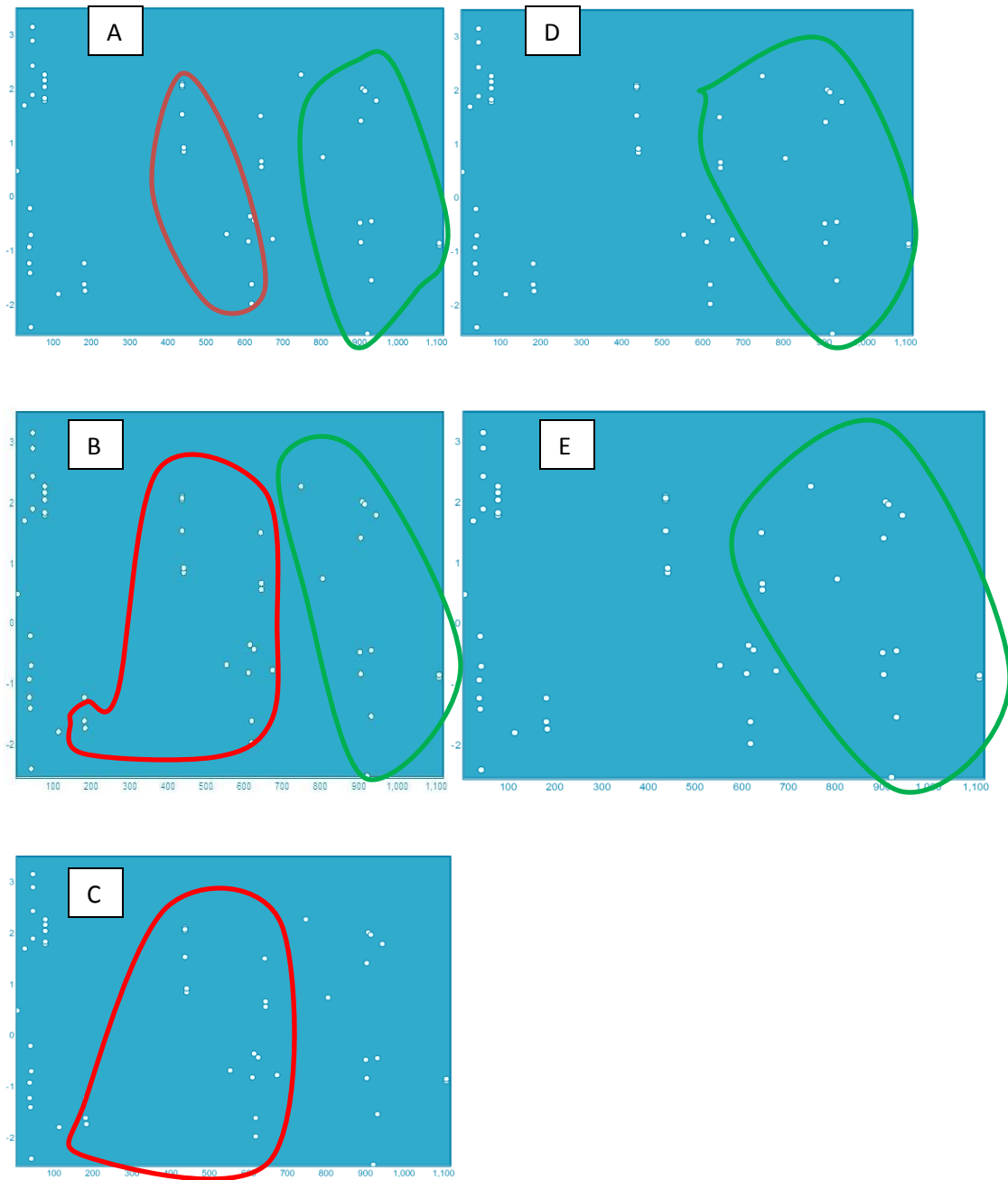


Figure 5: Representation of significant health clusters: A) initial, B) 8/4/2010, C) 8/18/2010, D) 9/1/2010, and E) 9/15/2010. y-axis is from -3 to 3 meters (- indicating south), x-axis is from 0-1100 meters, dots represent individual plants, and poor (red) and healthy (green) circles represent boundaries of the significant clusters.

The y-axis was between -3 and 3 meters (“-” indicating “south”) and the x-axis was the transect for a length of 1100 meters. The white dots show the individual Gray’s Lily per survey

time, while the green (healthy) and red (poor) circles show plants comprising significant clusters found in each time period. There were no significant clusters for height, fruit, whorls, or browsing. Spatial autocorrelation determined if plants closer to each to be more alike than expected, an approach used to confirm the cluster results (Table 7).

Height				
D.	Count	DistCntr	Moran's I	P
1	458	21.124	0.087	0.131
2	458	124.388	-0.037	0.523
3	458	302.361	-0.095	0.136
4	456	482.717	0.039	0.467
5	458	692.048	-0.106	0.121
6	458	964.645	0.001	0.965

Flowers				
D.	Count	DistCntr	Moran's I	P
1	458	21.124	0.075	0.186
2	458	124.388	-0.073	0.221
3	458	302.361	-0.105	0.116
4	456	482.717	0.035	0.487
5	458	692.048	-0.076	0.161
6	458	964.645	0.034	0.367

Whorls				
D.	Count	DistCntr	Moran's I	P
1	458	21.124	0.088	0.095
2	458	124.388	-0.159	0.01
3	458	302.361	0.049	0.281
4	456	482.717	0.153	0.035
5	458	692.048	-0.121	0.075
6	458	964.645	-0.135	0.03

Table 7. Results of spatial autocorrelation analysis for height, flowers, and whorls. Moran's I is the autocorrelation coefficient, "D" represents distance classes, "Count" is the number of pairs of plants, "DistCntr" is the distance center.

Health (7/3/2010-7/22/2010)				
D.	Count	DistCntr	Moran's I	P
1	458	21.124	0.192	0.005
2	458	124.388	0.142	0.015
3	458	302.361	0.012	0.724
4	456	482.717	-0.226	0.005
5	458	692.048	-0.104	0.065
6	458	964.645	-0.114	0.085

Health (8/4/2010)				
D.	Count	DistCntr	Moran's I	P
1	458	21.124	0.117	0.02
2	458	124.388	0.268	0.005
3	458	302.361	-0.058	0.261
4	456	482.717	-0.118	0.08
5	458	692.048	-0.105	0.08
6	458	964.645	-0.203	0.015

Health (8/18/2010)				
D.	Count	DistCntr	Moran's I	P
1	458	21.124	0.163	0.015
2	458	124.388	0.254	0.01
3	458	302.361	-0.103	0.085
4	456	482.717	-0.136	0.04
5	458	692.048	-0.204	0.02
6	458	964.645	-0.081	0.156

Health (9/1/2010)				
D.	Count	DistCntr	Moran's I	P
1	458	21.124	0.202	0.01
2	458	124.388	0.13	0.04
3	458	302.361	-0.163	0.035
4	456	482.717	-0.098	0.101
5	458	692.048	-0.101	0.095
6	458	964.645	-0.074	0.141

Health (9/15/2010)				
D.	Count	DistCntr	Moran's I	P
1	458	21.124	0.207	0.005
2	458	124.388	0.199	0.005
3	458	302.361	-0.127	0.05
4	456	482.717	-0.04	0.417
5	458	692.048	-0.096	0.095
6	458	964.645	-0.251	0.005

Table 8. Results of spatial autocorrelation analysis for health. Moran's I is the autocorrelation coefficient, "D" represents distance classes, "Count" is the number of pairs of plants, "DistCntr" is the distance center.

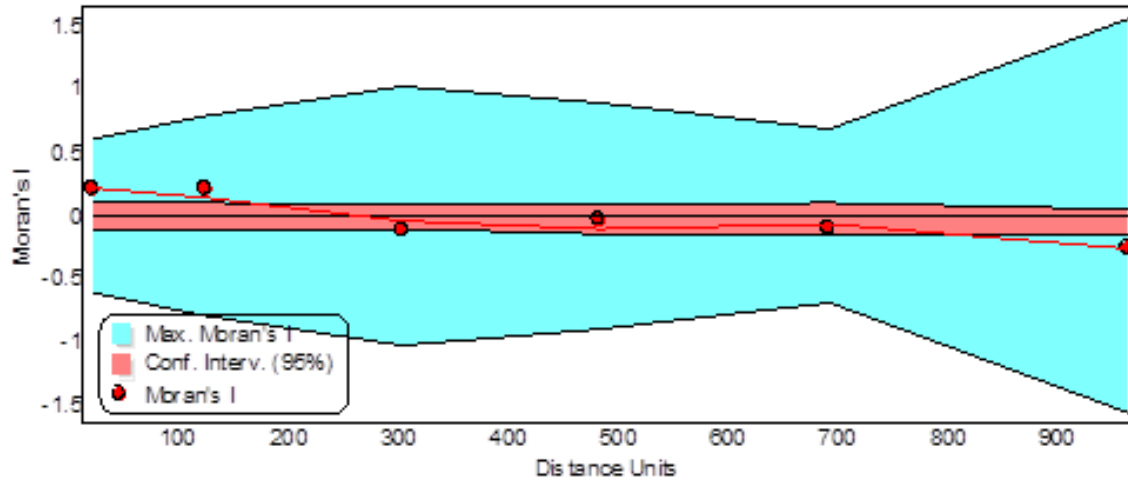


Figure 6. Visual representation of the results from spatial autocorrelation. This figure represents an example of the patterns observed when there was significant autocorrelation. Data shown is for health at 9/15/2010.

Figure 6 showed the results that were generally similar for each survey period for health. The first two distance centers were significant, while the last distance class was significant for 2 out of the 5 survey periods (Fig. 6 and Table 8). There were no significant autocorrelations for flowers, whorls, or height (Table 7).

Discussion

Effects of Browsing

Active management has been one of the major concerns facing the balds for at least the past ten years. Based on time and cost effectiveness, the land managers consider mowing the primary management option (Lindsay & Bratton 1979). The Baatany Goat Project is based on a strategy consistent with the “Megaherbivore Theory” using goats, a browsing species, and based on the hypothesis that goats can functionally replace the missing herbivores, and thereby create a habitat more closely resembling the balds of the past (Donaldson 2009).

Gray’s Lily does not appear to compete well with Canada blackberry and other woody plants invading the balds. Optimal habitat would increase the population size of Gray’s Lily, with no negative effects on morphology, vigor, reproduction, and health. If the “Megaherbivore Theory” is correct, then goat browsing would provide more optimal habitat for Gray’s Lily and create conditions more closely resembling the past habitat and overall composition of the balds. By focusing on the woody shrubs, the goats are able to open up more ideal habitat for seedlings and juvenile Gray’s Lily to take root, which was our hypothesis.

We did not expect to find any difference in plant characteristics between browsed and control plots due to the perennial nature of the plants and lack of an association between morphology, vigor, health and reproduction from one year to the next in browsed and control plots (Table 2). The health characteristic gradually became less and less correlated, which was unusual and difficult to explain (Table 1). We found a few treatment effects for different years for health, height, browsed and whorls, so we concluded that the results were not significantly

different from control plots because the variables were not reproducible for each year (Table 2). However, there were significant plot-to-plot differences between the measurements of morphology, reproduction and health (Table 2). Both of these findings were expected, because we did not expect plants to grow differently based on whether or not they were browsed in the previous year. Although plants in browsed plots may store fewer resources if browsed in early season, disease causes early collapse of many plants in all plots and probably had a larger effect on vigor. Therefore, the lack of a treatment effect was not unexpected. We also expected to find significant plot-to-plot differences within treatments due to the different habitats provided by each plot. Because of the heterogeneous nature of natural ecosystems and the clumped distribution of plants, it can be assumed that some plots would be better habitat for Gray's Lily and that they would not all be similar in habitat quality.

The hypothesis was that the Baatany Goat Project would significantly increase the available habitat for Gray's Lily and thus increase population size through increased recruitment of seedlings. In fact, the magnitude of the rapid decline in total population size in the control group, which was quite severe, was diminished in the browsed plots by the increase in juveniles, presumably caused by seedling recruitment due to the better habitat provided by the goats browsing. Compared to the baseline year of 2008, there was a steady decline of Gray's Lily except for the browsed juveniles and browsed total, which would both be affected by the overall better habitat for recruitment. These findings supported the hypothesis of an increase in recruitment. The increase in juveniles is important because the amount of new juveniles in the plots was able to quell the dramatic decrease of Gray's Lily by introducing younger plants

that hopefully will eventually increase the adult numbers. The three years of data indicate that browsing appears to have mitigated the decline in adult Gray's Lily.

Pathogen

Pseudocercospora inconspicua was found to be the plant pathogen likely causing the disease of Gray's Lily. The genus *Pseudocercospora* is comprised of common plant pathogens, mostly with lily species hosts and with a distribution centred in Eastern Europe (www.mycobank.com). There are two major hypotheses when accounting for the decline of Gray's Lily. It could be due to an infectious process or due to poor environment/habitat on Roan Mountain. With the identification of an association between visible diagnoses of plant health with a pathogen, a candidate pathogen has been identified, allowing the conservation effort to focus on a main fungal pathogen rather than a wide array of problems which could be hard to diagnose and treat. The demography study was able follow the plants through the season and examined the spread of the pathogen of one plant and to other plants, and specifically the effect *Pseudocercospora inconspicua* was having on the population as a whole.

Adult Demography, Temporal and Spatial Patterns

Whether it was bad environment or an infectious process that spread disease from plant to plant, we expected the lilies to show clustering in both numbers and health. The health variable was expected to be significantly clustered because the proximity of the plants made it

easier for the pathogen to spread. If there were “bad” habitats, the lily’s clustered in that area would be expected to be similar in health. An infectious process would follow a similar clustering pattern. The cluster results showed both a poor and good health cluster throughout much of the season. The cluster results provide evidence that there is, in fact, some effect that could be due to either the infectious process or an environmental effect, but this clustering pattern showed disease was not narrowly distributed. There were no significant clusters for any of the morphology, vigor or reproduction variables which provided evidence that the clustering based on health was a real and significant effect caused by an environmental or infectious problem.

Plants closer to each other would be more alike in health than expected due to the availability for the fungal pathogen to spread more easily and rapidly among closer plants. The spatial autocorrelation test provided additional support for the hypothesis that plants in spatial proximity and were more alike than expected. Spatial autocorrelation showed that there were significant similarities in health phenotype of plants in close proximity. Moreover, no significant autocorrelation was found for morphology, vigor or reproduction measurements. Thus, spatial autocorrelation supports the result obtained based on cluster analysis that uncovered significant clusters only for plant health status. Since health was the only variable that showed a positive autocorrelation, it provides evidence for clustering due to an infectious pathogen rather than an environmental effect. With a poor environment it would be expected that some of the morphology and vigor characteristics would be autocorrelated, but this was not the case for our results. Only the health was autocorrelated which provides evidence for

the prevalence of an infectious disease that is affecting the lilies on the balds of Roan Mountain.

The preceding discussion of the results showed that there was significant clustering and spatial autocorrelation of plants based on health status, two results indicative of a spatial pattern of disease due to, more probably, an infectious process rather than environmental conditions. These results provide important insight on the problem facing Gray's Lily on Roan Mountain. The prevalence of a disease/pathogen appears to be causing the death of the plants during the season. Already a rare plant, conserving the Gray's Lily is important not only for the population, but also for the balds, in general, because Gray's Lily is such a high profile species. Little is known about *Pseudocercospora inconspicua* in this habitat, geographical location or on this host plant, but it is a member of a genus whose species are high profile fungal pathogens. Comparisons among the species in the genus could help in providing a more specific and detailed treatment plan. The next step in the analysis of the pathogen problem is inoculating several plants to make sure that *Pseudocercospora* is actually the major pathogen and to study the infection process and eventual decline in more detail.

A similar study of Gray's Lily and a pathogen has been underway for eight years and is being conducted by Chris Ulrey of the Blue Ridge Parkway. That study monitors Gray's Lily over 8 years and our analysis of the data showed that health clusters were also present in those populations (data not shown). Ulrey's study used in combination with the seasonal patterns of Gray's Lily could help in understanding the entire lifecycle of Gray's Lily, which would provide valuable information in the conservation of Gray's Lily.

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