HABITAT QUALITY ASSESSMENT OF THE HORSEPASTURE RIVER IN WESTERN NORTH CAROLINA

OLIVIA ARNOLD, COREY BUHAY, ROBERT CURTIS, KATIE FUREY, KATHERINE HARRELL, CHARLOTTE HOPSON, ROBERT MCMAHAN, EMILY WATSON-COOK, SARAH WELLISH

Abstract. The Horsepasture River is a major river system in southwestern North Carolina, but there have been few comprehensive surveys of habitat quality in this watershed. We assessed the habitat quality of the Horsepasture River using common stream habitat evaluation techniques along with macroinvertebrate surveys. We found that habitat quality varies widely throughout the watershed. While some areas of intact riparian habitat remain, it has been heavily impacted by human activity. We found that areas subject to nearby human development consistently scored lower in stream health assessment protocol than areas without evidence of habitat alteration.

Key words: Horsepasture River, quality, habitat, assessment, SVAP, BEHI, benthic, macroinvertebrate, EPT, pebble count, southern Appalachians

INTRODUCTION

The Horsepasture River is approximately 14.8 miles in length and flows from the Blue Ridge Escarpment through Jackson and Transylvania Counties (NC) (Fig 1). The stream drops approximately 2780 feet in elevation along its course and eventually flows into Lake Jocassee in South Carolina. There are four impoundments in the river's upper drainage basin, two of which are in the main channel and two of which are in nearby tributaries. A total of 4.2 miles of the Horsepasture River are protected as a part of the National Wild and Scenic Rivers System, with 3.6 miles classified as scenic and an addition 0.6 miles classified as recreational (USDA 2000).

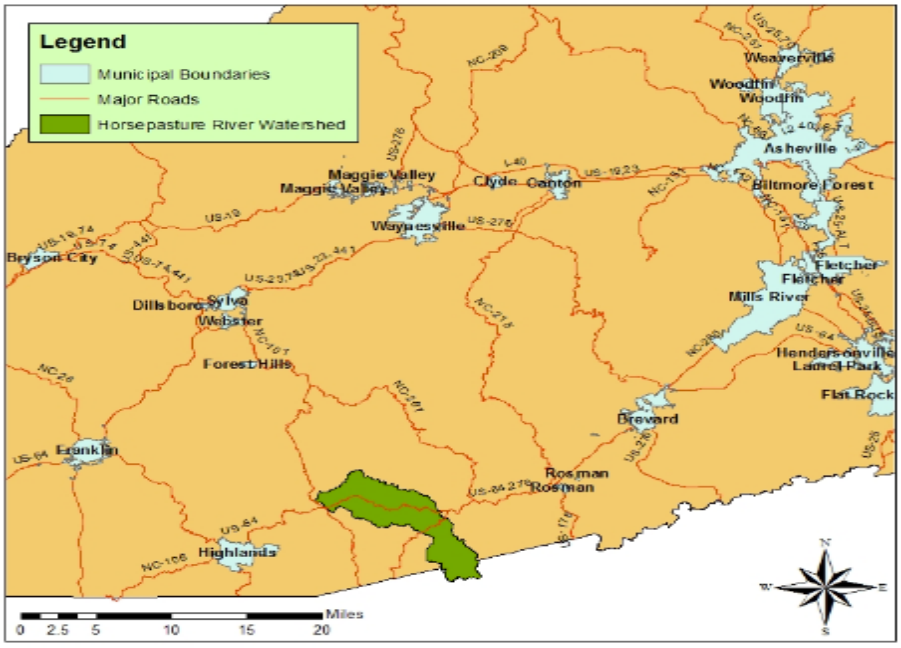

FIG. 1. Horsepasture River Watershed in Western North Carolina.

Very little data could be found on the current quality of the Horsepasture, despite its status as a significant waterway in the region. This is a potentially serious deficiency given the recent development in the watershed. Urbanization is associated with decreased water quality due to sedimentation, increased water temperatures, habitat alteration, and the loss of aquatic vertebrates and invertebrates (Stuart et al. 2012). Because the Horsepasture River headwaters feed populated valleys below, as well as a number of fragile ecosystems, determining the health of the waterway is important for effective future conservation and land management. We therefore undertook this study using a range of methods to assess the impacts on the river and the quality of its present riparian habitat.

During our assessment, we investigated the river channel morphology, substrate characteristics, probability of bank erosion, and surrounding habitat quality. We further relied on surveys of aquatic macroinvertebrate populations to assess stream health. Benthic macroinvertebrate diversity is a reliable predictor of water quality, and the larvae of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) are useful indicators of in-stream conditions (Stoyanova et al. 2014). Furthermore, more diverse macroinvertebrate assemblages tend to occur in areas with low levels of disturbance or habitat loss (Death and Winterbourn 1995). It is important to note that macroinvertebrate populations vary seasonally, with the lowest populations occurring in August and September, during which we were actively assessing the Horsepasture (Penrose, 2008).

METHODS

We sampled an experimental reach of approximately 100 meters along the streambed at seven locations in the Horsepasture River (Fig. 2, Table 1). Sites were located in the Horsepasture watershed, five of which were in Jackson County and two of which were in Transylvania County. All but one of the reaches investigated were located on the Horsepasture River with one reach located on Cashiers Creek in the headwaters of the watershed. Two of our sites were located along Cashiers Creek near Horse Barn Rd., which is north of Cashiers, NC. One of our sites was adjacent to the Cedar Creek Racquet Club and one was less than a mile from Getaway Ridge Rd., both of which were northwest of Cashiers, NC near Highway 64. One site was off of Cherokee Trail near the Country Club of Sapphire Valley. One site was near Burlingame Country Club and Upper Whitewater Rd. One site was farther southeast in Gorges State Park. We chose our reaches with a preference for safety and ease of access to the river. At each site we performed the following 6 assessments:

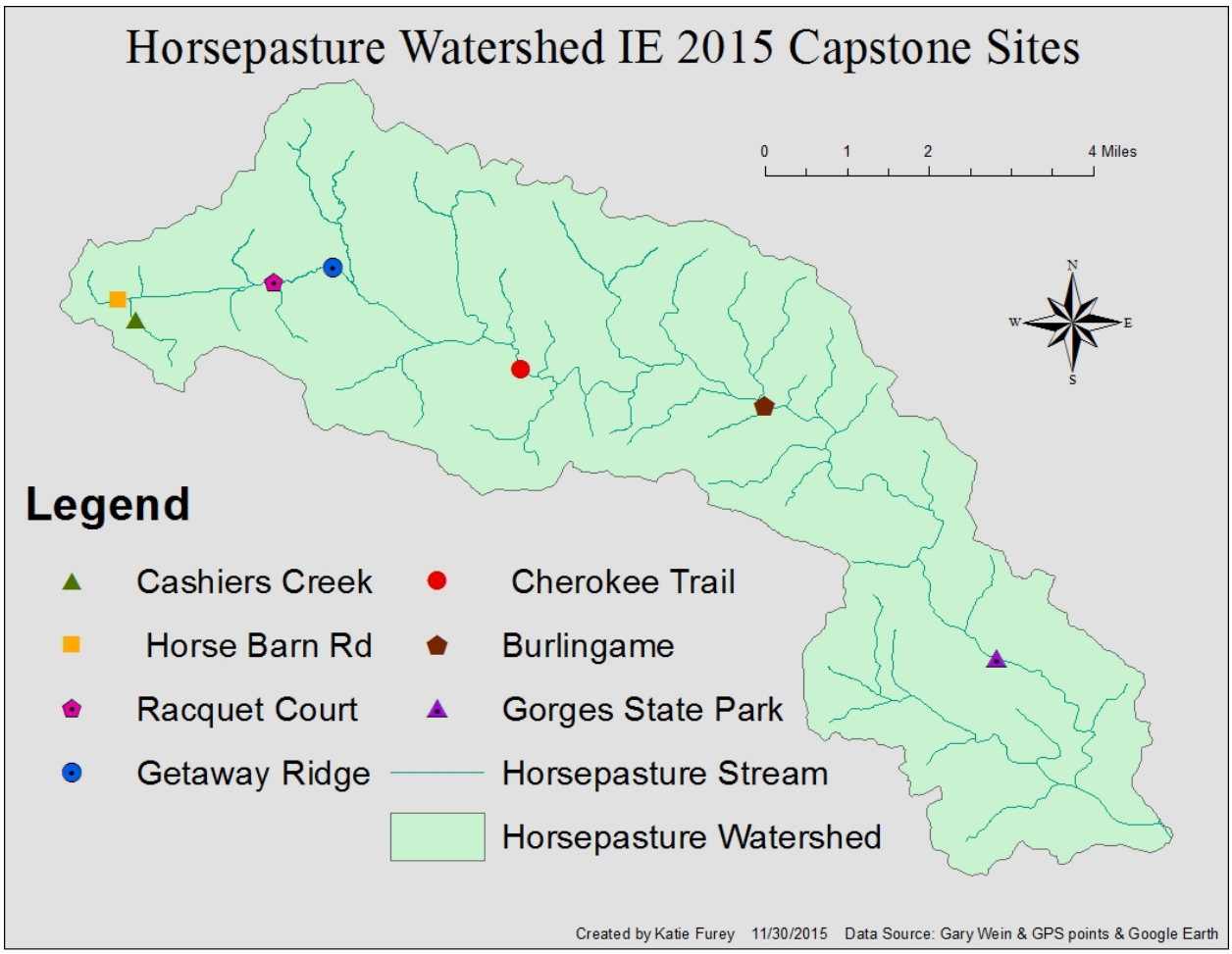

FIG. 2. Horsepasture watershed IE 2015 Capstone sites

TABLE 1. Site locations and coordinates.

Site Location	Abbreviation	Latitude	Longitude
Cashiers Creek	CC	N 35 7’ 25”	W 83 5’ 59”
Horse Barn Road (Hiron’s Property)	HB	N 35 7’ 27”	W 83 6’ 6”
Racquet Club	RC	N 35 7’ 39”	W 83 4’ 28”
Getaway Ridge	GR	N 35 7’ 81”	W 83 3’ 92”
Cherokee Trail	CT	N 35 7’ 26”	W 83 3’ 28”
Burlingame	BG	N 35 6’ 22”	W 82 6’ 16”
Gorges State Park	GSP	N 35 3’ 40”	W 82 6’ 48”

Stream Visual Assessment Protocol (SVAP)

We completed one Stream Visual Assessment Protocol (SVAP) for each site to compare to the North Carolina Habitat Assessment Field Data Sheet (Newton 1998) results. We used an SVAP that had been modified from the USDA version (2009) with a scoring system between one and four, one being very poor and four being excellent. For the SVAP we looked at elements such as bank condition, vegetation, pollution, and habitat cover. The SVAP is a less rigorous assessment of stream health intended for citizen science. It provides a preliminary qualitative assessment of overall stream conditions (USDA 2009), which makes it a suitable complement to the North Carolina Habitat Assessment Field Data Sheet.

Habitat Assessment

The North Carolina Habitat Assessment Field Data Sheet (NCHAFDS) was completed for each site (Barbour 1999). This assessment allowed us to use stream characteristics to determine a quantitative habitat score for each site. We first made quantitative observations on the location of the reach, visible land use, channel morphology, flow conditions, turbidity, channel flow status, and weather conditions at the time of sampling. In the second part of the analysis, we made qualitative observations of channel modification, instream habitat, bottom substrate and embeddedness, pool variety, riffle habitats, bank stability and vegetation, amount of light penetration, and riparian vegetation zone width. We then used these to calculate the site’s total quantitative score.

Canopy Cover and Stream Depth

We used a densiometer to determine the percentage of the stream shaded by canopy cover. We measured canopy cover in four directions at three different locations and calculated the mean of these results. We measured stream depth at 20 sites that were selected at random within the reach, and averaged these values to determine mean stream depth.

Reach-wide pebble count

We conducted a Wolman Pebble Count (Wolman 1954) to determine median particle size by performing ten evenly-spaced observations along ten stream-wide transects across the span of the 100-meter reach. For all sites but the Gorges State Park location, we blindly reached into the stream and measured the first material that came into contact with the sampler's index finger. At Gorges State Park, high currents necessitated a modification to this procedure, and we measured the first material that contacted the toe of the sampler's boot.

Bank Erosion Hazard Index

We calculated a Bank Erosion Hazard Index (BEHI) for each site by examining a portion of stream bank that we visually determined to be the most susceptible to or damaged by erosion (McQueen 2011). We measured bank height ratio and root depth with a pocket rod or a measuring tape. We measured root density and surface protection percentage with visual estimations. Finally, we adjusted this score with a field observation index based on substrate composition.

Macroinvertebrate Collection and Assessment

We collected macroinvertebrates via four different techniques. We conducted four 60-second kick-net samples, four 60-second D-net samples, four leaf pack searches, and four 5-minute visual assessments of rocks or organic matter collected from the streambed. We identified the collected macroinvertebrates using microscopes and dichotomous identification keys (Brigham 1982, Merritt 2008). The collections are housed at the Highlands Biological Station.

Biotic Indices

We identified aquatic macroinvertebrates to the family level. We counted the total number of organisms in each family (N) for each site (Appendix 1). We then used the Hilsenhoff Biotic Index (Hilsenhoff 1988, Zimmerman 1993) to determine the pollution tolerance value (TV) of each family. It is possible that a better index or adjustment system exists to convert these values to some more suitable for North Carolina (Lenat 1993). The sum of N*TV for each organism was then divided by the total number of organisms classified to establish a biotic index for each site (Lenat 1993).

$$BI = \frac{\sum(N_i * TV_i)}{\sum N_i}$$

We also calculated species richness—the total number of species found—for each site.

RESULTS

SVAP

The HB site scored the highest on the SVAP form. The lowest score occurred at the CT site. A high SVAP score indicates higher stream quality while a low SVAP score signifies poorer quality. There is no clear correlation between location of the site and the SVAP score (Table 3).

TABLE 3: SVAP scores for each of the seven locations sampled.

Site	CC	HB	RC	GR	CT	BG	GSP
Bank Condition	2.5	4	3.5	3	2	3.5	4
Vegetation Quantity	1.5	4	2	3	1	2	3.5
Vegetation Quality	2	4	3.5	3.5	4	4	4
Canopy Cover	2	4	3	2	1	2	1
Riffle Embeddedness	3	3.8	3	3	3	3	3
Trash and Garbage	4	3.7	3	3	3	4	4
Non-Trash Pollution	4	4	3.5	3.5	2.5	4	4
Livestock	4	4	4	4	4	4	4
Pools	4	3.7	3	3	4	4	4
Available Habitat/Cover	2	4	2	2.5	2.5	3	4
Barriers to Fish Movement	3	2	4	2.5	2.5	3.5	4
Overall	2.909	3.745	3.1	3	2.682	3.364	3.591

Habitat Assessment

The sites with the highest NCHAFDS scores were HB and GSP, with total scores of 97 and 86, respectively. The lowest score, 56, was recorded at the CT site (Table 4). NCHAFDS and SVAP scores are significantly correlated (R^2=0.89736) (Fig. 3).

TABLE 4. NCHAFDS scores for each site

Criteria	Sites						
	CC	HB	RC	GR	CT	GSP	BG
Channel Modification	5	5	4	5	3	5	4
Instream Habitat	15	19	11	14	15	19	20
Bottom Substrate	14	15	12	8	11	15	8
Pool Variety	10	10	10	8	8	6	10
Riffle Habitats	16	14	3	7	7	10	3
Bank Stability and Vegetation	5	14	12	11	8	14	11
Light Penetration	7	10	10	2	4	10	2
Riparian Vegetative Zone Width	0	10	5	8	0	7	2
Total Score	72	97	67	63	56	86	60

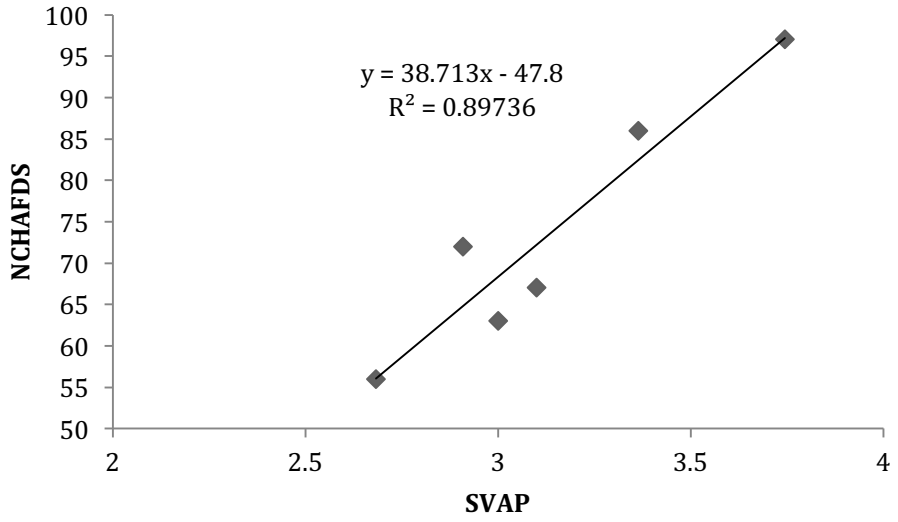

FIG. 3. Relationship between NCHAFDS and SVAP scores for six of the seven sites surveyed.

Canopy Cover and Stream Depth

The largest percentages of open canopy were recorded at the CT, GSP, and BG sample sites. The lowest percentage of open canopy was recorded at the HB sample site (Table 5).

TABLE 5. Percentage of canopy cover open for each location.

Sample Site	Percent Canopy Open
CC	6.80%
HB	2.95%
RC	7.60%
GR	13.17%
CT	35.27%
BG	52.52%
GSP	44.89%

The highest values for minimum, maximum, and average depths were measured at BG and in GSP. BG was slightly deeper on average but GSP had the deepest minimum value. Conversely, the lowest depths occurred at CC and HB, with CC being slightly deeper (Fig. 4). Depth tends to increase with progression downstream (Fig. 4).

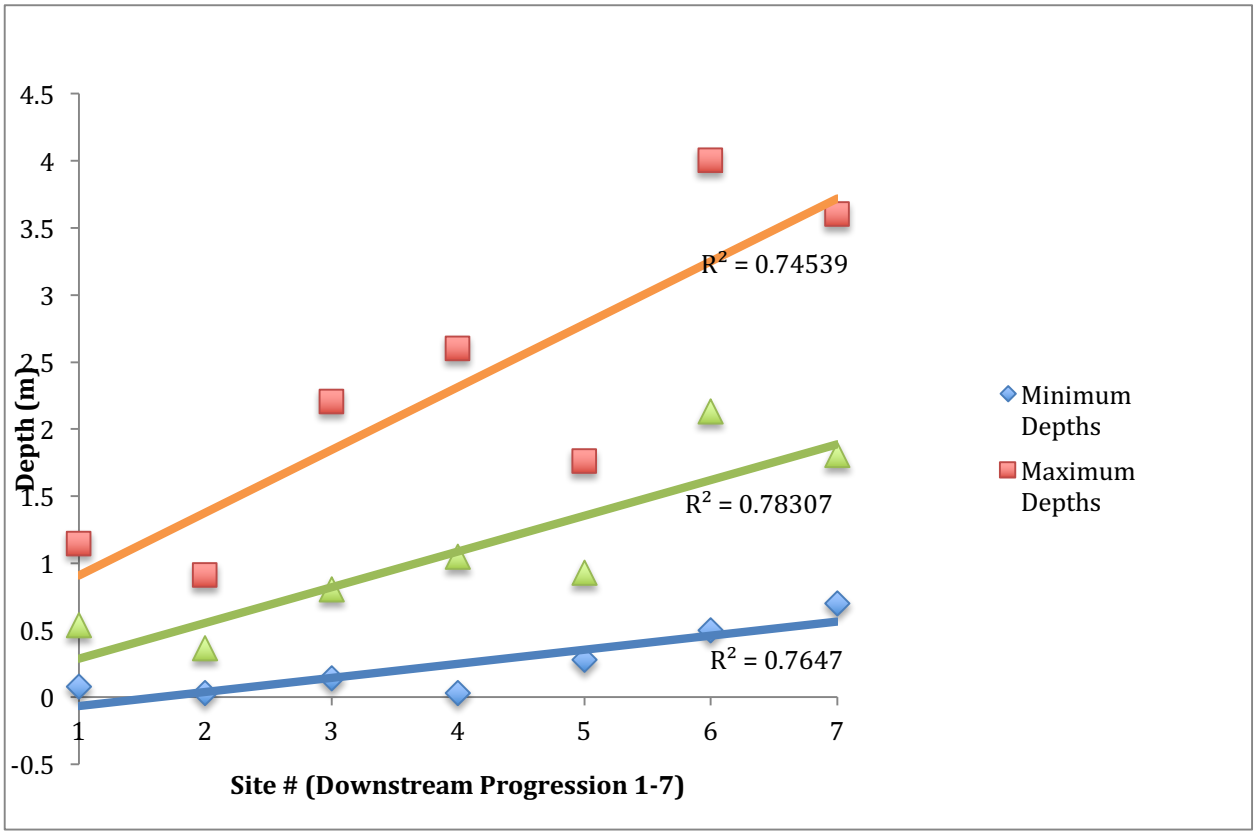

FIG. 4. Comparison of site minimum, maximum, and average depths with site position downstream (numbered from farthest-upstream site), with regressions and correlation coefficients.

Pebble Count

GSP, dominated by bedrock and boulders, had the largest median particle size of all the sites sampled. GR had the smallest median particle size of the seven sites sampled. On average, medium to very coarse gravel comprised the majority of streambeds studied (Table 6).

TABLE 6. Median particle size at each location.

Stream	Median Particle Size (mm)	Particle Description
CC	32-45	very coarse gravel
HB	16-22.6	coarse gravel
RC	64-90	small cobble
GR	0.25-0.5	medium sand
CT	11.3-16	medium gravel
BG	128-180	large cobble
GSP	256-362	small boulder

BEHI

CT had the highest Bank Erosion Hazard Index (BEHI) value, closely followed by BG. GSP had the lowest BEHI due to adjustment for bedrock, the main component of the bank in the area we examined. Only two sites, HB and GSP, had BEHI classifications as low or very low, all other sites had high BEHI classifications (Table 6). Most adjustments were due to the presence of sand at the site, which resulted in higher BEHI scores (Table 7).

TABLE 7. BEHI, adjusted BEHI, and classification for each site.

Site	BEHI	BEHI_adj	Class.	Class_adj	Reason_for_adj
CC	23	33	Moderate	High	Sand (+10)
HB	16.5	16.5	Low	Low	N/A
RC	33.7	43.7	High	Very High	Sand (+10)
GR	30	40	High	Very_High	Sand (+10)
CT	35.5	45.5	High	Extreme	Sand (+10)
BG	35.4	45.4	High	Extreme	Gravel (+10)
GSP	20	10	Moderate	Very_Low	Bedrock (-10)

Biotic Indices

HB and GSP had the lowest biotic indices while CT had the highest, closely followed by GR and CC. There is no strong correlation between biotic index and species richness (Table 8).

We found no relationship between biotic index and species richness (Table 8). However, SVAP and biotic indices were significantly inversely correlated ($R^2 = 0.88038$) (Fig. 5). HB and GSP had the lowest biotic indices and the highest SVAP scores while CT, which had the lowest SVAP score, had the highest biotic indices. At CT, GR, and RC – all of which had low SVAP scores – we found two to three times more Heptageniidae, a pollution-tolerant species, than at the other sites (Appendix 1). RC and GR also had extraordinarily high numbers of Hydropsychidae, another pollution-tolerant species: at RC we caught 182 specimens, and at GR we caught 275 (Appendix 1). However, we also found some pollution-intolerant species at high-SVAP sites. For example, GR and CT were the only sites surveyed where we found Leuctridae, which had a tolerance value (TV) of 0. Both HBR and CT had twice as many Glossosomatidae (TV 0) as any other site even though HBR had the highest SVAP value and CT had the lowest. NCHAFDS scores also exhibited a strong inverse correlation with biotic indices (R^2=0.9139) once the outlier point for BG was removed (Fig. 6).

TABLE 8. Species richness and biotic index for each location.

	CC	HB	RC	GR	CT	BG	GSP
Species Richness	17	10	15	12	12	18	15
Biotic Index	3.59	2.51	3.55	3.60	3.73	2.80	2.68

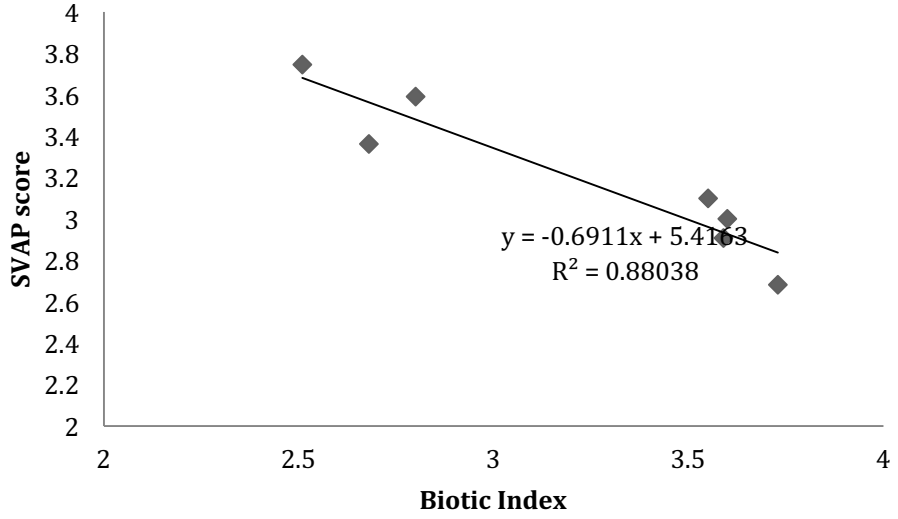

FIG. 5. Relationship between SVAP score and Biotic Index for each of the sites surveyed.

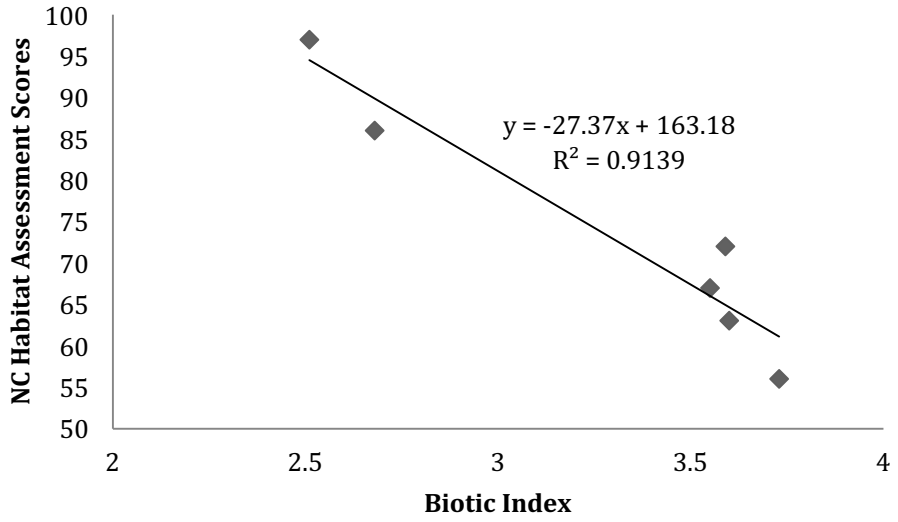

FIG. 6. Relationship between NC Habitat Assessment Scores and Biotic Index for six of the seven site surveyed.

DISCUSSION

The NCHAFDS and SVAP produce nearly identical results. Habitat assessment and SVAP scores are significantly correlated (R^2=0.89736). CT had the lowest NCHAFDS and SVAP scores. HB received the highest of both scores. The greatest divergence between the two scores was at BG. This disparity for BG is accounted for by low NCHAFDS scores in riparian vegetative zone width and canopy cover. While the type of riparian vegetation was desirable (deep rooting depth, intact riparian zone, and diverse vegetation), the area that it covered was narrow on each side of the river due to a dog park and road on either side of the stream. The BG site also had a high open canopy percentage but this was to be expected due to the expansive width of this river at downstream sites. When placed into context, the SVAP score is more indicative of the river quality at this site than other sites. This close match of SVAP and NCHAFDS protocol scores confirms our hypothesis that these two methods are nearly equivalent methods of stream evaluation when performed consistently.

The highest SVAP and NCHAFDS scores were found at HB due to its large, intact riparian zone, dense canopy cover, and diverse substrate. The large, intact, riparian zone leads to greater bank stability and a lower risk for erosion. Furthermore, the diverse substrate and large amounts of canopy cover provided ample habitat for fish and macro invertebrates. CT had the lowest SVAPs and NCHAFDS. There are many factors that could have contributed to the low SVAP and Habitat Assessment scores at CT. The site was in the midst of a residential area with large swaths of open grass and a very small riparian zone. Previous erosion prevention attempts like the addition of riprap to the banks and the stream had proven ineffective. Attempts had also been made to redirect the channel away from undercut bank portions. This could have been the

cause of the sediment deposition in the middle portions of the stream as well as the continual erosion and undercutting of the banks.

SVAP and NCHAFDS scores did not correlate significantly with stream depth or median particle size. CT had the second lowest median particle size but HB had the third lowest median particle size. Pebble count tended to be lower for sites where sediment deposition was a problem. For example, sand and silt were prevalent at RC where a dam slightly upstream of our reach prevented larger particles from entering the system as well as artificially decreasing stream velocity, which led to sediment deposition. Particle embeddedness was high for this site. GR, a similarly impacted site, also had a low median particle size. SVAP and NCHAFDS scores did correlate with BEHI. CT had the highest BEHI, indicating high risk for erosion, while HB had the lowest BEHI, indicating relative bank stability. Streams with greater vegetative cover and riparian habitat have greater root density, ground cover, and rooting depth, all of which contribute to a low BEHI. Better bank stability leads to higher SVAP and NCHAFDS scores.

HB had the lowest percentage of open canopy while BG had the highest. In general, sites more impacted by human development like BG and CT had higher percentages of open canopy. GSP had the second highest percentage of open canopy even though we sampled on protected state park land. Despite the protected nature of the site, the river banks had been cleared for a campsite and a gravel road that crossed a bridge over the river. Also, the river was wide at GSP, since this was the site furthest downstream. Canopy cover is less of a reliable measure of surrounding riparian habitat further downstream in lower valleys because the surrounding slope opens up, leaving less vegetation overhanging the stream. Furthermore, the channel widens creating larger gaps in the canopy cover. Although canopy cover often indicates greater habitat quality, these data may be skewed due to loss of leaf cover in October and November.

HB and GSP had the lowest biotic indices (Table 8), which corresponds to high habitat assessment and SVAP scores for those sites and support the conclusion that these were the healthiest of the sites surveyed. CT and GR, with biotic index of 3.75 and 3.60, respectively, were among the most impacted. Species richness and biotic index did not appear to be correlated. This is not what we expected, but the lower-than-predicted species richness at the healthy sites could have been due to seasonal variability in macroinvertebrate populations.

There was a strong inverse correlation between SVAP scores and overall biotic index scores at each site ($R^2 = 0.88038$). This inverse correlation indicates that more pollution intolerant species live in high quality stream habitats. NCHAFDS scores also exhibited a strong inverse correlation with biotic indices ($R^2=0.9139$) once the outlier point for BG was removed (FIG. 5). This inverse correlation indicates that more pollution intolerant species live in high quality habitats. The NCHAFDS score for BG was significantly lower than the SVAP score due to its narrow riparian vegetative zone and lack of canopy cover. Although the sites with higher SVAP and NCHAFDS score have low biotic indices, these sites did not have the highest total numbers of macroinvertebrates, indicating that macroinvertebrate diversity is more important than total numbers of macroinvertebrates.

Conclusion

Both SVAP and NCHAFDS scores exhibited a significant inverse correlation with biotic index, which demonstrates the susceptibility of macroinvertebrate populations to human impacts on streams. This confirms our hypothesis that we would find more pollution intolerant macroinvertebrate species in sites less impacted by human development. Based on assessments of stream and riparian zone habitat quality, the Horsepasture River displays a high degree of variability in the portion of the watershed that we studied. While the undisturbed headwaters and protected area of GSP appear to be relatively intact, sites near areas of human development show evidence of degradation. Large-scale habitat alteration, such as the construction of bridges and dams, negatively impacted habitat quality along populated areas of the watershed which, in turn, had a negative impact on the diversity of macroinvertebrate species at the impacted sites.

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APPENDIX 1

APPENDIX 1. Macroinvertebrate specimen counts and BI, by family, for each sampling site

Taxon		Site						
	Biotic Index	CC	HB	RC	GR	CT	BG	GSP
EPHEMEROPTERA								
Baetidae	4		1	10	9		21	
Baetiscidae	3				1			2
Ephemerellidae	1		1	1	1			12
Heptageniidae	4	28	5	110	75	130	39	32
Leptophlebiidae	2	1					3	2
Siphloneuridae	7	4		12		2	1	5
Isonychiidae	2			14	5		7	
Ephemeridae (*Hexagenia* sp.)	6		1					
PLECOPTERA								
Capniidae	1			1				1
Chloroperlidae	1	1			4			
Leuctridae	0			2	1			
Nemouridae	2	1						
Perlidae	2	33	21	1	10		30	8
Perlodidae	2		2	12			2	176
Pteronarcyidae	0	13	7		1		12	
Peltoperlidae	2		29		2	2	2	2
Chloroperlidae (*Haploperla* sp.)	2					4		
Psychomyiidae	2						1	

TRICHOPTERA								
Brachycentridae	1			1				
Glossosomatidae	0						3	4
Helicopsychidae	3					1		
Hydropsychidae	4	64	44	275	52	182	16	40
Leptoceridae	4						1	
Limnephilidae	1	1						
Odontoceridae	4	2						
Philopotamidae	0	14	23	50		17	14	
Polycentropodidae	3	3	4				1	
Rhyacophilidae	4		1	1			2	
Sericostomatidae	6						3	
Dipseudopsidae	3	3		3				
ODONATA	4	18	7		7	3	2	2
COLEOPTERA	4	1		3				3
Psephenidae	4	23	5	2	1		10	4
Elmidae	4	17	1	27	2	5	2	
DIPTERA	4.75	47					10	
Athericidae	2		1					1
Chironomidae	8	1		18	7	10		12
Empididae	6	1						
Simuliidae	6	4		8		5		
Tipulidae	3	4	1		6			1
MEGALOPTERA	4.78					13		

Corydalidae	0	11		4	4		2	7
OLIGOCHEATA	8	3	1		1	5		
AMPHIPODA	6		1					
HEMIPTERA	5		1					
DECAPODA (crawfish)	5				1			

PRESENCE OF *DESMOGNATHUS FOLKERTSI IN THE CHATTOOGA DRAINAGE OF WESTERN NORTH CAROLINA*

Corey Buhay

Abstract. With global amphibian populations declining, conservation of salamander biodiversity requires more attention than ever to prevent extinction. The dwarf black-bellied salamander is a rare, recently described species cryptic with black-bellied salamanders but genetically and morphologically distinct. I surveyed 11 first- and second-order streams in the Chattooga drainage of western North Carolina to establish the presence of *D. folkertsi,* which is currently a species of special conservation interest. I found potential *D. folkertsi* specimens in 10 of the 11 streams studied, establishing the species' presence in the Chattooga drainage.

Key words: black-bellied; Chattooga; cryptic; Desmognathus; dwarf; folkertsi; eDNA; mtDNA; salamander

INTRODUCTION

The dwarf black-bellied salamander *(Desmognathus folkertsi*) is a cryptic and recently described species of the black-bellied salamander (*Desmognathus quadramaculatus)* complex (Camp 2002). It is known to inhabit several Appalachian streams in northern Georgia and one stream in South Carolina, syntopic with *D. quadramaculatus. Desmognathus folkertsi* has been found at only two sites in North Carolina – in the Tennessee River and Savannah River drainages in Clay County (Wooten 2009, Wooten 2011).

The Chattooga drainage is rich in undeveloped, high-velocity first- and second- order streams characterized by riffles and cover objects such as cobbles and woody debris. This is the preferred habitat of *D. folkertsi*, but populations have yet to be confirmed within the Chattooga drainage (Wooten 2009).

Because of its rarity, the US Forest Service has named *D. folkertsi* a Species of Conservation Concern (SCC), and the 2015 revision of the *NC Wildlife Action Plan* will designate it a Species of Greatest Conservation Need (NC Wildlife Resources Commission). The state of North Carolina is currently considering it for state listing. This survey will contribute to that assessment.

Since salamander population health is a good indicator of stream health, survey information can help pinpoint streams that require the focus and funding of conservation projects (Welsh 2001, Southerland 2004). The 3200-acre Blue Valley, also known as the Overflow Wilderness Study Area, between Highlands and Scaly Mountain, NC, is currently under consideration for wilderness designation (Carpenter 2011). The discovery of a rare salamander in the creeks within this area would contribute to evidence supporting the designation of Blue Valley as a refuge for biodiversity.

Conserving biodiversity is essential for maintaining stocks of unique genetic material for potential future benefits to biological or medical research. High biodiversity also ensures that an ecosystem functions properly and remains robust in case of disease or disturbance. A diverse ecosystem will be able to recover from the loss or decimation of one species if it has several species performing similar roles within the ecosystem. The resulting ecosystem stability ensures that ecological services – like a wetland's role in water filtration – will be rendered (Randall 1991). With many species of amphibians currently in decline due to disease epidemics and possibly climate change, the preservation of rare species requires even more vigilance. Now that

global amphibian populations are low, there is less room for error in protecting remaining individuals (Rohr 2008).

Frequent speciation within salamander species is common and contributes to the extensive number of cryptic species in the southern Appalachians. However, patterns of differentiation are not well understood (Wooten 2009). Accurately determining the distribution of *D. folkertsi* may contribute to the understanding of these patterns and their ability to predict the formation of pockets of genetic diversity resulting from cryptic speciation.

Like rapid speciation, hybridization is not uncommon among Appalachian salamanders (Wooten 2009, Wiens 2006). I also hope to find out from the mitochondrial DNA (mtDNA) analysis whether *D. folkertsi* and *D. quadramaculatus* have been able to hybridize in the streams where they are found syntopically. Past studies have found success in identifying *Desmognathus* species by sequencing mitochondrial cytochrome oxidase c (*cox1)* (Beamer 2008). The *cox1* gene was proposed as a genetic barcode because of its variability within species (Robideau 2011). Genetic analysis will definitively determine whether *D. folkertsi* exist in the Chattooga Drainage. It will also provide evidence for confirming certain diagnostic features like a maximum SVL of 85 mm, a blotchy brown and black dorsal patterning, and the lack of a reddish tail stripe on young individuals (Camp 2002).

Environmental DNA (eDNA) is another method of rare species identification that has proven successful in past studies (Davy 2015, Fukumoto 2015). Comparing the results of our eDNA analysis with mitochondrial DNA sequencing collected directly from captured salamanders will allow us to determine the effectiveness of eDNA for salamander stream surveys (Townsend 2011). Environmental DNA will allow us to determine species presence while tail snip DNA will provide information about specific salamanders and confirm diagnostic features mentioned in previous papers (Camp 2013).

My goal in this study is to contribute to the effort to determine the geographic range of *D. folkertsi* and to determine its rarity for considerations of special concern status. I also wish to determine *D. folkertsi* presence in Blue Valley as part of the area's evaluation for wilderness designation.

METHODS

I surveyed 10 creeks in the Chattooga River drainage for *Desmognathus folkertsi*. At each creek, I selected two analogous sites at least 200 m apart as measured along the stream. This resulted in two survey sites at each creek with the exception of Clear Creek; I sampled two different sections of Clear Creek (four sites total) due to the length and variability of the habitat along that stream.

I first surveyed the downstream of the two analogous sites to avoid any DNA-contamination of the water destined for our second site. I sampled a 30 m reach at each site. I proceeded from the downstream limit to the upstream limit of the reach, recording the species of each salamander I encountered. The capture data were later used to calculate species richness and relative abundance. At each site I took three stream width and bank width measurements at random. I recorded GPS coordinates of the upper and lower limits of the reach and photographed the area. Over the length of the reach, I collected 1 L of water in sterile plastic bottles for environmental DNA (eDNA) analysis. I also recorded start and end times for a reach-wide search of all banks, shoals, and likely cover objects. Start and end times were later used to calculate captures per unit effort.

For each potential *D. folkertsi* captured, I measured snout-vent length (SVL) and total length, recorded the cover type and distance from water at the time of capture, photographed the specimen, and took approximately 3 mm of tail tip for DNA sampling. I immediately placed tail snips in vials of 80% ethanol and refrigerated them at approximately 35 °F within 8 hours of capture.

In the lab, I extracted eDNA from the water samples by filtering each through a 0.45μm-pore diameter cellulose nitrate filter membrane with a manual hand pump. I then transferred filters to vials of 80% ethanol refrigerated at about 35 °F for storage. Samples were stored for 1 to 14 weeks before shipment to Nash Community College, N.C., for DNA analysis by Dr. David Beamer. The analyses of the mitochondrial gene, cytochrome c oxidase subunit I (COX1) will take several months to conduct and, due to the limits of the study, will not be discussed in this paper.

RESULTS

TABLE 1. Site names, site abbreviations, and survey dates.

Survey Date	Site Name	Site Abbreviation
9/3/15	Scottsman Creek Downstream	SCD
9/3/15	Scottsman Creek Upstream	SCU
9/9/15	Clear Creek Downstream	CCD
9/9/15	Clear Creek Upstream	CCU
9/11/15	Clear Creek 2 Downstream	CC2D
9/11/15	Clear Creek 2 Upstream	CC2U
9/17/15	Overflow Creek Downstream	OCD
9/17/15	Overflow Creek Upstream	OCU
9/18/15	Chinquapin Creek Downstream	ChCD
9/18/15	Chinquapin Creek Upstream	ChCU
9/24/15	Brooks Creek Downstream	BCD
9/24/15	Brooks Creek Upstream	BCU
10/8/15	Wilson Lakes Downstream	WLD
10/8/15	Wilson Lakes Upstream	WLU
10/9/15	Edwards Creek Downstream	ECD
10/9/15	Edwards Creek Upstream	ECU
8/28/15	Fowlers Creek Downstream	FCD
8/28/15	Fowlers Creek Upstream	FCU
10/23/15	Unnamed Tributary Downstream	UTD
10/23/15	Unnamed Tributary Upstream	UTU
10/30/15	Overflow Proper Downstream	OPD
10/30/15	Overflow Proper Upstream	OPU
11/5/15	Abes Creek Tributary Downstream	ACTD
11/5/15	Abes Creek Tributary Upstream	ACTU

TABLE 2. Average stream width and depth at each site.

Site	Average Stream width (m)	average bank width (m)	Site	Average Stream Width (m)	Average Bank Width (m)
SCD	4.33	0.63	WLD	3.33	2.07
SCU	3.75	1.81	WLU	2.21	0.40
CCD	3.47	1.17	ECD	2.27	0.77
CCU	1.63	1.08	ECU	5.28	1.63
CC2D	1.47	2.07	FCD	5.63	2.13
CC2U	1.85	0.76	FCU	3.70	2.10
OCD	3.53	1.37	UTD	3.05	0.57
OCU	2.80	1.87	UTU	1.84	1.33
ChCD	2.46	1.23	OPD	4.53	1.51
ChCU	2.10	1.82	OPU	3.74	1.23
BCD	3.35	2.82	ACTD	2.83	0.41
BCU	0.79	2.30	ACTU	5.29	2.57

FCD and ECU had the greatest average stream width while BCU and CC2D were the most narrow. BCD had the greatest average bank width. Banks at WLU and ACTD were the most narrow on average (Table 2).

TABLE 3. The type of cover under which each *D. folkertsi* specimen was found.

Specimen	Cover Type	Specimen	Cover Type
SCD_1	under cobble	ECU_2	under fallen log, midstream
CC2U_1	NA	FCD_1	under cobble
OCD_1	under cobble	FCD_2	under cobble
OCU_1	bedrock crevice	FCU_1	under cobble
ChinCD_1	under cobble	UTD_1	under cobble
ChinCU_1	bedrock crevice	UTD_2	under cobble
ChinCU_2	crevice	UTD_3	under cobble
BCD_1	crevice	UTU_1	under cobble
BCD_2	under cobble	UTU_2	on bedrock overhang
BCD_3	under cobble	OPD_1	between cobbles, midstream
BCU_1	under bedrock overhang	OPU_1	between boulders, midstream
BCU_2	under bedrock overhang	ACTD_1	bank
BCU_3	under boulder	ACTU_1	under undercut bank
BCU_4	on boulder, midstream	ACTU_2	on fallen log, midstream
ECD_1	under cobble	ACTU_3	under fallen log, midstream
ECD_2	under cobble	ACTU_4	under small cobble
ECU_1	under fallen log, midstream		

Most (54%) of the captured *D. folkertsi* were found under or between rocks, usually cobbles. I found 11% of captures in crevices, 11% under overhanging bedrock or undercut banks, and 11% on or under fallen logs (Table 3).

TABLE 4. Capture data, species richness, and relative abundance of *Desmognathus folkertsi* for each site.

Site	*D. folkertsi* captures per hr effort	*D. folkertsi* captures	Species Richness	relative species abundance of *D. folkertsi*
SCD	0.24	1	2	0.20
SCU	0.00	0	0	0.00
CCD	0.00	0	1	0.00
CCU	0.00	0	3	0.00
CC2D	0.00	0	2	0.00
CC2U	0.75	1	2	0.20
OCD	NA	1	4	0.07
OCU	NA	1	4	0.07
ChCD	0.45	1	4	0.13
ChCU	0.68	2	4	0.09
BCD	0.56	3	6	0.08
BCU	NA	5	4	0.17
WLD	0.00	0	0	0.00
WLU	0.00	0	1	0.00
ECD	0.73	3	4	0.33
ECU	0.59	2	5	0.13
FCD	0.33	2	5	0.13
FCU	0.38	1	4	0.08
UTD	0.71	3	6	0.19
UTU	0.47	2	5	0.25
OPD	0.45	1	5	0.20
OPU	NA	1	4	0.08
ACTD	0.63	1	4	0.08
ACTU	0.84	5	2	0.50

I captured the highest number of potential *D. folkertsi* at the upstream sites on Brooks Creek and Abes Creek Trib. Both sites were relatively undisturbed by human development and were dominated by high bedrock waterfalls. ACTU (the site with the waterfall) had the highest relative abundance of potential *D. folkertsi* specimens, while BCU had a relative abundance of 0.17, only slightly higher than the average of 0.12 (Table 4).

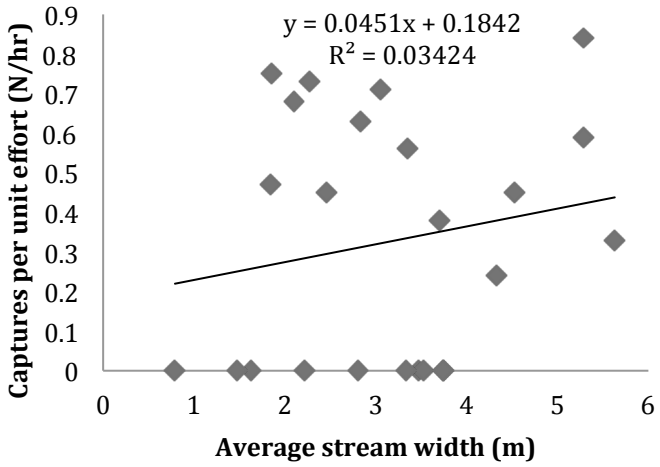

FIG.1. Relationship between stream width and the number of potential *D. folkertsi* specimens captured.

At 0.03, the correlation coefficient of the relationship between stream width and *D. folkertsi captures* indicates the two have no significant correlation (Fig. 1).

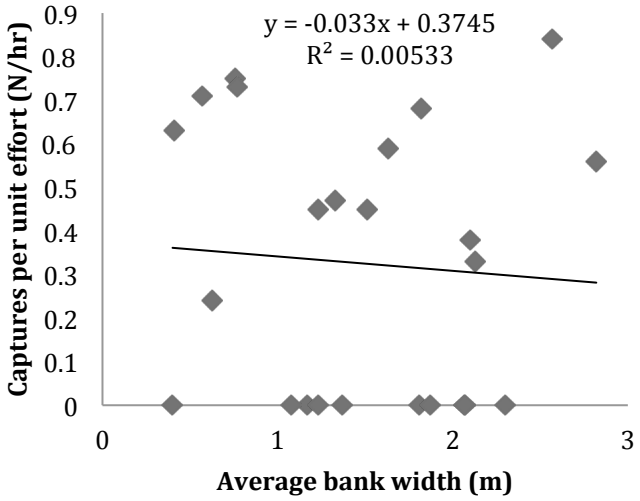

FIG. 2. Relationship between bank width and the number of potential *D. folkertsi* specimens captured.

At 0.005, the correlation coefficient of the relationship between bank width and *D. folkertsi* captures indicates the two have no significant correlation (Fig. 2).

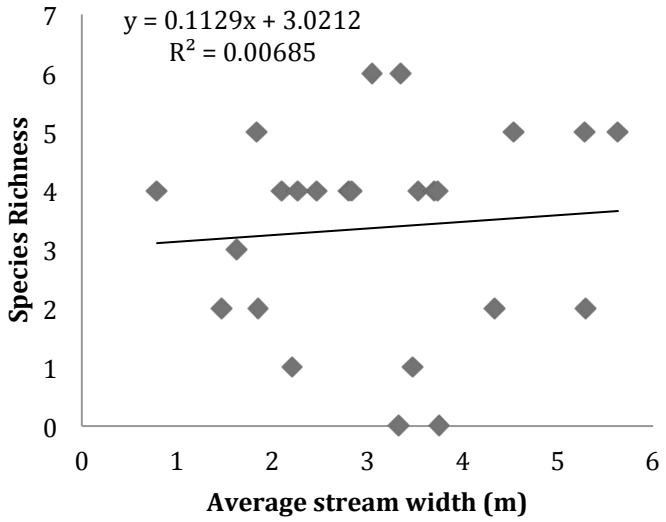

FIG. 3. Relationship between stream width and the species richness of all captured specimens

The correlation coefficient between species richness and average stream width was 0.006, indicating that the two have no significant correlation (Fig. 3).

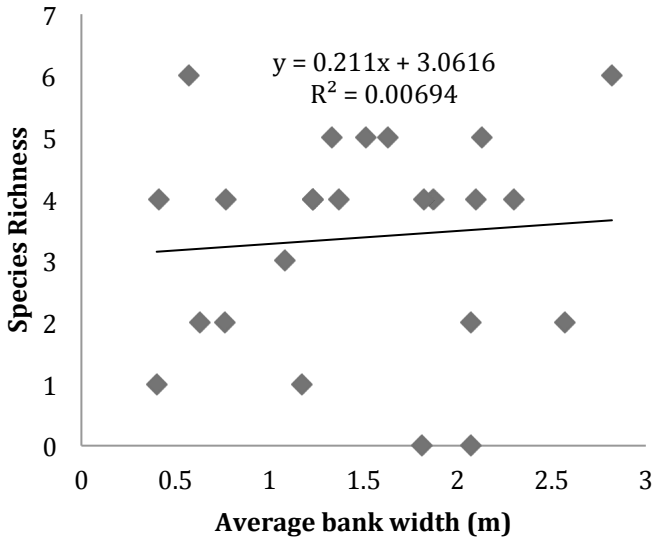

FIG. 4. Relationship between bank width and species richness of captured specimens.

I also found no significant correlation between bank width and species richness. The correlation coefficient here was 0.007 (Fig. 4).

DISCUSSION

Captures per unit effort did not appear to be correlated to stream width or bank width (Figs. 1-4). Instead captures per unit effort seemed most correlated to amount of human impact

evident at sites and the types of cover available. I found most of the potential *D. folkertsi* under partially submerged cobbles in running water, though this could be due to some bias associated with the researchers' inability to move boulders by hand. Significant percentages were also found in in bedrock crevices several centimeters from running water, or perched on rocks jutting out from waterfalls (Table 3). I found few salamanders (and no *D. folkertsi*) at CC and WL. Both the upstream and downstream CC sites were within sight and earshot of the road with poor vegetative cover and a relatively open canopy.

I had the highest number of potential *D. folkertsi* captures at BC and ACT. These were both remote sites with high bedrock waterfalls. The stream channel at CCU, which had both a low species richness and capture rate, contained old tires and other human refuse, and it abutted well-maintained fields that were likely treated with pesticides. The reach at CCD followed a quiet but regularly trafficked roadway. WLU featured wire cages full of rip rap and erosion management tarp. This site was also just downstream of a dammed pond, which could have contributed to deoxygenation of the water. This would explain the poor species diversity at WL. I encountered two adult eastern newts and no salamanders at either the upstream or downstream site.

Scottsman Creek was relatively isolated; it was out of sight and earshot of the road except for a high bridge passing over the downstream limit of SCD. Vegetative cover was prolific, and the stream was composed primarily of riffles passing over partially submerged cobbles. However, I discovered poor species richness and low species abundance at both SCU and SCD. A logging operation occurring farther upstream could have contributed to this.

CONCLUSION

The range of *D. folkertsi* does extend into the Chatooga River drainage. I encountered *D. folkertsi* sympatrically with other members of the *Desmognathus* genus, especially *D. quadramaculatus*. These results must be confirmed with genetic testing, but many of the sampled specimens possessed clear diagnostic features that distinguish them from *D. quadramaculatus*, including a dark belly at small size, a brown V-shaped patch on the front of the head, and brown and black dorsal patterning. I have also confirmed that the habitat preferences of *D. folkertsi* consist of partially submerged cobbles and small boulders in fast-flowing water, bedrock crevices, and high-energy waterfalls.

ACKNOWLEDGEMENTS

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HABITAT AND DISTRIBUTION OF FRASER MAGNOLIA

ROBERT NEIL CURTIS

Abstract. The species *Magnolia fraseri* is found only within the southern Appalachians but would only be described as common within microhabitats. It requires very specific conditions in order to thrive but in-depth literature detailing these conditions and the species' distribution is not available. The objective of my study was to visit the locations in which its populations occur and describe what factors gave rise to its presence there. I collected data on 183 individuals in 12 sampling sites including circumference, coordinate location, and elevation. I then mapped and graphed this data using ArcGIS 10.3 and Microsoft Excel in order to study the specifications of its distribution. Its ideal growing conditions are moderate to high elevation, on north to west facing slopes, and in moist but well-drained soils on slopes. This species absolutely must have a wealth of sunlight to thrive and can outcompete other woody species if given these conditions. This study helps to understand the niche of this species in the southern Appalachian region.

Key words: distribution; Fraser magnolia; Magnolia fraseri*; habitat analysis; southern Appalachians; western North Carolina.*

INTRODUCTION

During the last glaciation diversity in the *Magnolia* genus boomed, especially in places like China and the subtropics. Following this period, many of the magnolia species in what is now the United States were largely lost due to the drying of the west (J. Johnston, pers. communication). *Magnolia fraseri*, commonly referred to as Fraser magnolia or mountain magnolia, is one of three magnolia species remaining in western North Carolina. While many people who reside in the region may describe the species as common, this is not wholly true. *Magnolia fraseri* is more accurately described as locally common or common within specific habitats as it is not widespread nor is it evenly distributed throughout its range (J. Johnston, pers. communication). The species' range is restricted almost exclusively to the Appalachians of the eastern part of West Virginia, western Virginia, east Tennessee, and northeastern Georgia, and western North Carolina is the epicenter (*Magnolia fraseri* Walt).

Magnolia fraseri accounts for less than 10% of all canopy tree species in the southern Appalachians, yet it is not easily overlooked (*Magnolia fraseri* Walt). *Magnolia fraseri* is a moisture-loving species and mature trees often have several large trunks, which are covered by smooth bark. Its most distinguishing characteristic is its large leaves, usually 10 to 15 inches long, which are drawn into lobes commonly referred to as "ears" at the base of each leaf. The tree produces large, saucer-shaped white flowers that are followed by 3-4 inch cone-shaped fruits containing red, nutritious seeds that are eaten and dispersed by wildlife such as birds and rodents (Weaver 1981). These features make it one of the best ornamental species in the magnolia family.

While the presence of this species has been long documented, first by William Bartram c. 1775, little research has been conducted on the details of its occurrence in the region (Weaver 1981). As specialist species such as *M. fraseri* could be in danger from climate change, a scientific inquiry into this species is both warranted and potentially rewarding. This study aims to quantify and describe the distribution of *M. fraseri* by investigating the elements of its habitat as it occurs in the southern Appalachians.

METHODS

I surveyed areas in Macon and Jackson counties, North Carolina, and Rabun and Habersham counties, Georgia for *Magnolia fraseri* (Fig. 1). The three points in Habersham County, GA are the southernmost extent of this magnolia species. I collected data from late August through November 2015.

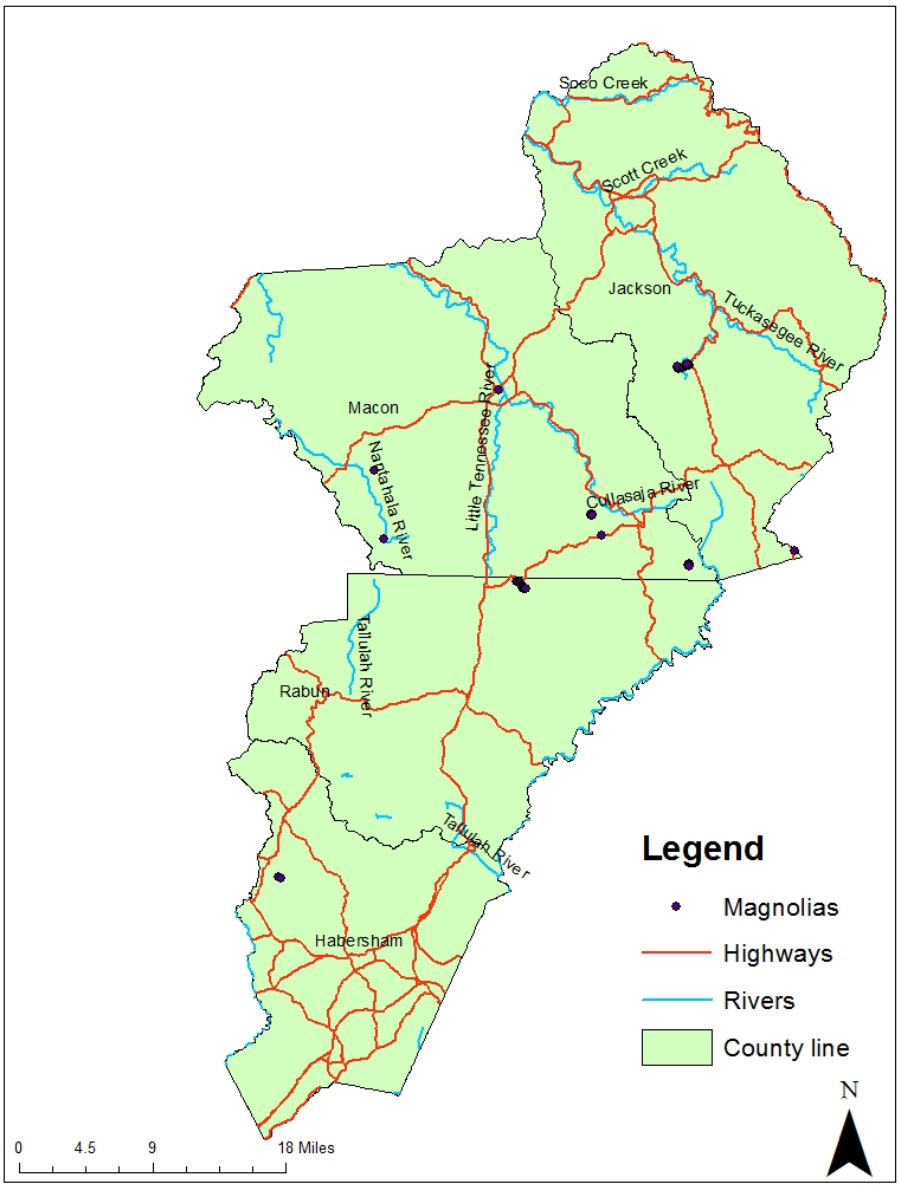


FIG. 1. Extent of sampling area showing counties in the southern Appalachians. Locations of *M. fraseri* in this study represented by black points.

My research partner and I surveyed sites selected by our mentor, Mr. Jack Johnston, or recommended by others. At each sampling location, we followed either an established trail or one blazed by our mentor and located target trees for data collection. I tried to reach trees that

were present or expected to be so based on local conditions. I collected data on mature trees with a diameter at breast height (dbh) of four inches or larger. When I reached a magnolia large enough to have its data collected, I first marked its location with a (Garmin® Montana) GPS unit and measured the circumference with a measuring tape approximately 1.5 m from the ground. For each site, I took note of slope and aspect. The GPS points were exported using DNR Garmin and mapped using ArcGIS 10.3.

I conducted a survey of the habitat and plant community in the area where I found each Fraser magnolia tree. In particular, I noted what made that site hospitable for growth of the target species, including amount of sunlight to reach the ground during daylight hours, the quality and quantity of leaf litter, elevation, and proximity to water.

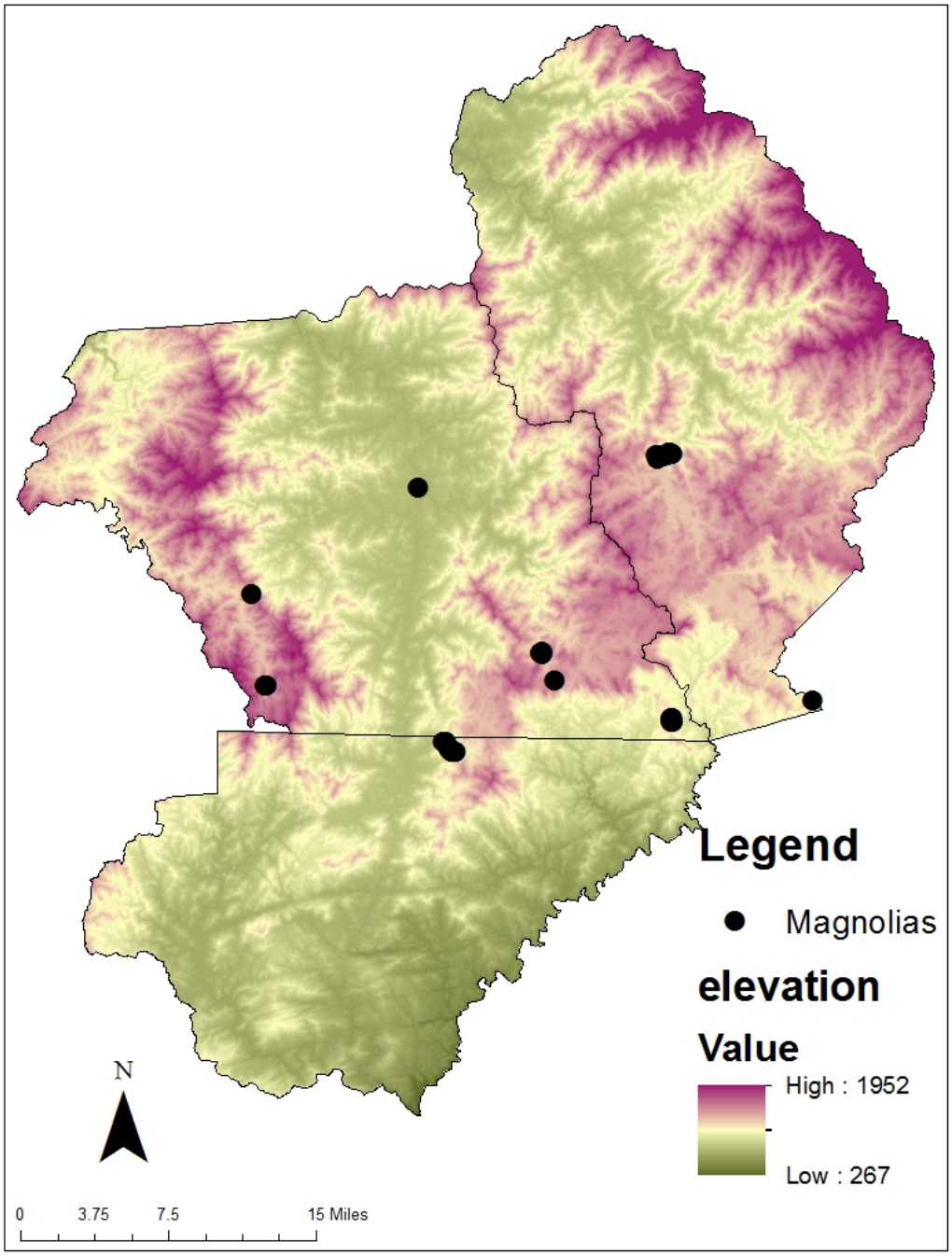
I collected seeds from each magnolia while seeds were present in the early fall. I used a pole pruner to cut seed pods from the branches while a second person caught the seed pods as they fell from the tree. I also collected pods and individual seeds from the forest floor and used these to gather seed viability data for each seed collection site. In order to determine seed viability, I extracted the seeds from the pods and soaked them in a container of water for an hour. After an hour, floating seeds were deemed unviable, counted, and discarded. Viable seeds were those that sank and these were stored and given to the Magnolia Society. Due to an accidental occurrence, much of the seed data was lost and will not be included in the remainder of this study.

Table 1. Sample site tree data.

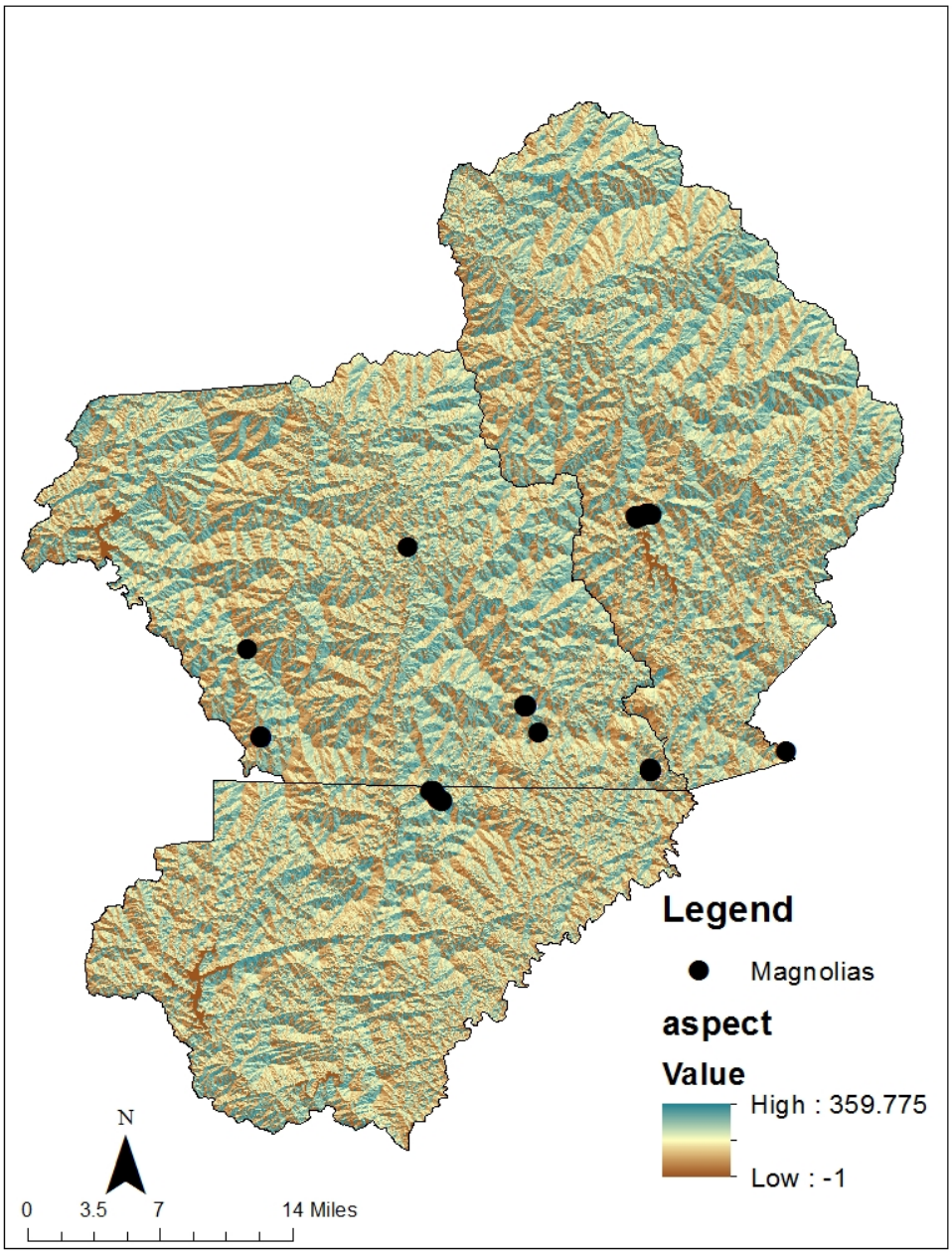
Site Number	Date	Location	Trees Sampled	Largest Circumference (cm)	Average Circumference (cm)
1	9/3/2015	Lee Creek Road Franklin, NC	17	128	70.88
2	9/4/2015	Wayah Gap Road Franklin, NC	3	145	99
3	9/4/2015	Forest Service Road (711) Franklin, NC	4	172	115.5
4	9/4/2015	Needmore Road Franklin, NC	3	118	84.33
5	9/18/2015	North Fork Creek Franklin, NC	33	121	82.06
6	9/18/2015	Hodge's property Franklin, NC	31	174	88.23
7	9/24/2015	State Road 1158 Cullowhee, NC	17	141	85.76
8	10/9/2015	Tahoe Lane Sky Valley, GA	6	132	85.67
9	10/23/2015	Turtle Pond Road Highlands, NC	13	115	57.31
10	10/30/2015	Ellicot's Rock Highlands, NC	23	157	80.09
11	11/6/2015	Georgia Piedmont, GA	3	26	20.33
12	11/19/2015	Standing Indian Franklin, NC	10	174	118.2

RESULTS

I collected data on a total of 163 mature trees across 12 sampling sites. I attained GPS data for 83 individuals, just over half of the measured trees. Using DEM data from Landfire (2010) I was able to plot the magnolia populations in relation to elevation, aspect, and slope (Fig. 2).



(a)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14 Miles

(b)

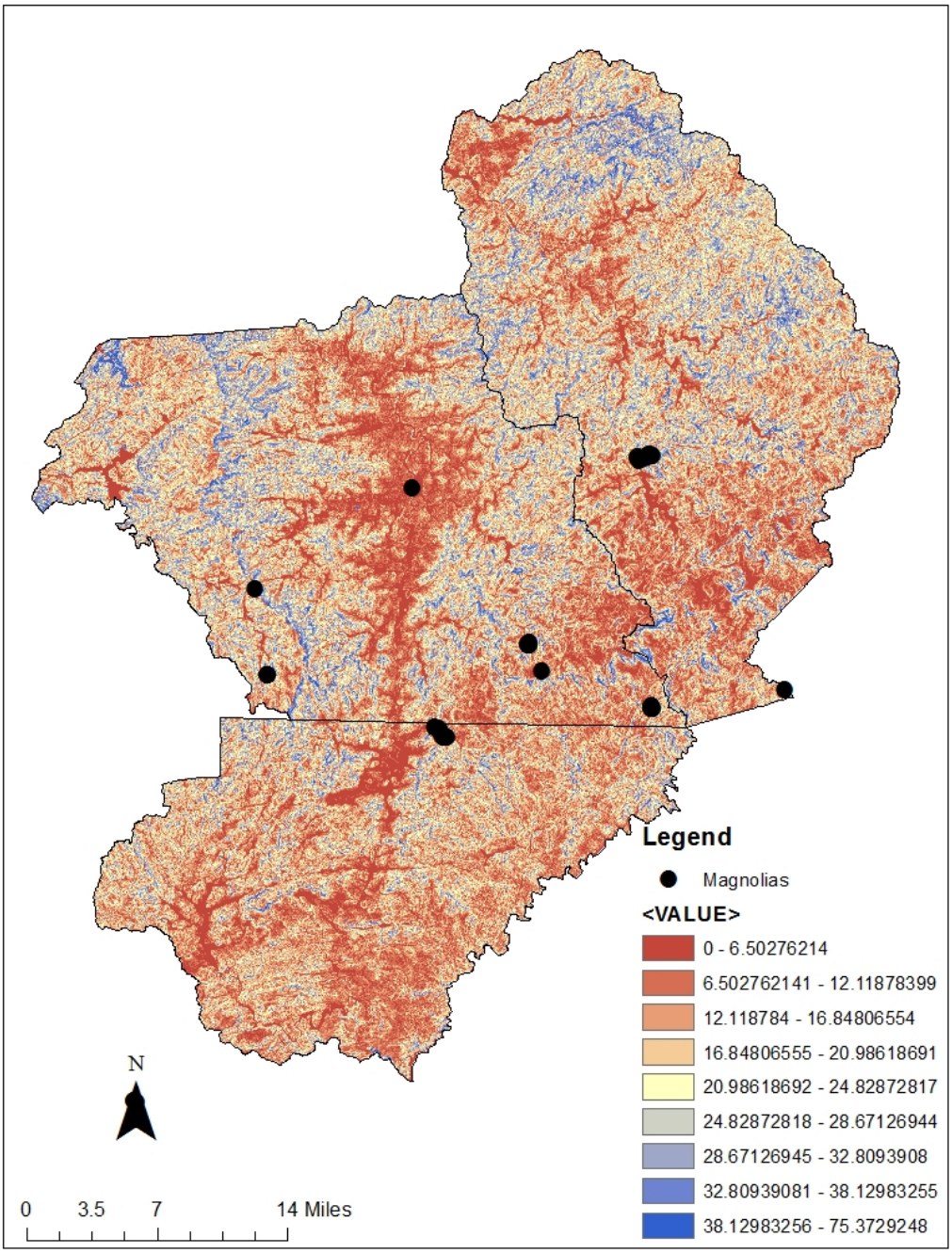

FIG. 2. *Magnolia fraseri* habitat: (a) elevation (M), (b) aspect (degrees from North), (c) slope (degrees). Black points represent *M. fraseri* individuals.

I used the maps to extrapolate and analyze the range and mean for slope, aspect, and elevation. The minimum elevation was 647 m while the maximum elevation was 1200 m averaging about 939 m. Aspect was predominantly western, or northern while also ranging towards the southwest. The average slope was 15.65° and ranged from 0.95° – 32.9°.

Table 2. Habitat ranges of *M. fraseri* in the southern Appalachians

Habitat	Range	Mean
Elevation (m)	647 -1200	937.5
Aspect (degrees from North)	0 - 351.7	232.38
Slope (degrees)	0.95 – 32.9	15.65

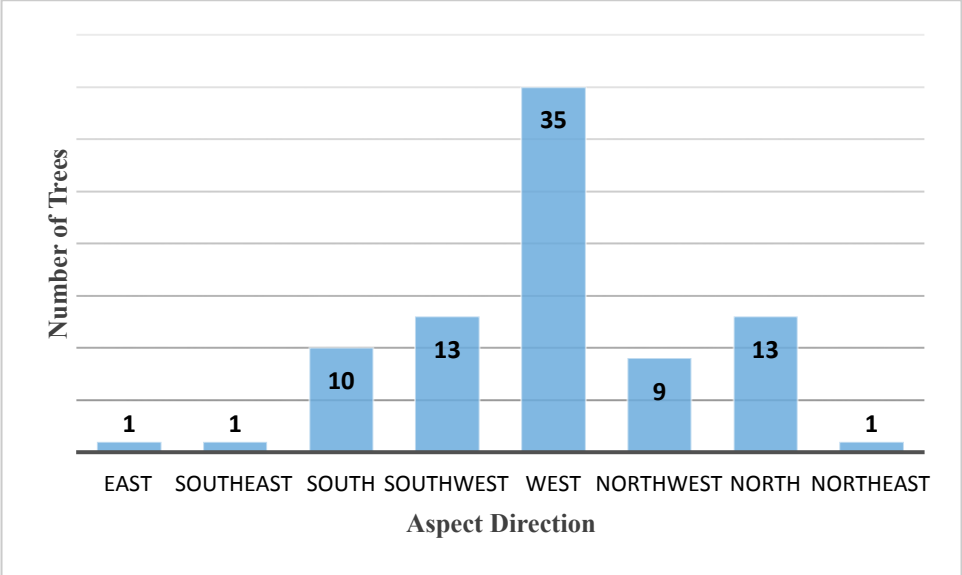

FIG. 3. Aspect frequency of *M. fraseri.*

DISCUSSION

Magnolia fraseri requires a complexly specialized set of conditions in order to become established. The species is found in the southern Appalachians at moderate to high elevations (647 m -1200 m), although the three Georgia piedmont trees are at lower elevations. This may indicate that this magnolia thrives in the cooler growing season temperatures of higher elevations, which would keep the forest floor, and therefore its roots from drying out, and this would be an interesting hypothesis to test. The two sites with trees with the largest average circumference (Forest Service Rd 711 and Standing Indian) were also the two highest elevation areas.

Aspect is very important for the habitat of this tree. The peak abundance according to mapping was on western facing slopes with numbers ranging to both the north and south. This result is somewhat unexpected as the field analysis of aspect indicated that trees were located on north to northwestern slopes. This difference in results may be partially attributed to the lack of GPS data for all sites and that south or southwestern slopes can sometimes function as northern slopes in terms of forest conditions (J. Johnston, pers. communication). This can arise from the mountain shadow effect that can reduce sunlight on typically sunnier southern slopes. Moisture levels usually indicative of northern slopes can also occur on southern slopes with proximity to water sources such as streams or seeps. North (and northwestern) facing slopes are usually

preferred by these trees because this aspect decreases wind exposure and permits the tree base to remain out of the sunlight for longer. This is meaningful because the canopy still receives sunlight while the forest floor retains moisture. This increased moisture results in all trees producing higher biomass leading to more decaying leaves meaning that there will be greater nutrition available in the future and further moisture retention (J. Johnston, pers. communication). Future research might focus on the functional features of these aspects.

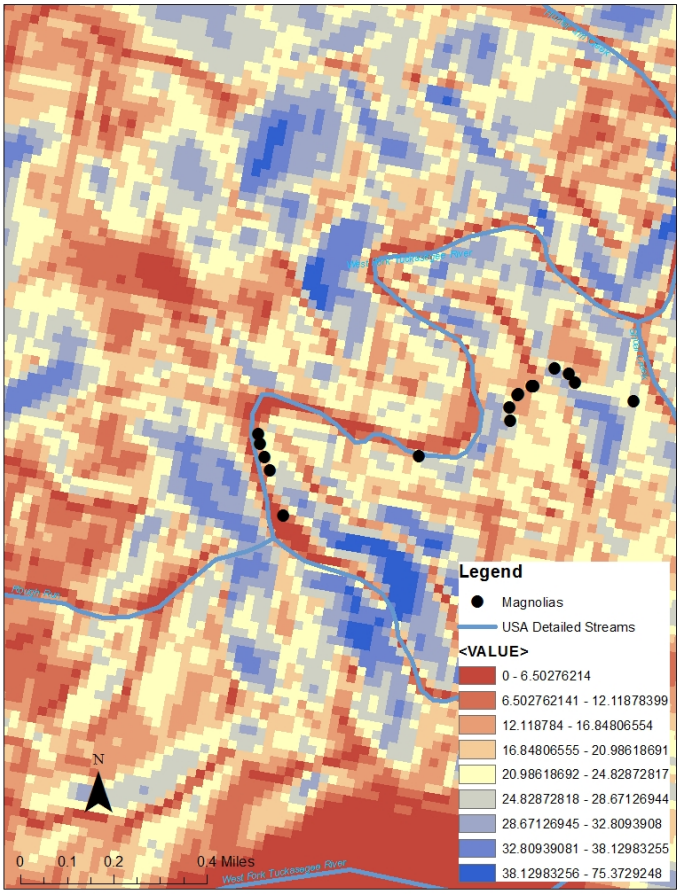

FIG. 4. State Road 1158 site showing magnolia proximity to stream and steeper slopes.

A relatively moderate slope is necessary for the magnolias because while they require moist earth, the soils also need to be well drained (J. Johnston, pers. communication). Slope analysis revealed that Fraser magnolias occur on a range of slopes, from gentle to moderate slopes, with an average of 15.65 degrees. This is a shallower slope average than I expected as field excursions usually involved scrambling up mountainsides on all four limbs to measure our trees. However, upon further investigation, this is due, in part, to the locations of our sampled trees. They occurred on the edges of much steeper slopes as is indicated in both field notes and a fine scale inspection of the slope maps (Fig. 4). *Magnolia fraseri* prefers these areas because they often contain either a water body (such as the stream in Fig. 4) or increased canopy exposure to sunlight. Water and sunlight are two limiting resources that for this species as they become established in the forest. Fraser Magnolia is an opportunist species seeking to use any advantage (such as a canopy opening) over its competition. When forest patches were cleared,

M. fraseri was one of a few minor canopy trees, including black locust and Carolina silverbell, that accounted for the largest portion of net primary production in the clearing (Phillips and Shure 1990). This species absolutely must have a wealth of sunlight to thrive and can outcompete other woody species if given these ideal conditions.

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EXPLORING THE CORRELATION BETWEEN *CASTANEA DENTATA* SIZE (DIAMETER AND HEIGHT) AND THE PRESENCE OF *CRYPHONECTRIA PARASITICA*

KATIE FUREY

Abstract. American chestnuts (*Castanea dentata*), a keystone forest tree species in the Appalachian Mountains during the turn of the century, have been functionally extinct for a century in their native range due to the invasive chestnut blight fungus (*Cryphonectria parasitica*). The roots, however, remain alive and the American chestnut continues to form sprouts in clumps (coppice) that are later killed by the chestnut blight fungus. Understanding where natural population levels of the chestnut blight pathogen inoculum is heavy may be important to restoring resistant hybrids of American chestnut to its native range. Knowledge of pathogen establishment on sprouts, especially in heavily concentrated areas, may provide valuable information for avoiding these areas when planting resistant American chestnut hybrids. To accomplish this objective, average numbers of sprouts per location, sprout height and diameter, and disease levels (cankers) were collected from up to 150 coppice clusters per site. I sampled 450 American chestnut coppice sprouts across three sites: two in Highlands, NC, and one at in the Great Smoky Mountains National Park. Results from the study, across locations and by locations, showed American chestnut sprouts of greater height and diameter had significantly greater disease levels than smaller American chestnut sprouts. This indicates that disease levels increase with larger coppice and potentially provide heavy inoculum concentrations if not managed. Furthermore, a bark sampling and pathogen isolation study in the laboratory confirmed that the visual disease ratings in the field were strongly supported by laboratory isolation data based on a Chi-square analysis. If American chestnut trees with resistance can grow large enough to fruit before succumbing to blight, the resistant trees can spread and eventually extend through their former range in eastern hardwood forests.

Key words: American chestnut; chestnut blight; Castanea dentata, Cryphonectria parasitica*; height; diameter; disease; fungus; fungal disease*

INTRODUCTION

The American chestnut (*Castanea dentata)* once composed 25% or more of eastern hardwood forests (Burnham 1988) and was immensely important for wildlife and people (Anagnostakis 2000), producing a reliable yearly nutritious mast which is rare among nut bearing trees (Burnham 1988). American chestnut wood is highly rot resistant, straight-grained, easily worked, and high in tannin (Burnham). These qualities made American chestnut timber valuable and useful for a variety of purposes such as, fencing, heavy construction, furniture, musical instruments, and tanning heaving leathers (Burnham). American chestnuts have strong coppicing ability and sprouts grow from stumps rapidly producing high quality wood, thus replanting after logging was unnecessary (Burnham).

In 1904, American chestnuts began dying in New York City. It was determined that chestnut blight (*Cryphonectria parasitica,* formally *Endothia parasitica)* was the culprit (Burnham), accidentally introduced on imported blight-resistant Asian chestnut trees sometime in the late 1800's (Mannion 2011). Chestnut blight is an ascomycete fungus that enters through a wound in the bark, quickly producing a canker that kills the cambium all the way around the twig, branch, or trunk (Burnham, 1988, Anagonstakis 1992). The fungus releases two kinds of spores: short-lived condia, produced in sticky masses and spread by insects, animals, and rain and wind-borne ascospores, which are expelled for years after the tree is dead (Burnham, 1988). All efforts to control the chestnut blight – including chemical treatments and clearing and

burning chestnut trees around infection sites – were unsuccessful (Anagnostakis 1992). Within 50 years of the discovery of blight it had spread throughout the entire natural range of the American chestnut and killed virtually all of the trees, leaving the American chestnut functionally extinct (Childs, 2002, Vandermastm 2007). Once an overstory tree, the American chestnut has been reduced to a shrub, drastically altering the ecology of eastern hardwood forests (Childs 2002). Chestnut blight doesn't affect the root system, therefore American chestnut is not likely to vanish completely, however new shoots sprouting from the stump rarely survive to fruiting age before becoming blighted and dying (Childs). Many chestnut seedlings have survived for at least a century and have root collar sprouting characteristics designed to insure that "old seedlings" remain juvenile (Paillet 2005).

Current efforts to reintroduce the American chestnut to eastern hardwood forests include backcrossing with Chinese chestnuts (*Castanea sativa*) to produce a hybrid that has the physiological characteristics of the American chestnut and the blight resistance of the Chinese chestnut (TACF, 2012). The American Chestnut Foundation began this program in 1989, and the goal is to produce a tree that is 94% American and 6% Chinese (TACF). Each backcross reintroduces the non-resistance of the American chestnut into the genome, so intercrosses of two American chestnuts are required to minimize this effect and increase blight resistance (TACF). After each backcross, trees that have Chinese chestnut characteristics are removed, and the remaining trees are tested for blight-resistance between two and five years of age (TACF). Those that have the most resistance are selected for breeding and all others are removed (TACF). Under careful breeding protocols, three backcrosses and two intercross are required equaling six generations or about 30 years (TACF). Fifth-generation trees are growing, and the final step occurred when these trees open pollinated with each other to create the sixth generation of trees that are 94% American chestnut which are morphologically similar to wild American chestnuts and have significant blight resistance (TACF). In 2009, the first of the sixth generation American chestnuts were planted in real forest environments as a test phase to study how they survive in the wild (TACF). Planting of the sixth generation trees has occurred in national parks, forests and private lands (Horton, 2010). Efforts to reintroduce the American chestnut to its former range not only include developing blight resistance but also weakening chestnut blight by introducing hypovirulence strains that dampen the effect of chestnut blight (Horton). This effort has been very effective in Europe but less effective in the United States, perhaps because there are more strains in which both blight and hypovirulence occur in the US than in Europe and because European chestnuts have more resistance to blight than American chestnuts (Horton).

There has been little research on factors that affect the presence of chestnut blight in American chestnuts, particularly on the correlation between American chestnut size and the presence of blight. However, there have been several studies that show evidence for the positive correlation between tree size (height, diameter, or height and diameter) and the infection of fungal diseases (Falk et al. 1989, Griffin et al. 2003, McCann et al.).

I explored the correlation between American chestnut size – based off height and diameter – and the presence of blight. Chestnut blight infects the tree by entering through injuries and wounds in the tree (TACF 2012). I predicted that American chestnut size (height and diameter at one foot height) will be positively correlated with presence of blight, because the older and larger the chestnut trees get, the more likely they will be wounded, allowing for blight infection. These findings can be useful to further our fundamental understanding of chestnut blight infections and restore the American chestnut to its former range. Specifically, these findings can aid researchers studying hybrid and resistant American chestnuts to determine the

appropriate size range of the tree to test for blight resistance, instead of testing for resistance at a certain age.

MATERIALS AND METHODS

Data Collection

I collected field data at three research sites. Site #1 is located on the south side of Satulah Mountain and owned by Robert Haynes (BH) in Highlands, North Carolina; site #2 is located in the gated community of Ravenell on Little Creek Road and owned by a private property owner (LC) in Highlands, North Carolina; site #3 is located at Purchase Knob (PK) in the GSMNP, Waynesville, North Carolina.

At BH and PK I randomly sampled 150 American chestnut coppices and at LC I randomly sampled only 100 American chestnut coppices. LC was a smaller site, and thus had fewer available American chestnut coppices to sample. A coppice was defined as all of the individual stems greater than one foot in height that originated from the same root cluster. I used a Garmin Montana 650t hand-held GPS to record a location for each chestnut coppice sampled and flagged the clump with an identification number (1-100 or 1-150) so I could easily identify it for future data collection. Stem height was scored based on a five-foot scale score [1= 1-5ft 2= 6-10ft 3= 11-15ft 4= 16-20ft 5= 21-25ft 6= >25ft], and diameter at one foot height was recorded for each stem in the coppice. Since many of our stems were below breast height, I decided that measuring diameter at 1ft height would allow us to have a uniform sampling technique. Each stem in a coppice was assessed for the presence of chestnut blight based on the chestnut blight index I created(Table. 1). If I was unsure whether a stem had blight or not I rated the stem as 'other'.

TABLE 1. Chestnut blight index

Rating	Blight Description
1	Canker with sporulation
2	Canker without sporulation
3	Swollen
4	Sunken
5	Other
6	Sporulation

Following the initial field data collection – to verify the reliability of the visual chestnut blight identification – all chestnut coppices determined to have blight were entered into a number randomization tool to create a simple random sample of 20 chestnut clumps with blight. For each of the clumps randomly selected I took three bark tissue samples from blight-infected areas for a total of 60 samples. For all chestnut clumps that were rated as 'other', bark tissue samples were taken to verify with culturing whether the sample contained chestnut blight or not. To do this, I sterilized a box cutter, cut a small chunk of bark tissue out, and placed in a box corresponding to the correct tree number. This was repeated twice for a total of three bark tissue samples per chestnut clump. Bark samples were stored for 1-5 days in a refrigerator, then were placed in 20% sodium hypochlorite solution for 1 minute, then plated on to potato dextrose agar plates. The cultures were incubated at room temperature for 14-21 days and then visually confirmed for the presence of the *Cryphonectria parasitica*.

Statistical Analysis

Means of American chestnut sprout heights, diameters, and chestnut blight hits were analyzed as a series of combined experiments – combined across site – using the GLM procedure of SAS (SAS Institute, Cary, NC). Means of American chestnut sprout heights, diameters, and chestnut blight hits were separated using Fisher's protected least significant difference if significant (LSD, p=0.05). Chi-square analysis was run to compare 20 randomly selected coppices as visually identified as blighted versus identification by culture bark isolation data. The purpose of this last analysis is to determine the accuracy of the visual blight observations.

RESULTS

A total of 150 sprouts are found at BH and PK sites, but due to limited forest I only recorded 100 at LC (Table 2). LC site has the greatest percentage of infected coppices (46%), and also has the greatest height and diameter means (Table 2). BH site has the second greatest percentage of infected coppices (44%), but had the smallest height mean and the second greatest diameter mean (Table 2). PK has by far the lowest percentage of infected coppices (25.33%), but has the second largest height mean and the smallest diameter mean (Table 2).

TABLE 2. Number of American chestnut coppices, number of coppices infected, the percent of the coppices infected with chestnut blight, and the height and diameter means at each sampling site.

Plot Type	Number of Coppices	Number Infected	Percent Infected (%)	Height[A]	Diameter (cm)
LC	100	46	46	2.38A[B]	3.06A[B]
PK	150	38	25	1.90B	1.31C
BH	150	66	44	1.45C	2.04B
(LSD)				0.2124	0.2881

[A] Height was measured using a scale: 1=1- 5 ft, 2= 6-10ft, 3= 11-15ft, 4=16-20ft, 5=21-25ft, 6>25ft.
[B] Mean data having different letters for heights or diameters between locations were significantly different ($P \leq 0.05$) using Fisher's LSD.

The cultures from the bark samples confirmation study were used to verify the reliability of the field identifications of blight infection. Based on the cultures, the Chi-squared analysis was non-significant from each location, indicating that field blight identification and culturing results were closely aligned, as in they did not differ from a 1:1 ratio (Table 3). Also, following a greater than 70% (14/20) accurate visual blight identification for our cultured samples, I assumed that our visual blight identifications in the field were correct (Table 3). Therefore the field blight identifications are considered to be accurate.

TABLE 3. Chestnut blight field identification accuracy based on cultures and Chi-squared values for each sampling site.

Plot Type	Accuracy	X^2 Value
LC	20/20	1
PK	19/20	0.6219
BH	17/20	0.8728

According to the Fisher's LSD test, means data for heights or diameters between locations were significantly different (P≤ 0.05) for American chestnuts with no chestnut blight (Table 4). According to statistical analysis, American chestnuts with blight sampled at LC have significantly greater (P≤ 0.05) heights and diameters than at PK or BH, and PK diameters are significantly greater (P≤ 0.05) than at BH (Table 5).

TABLE 4. American chestnuts with no chestnut blight stem height and diameter means.

Plot Type	Height [A]	Diameter (cm)
LC	2.07A[B]	2.45A[B]
PK	1.39B	1.25C
BH	1.81A	1.99B
(LSD)	0.2588	0.3611

[A] Height was measured using a scale: 1=1- 5 ft, 2= 6-10ft, 3= 11-15ft, 4=16-20ft, 5=21-25ft, 6>25ft.
[B] Means data for heights or diameter between locations having different letters were significant different (P>0.05) using Fisher's LSD Test.

TABLE 5. American chestnut with chestnut blight stem height and diameter means.

Plot Type	Height[A]	Diameter (cm)
LC	2.74A[B]	3.78 A[B]
PK	1.63B	1.51C
BH	2.01B	2.09B
(LSD)	0.3901	0.5098

[A] Height was measured using a scale: 1=1- 5 ft, 2= 6-10ft, 3= 11-15ft, 4=16-20ft, 5=21-25ft, 6>25ft.
[B] Mean data having different letters for heights or diameters between locations were significant greater (P>0.05) using Fisher's LSD Test.

When American chestnut tree height was used as a dependent variable against height and diameter means, tree data at all three locations were significantly different (P≤ 0.05), indicating a strong correlation between height and diameter (Table 6). Thus, as height increases, diameter increases except for PK which tended towards smaller sprout diameters that at other locations (Table 6). When American chestnut height was used as a dependent variable against the presence of blight, the presence of blight was significantly different (P≤ 0.05) (Table 7). Therefore the statistical analysis shows increased height is strongly correlated with increased diameter and presence of blight (Table 6; Table 7).

TABLE 6. American chestnut stem height and diameter means at each sampling site.

Plot Type	Height[A]	Diameter (cm)
LC	2.38A[B]	3.06A[B]
PK	1.90B	1.31C
BH	1.45C	2.04B
(LSD)	0.2124	0.2881

[A] Height was measured using a scale: 1=1- 5 ft, 2= 6-10ft, 3= 11-15ft, 4=16-20ft, 5=21-25ft, 6>25ft.
[B] Means data followed by a different letter for each locations were significantly different (P≤ 0.05) using Fisher's LSD Test.

TABLE 7. American chestnut stem height and diameter means based on presence of chestnut blight.

Presence of Blight	Height[A]	Diameter (cm)
Yes	2.10A[B]	2.48A[B]
No	1.67B	1.75B
(LSD)	0.1763	0.2392

[A] Height was measured using a scale: 1=1- 5 ft, 2= 6-10ft, 3= 11-15ft, 4=16-20ft, 5=21-25ft, 6>25ft.
[B] Mean plant height and diameter followed by a different letter were significantly different (P≤ 0.05) using analysis of variance (ANOVA).

DISCUSSION

Overall, the results of this study supported my hypothesis that there is a positive correlation between American chestnut size and the presence of chestnut blight. The height and diameter means of all 450 trees sampled are both greater for trees that were infected with blight than for trees that were not infected with blight. However, out of the 450 trees sampled, 150 trees were infected with blight, so the data may be skewed due to the smaller sample size when compared to the 300 trees sampled not infected with blight. Statistical analysis shows that increased American chestnut height is strongly associated with increased diameter and the presence of blight. Thus, American chestnut tree size (height & diameter) is strongly correlated with the presence of chestnut blight. As American chestnut size increases, the presence of chestnut blight increases on average. Larger trees have more surface area to sustain bark injuries for blight to enter through and infect the tree.

Further study of the American chestnut is needed to ensure an effective reintroduction of the American chestnut back into the eastern hardwood forests. Specifically, further scientific research efforts should look at the habitat preference of American chestnuts so that resistant chestnuts can be planted in their preferred habitat type, which will allow them the best chance at survival. During my field work, I also observed that the bark type between American chestnuts varied to some extent and that smoother darker bark tended to have less blight, however further research needs to be conducted to confirm this. This could be beneficial to chestnut restoration efforts as smoother, darker-barked American chestnuts may have some form of resistance to blight or may delay the infection of blight due to fewer cracks in the bark. I believe it is also

worthwhile to study the effects blight has on other species it can infect, such as chinquapin (*Castanea pumila*) and some oak species (*Quercus spp*.), because there are few scientific studies on the effect of blight on these species; it could enhance the knowledge on chestnut blight (Missouri Botanical Garden). Knowing how chestnut blight affects species with some resistance to it will be useful in many ways when studying resistant American chestnuts. Determining the effects of chestnut blight on chinquapin and oak species with some resistance in different scenarios – such as high/low levels of blight, the presence of different strains of blight, and different environmental conditions – can be useful for predicting how certain scenarios will affect resistant American chestnuts in nature. For example, knowing how chestnut blight affects resistant oak species in cold versus warm conditions can provide predictions for how chestnut blight may affect resistant American chestnuts in different climatic conditions. Studying blight resistant species can also help develop a scale of resistance to blight, which can help determine the resistance-level American chestnuts should be in order for American chestnuts to thrive in the wild.

There has been little to no research on the correlation between American chestnut size and the presence of blight, so this research sheds light on blight characteristics and may build on prior information to better understand the infection of blight on American chestnuts. This study gives evidence for the positive correlation between American chestnut size and chestnut blight infection, which can aid researchers studying hybrid and resistant American chestnuts determine the appropriate size of the tree to test for blight resistance.

Conclusion

American chestnuts are functionally extinct in their native range due to the invasive chestnut blight for a century and current efforts to return the American chestnut to its former glory are promising(Anagnostakis 2000). Understanding chestnut blight is essential to restoring the American chestnut to its native range and historical numbers. This study gives evidence for the correlation between American chestnut size and chestnut blight infection. If American chestnut trees with resistance can grow large enough to fruit before succumbing to blight, the resistant trees can spread and eventually extend through their former range in eastern hardwood forests.

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TEMPORAL AND SPATIAL SOIL MOISTURE PATTERNS ON TWO SOUTHERN APPALACHIAN HILLSLOPES

CHARLOTTE HOPSON

Abstract. Spatial and temporal characteristics of soil moisture patterns are key elements in the movement of water through an ecosystem. These characteristics can help create soil moisture gradients that tell us how heterogeneous the spatial distribution of moisture is across a landscape. This study looks at two forested hillslopes in two different catchments within the Coweeta Basin in the southern Appalachians. Point moisture observations were collected in the watersheds in fall to winter 2015. The literature shows that, on hillslopes, wetter areas are found closer to streams (spatial pattern) and steeper moisture gradients are found during dry spells (temporal pattern). This study supports the common spatial pattern found regarding distance to stream and the rest of the research explores the relationship between mean soil moisture, elevation, time, slope, and aspect.

Key words: *catchment; Coweeta LTER; hillslope; soil moisture; southern Appalachians; watershed*

INTRODUCTION

Soil moisture patterns can show great variability or heterogeneity across spatial and temporal scales. Understanding these patterns and the processes that create them is necessary for understanding the movement of water between terrestrial and aquatic systems, the flow of water through a catchment, and the storage of groundwater (Grayson et al., 1997; Tenenbaum et al., 2006). Beyond the movement of water, soil often acts as an intermediary between precipitation and water in important biogeochemical processes, such as nitrogen cycling. Understanding the patterns of soil moisture in a landscape can also point to likely areas of biogeochemical activity (Tague et al., 2010). Additionally, mapping the distribution of soil moisture in systems can help improve environmental models focusing on climate or water circulation by displaying where water is physically within the soil and temporally before, after, and during precipitation events (Grayson et al., 1997; Lin et al., 2006).

It has been shown that the organization of these spatial and temporal patterns can be influenced by a number of factors, including vegetation, precipitation, slope, aspect, etc. Debates over which soil properties or topographic features most control the moisture organization fill the literature, typically concluding that topographic features are more dominant in the formation of moisture patterns, especially during dry periods (Yeakley et al., 1998). Additionally, there is evidence for soil moisture gradients along hillslopes in shallow soil layers, often affected by precipitation events (Yeakley et al., 1998). Like Yeakley et al. (1998), I looked at shallow soil layers on a hillslope, and out of all of the potential variables I chose to focus on the effects of topographic factors on mean soil moisture, including slope, elevation, aspect, and distance to stream. I also looked at season as a temporal factor, as my study period went through fall to the beginning of a winter recharge.

My study involved taking point observations of soil moisture on two hillslopes in two different watersheds and correlating the values with slope, aspect, and distance to the stream measurements. A similar study was done by Lin et al. (2006) in Pennsylvania, but my study differs from that of Lin et al. because I used straight-line transects rather than the scattered-plot method that they employed, so as to better highlight the measured variables. Following Lin et al. (2006) and Yeakley et al. (1998), I expected to find wetter areas near the stream, on flatter slopes, and on north-facing slopes, along with steeper moisture gradients during drier periods.

MATERIALS AND METHODS

Study Site

The Coweeta Hydrologic Laboratory (Coweeta) is located in the Nantahala Mountain Range of the southern Appalachian Mountains in North Carolina (35'N, -83'W) and is home to many forest and hydrologic experiments. The lab itself sits in a bowl-shaped basin (Fig. 1) and is divided into 24 present watersheds. Of the 24 watersheds, 16 are gauged to measure streamflow every 5 minutes, although 32 total weirs exist for this purpose (Swank and Crossley, 1988).

The climate at Coweeta varies from a marine classification to humid subtropical, based on high moisture and milder temperatures. The lab takes climatic measurements, including precipitation, air and soil temperature, relative humidity, wind travel, solar radiation, evaporation, and cloud cover, using climate stations. Overall, Coweeta gets 152 mm of precipitation a month, with greater values in the late winter, as measured by Climate Station 01. Precipitation generally increases with elevation (Swank and Crossley, 1988).

The soils at Coweeta are immature Inceptisols and developed Ultisols. They are moderately acidic and high in organic matter (Swank and Crossley, 1988).

My study focuses on two watersheds within the basin (Fig. 2). The first site, Watershed 14 (WS14), is a 61 ha catchment with elevations ranging from 707 m to 992 m. It is a mixed hardwood riparian zone that serves as a control for many studies at Coweeta, since it has not been disturbed since 1927. Watershed 18 (WS18) is a 12 ha catchment with elevations ranging from 726 m to 993 m. It is also a control watershed with a mixed hardwood community type.

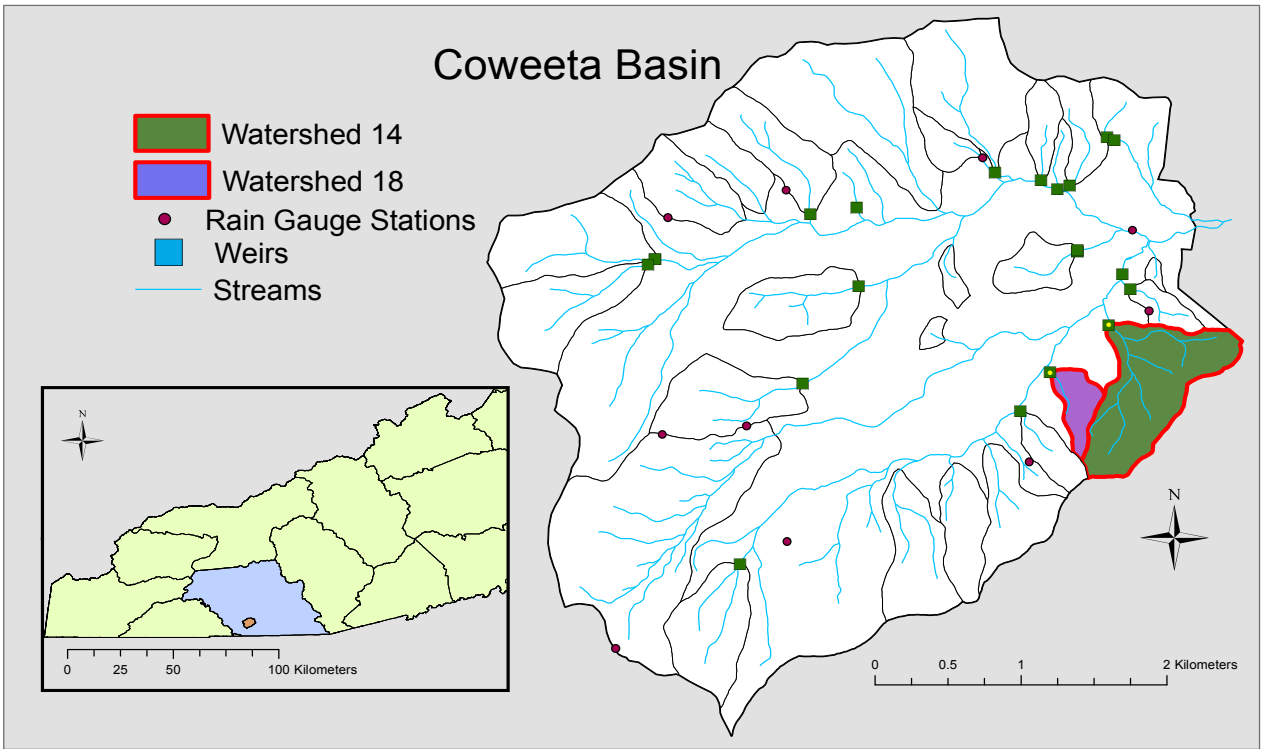

FIG. 1. Map of Coweeta Basin with Watersheds 14 and 18 highlighted (Coweeta Dataset, ArcMap10.3.1™, ESRI 2015).

Field Measurements

To measure synoptic soil moisture content, I looked at 18 plots within WS14 and six in WS18. In WS14, the plots were arranged in rows of six across three transects perpendicular to the stream and running up the west-facing ridge. In WS18, the six plots ranged from the upper half of the ridge down to the bottom of the catchment. I used a Thetaprobe ML2x (Delta-T Inc.) to take 10 soil moisture measurements at each plot per sampling date. The process was as follows: choose at random a spot within a 5 m radius of the plot center, remove leaf litter from the top of the soil, insert probe into the soil, take the reading, replace the leaf litter, and repeat. This is a common method for this instrument (Tague et al., 2010). The instrument gives both a water volume content and a saturation percentage. Measurements were only taken when the watersheds had not received precipitation within the last 24 hours.

Dr. Larry Band's lab has taken soil moisture data at irregular intervals since August 2011 at WS14, which I used in some of my analyses. I took almost weekly data measurements, exceptions following precipitation events, from August 2015 to November 2015 in WS14 to test the effects of most of topographic features. I took data at the plots in WS18 on three dates for this study; these data stand to serve as comparison between southwest- and northwest-facing slopes.

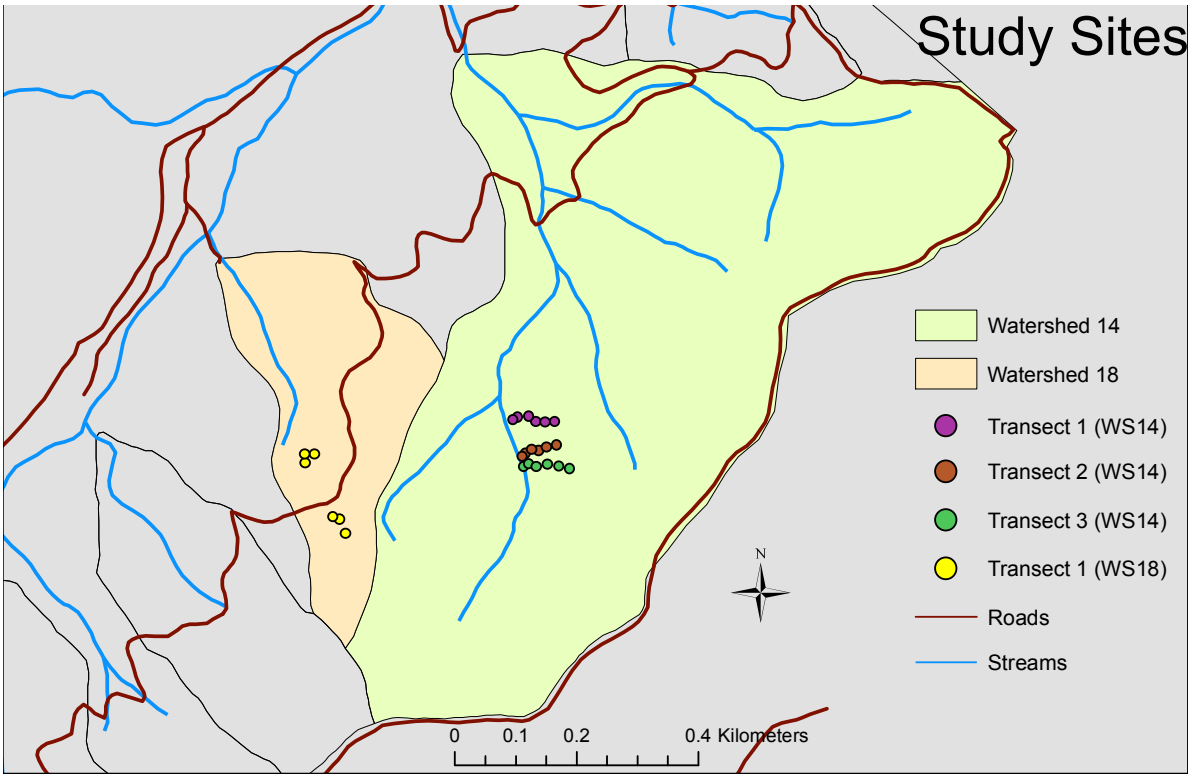

FIG. 2. Map of study sites in Watersheds 14 and 18 including transects and plots within transects (Coweeta Dataset, ArcMap10.3.1™, ESRI 2015).

Analysis

I created watershed maps using ArcMap 10.3.1 and map data taken from Coweeta's LTER website. I additionally used this software to calculate topographic parameters for each of the plots, including aspect, slope, upslope area, elevation, and distance to stream. I used Excel to create comparison charts between variables. All soil moisture values are percent saturation measurements (%).

RESULTS

Distance to Stream, Elevation, and Time

Because the plots were in transects from the top of ridges down to the stream in the valley, as distance to stream increased, so did elevation. Therefore, although only graphs are shown for distance to stream against soil moisture measurements, it can be assumed that similar patterns would be found with elevation plotted against moisture.

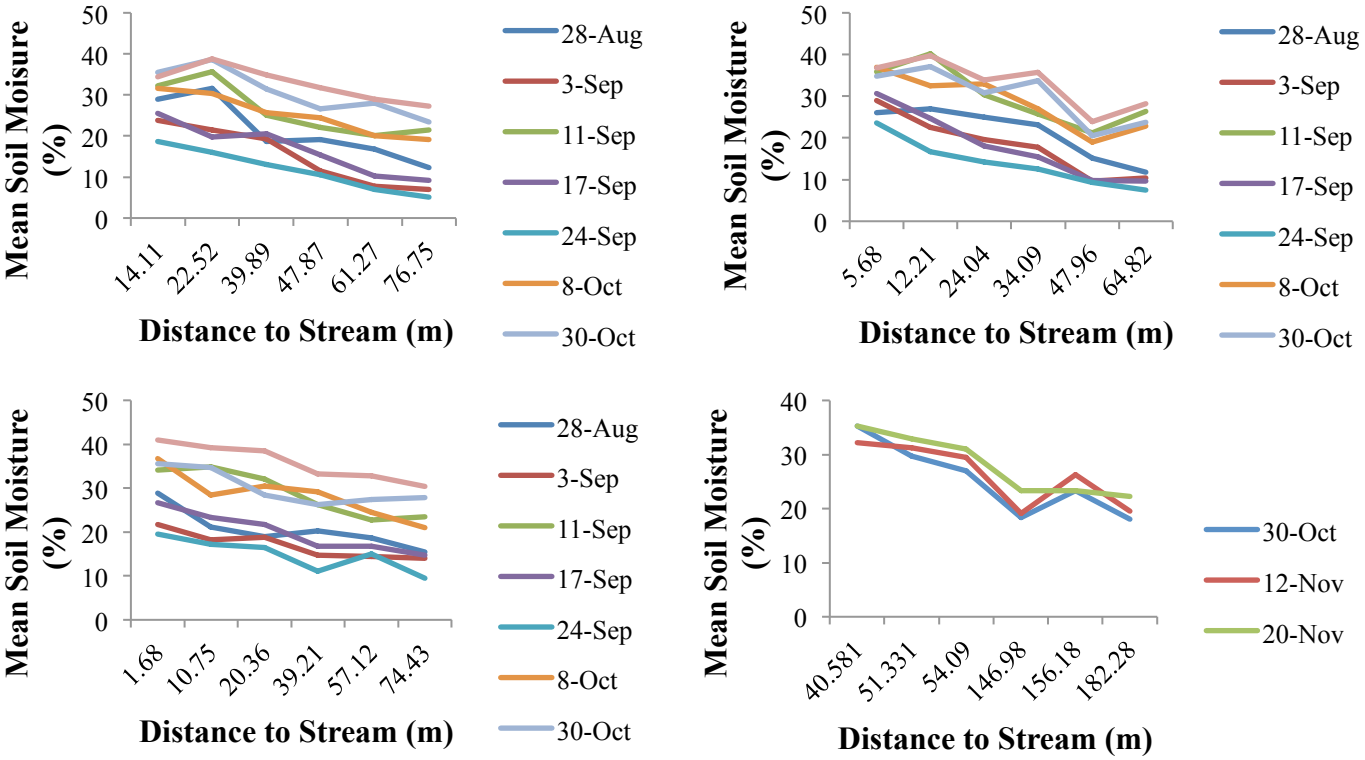

FIG. 3 (a-d). Soil moisture measurements (%) during the 2015 observation period in transects 1-3 in WS14 (top left, top right, bottom left) and transect 1 in WS18 (bottom right). The six distance to stream measurements (m) represent the six plots in each transect.

Over the 2015 observation period, average soil moisture values in WS14 varied from 5% to 40% in both watersheds (Fig. 3). Consistently, plots A-B (those at the lowest elevation and closest to the stream) had the highest means while plots E-F (those at the highest elevation and closest to the ridge) had the lowest means. WS18, although only observed on three different occasions, demonstrated this pattern as well, with mean soil moisture values ranging from 18%

to 35% (Fig. 3). This pattern was evident during dry periods, such as September 24, when soil moisture measurements were the lowest overall and during wet periods (presumably soon after a precipitation event), such as November 20 (Fig. 3).

The main temporal trend observed showed that the later dates correlated with the highest soil moisture values. Additionally, I created trend lines on each of these plots (not shown) and found that there was no trend relating a steeper moisture gradient to time of year (Fig. 3).

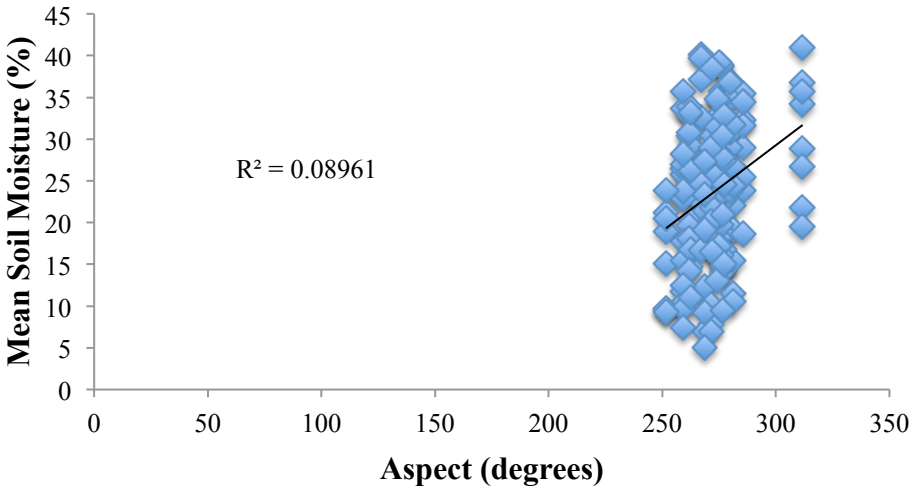

FIG. 4. Aspect plotted against mean soil moisture (%) for WS14. As degrees increase, the plots are more NW-faced, rather than SW-faced. No correlation was found between aspect and mean soil moisture.

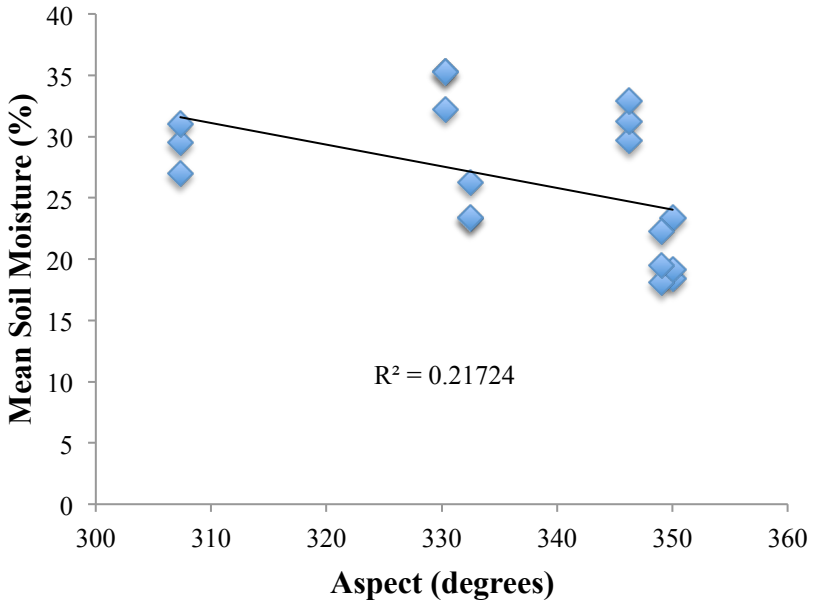

FIG. 5. Aspect plotted against mean soil moisture (%) for WS18. As degrees increase, the plots are more NW-faced, rather than SW-faced. An extremely low correlation was found between more north-facing slopes and lower mean soil moisture values.

Aspect

WS14's plots range from an aspect of 251.9 (southwest) to 311.5 (northwest). Fig. 4 plots aspect against mean soil moisture, showing a weak positive trend and no correlation between aspect (becoming more north-facing) and mean soil moisture ($R^2 = 0.09$).

WS18's plots range from an aspect of 307.3 (northwest) and 350 (almost completely north). Fig. 5 plots aspect against mean soil moisture again, showing a weak negative trend and slightly higher correlation than WS14, although still not near significant ($R^2 = 0.22$).

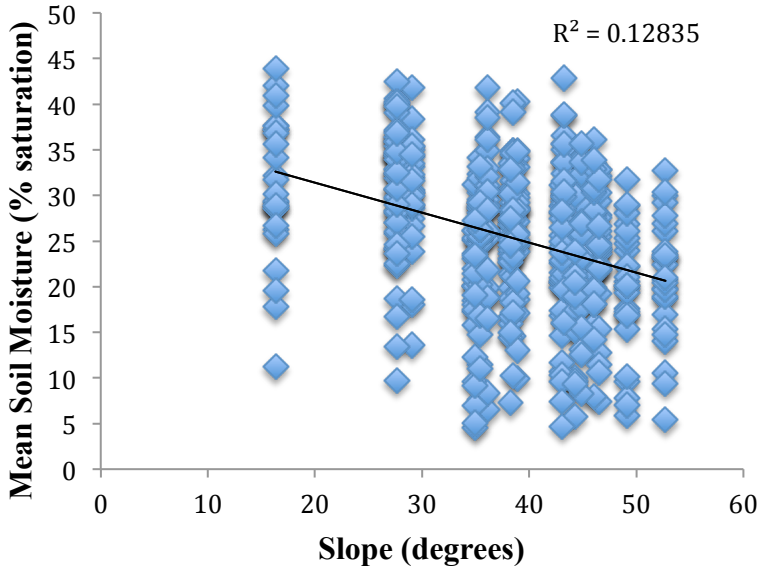

FIG. 6. Slope (degrees) plotted against mean soil moisture (%). Values include both 2015 observations and historical data since 2011. Trend line shown although R^2 value is only 0.128.

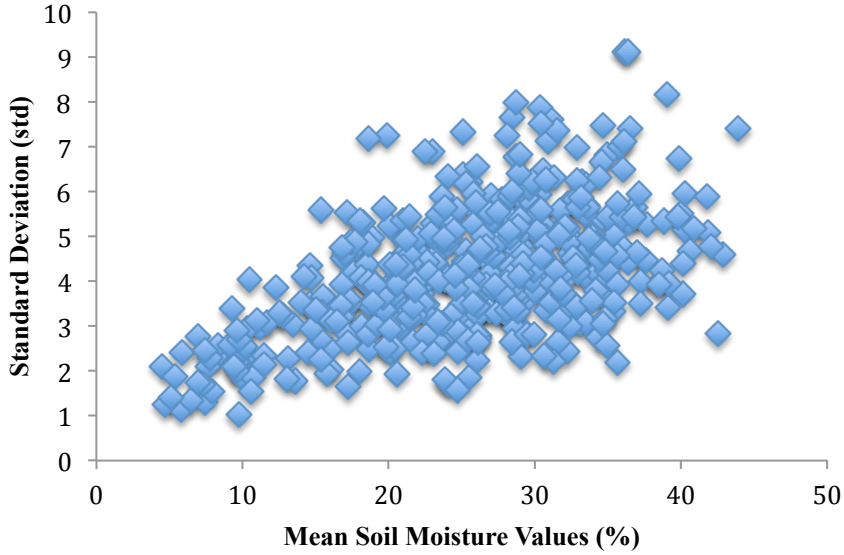

FIG. 7. Mean soil moisture plotted against standard deviation. Values include both 2015 observations and historical data since 2011.

Slope

The slopes of the plots in WS14 range from 16.3 degrees (Transect 3, Plot A) to 52.7 degrees (Transect 3, Plot F). However, as Fig. 6 shows, a range of soil moisture values occur at each slope. There is almost no correlation between the variables ($R^2 = 0.128$).

Mean and Standard Deviation

Fig. 7 plots mean soil moisture values from the 2015 observations in WS14 against the matching standard deviations. The general trend is that as mean soil moisture increases, so does standard deviation.

DISCUSSION

Topographical Results

My results on the effects of elevation in both WS14 and WS18 support the common hillslope trend of surface soil moisture increasing from the shoulder of a ridge to the footslope (Lin et al., 2006). Additionally, the range of my data in WS14 (5%-40% saturation) closely matched values found by Yeakley et al. (1998) in Coweeta's Watershed 2.

Slope, as a topographic feature, did not appear to correlate with mean soil moisture content, going against my initial hypothesis that flatter slopes would result in higher saturation measurements. This suggests that slope may not be a useful indicator in determining soil moisture patterns on hillslopes.

A comparison of the aspect vs. mean soil moisture plots gives inconclusive results about the effects of direction on soil moisture values. The WS14 plot shows that as aspect changes from southwest to northwest, mean soil moisture may have an increasing trend. The WS18 plot, however, suggests a trend that as aspect turns more north, soil moisture content decreases. Of course, the small sample size of only six plots and three sample days at WS18 gives a little more credibility to the WS14 trend, although that is not strong either. Therefore, more data is needed, possibly comparing hillslopes with more distinctive aspects.

Temporal Results

Temporally, my results reflect the beginning of the seasonal water recharge in November, as this is when mean soil moisture was at its highest in most of the plots. However, the data did not depict a strong timeline of lower moisture values to higher as time went on. This is potentially due to the unusually high amount of precipitation received in the region this year. Additionally, steeper moisture gradients were not found during wetter or drier periods. This goes against my initial hypothesis and Yeakley, et al. (1998), although this may be because many of my furthest plots were further away from the stream than the furthest plots in that study and therefore would not show as part of the gradient.

Mean and Standard Deviation

Finally, the comparison between mean soil moisture and standard deviation at WS14 show that as moisture content increases, so does standard deviation, signifying larger spatial variability during wetter periods and in wetter regions. Considering this, the data suggests that the plots found closer to the stream, since they tend to have higher soil moisture values, also have higher standard deviations than those plots closer to the ridge.

CONCLUSION

Shallow soil moisture organization is complex and highly variable, depending on topographical features and temporal scales. To better characterize hillslope soil moisture patterns for the purpose of mapping out potential moisture content, both a yearlong study and one that better shows the effects of aspect on soil moisture should be completed and compared to the current literature.

ACKNOWLEDGEMENTS

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ANALYZING THE POPULATIONS OF TWO LOCUST SPECIES, *ROBINIA HARTWIGII* AND *R. HISPIDA*, ON SATULAH MOUNTAIN AND LAUREL KNOB (MACON AND JACKSON COUNTIES, NC)

ROBERT K. M[C]MAHAN III

Abstract: I investigated properties on Satulah Mountain and Laurel Knob in southwestern North Carolina for the presence of Hartwig's locust (*Robinia hartwigii*) and hairy locust (*R. hispida*). I then gathered data on stem height and soil depth from these populations, as well as data on surrounding vegetation and the number of stems with seedpods. I used these data to analyze the health and prospects of the populations, as well as make recommendations for their future management. I found that the biggest issue facing the locusts was crowding and shading by competition, which severely hindered population expansion. I therefore recommended that the locusts be propagated in specially cleared plots, to ensure better reproductive success in future.

Keywords: hairy locust; Hartwig's; hartwigii*;* hispida; *laurel; North Carolina; population*; Robinia; *Satulah*

INTRODUCTION

Locusts are a group of small trees belonging to the genus *Robinia,* of the family Fabaceae (which also includes the similar acacia) (K. Pursel pers. comm.; Frankis et al 2015). There are several species and varieties of locust trees native to the southern Appalachians (Isley and Peabody 1984). These plants have long played a significant role in the ecology of the Appalachians as common early-successional species (K. Pursel, pers. comm.).

Robinia all have broadly the same habitats and characteristics; however, there are a number of significant morphological differences among the species (Isley and Peabody 1984). They thrive in conditions of high sunlight and drier soils (Van Dersal 1907). *Robinia* have compound leaves and "often [spread] by underground stems" (Radford et al 1968). A commonly occurring native species of *Robinia* is the black locust (*R. pseudoacacia*) (Isley and Peabody 1984). It is also one of the largest, growing into medium-large trees (Isley and Peabody 1984; Radford et al 1968). Another common species is the hairy locust (*R. hispida*), a shrub-sized tree with dense, coarse pubescence, which is often sterile; this sterility has been linked to frequent triploidy (Isley and Peabody 1984; Radford et al. 1968).

Much rarer is Hartwig's locust (*R. hartwigii*), another small tree that is distinguished by its thorn-like stipules, pubescence on the stem, and tacky glands on new twigs and seed pods (K. Pursel pers. comm.; Isley and Peabody 1984; Radford et al. 1968). This species, or variety as some researchers believe, is a narrow endemic that occurs primarily on a small number of slopes on the Highlands Plateau, in the southwestern part of North Carolina (K. Pursel pers. comm.; Isley and Peabody 1984; Radford et al. 1968). Virtually no detailed studies of this plant exist. A literature review reveals that most treatments of *R. hartwigii* are short, general descriptions of the plant, and most sources confine themselves to the species' taxonomic classification. Little information is available on *R. hartwigii*'s present distribution or projected future (K. Pursel pers. comm.).

I conducted my investigation on *R. hartwigii* and on *R. hispida*; *R. hispida* was included because several rare varieties exist on the Highlands Plateau, and data gathered on *R. hispida* specimens is of potential conservation value (K. Pursel pers. comm.). I first located and mapped all populations of *R. hartwigii* and *R. hispida* located on two Highlands-Cashiers Land Trust properties (Satulah Mountain in Highlands, Macon County, NC, and Laurel Knob in Jackson County, NC). Subsequently, I divided the plants I found into clusters and took data from each cluster on stem morphological features and surrounding flora/habitat. I used these data to evaluate the status of the two species on these properties. The Highlands-Cashiers Land Trust (HCLT) will be able to use these data and analysis to inform decisions on management and possible propagation of these species (K. Pursel pers. comm.).

METHODS

Study Sites

I conducted research on two separate holdings of the Highlands-Cashiers Land Trust, the conservation easements on Satulah Mountain (Macon County) and Laurel Knob (Jackson County). I first surveyed these sites for clusters of Hartwig's locust and hairy locust (*R. hartwigii* and *R. hispida*). I considered clusters to be more-or-less well-defined groups of stems; most of these were made up of stems immediately adjacent to each other (and thus likely the result of stem propagation), but some featured stems at up to a few meters' distance. I marked these clusters with flagging tape, and found their GPS coordinates using Garmin® Montana 650t (Hartwig's locusts) and GPSmap 62sc (hairy locusts) handheld devices.

Determining Samples

Since the search for plants was undertaken from August to November 2015, I used a number of different characteristics to identify the two species. For *R. hartwigii*, I used the leaves, hairy and sticky seedpods, sticky branches, and the general shape (branches grow in a distinctive series of angles) as primary identifiers. After the leaves and seed pods had mostly dropped off and the glands on the limbs had dried, I used the general shape, the hairy bark, and the residual branch glands to identify the species, along with residual seed pods and leaves where applicable. For the *R. hispida*, the identifying traits were the leaves and hairs; after the plants lost their leaves, the hairy stems served as the primary identifying trait.

Following the completion of surveying, I selected from each cluster a random sample of up to 3-5 stems; I selected samples of 1-2 stems for groups of less than four total stems. I used a variety of methods to arrive at the randomized selections. I chose all but two samples with random numbers. I made use of assistance with these – one experimenter numbered the plants, and the other (without knowing which plants had which numbers) picked a set of random numbers, which corresponded to the individuals to be used in that sample. I used a series of randomization techniques when sampling without assistance.

Data Collection

I examined the identified samples with respect to the following variables: surrounding vegetation type/thickness, soil depth (cm), and total plant height (cm).I measured soil depth, after

removal of leaf litter, using the following method: I inserted the rod into the soil next to the stem (perpendicularly to the ground) until it hit an obstruction (presumed to be bedrock). Upon reaching bedrock, I used my thumb and index finger to mark how deep the rod had penetrated, and I measured the length of rod between the marked point and the tip. I used a 50-ft measuring tape to make all measurements. I then made a second unofficial observation to check for large depth variations (e.g. 4 cm and 15 cm) on different sides of the stem. When such variations were found, I took a second formal observation and used the average of the two as my result, then checked once more to confirm accuracy.

I measured stem height in two ways. The *R. hartwigii* stems were measured from the highest twig on the plant to the ground directly beneath it (the "ground-point"). For plants on a slope, I approximated a horizontal line from the stem's base to above the ground-point. When a ground-point was downslope from the stem base, I measured the height *h* from the horizontal line down to the ground-point. For ground-points upslope from the stem base, the horizontal line was measured from the ground-point. This value *h* was then added or subtracted from the twig/ground-point height *H* to find the correct stem height, based on the ground-point's uphill or downhill position relative to the stem base. All but one of the *R. hispida* had small, roughly linear stems. I measured these by straightening the stem (to minimize bends) and then measuring it directly. The single non-linear *R. hispida* stem was measured using the same method as the *R. hartwigii* stems.

I tallied the cluster's total number of stems with seedpods or traces of seedpods (the seedpods' stems, remnants of eaten seedpods, or fallen seedpods underneath a stem). I also made observations of the proportions of small stems (small stems ranged up to ~0.3 m tall) and surrounding plant types and densities. Mr. Kyle Pursel from the HCLT identified surrounding plants; Latin names were taken from Radford et al (1968).

Data Analysis

I used the soil depth and stem height data in a regression analysis (depth vs. height) to examine effects of soil depth on stem size. I analyzed the qualitative data for patterns within this data and for potential relationships with the quantitative data trends. Finally, I tabulated all quantitative data and overlaid the GPS coordinates onto maps of the Satulah Mountain and Laurel Knob properties (provided by Dr. Gary Wein of the HCLT) using ESRI ARCMap 10.3.1; these are included in Appendix A.

RESULTS

Stem Heights and Soil Depths

I used these data to perform regression analyses of stem height vs. soil depth. Samples were analyzed by cluster, in order to avoid lurking variables such as surrounding vegetation. In these two graphs, the correlation coefficients for each cluster's regression are at the end of the corresponding line.

TABLE 1: Soil Depth Measurements (cm) of 8 *Robinia hartwigii* clusters, Satulah Mountain site, Macon Co., NC

Cluster	Depth 1	Depth 2	Depth 3	Depth 4	Depth 5	Median
1	6.4	8.2	5.4	13	6.2	6.4
2	4.6	5.2	8.6	13.4		6.9
3	5.4	6.3	8.2			6.3
4	19.2	16	41.2			19.2
5	5	19.4	16.2			16.2
6	7	7.6	13			7.6
7	17.2	2	19.6	9.8		13.5
8	15.6					15.6

Table 2: Soil Depth Measurements (cm) of 6 *Robinia hispida* clusters, Laurel Knob site, Jackson Co., NC.

Cluster	Depth 1	Depth 2	Depth 3	Depth 4	Depth 5	Median
1 (Satulah)	16.4	16	16.8			16.4
2 (Laurel)	11					11
3 (Laurel)	14.47	8.8				11.63
4 (Laurel)	2	3.6	9.4			3.6
5 (Laurel)	19.5					19.5
6 (Laurel)	24	14.1				19.05

TABLE 3: Stem Height Measurements (cm) of 8 *Robinia hartwigii* clusters, Satulah Mountain site, Macon Co., NC.

Cluster	Height 1	Height 2	Height 3	Height 4	Height 5	Median
1	110	42	100	128	112.2	110
2	38.6	46	153.8			46
3	60	58	145			59
4	49	72	75			72
5	212	73.4	80			80
6	105.6	64	53			64
7	203.4	183.4	223.6	99		193.4
8	179					179

TABLE 4: Stem Height Measurements (cm) of 6 *Robinia hispida* clusters, Laurel Knob site, Jackson Co., NC.

Cluster	Height 1	Height 2	Height 3	Height 4	Height 5	Median
1 (Satulah)	147.4	173.8	45.6			147.4
2 (Laurel)	58					58
3 (Laurel)	15	12				13.5
4 (Laurel)	36	30	4.6			30
5 (Laurel)	30					30
6 (Laurel)	64	79				71.5
Overall						44

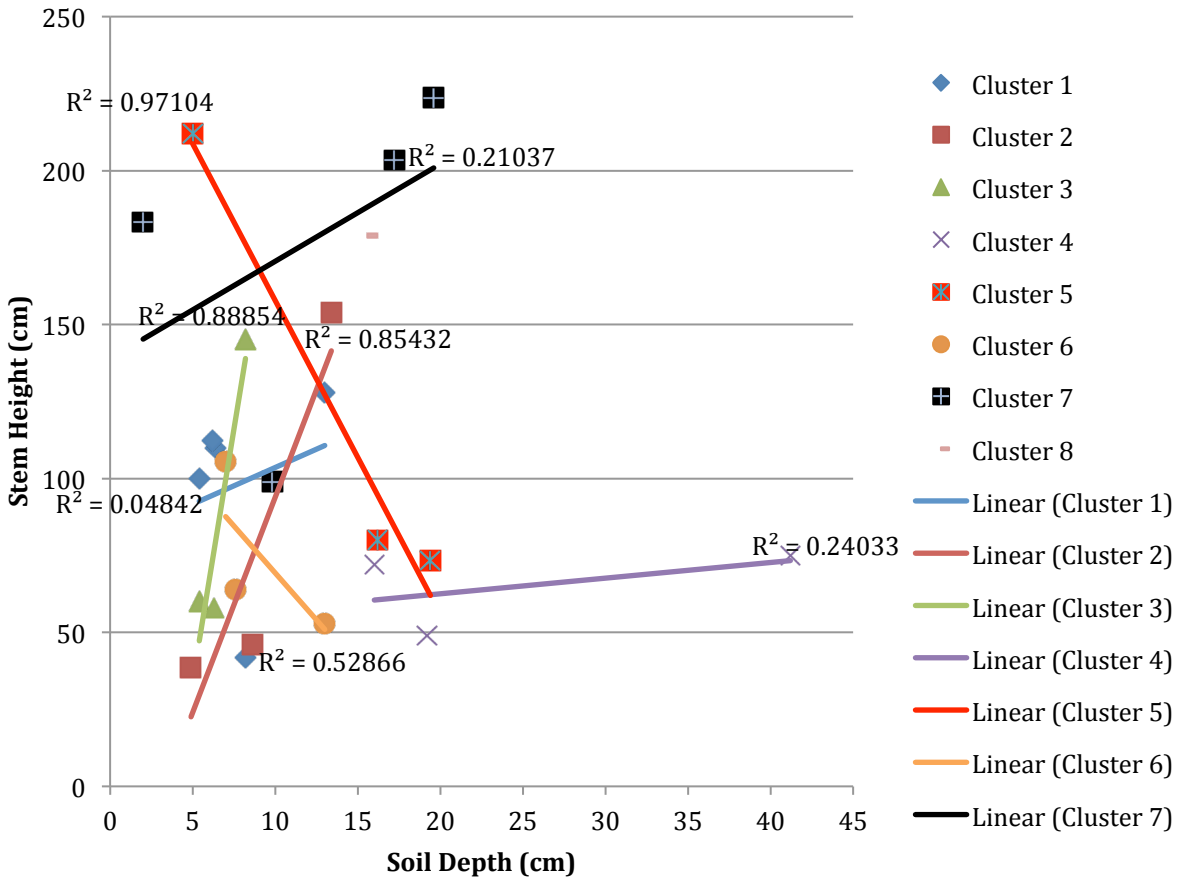

FIG. 1: Linear Regressions of stem height vs. soil depth measurements for *R. hartwigii*.

The cluster regression lines show a wide slope magnitudes and signs. The strength of the correlations between soil depth and stem varies widely from cluster to cluster. Most clusters have weak correlations; some have quite strong ones. There is not a strong overall correlation between these data groups. If the regression lines had broadly similar slopes/correlation coefficients, there would be evidence for a relationship between the two variables; variations in such a situation could then be the result of inter-sample variation. However, the various regressions are quite dissimilar, (and mutually contradictory in the case of Clusters 5 and 7), so the data do not return evidence of a relationship.

Since Clusters 3 and 6 are only 2 individuals apiece, they are of limited utility in a regression analysis. Based on the analyses of Clusters 1 and 4 and the slopes of Clusters 3 and 6, the height-depth correlation is tenuous. While Clusters 1 and 4 seemingly display strong negative correlations, Cluster 1 has such little depth variation that its regression line is nearly 0; this causes the high correlation and shows that Cluster 1 actually gives evidence of no correlation. The conflicting testimonies of Clusters 1 and 4 and the high slope variation in the four clusters high provide evidence of no correlation for *R. hispida* either.

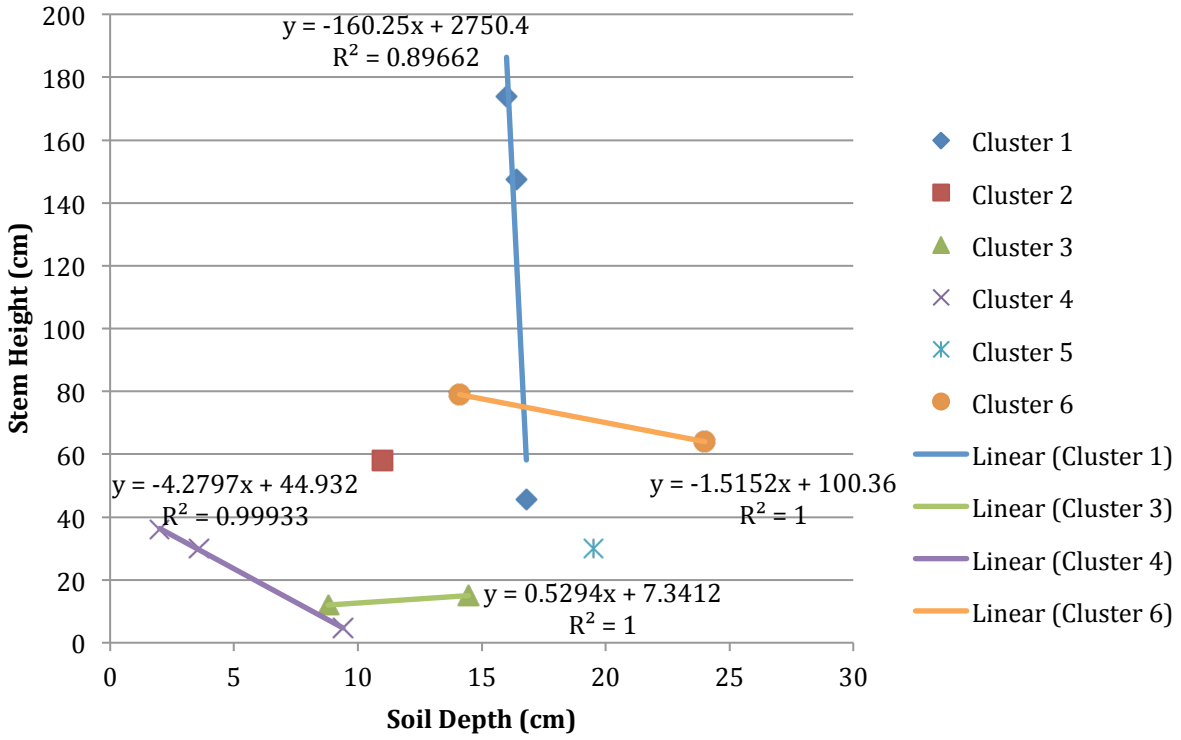

FIG. 2: Linear Regressions of stem height vs. soil depth measurements for *R. hispida*. As before, R^2 values for each regression line are placed at the line's edge.

Seedpods

The *R. hispida* I studied did not produce seeds at all (Tables 5 and 6). The *R. hartwigii* contained a total of 72 stems with seedpods, but the numbers of these stems varied considerably among individual clusters.

TABLE 5: Number of *Robinia hartwigii* Stems with Seedpods, Satulah Mountain site, Macon Co., NC.

Cluster	No. Bearing Pods
1	13
2	6
3	4
4	0
5	5
6	5
7	38
8	1
Total	72

TABLE 6: Number of *Robinia hispida* Stems with Seedpods, Satulah Mountain (Macon Co.) and Laurel Knob (Jackson Co.) Sites.

Cluster	No. Bearing Pods
1 (Satulah)	0
2 (Laurel)	0
3 (Laurel)	0
4 (Laurel)	0
5 (Laurel)	0
6 (Laurel)	0
Total	0

Surrounding Vegetation and Seedling Presence

Surrounding vegetation of each cluster varied predictably in conjunction with the number of small stems observed; this was particularly true when rhododendron and/or laurel were a significant portion of the surroundings. Almost all small *R. hartwigii* stems were in Cluster 1, in a large grassy patch next to the shrubbery. In Cluster 7, there were (many fewer) small stems in the forest where there were no laurel or rhododendron, but only large plants/stems where there were rhododendrons, laurel or other thick underbrush (which included the center of the cluster).

TABLE 9: Surrounding cover for each *Robinia hartwigii* cluster, Satulah Mountain, Macon County, NC.

Cluster	Surrounding Vegetation/Habitat
1	Growing in and around a thick patch (around three feet high) of blackberry (*Rubus*) and other shrubs, adjacent to a large patch of grass. Surrounded on one side by bare stone and on the other by laurel (*Kalmia latifolia*) and rhododendron (*Rhododendron*). Some stems in the cluster were growing out of the laurel. Almost no trees.
2	Same as above, though shrubbery was less dense and the patch of grass was small and interspersed with large rocks
3	Dense thicket of shrubs with some grass on margins
4	Cleared ground in a reclamation area; a few adjacent pines (*Pinus*), with remains of blueberry and laurel/rhododendron
5	Growing in and around a patch of blackberry adjacent to a small patch of exposed stone, with scattered laurel. Oak (*Quercus*) dominated forest, surrounding the cluster at ~1 m distance.
6	Growing in medium-thickness forest with laurel, azalea (*Azalea*), pine, oaks, and Carolina rhododendron (*R. minus*). Only large plants growing among rhododendron. Trees scattered.
7	Growing adjacent to exposed stone, in and around two patches of ground surrounded by catbriar (*Smilax*), laurel, rhododendron, azalea, and oak. As elsewhere, only the largest plants were extant in rhododendron/laurel; varying sizes elsewhere. Canopy cover moderate to full.
8	Primarily laurel and rhododendron (*R. catawbiense*, *R. minus*); also briar, various shrubs, blackberry, oak, pine

In Cluster 5 there were one or two small stems where the ground was not overshadowed by blackberries or mountain laurel, but only large stems elsewhere. Finally, Cluster 4 – a cleared reclamation area – had essentially bare ground, and all but one of the stems were small. Thus, the distribution of small stems was heavily slanted towards clusters with significant portions of uncovered ground – the more exposed the ground, the more small stems.

This same pattern was observed for *R. hispida* – small stems were only seen in places with lighter cover or gaps in the cover (such as on pathways). Only the largest specimens (in Cluster 6) were observed among high cover; however, all these were growing in a clearing, on or near a path.

DISCUSSION

Reasoning tells us that a correlation between soil depth and stem height is likely – tall trees need stronger roots and more nutrients, both of which are obviously found more easily in deep soil than shallow. However, the actual analyses support quite a different conclusion. There is also no justification for excluding outliers like those of Cluster 7 (such as 9.8 cm depth/99 cm height) – the samples did not have enough individuals to exclude the effect of natural variation. It is possible, however, that the sample size was too small to capture an existing relationship, particularly in the case of clusters with many individuals (>40, for instance). In this study soil depth and stem height were not correlated for either species of *Robinia*.

Table 10: Surrounding cover for each *Robinia hispida* cluster; all but Cluster 1 on Laurel Knob, Jackson Co., NC.

Cluster	Surrounding Vegetation/Habitat
1 (Satulah)	Growing in and around a large patch of Carolina rhododendron and scattered laurel, either enmeshed in the rhododendron (large *hispida* stems) or in exposed areas within the patch (small *hispida* stems); also scattered azalea, blackberry, catbrier, fetter-bush (*Pieris*); oak surrounding patch
2	Fetterbush, lichensv, blueberry (*Vaccinium*), sand myrtle (*Leiophyllum*), Catawba rhododendron, table mountain pine (*P. pungens*), club mosses. Individual is on edge of path, growing above ground cover.
3	Teaberry (*Gaultheria*), sand myrtle (thick), blueberry; plants flanking a path next to clearing
4	1 table mountain pine, sand myrtle, lichens, teaberry, mosses, club mosses, blueberry; site was thin ground surrounding a lone table mountain pine, with thick laurel growing on one side about 6 feet away.
5	Sand myrtle, teaberry, laurel
6	Ibid; plants growing next to thick patch of laurel and rhododendron
7	Growing in and around a large patch of Carolina rhododendron, either in the rhododendron (large plants) or in bare areas within the patch (small plants)
8	Fetterbush, lichens, blueberry, sand myrtle, Catawba rhododendron, table mountain pine, club mosses. Individual is on edge of path, growing above ground cover.

The surrounding vegetation and seedpod-stems data are most strongly correlated to the current distributions of *Robinia*. The lack of small stems in all but a few clusters is in sharp contrast to the number of *R. hartwigii* seedpod-bearing stems– only one cluster had no observed

seedpods. Moreover, Cluster 1 with 13 seedpod-bearing stems and its large patch of open grass had many more seedlings than Cluster 7 with 38 seedpod stems and scattered patches of forest floor. My late October-early November sampling could have overlooked some stems which had previously lost their seedpods. However, the seedpod stem counts would have to be very different to support an alternate conclusion. For example, Cluster 1 would have to have more seedpod stems than Cluster 7, commensurate with its greater number of small stems, to raise the possibility of a correlation between the two. Therefore, I conclude that surrounding vegetation has the strongest correlation with *R. hartwigii* distribution of the variables considered.

The *R. hispida* did not have any seedpods; nevertheless, the above conclusion applies to them as well. The plants were exclusively found in locations with access to frequent direct sun – on or near paths and/or gaps in tree- and ground-cover, for the most part.

Because of these observations, and because *Robinia* are known to be early-successional species (K. Pursel pers. comm.), it is most likely that *R. hartwigii* and *R. hispida*'s shade-intolerance (K. Pursel pers. comm.) is strong enough that the species are effectively denied propagation when surrounded by thick shrubbery (especially rhododendron and mountain laurel).

While the data indicate a strong correlation between surrounding cover and small stem presence, these results are only qualitative. A quantitative metric for ground cover (e.g. percent bare ground, percent of cover rhododendron/laurel, etc.) would allow future studies to perform regression analysis against the numbers of small stems, and thus gain a more precise understanding of the relationship between the two.

CONCLUSION

While there are several very large *Robinia* in the Laurel Knob and Satulah populations, the populations' long-term flourishing is by no means assured. Comparison in particular of the low number of *R. hartwigii* clusters with many small stems to the high number of clusters with seedpod-bearing stems indicates a low level of successful propagation. This appears to be primarily caused by competition from other small ground-level plants, which shade the *Robinia* and make it difficult for them to grow. This is particularly true for mountain laurel and rhododendron – in one memorable instance, a stem of *R. hartwigii* was found sprouting out of a patch of laurel, and was growing almost horizontally to escape the patch. Clearing the ground of other competing plants – especially mountain laurel and rhododendron – and keeping them clear until the desirable *Robinia* become established is the best way to encourage the growth of *Robinia*. Best growth will occur when the locusts are adjacent to a tree-fall or patch of bare stone, which will maximize the amount of incoming light. In addition, if these plots are allowed to regenerate following locust propagation, the locusts must remain taller than their competition in order to not be starved of light.

ACKNOWLEDGEMENTS

I would like to thank my mentors Mr. Kyle Pursel and Dr. Gary Wein of the Highlands Cashiers Land Trust for their endless patience and guidance throughout my internship. I would also like to thank Dr. Karen Kandl, Dr. Jim Costa, Alyssa Fuller, and the rest of the staff at the Highlands Biological Station for all of their support and encouragement throughout the semester.

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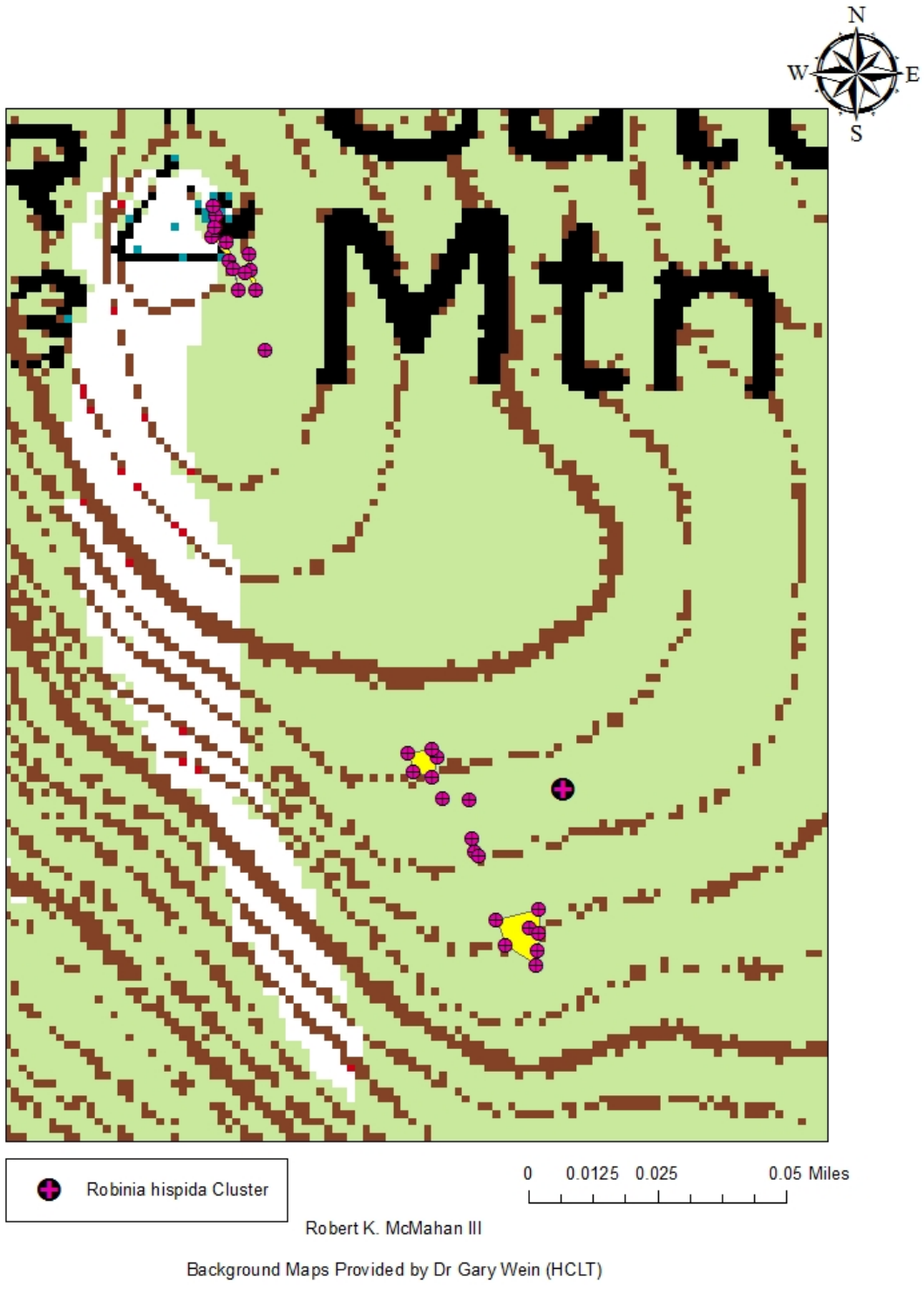
APPENDICES

APPENDIX 1: Coordinate chart for *Robinia hartwigii* clusters (decimal degrees) on Satulah Mountain.

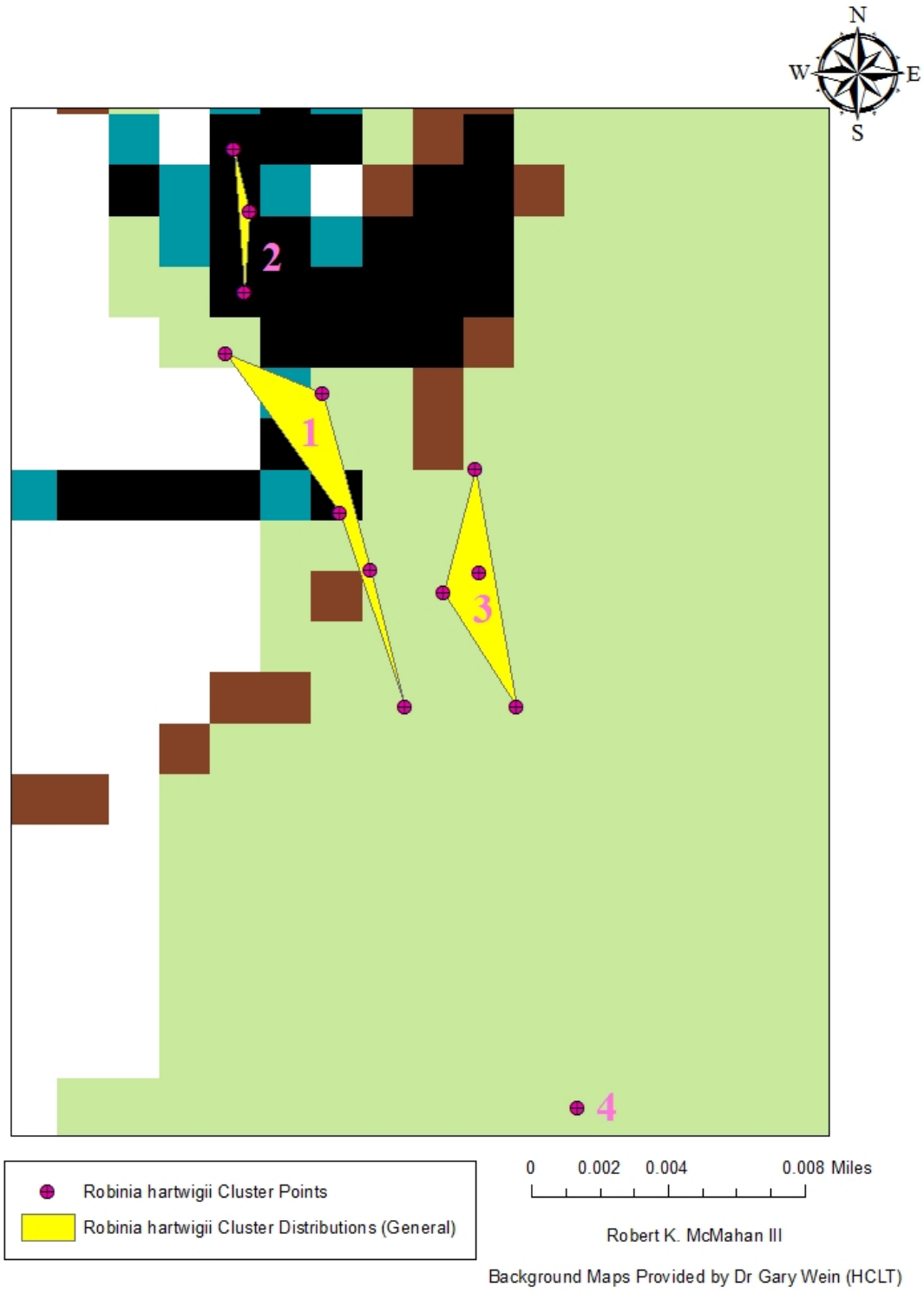
Cluster	Latitude (*N)	Longitude (*W)
hartwigii 1	35.03604300	-83.19220600
hartwigii 2	35.03617100	-83.19225600
hartwigii 3	35.03601900	-83.19213300
hartwigii 4	35.03579200	-83.19207600
hartwigii 5	35.03465400	-83.19145000
hartwigii 6	35.03438600	-83.19131800
hartwigii 7	35.03417400	-83.19112200
hartwigii 8	35.03453500	-83.19134000

APPENDIX 2: Coordinate chart for *Robinia hispida* clusters (decimal degrees) on Laurel Knob (LK) and Satulah Mountain (SM).

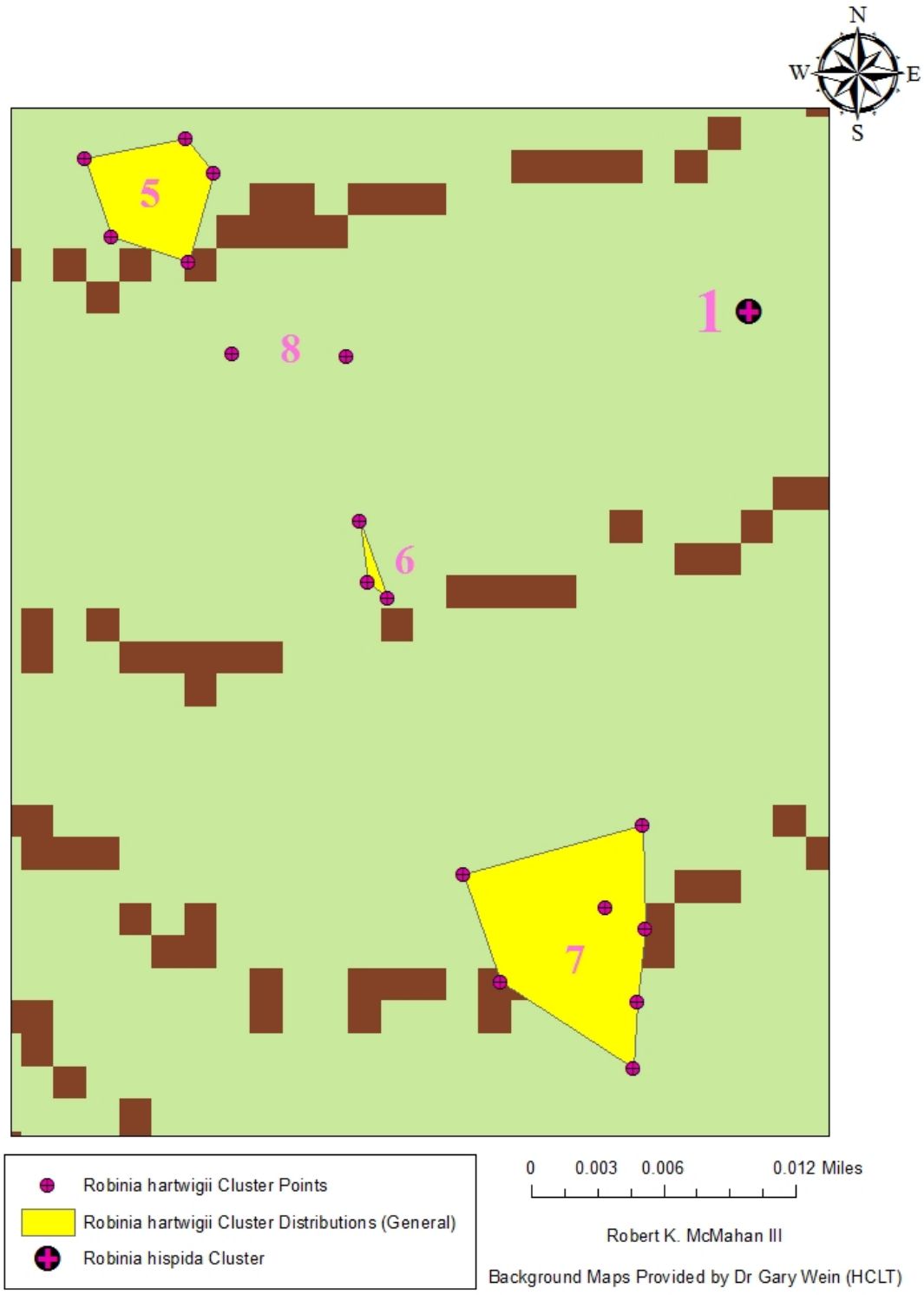
Cluster	Latitude (*N)	Longitude (*W)
LK Hispida 2	35.153922	-83.055355
LK Hispida 1	35.153961	-83.055260
LK Hispida 4	35.153767	-83.055532
LK Hispida 3	35.153159	-83.055724
LK Hispida 5	35.154558	-83.055586
SM Hispida 1	35.034577	-83.191021



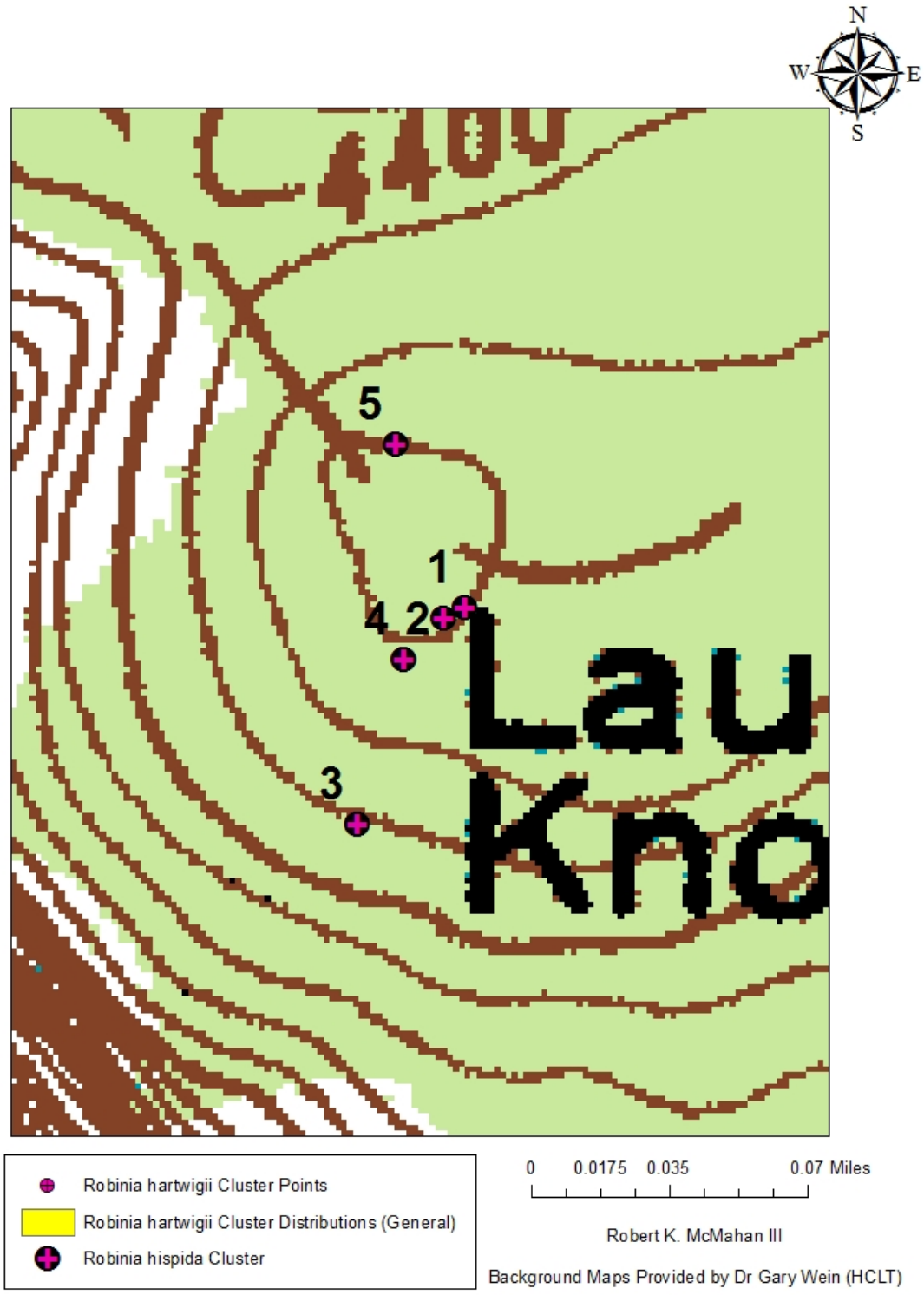
APPENDIX 3: Distribution map for *Robinia hartigii* (yellow/small dots) and *Robinia hispida* (large dots) on Satulah Mountain, Macon Co., NC.



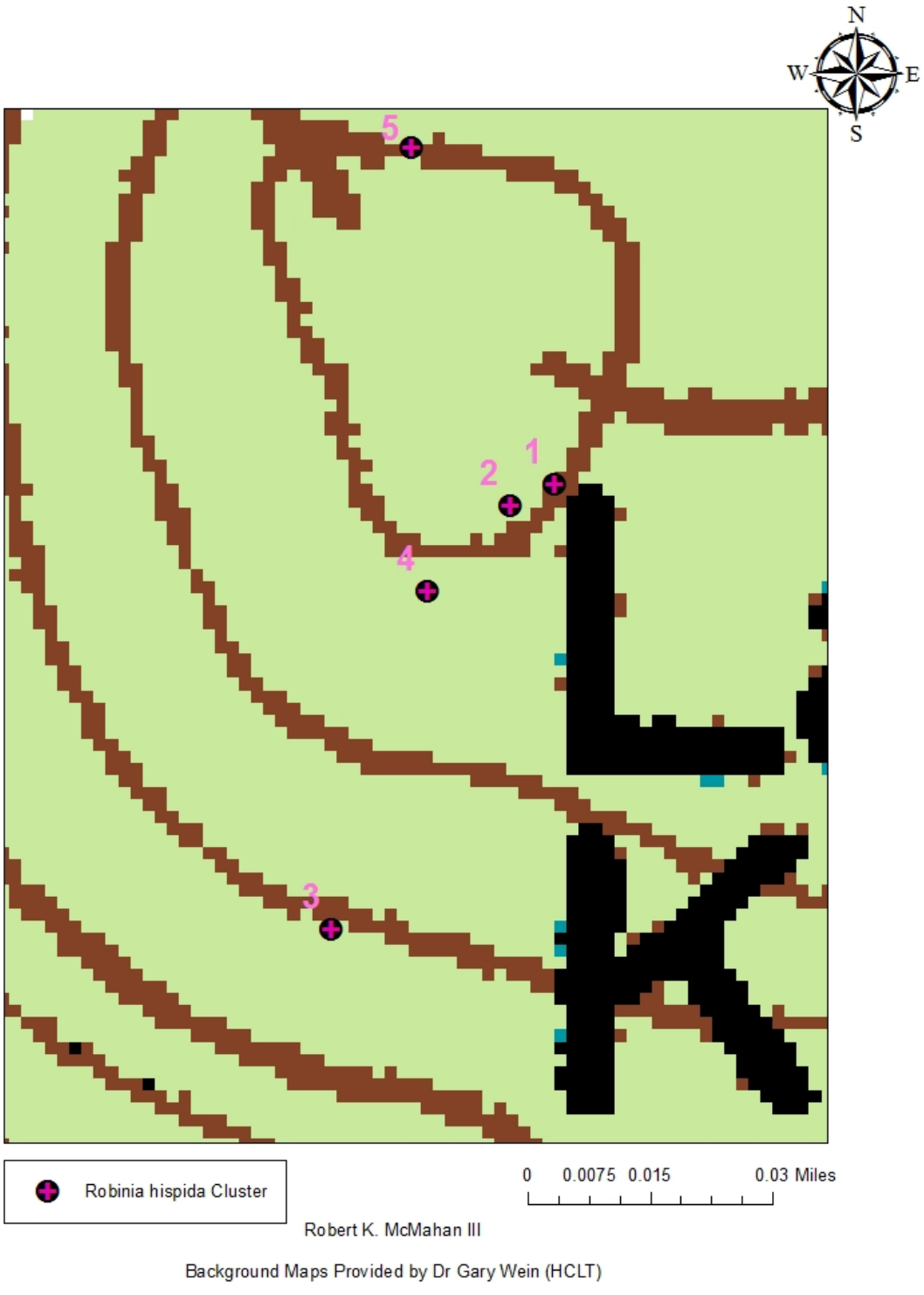
APPENDIX 4: Distribution map for *Robinia hartigii* Clusters 1-4 (yellow/small dots) on summit of Satulah Mountain, Macon Co., NC.



APPENDIX 5: Distribution map for *Robinia hartigii* Clusters 5-8 (yellow/small dots) and *Robinia hispida* (large dots) on Satulah Mountain, Macon Co., NC.



APPENDIX 6: Distribution map for *Robinia hispida* (large dots) on Laurel Knob, Jackson Co., NC.



APPENDIX 7: Detailed distribution map for *Robinia hispida* (large dots) on Laurel Knob, Jackson Co., NC.

ESTABLISHING THE DISTRIBUTION OF *DESMOGNATHUS FOLKERTSI* IN THE NORTH CAROLINA SEGMENT OF THE CHATTOOGA RIVER DRAINAGE

OLIVIA ARNOLD

Abstract. The southern Appalachians are home to the greatest salamander diversity in North America. Habitat and niche separation leads to regular speciation in salamanders which often results in highly cryptic species. *Desmognathus folkertsi*, a recently described species, and *Desmognathus quadramaculatus* are two cryptic species in the black-bellied salamander complex. Currently, *D. folkertsi* has only been found at two sites in North Carolina. Because of its rarity, it is important to further establish its range and relative species abundance to aid in conservation efforts. I surveyed the North Carolina segment of the Chattooga river drainage for the presence of *D. folkertsi*. I found 36 potential specimens throughout the headwaters of the drainage, suggesting there is a population of *D. folkertsi* in North Carolina with a relative species abundance of 13.58%. This research will contribute to the assessments of the North Carolina Wildlife Resource Commission as they determine whether or not *D. folkertsi* will become a state-listed endangered species.

Key words: black-bellied salamander complex; Chattooga River drainage; Desmognathus; Desmognathus folkertsi; Desmognathus quadramaculatus; *distribution; North Carolina; relative abundance; southern Appalachians*

INTRODUCTION

Desmognathus folkertsi, the dwarf black-bellied salamander, is a recently acknowledged species of dusky salamander. It is cryptic with *Desmognathus quadramaculatus,* the black-bellied salamander (Wooten and Rissler 2011). Both of these species are native to the southern Appalachians, however, due to the cryptic nature of the species and the recent discovery of *D. folkertsi*, the actual range of *D. folkertsi* is largely unknown. *D. folkertsi* has been found in several sites in Georgia, one site in South Carolina, and only two sites in North Carolina. Because of the rarity of the species, I sought to establish its distribution in the Chattooga River drainage, describe its stream habitat, and determine its relative abundance. *D. folkertsi* is being considered for state listing and the NC Wildlife Research Commission is assessing the status of the species. This research will contribute to their assessments.

One of the reasons so little is known about the range of *D. folkertsi* is the fact that it is very difficult to identify. *D. folkertsi* and *D. quadramaculatus* are very cryptic and misidentification of both species has led to confusion regarding the presence of *D. folkertsi* in some streams. There are distinguishing characteristics between the two: in the larval form, *D. folkertsi* has a smaller body size than *D. quadramaculatus. D. quadramaculatus* larvae also have redder tail fins than *D. folkertsi* (Barbour et al. 2014). The two species also exhibit morphological differences as adults. Adult male *D. folkertsi* measures 58-85mm SVL and adult females measure 56-76mm SVL. Adult male *D. quadramaculatus* are typically at least 120mm SVL and females are around 102mm SVL with the minimum size at adulthood being 80mm SVL (Camp et al. 2013). *D. quadramaculatus* tend to have longer limbs and toes than *D. folkertsi* with taller narrower tails. The species can also be distinguished by dorsal color and pattern. The dorsum of *D. folkertsi* tends to have vermiculate patterns of brown and black while *D.*

quadramaculatus is a more uniform brown or dark green, with occasional black spots. *D. quadramaculatus* also has a reddish tint to the rib of its tail, especially when newly metamorphosed (Camp et al. 2002). Furthermore, *D. folkertsi* has a discolored portion of its head that often appears to be wine colored in the light. Both species have black bellies in adulthood.

METHODS

We began surveying the distribution of *D. folkertsi* by establishing a rubric for stream selection. We surveyed first and second order tributaries of the Chattooga River that were at least one meter wide (Fig. 1, Fig. 2). Once we selected a stream, we surveyed two 30-meter reaches, each between 100 yards and a half-mile apart. Within the reach we took three measurements of stream width and three measurements of bank width. We defined bank as usable habitat interface. Then we collected one liter of water from each reach to use as environmental DNA (eDNA) samples in order to test for *D. folkertsi* presence. We also took GPS coordinates and photographs of the reach in order to document and map site locations. Each stream surveyed has an abbreviation followed by a number or the letter U/L. This is the code for specimens and locations presented in the data (Table 1). We surveyed the downstream reach first to prevent contamination from human DNA in the upstream reach.

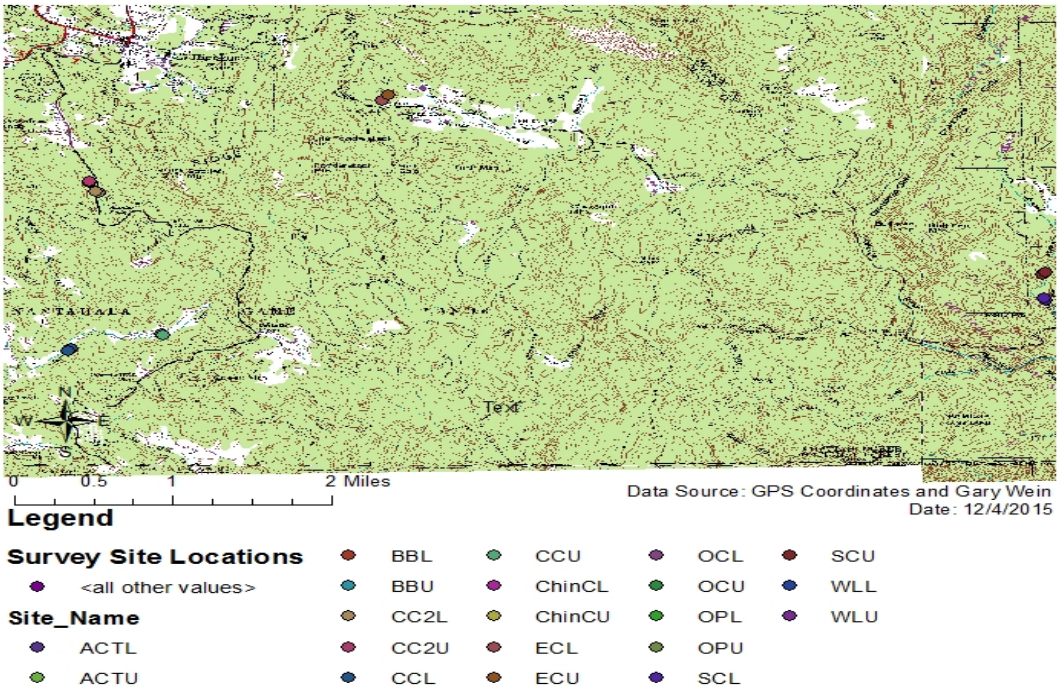


FIG 1. Survey sites in the eastern portion of the Chattooga drainage, Macon County, North Carolina.

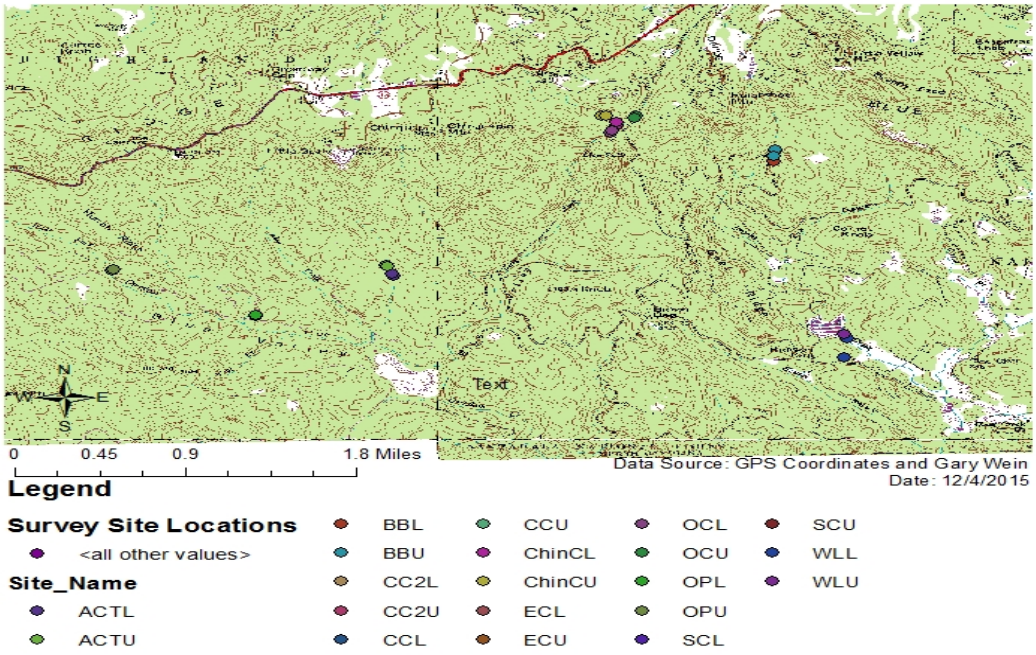


FIG 2. Survey sites in the western portion of the Chattooga drainage, Macon County, North Carolina.

TABLE 1. Abbreviations for all surveyed sites.

Abbreviation	Site Name
FC	Fowler's Creek
SC	Scottsman Creek
CC	Clear Creek
CC2	Clear Creek 2
OC	Overflow Creek
ChinC	Chinquapin Creek
BB	Brooks Branch
WL	Wilson Lakes
EC	Edwards Creek
UT	Unnamed Tributary
OP	Overflow Proper
ACT	Abe's Creek Tributary
L	Lower (downstream) Site
U	Upper (upstream) Site

After we collected the stream data, we began to survey for *D. folkertsi.* We overturned rocks and searched all usable habitat within the reach. In order to establish the relative abundance of *D. folkertsi*, we documented the number and species of any salamander captured. When we found a prospective *D. folkertsi* we measured its SVL and

full length. Then we recorded what type of cover each specimen was found in or under (e.g. cobble, rock crevice, or rotting log). If it was found under a rock, we measured the intermediate axis of the cover type when possible. We also recorded the specimen's distance from running water at time of capture. Before release, we took a tail snip of each prospective *D. folkertsi*. We sent the tail snips and the eDNA samples to Piedmont College where they will be genetically analyzed to confirm species.

RESULTS

Seven species of salamander were collected throughout the survey: *D. folkertsi, D. quadramaculatus, Desmognathus ocoee, Desmognathus monticola, Desmognathus marmoratus, Eurycea wilderae, Notophthalmus viridescens*, and *Plethodon teyahalee.* There were also several unidentified escapees. Accounting for the possibility that the

TABLE 2. Species richness at each site.

Site	**Species Richness**
FCL	5
FCU	5
SCL	2
SCU	0
CCL	1
CCU	3
CC2L	2
CC2U	2
OCL	6
OCU	5
ChinCL	5
ChinCU	5
BBL	7
BBU	5
WLL	0
WLU	1
ECL	5
ECU	6
UTL	7
UTU	5
OPL	3
OPU	5
ACTL	5
ACTU	3
TOTAL	**8**
AVERAGE	**4**

escaped salamanders were new species or that they were not, we calculated the total species richness to be eight. Individually, the species richness of each site varied. The highest species richness values were found at BBL and UTL, each with seven species.

The sites with the least amount of species richness were WLL and SCU—no salamander species were found at these sites. The average species richness was four (Table 2).

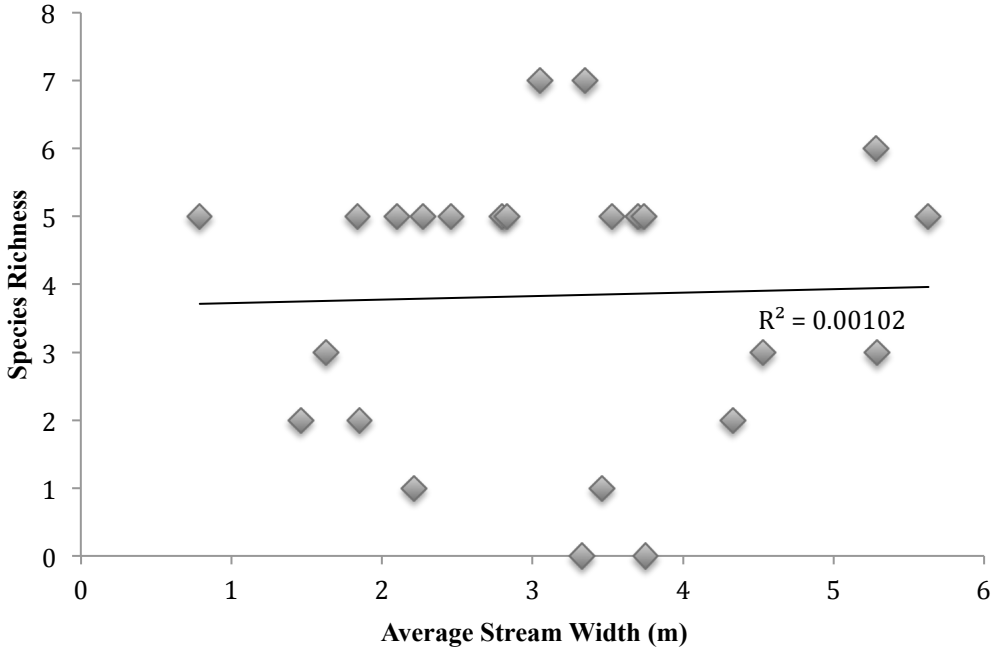


FIG. 3. Average stream width (m) plotted against species richness. There is no significant correlation between the two.

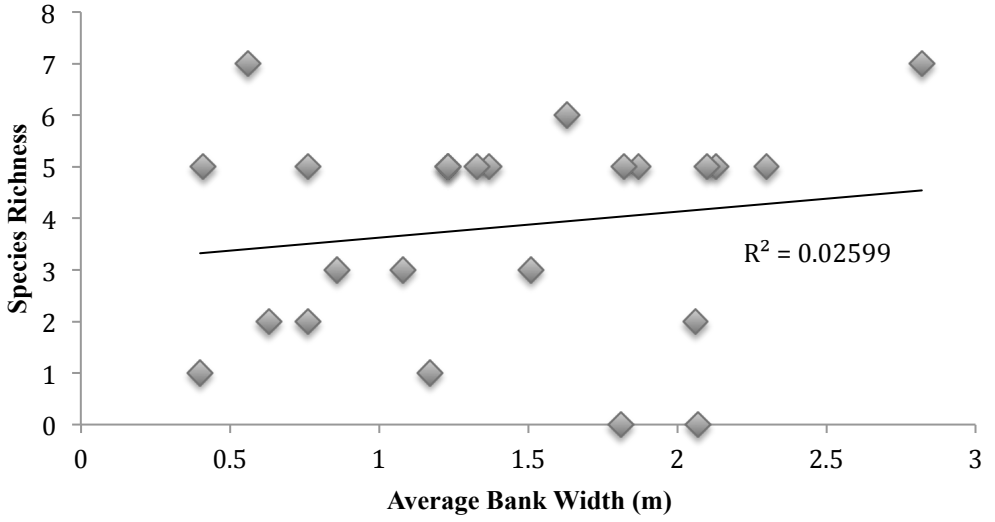


FIG. 4. Average bank width (m) plotted against species richness. There is no significant correlation between the two.

When the average stream width and species richness were compared for each site. The correlation coefficient was 0.0319 and an R^2 value of 0.00102 (Fig. 3). Average bank width and species richness were also compared resulting in a correlation coefficient of

0.1612 and an R^2 value of 0.02599 (Fig. 4). Species richness has no strong correlation with either stream width or bank width.

A total of 36 possible *D. folkertsi* specimens were collected out of the total 265 salamanders observed. The overall relative abundance of *D. folkertsi* in the area surveyed was 13.58% (Fig. 5). Its relative abundance differed from site to site. ACTU had the highest relative abundance, with 50% of species observed initially identified as *D. folkertsi.* The lowest relative abundance, excluding sites in which no species were found, was 0% at sites WLU, CCL, CCU, and CC2L. This was followed by an approximately 7% abundance at OCL, OCU, BBL, FCU, and OPU (Fig. 6).

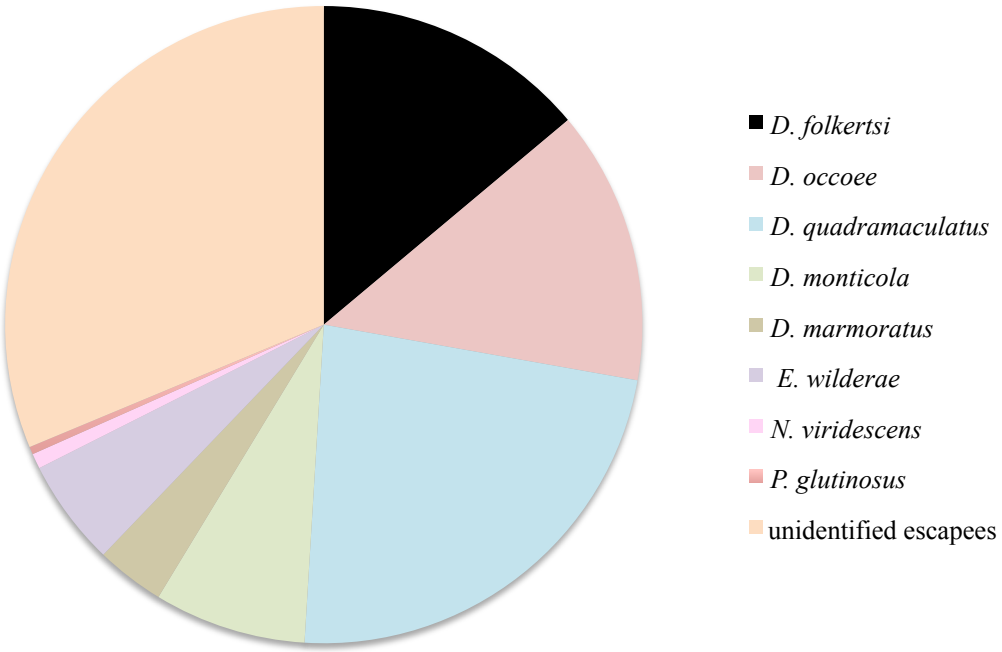


FIG. 5. Relative abundance of salamander species collected throughout entire survey. *D. folkertsi* had an overall relative abundance of 13.58%.

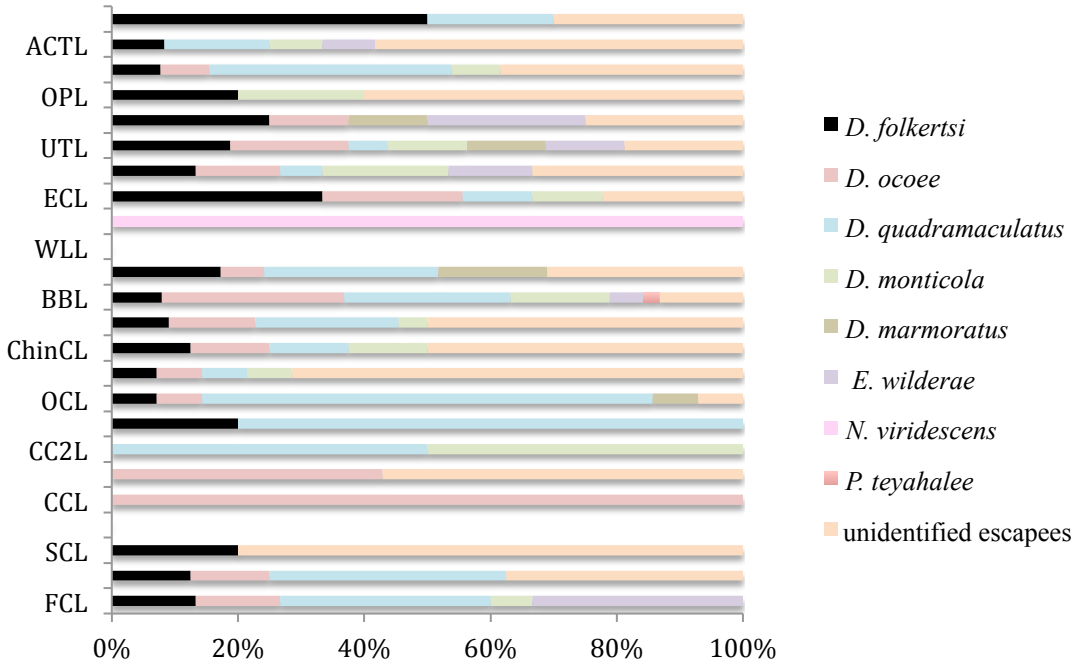


FIG. 6. Relative abundance of salamander species within each site. *D. folkertsi* is most abundant at ACTU and absent from WLU, CCL, CCU, and CC2L.

When average stream width and the relative abundance of *D. folkertsi* are compared at each site the correlation coefficient is -0.3949 and the R^2 value is 0.00522 (Fig. 7). Average bank width and relative abundance were also compared resulting in a correlation coefficient of -0.4095 and a R^2 value of 0.01091 (Fig. 8). Neither stream width nor bank width correlate with the relative abundance of *D. folkertsi.*

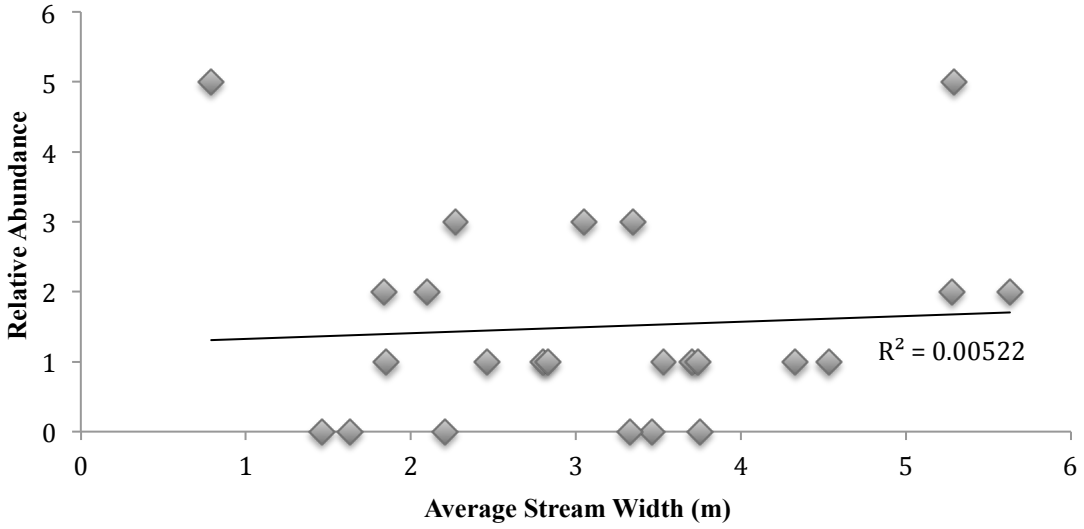


FIG. 7. Average stream width (m) plotted against the relative abundance of *D. folkertsi.* There is no significant correlation between the two.

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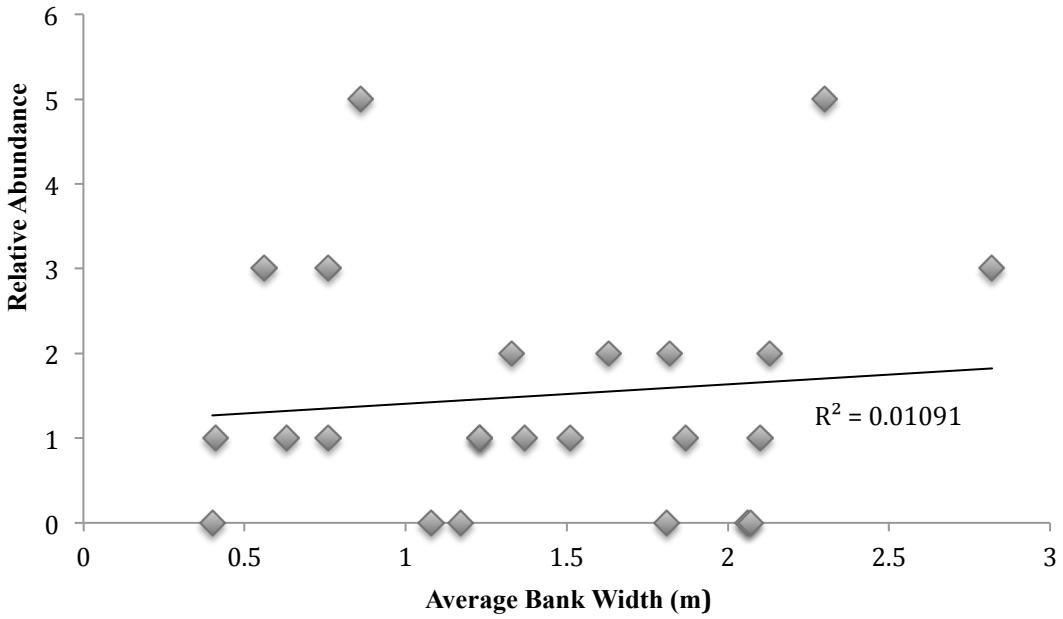

FIG. 8. Average bank width (m) plotted against the relative abundance of *D. folkertsi.* There is no significant correlation.

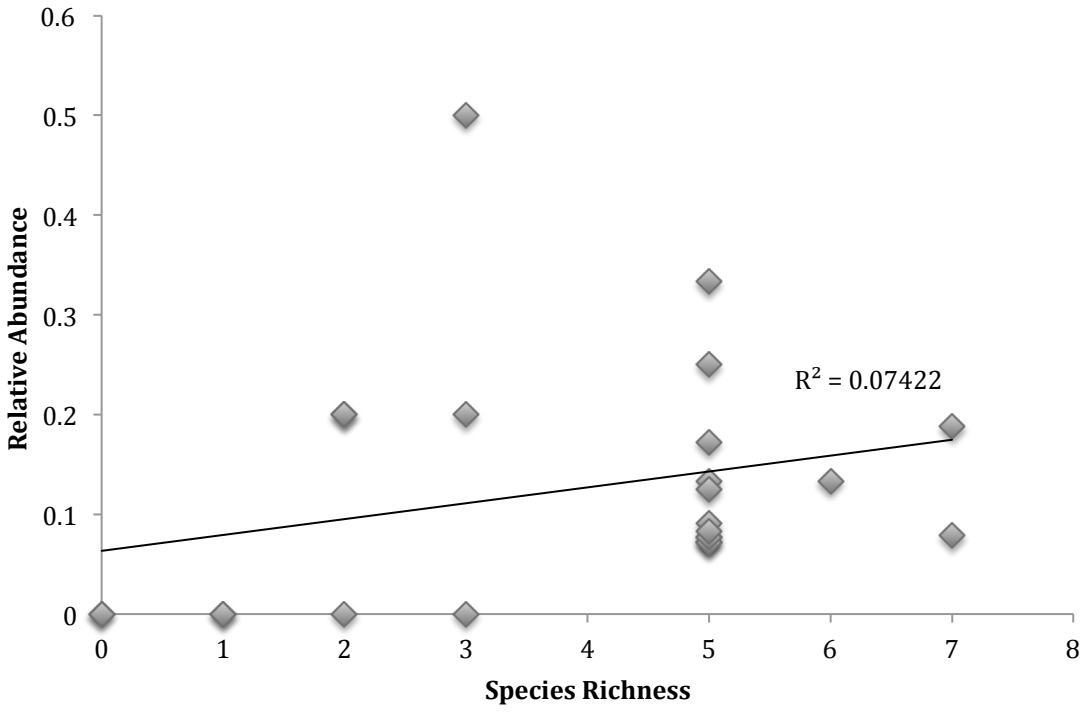

FIG. 9. Species richness plotted against *D. folkertsi*. There is no major correlation.

When species richness and the relative species abundance of *D. folkertsi* were compared the resulting correlation coefficient was 0.2724 and the R^2 value was 0.07422 (Fig. 9). There was no significant correlation between them.

DISCUSSION

Though there were slight positive correlations between species richness, stream width, and bank width, these results were not significant. The same held true when relative abundance of *D. folkertsi* was compared with the same habitat factors. This suggests that stream dimensions do not play a major role in the presence of *D. folkertsi.* The greatest relative abundances of *D. folkertsi* specimens were found at ACT and BB. These sites contained lots of fast moving water and plenty of hiding places. They also offered large waterfalls. Throughout the survey, I noted that *D. folkertsi* was typically found in locations that had small waterfalls or cascades. They seemed to prefer hiding under rocks or in crevices at the base of the falls. This could be a good indicator of possible *D. folkertsi* habitats in the future. The sites with low species richness could be a result of pollution or inadequate stream cover. WL, CC, and SC all showed signs of habitat disturbance. Upstream of the SC sites there was a private logging event occurring. At CC and WL there was human pollution, such as tires and other metal, inside the stream as well as poor canopy cover. More successful sites had large amounts of canopy cover shading the stream. This could suggest that *D. folkertsi* requires undisturbed habitat with high amounts of shade. If this holds true, canopy cover may be a good indicator of potential habitat in future surveys. Overall, specific habitat features and water quality appear to play a greater role in *D. folkertsi* abundance and species richness than stream dimensions. Further research should be conducted that explores the relationship between habitat features and the presence of *D. folkertsi.*

There was no correlation between species richness and the relative abundance of *D. folkertsi*. The largest relative abundance occurred at a site with a species richness of three. This may indicate that *D. folkertsi* may not be as successful in areas where there is competition for resources. As with other species of *Desmognathus, D. folkertsi* may fall into a strict community assembly (Bruce 2011). Once its North Carolina range is established, further studies should be conducted to determine what species are most likely to coexist with *D. folkertsi,* and in what habitats.

The data collected will help expand the known North Carolina range of *D. folkertsi.* Not only will this contribute to the assessment, by providing habitat information and relative abundance, of *D. folkertsi* as it is considered for state listing, it may also play a role in the North Carolina Wildlife Action Plan. The plan identifies the Chattooga River, a part of the Savannah River drainage, as an area of special concern. One of the ways proposed to protect this area is to grant the Overflow creek region, also known as Blue Valley, of the watershed Wilderness Designation. Part of the research that plays into this decision is the identification of rare or at risk species in the region as well as their habitats (NC Wildlife Resources Commission). We found several potential *D. folkertsi* in the area. If they are confirmed as *D. folkertsi*, the data we have collected may contribute to the research deciding whether or not it will be awarded Wilderness Designation.

Conclusion

Thirty-six *D. folkertsi* specimens were collected and identified from 10 tributaries in the Chattooga river drainage. If these specimens are confirmed as *D. folkertsi* with genetic analysis, the known distribution of *D. folkertsi* in North Carolina will be expanded. The data collected in this survey will contribute to the North Carolina Wildlife Resource Commission's assessment of *D. folkertsi* while it is being considered for state listing.

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THE VEGETATION AND SEED BANK OF DULANY BOG, A SOUTHERN APPALACHIAN FEN

EMILY WATSON-COOK

Abstract. Southern Appalachian bogs and fens are relatively rare communities that are becoming increasingly uncommon due to the inhibition of water flow and natural disturbance regimes. We sampled vegetation type (grasses, sedges, rushes, forbs, and woody plants) and examined differences in seed bank and community composition between closed and open canopy areas of a southern Appalachian fen in western North Carolina. The densities of sedges and rushes were significantly higher in open canopy areas relative to closed canopy areas. *Alnus serrulata* (smooth alder) also had a significantly higher density in open canopy plots than in closed canopy plots. The number of emerged seedlings was greater in open canopy soil samples than in those of the closed canopy. As lack of disturbance allows fast-growing woody species like alder to encroach upon wetland areas, examination of these differences is important to understanding the process of woody encroachment and predicting seedling emergence following management efforts.

Key words: Alnus serrulata*, canopy cover, Dulany Bog, plant community composition, seed bank, southern Appalachian fen, woody encroachment*

INTRODUCTION

Wetland communities are an uncommon occurrence in the Blue Ridge Province. Despite the relative rarity of these areas, they are highly diverse in terms of vegetation (Warren et al. 2007). The atypical topography, hydrology, and soil composition of these montane wetland ecosystems supports many endemic and disjunct plant species, several of which are rare, threatened, or endangered (Weakley and Schafale 1994). The scarcity of southern Appalachian wetlands is increasing as open water and marsh communities transition to shrub swamps and woodlands. Due to the persistent degradation of these wetlands, there are now fewer than 500 left in the region (Moorhead and Rossell 1998, Rossell and Wells 1999). There is some debate regarding the cause of these changes; the human interference versus natural succession dilemma is at the heart of this question.

Human activity likely reduces or eliminates previous natural patterns of disturbance. Fire suppression, beaver trapping, and reduction of water level fluctuations can result in wetland transitions (Keddy 1983). As these transitions occur in bogs and fens, woody shrub and tree species encroach upon the previously open wetland area. Fast-growing woody plants that are already present in surrounding mesic forests have the potential to outcompete rare herbaceous wetland species (Warren et al. 2007). In recent years, management and restoration of southern Appalachian wetlands has become a priority of government agencies and some private landowners. Methods of bog and fen restoration that have been proposed include the cutting of woody species, prescribed burning, and the encouragement of beaver activity (Pitillo 1993). Burning and hand-removal can be effective (Clark and Wilson 2001), although slashing and burning methods have achieved only limited success in some cases (Pitillo 1993). The allowance of beaver activity where possible is also a potential method of wetland restoration. The presence of beavers increases the duration of periods of flooding, often preventing the development of forests in wetland areas (Little et al. 2012). In addition, they tend to forage on woody species such as *Alnus serrulata* (smooth alder) (Rossell et al. 2014).

The study of seed banks, which contain reserves of viable seeds within soil, can be useful in predicting the after-effects of wetland management and the future of the plant community

(Rossell and Wells 1999). The seeds contained in the seed bank are typically more tolerant of stress and disturbance than their adult forms, so a species that has been eliminated from an area can still persist in its soil (Chang et al. 2001). Incubating soils is a common technique used to estimate the composition of readily germinable seeds in the seed bank (Brown 1992). Seedling emergence can therefore indicate the species that will persist following disturbance.

In this study, I examined the plant community composition and seed bank of a southern Appalachian wetland. The purpose of this research was to study the differences in vegetation type between open, bog-like areas and closed, woodland areas. These differences could provide insight into the process of woody encroachment. I also compared the seed bank of open and closed canopy areas with the intention of predicting the potential post-management bog community and evaluating the potential effectiveness of future management efforts.

METHODS

Site Description

Dulany Bog is a 45 acre site in Jackson County near Cashiers, North Carolina. Although commonly referred to as a bog, the area is technically considered a fen. Many southern Appalachian wetlands are known inaccurately as "bogs". True bogs are typically ombrotrophic, while fens, which may be fed by streams and groundwater, are typically minerotrophic (Warren et al. 2004). The characteristics of Dulany Bog, which is fed by a stream and groundwater in addition to precipitation, more closely resemble those of a fen than those of a bog. The stream that intersects the fen is the East Fork of the Chattooga River, a portion of the Chattooga River watershed (Pitillo 1993).

The community of the true fen portion of the site would be most accurately classified as the Typic Subtype of a Southern Appalachian Bog, according to Schafale's Fourth Approximation (2012). This community is characterized by permanently saturated wetlands at the bottoms of steams or gentle slopes. Due to the substantial groundwater and runoff they receive, these areas would technically be classified as poor fens. The hydrology and nutrient dynamics of these wetlands remain largely unknown. Sedges and grasses are commonly found within this community type, as is *Sphagnum* spp. (peat moss), *Juncus subcaudatus* (woodland rush), and *Osmunda cinnamomea* (cinnamon fern). *Alnus serrulata* is common in these areas when the process of woody encroachment is occurring.

Surrounding the fen is a transition zone from open canopy, bog-like habitat to closed canopy, forested habitat. The surrounding forest would be considered the Typic Subtype of a Swamp Forest-Bog Complex (Schafale 2012). Sedges dominate the open canopy area, while the closed canopy area is dominated by large trees, particularly *Pinus strobus* (white pine), which were most likely planted. Much of the open canopy portion of the fen contains smooth alder, which may be increasing in growth and outcompeting rare plant species. To manage encroachment, the United States Forest Service (USFS) has attempted slash removal of the alder with hand tools. Additional management techniques that have been proposed include conducting prescribed burns and encouraging beaver activity (Pitillo 1993).

Field Methods

We sampled vegetation plots of both open and closed canopy cover for a period of 29 days, from 27 August to 24 September 2015. To facilitate sampling, I systematically established four 20 m transects, 10 m in closed and 10 m in open canopy, across the fen. Along each transect, for both the open and closed portion, I randomly selected three 1 x 1 m plots for sampling. This arrangement allowed me to sample the transition zone from forest to shrub-dominated fen. To determine the density of herbaceous and woody species, for each 1 x 1 m plot, I calculated the density of sedges, grasses, rushes, forbs, and woody plants.

I classified each species as grass, sedge, rush, forb, or woody plant. To determine density, I counted the total number of stems for each classification type and identified the species present when possible. I estimated the percent cover of *Sphagnum* moss in each plot and recorded the percent cover of canopy using a spherical densiometer. I took densiometer readings in the center of each plot, along the transect.

To determine the density of woody plant species, I randomly sampled one 3 x 3 m plot in the closed canopy and one 3 x 3 m plot in the open canopy. For each plot sampled, I recorded the total number of stems and species of woody plants that were at breast height (approximately 4.5 ft). I also recorded percent ground cover of *Sphagnum* moss and percent canopy cover in the center of each 3 x 3 m plot.

I collected soil samples in each of the 3 x 3 m plots for both open and closed canopy sections. I used a trowel to collect a sample approximately 5 cm in depth and 5 cm in width. I stored samples in sealed plastic bags and refrigerated them following collection. On 5 October 2015, I placed soil samples in 9 cm x 9 cm x 10 cm pots on top of sterile potting soil. I mixed the samples before potting and spread them in layers of approximately 3 cm on top of the potting soil. I stored the pots in a greenhouse and kept them damp by watering them weekly. I observed the seedlings for emergence weekly and recorded the total number after a period of seven weeks, from 5 October to 23 November 2015.

Data Analyses

I analyzed differences in observed maximum indicator values for species between open and closed canopy areas using a Monte Carlo test of significance. These indicator values were calculated using the method of M. Dufrene and P. Legendre (1997). I used Multi-Response Permutation Procedures (MRPP) to determine whether differences in the species present in open and closed canopy plots were significant. I used another MRPP to determine whether vegetation types differed between open and closed canopy areas. I used Statistical Analysis System programs (SAS Institute, Inc. 2011) for these analyses.

I used analysis of variance (ANOVA) to determine whether the number of individuals of a certain vegetation type (sedges, grasses, rushes, forbs, and woody plants) differed significantly in open and closed canopy areas. I adjusted the data by adding one to each value to eliminate zeros present in plots where certain vegetation types were not found to be present. I also took the square root of each number when conducting statistical analyses to adjust for the large differences in some of the values. When vegetation type failed the test for normality, I used Mann-Whitney Rank Sum Tests to determine significance instead. To test woody stem and seedling data for significance I conducted unpaired t-tests using GraphPad (GraphPad Software, Inc. 2015).

RESULTS

I identified 54 plant species (Appendix A) and found 2,729 individual stems at Dulany Bog during this study. The average percentage of canopy coverage for plots defined as closed was 92.51 % and the average coverage for those defined as open was 5.02 %. Estimated average *Sphagnum* ground cover was 34.58 % for closed canopy plots and 19.25 % for open canopy plots.

The density of stems was generally greater in open canopy plots than in closed canopy plots. This was true for all vegetation types except woody plants, which were more numerous in closed canopy plots than they were in open canopy plots (fig. 1).

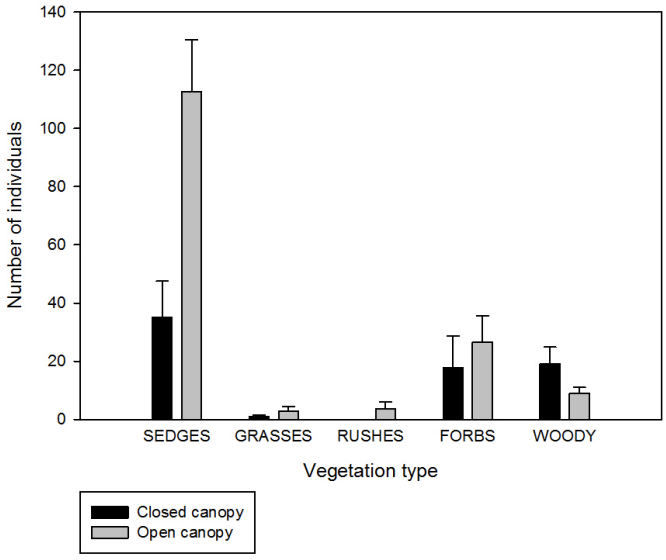

FIG. 1 Number of individual stems of each vegetation type in closed and open canopy plots

Based on analysis of observed maximum indicator values, *A. serrulata* density was significantly greater in open canopy plots than in closed canopy plots. *Smilax glauca* (cat greenbrier) and *Aronia arbutifolia* (red chokeberry) densities were significantly greater in closed canopy plots than they were in open canopy plots. Species most associated with closed canopy plots were *S. glauca*, *A. arbutifolia*, *Clintonia* sp., *Vaccinium* sp., *Viburnum lantanoides* moosewood), *Sarracenia pupurea* var. *montana* (southern Appalachian purple pitcher plant) and *Kalmia latifolia* (mountain laurel). *A. serrulata*, *Carex bullata* (button sedge), *Impatiens pallida* (pale jewelweed), *Dichanthelium dichotomum* (forked witchgrass), *Gallium* sp., *Rosa palustris* (swamp rose), and *Rumex* sp. were associated with open canopy plots (table 1).

TABLE 1. Indicator values and p* (alpha = 0.050) for species with the highest observed indicator values (IVs) in closed and open canopy

Closed Canopy			Open Canopy		
Species	Observed IV	p*	Species	Observed IV	p*
Smilax glauca	11.4	0.0388	*Alnus serrulata*	21.8	0.0066
Aronia arbutifolia	11.4	0.0390	*Carex bullata*	17.4	0.4271
Clintonia sp.	8.6	0.0894	*Impatiens pallida*	12.2	0.0594
Vaccinium sp.	8.6	0.0860	*Dichanthelium dichotomum*	9.9	0.2004
Viburnum lantanoides	8.6	0.0854	*Gallium* sp.	9.8	0.1292
Sarracenia purpurea var. *montana*	8.6	0.0912	*Rosa palustris*	9.2	0.5059
Kalmia latifolia	8.6	0.0916	*Rumex* sp.	7.5	0.3763

*Proportion of randomized trials with indicator values equal to or exceeding the observed indicator value

In the 3 x 3m plots, the mean density of woody stems was 13.25 in closed canopy plots and 27.50 in open canopy plots. I did not find the difference between these densities to be statistically significant ($t = 1.820$, $p = 0.119$). The mean density of *A. serrulata* was 2.50 stems per 3 x 3 m closed plot and 21.50 stems per open plot. I did not find the difference between average density of *A. serrulata* in closed and open plots to be significant ($t = 1.848$, $p = 0.1141$).

We compared the means of the square roots of numbers of stems in closed and open plots for each vegetation type. There were significantly more sedges in open canopy plots than in closed canopy plots (Table 1). I did not find significant differences in woody plant density between closed and open plots using a one-way ANOVA. Grass, rush, and forb data failed the test for normality, likely due to sample size and large variation between stem densities in plots.

I used a Mann-Whitney Rank Sum Test to analyze the vegetation types that failed the test for normality. The number of rushes in open canopy plots was significantly greater than the number found in closed canopy plots (Table 2). Differences in numbers of grasses and forbs between closed and open canopy plots were still found to be insignificant when corrected for non-normality.

TABLE 2. ANOVA and Mann-Whitney Rank Sum Test results comparing the square root of the number of stems of each vegetation type found in closed and open canopy plots (alpha=0.050)

Vegetation Type	P
Sedges	<0.001
Grasses*	0.335
Rushes*	0.037
Forbs*	0.311
Woody plants	0.158

*Failed test for normality and so were analyzed with a Mann-Whitney Rank Sum Test rather than ANOVA

I used MRPP to compare plant communities between closed and open canopy plots. Species composition of closed and open plots differed significantly ($A = 0.01175$, $p = 0.0132$). I also used MRPP to analyze differences in vegetation type between closed and open canopy plots, finding that vegetation type heterogeneity was not significantly greater than would be expected by chance ($A = 0.0010$, $p = 0.3455$).

Numbers of individual seedlings that emerged from open canopy soil samples were significantly greater than those that emerged from closed canopy soil samples ($t = 2.6027$, $p = 0.0405$) (Table 3).

TABLE 3. Number of emerged seedlings in closed and open canopy soil samples.

Transect	Closed Canopy	Open Canopy
1	4	3
2	4	28
3	0	14
4	1	28

DISCUSSION

Alnus serrulata was associated with open canopy plots, based on its observed indicator value. Since this species is the most prevalent woody species in the open, wetland areas of Dulany Bog, it is likely the primary player in the issue of woody encroachment and conversion of the fen to shrubland. Prevention of disturbance due to human activity in the area is likely contributing to the alder's success in competition with other wetland species. While sampling, I observed the inhibition of beaver activity. The stream under a low bridge leading to the residence of a private landowner was blocked by debris that has been assembled by beavers. The debris allowed portions of the fen to flood with standing water. The standing water on the bridge would prevent the landowner from safely accessing their property, so the debris was removed and the standing water drained. If the area were allowed to remain flooded, it is possible that the alder cover would lessen in favor of more aquatic bog species. However, the current height of the bridge and presence of the road prevent standing water from remaining in the area. Beaver activity could further aid in the prevention of woody encroachment by slowing the progression of alder growth because beavers selectively forage on *A. serrulata* (Rossell et al. 2014). Following the limited success of slashing methods (Pitillo 1993), fire management has also been proposed for Dulany Bog.

Central to the prevention of woody encroachment is the preservation of rare wetland species in Dulany Bog. Several populations of *S. purpurea* var. *montana* (southern Appalachian purple pitcher plant), the genus of which is listed in Appendix II of the Convention of International Trade in Endangered Species of Wild Fauna and Flora (2015), are found throughout the fen. One population happened to be present in some of the plots of one of our randomly selected transects. This population was not, however, located in the more open and mesic portion of the transect, as would have been expected of a wetland forb species. Instead, the population was primarily located in the closed canopy portion of the transect near the transition to open canopy. This may be due to the lack of competition from alder and sedges in the transitional, shaded area.

For all vegetation types except woody plants, I found a greater number of individuals in open canopy plots than in closed canopy plots. This could be a result of competition for light between shade intolerant bog species and shade tolerant, fast-growing woody species. If encroachment continues in the wetland, numbers and diversity of sedges, grasses, rushes, and forbs could potentially decrease due to competition with woody species. Open canopy areas are currently dominated by sedges but a variety of other vegetation types persist in the open canopy area as well. Rushes, for example, were found in several open canopy plots but were entirely absent in closed canopy plots, likely due to a lack of light availability.

Seedling density was significantly greater in open canopy plots than in closed canopy plots. Several studies of seed bank composition and characteristics have been conducted in the past (McGraw 1987, Rossell and Wells 2007). In Rossell and Wells' study (1987), 26 taxa

emerged in closed canopy soils and 19 emerged in open canopy soils. Although I did not identify seedlings to taxa in this study and thus did not examine richness, I found that a significantly greater number of individuals emerged in open canopy soils than in closed canopy soils. Due to the time limitations of this study, I was only able to observe seedling emergence for seven weeks. Dormancy requirements of some seeds were likely not fulfilled during this time period and thus these data cannot be considered representative of the complete composition of Dulany Bog's seed bank. These data are likely indicative of seedling emergence immediately following management action. Further studies of the seed bank, particularly the species composition, of Dulany Bog would be useful in predicting the effects of management on the plant community.

CONCLUSION

Clear differences in community composition are present along the gradient from open canopy fen to closed canopy woodland. It is possible that the process of woody encroachment is amplifying these differences. As *A. serrulata* and other fast-growing woody species continue to overtake the wetland, the plant community will likely experience changes that could result in a loss of unique aquatic species and diversity in general. Continued evaluation of changes in the area's vegetation, both pre- and post-management, will be necessary in the effort to maintain the unique character of Dulany Bog.

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APPENDIX A

Species presence data for sedges, grasses, rushes, forbs, and woody plants in closed and open canopy plots (X=present).

Family	Species	Closed Canopy	Open Canopy
Sedges	*Carex aestivalis*	X	
	Carex folliculata	X	X
	Carex gynandra	X	X
	Carex sp.	X	X
Grasses	*Dichanthelium dichotomum*	X	X
	Unknown grass		X
Rushes	*Juncus subcaudatus*		X
Forbs	*Aster* sp.		X
	Chelone sp.		X
	Clintonia sp.	X	
	Eupatorium perfoliatum		X
	Eupatorium sp.		X
	Eutrochium purpureum		X
	Galax urceolata	X	
	Gallium sp.		X
	Hypericum sp.		X
	Impatiens pallida		X
	Lobelia cardinalis		X
	Lobelia puberula		X
	Osmunda cinnamomea	X	
	Packera sp.		X
	Rubus hispidus		X
	Rubus sp.	X	X
	Rumex sp.1		X
	Rumex sp.2	X	X
	Sanicula sp.		X
	Sarracenia purpurea var. *montana*	X	
	Smilax glauca	X	
Forbs	*Smilax* sp.	X	X
	Thelypteris noveboracencis	X	X
	Viola sororia		X
	Viola sp.		X
	Unknown broad leaf		X
	Unknown fern	X	
	Unknown forb	X	
Woody plants	*Acer rubrum*	X	X
	Alnus serrulata	X	X
	Aronia arbutifolia	X	
	Cornus amomum	X	
	Ilex opaca	X	
	Kalmia latifolia	X	
	Leucothoe fontanesiana	X	
	Lyonia ligustrina	X	
	Oxydendron arboretum		X
	Pinus strobus	X	X
	Rhododendron maximum	X	
	Rhododendron sp.	X	
	Rosa caroliniana		X
	Rosa palustris	X	X
	Sambucus canadensis	X	
	Vaccinium staminium	X	X
	Vaccinium sp.	X	
	Viburnum cassinoides	X	X
	Viburnum lantanoides	X	

DISTRIBUTION PATTERNS OF CHESTNUT BLIGHT IN AMERICAN CHESTNUT POPULATIONS

SARAH WELLISH

Abstract The American chestnut, an ecologically dominant and commercially valuable tree, was functionally eliminated from the wild following the introduction of the ascomycete pathogen *Cryphonectria parasitica* (chestnut blight). The potential for reintroduction of resistant hybrid trees has led to a need for increased understanding of American chestnut distribution patterns and how they affect the spread of disease. I examined the relationship between chestnut coppice distribution and disease levels in three populations of American chestnut. Many coppices exhibited a clumped distribution pattern, and disease was more prevalent within clumps if a tree was heavily impacted. Isolated coppices were less frequently infected, suggesting that the risk of disease transmission is greatest over short distances.

Key Words: American chestnut; chestnut blight; clumped distribution; disease pathology; spatial analysis; vectors

INTRODUCTION

The American chestnut (*Castanea dentata*) was one of North America's most ecologically and commercially valuable tree species. It made up 25% of the trees in the forests where it was found at the height of its population (Jabr 2014). At the beginning of the 20th century, the American chestnut population was devastated by an introduced pathogen–the ascomycete fungus *Cryphonectria parasitica*. The fungus came to America along with nursery cuttings of Chinese chestnut; however, the American chestnut lacked the resistance of its Asian cousin and was subsequently devastated by the disease (Burke 2012). The fungus infects the tree through cracks in the bark, girdling the tree and cutting off the flow of nutrients through the phloem and outer xylem (Hebard 2001). The first incidence of the disease in wild American chestnut populations occurred in 1905, and the fungus rapidly spread across the trees' native range. By the 1970s, the majority of wild American chestnut populations–approximately ten billion trees–had been eliminated (TACF 2009). The surviving root systems will periodically send up new stems; however, the continued presence of the fungus in the environment prevents the majority of these stems from reaching reproductive age (Heinrich 2014).

Because of the American chestnut's ecological and commercial significance, reintroduction efforts have been extensive. The foremost organization dedicated to the preservation of the American chestnut is the American Chestnut Foundation, founded in 1983. This organization focuses on the hybridization of American and Chinese chestnut trees in hopes of breeding viable, disease-resistant hybrids (Hebard 2001). In 2005, the American Chestnut Foundation harvested its first crop of potential blight-resistant trees. These trees, produced from multiple backcrosses to American chestnut stock, are 15/16 American chestnut (TACF 2009).

Now that potential exists for successful American chestnut re-introduction, it is critical that further research be conducted into fungal distribution patterns within existing populations of wild American chestnuts. Such data will help to determine whether sites may be suitable for reintroduction and provide information that can maximize survivorship of planted hybrids. While potential disease vectors and transmission methods have been studied, there is little research on distribution patterns themselves. Furthermore, there is a substantive lack of data on chestnut populations within Macon County and the greater Southern Appalachians. While this study is not intended to serve as a comprehensive examination of blight distribution patterns, it can provide

the necessary initial data on chestnut blight in Macon County and the Great Smoky Mountains National Park.

METHODS

Field data were collected from three sites, two of which were located in privately-held property on Satulah Mountain in the township of Highlands, North Carolina (South Satulah and Little Creek Road). The third site was located at Purchase Knob in the Great Smoky Mountains National Park (Purchase Knob).

At each research plot, we sampled between 100 and 150 randomly selected American chestnut coppices. A coppice was defined as all of the individual stems greater than one foot in height that originated from the same root cluster. Coppices were determined to originate from separate root clusters if they were further than one meter apart (Burke 2012). We used a Garmin Montana 650t GPS to record a location for each chestnut coppice, and we sampled and flagged the coppice with an identification number so we could easily identify it for future data collection. Stem height – estimated based on a five-foot scale – and stem diameter at one foot in height were recorded for each stem within the coppice. Each stem was also assessed for the presence of the chestnut blight fungus *(Cryphonectria parasitica)* based on evidence of sporulation as well as other visible signs such as cankers and flagging limbs.

Following initial data collection, all chestnut coppices determined to have blight were entered into a number randomization tool. We then selected a simple random sample of twenty chestnut coppices from each study site. From each of these twenty coppices, we took three bark tissue samples from blight-infected areas; all coppices with uncertain disease status were also sampled. Bark samples were then cultured in agarose gel and the cultures were examined for the presence of *C. parasitica* after one week. The identity of the cultured fungus was confirmed by Dr. Rich Baird using molecular identification techniques. Genomic DNA was extracted from the samples, amplified using PCR, and compared to existing data on the genome of *C. parasitica* through the NCBI GenBank.

To study distribution patterns of *C. parasitica*, I imported GPS coordinates into ArcGIS 10.3, where they were combined with physiological data, and disease pathology data contained in Microsoft Excel spreadsheets. I then made a disease percentage index by calculating the percentage of infected stems within each coppice:

Disease percentage index score = Number of infected stems / Total stems

Based on this data, I created maps displaying location data and disease percentage for the two Satulah Mountain sites and for Purchase Knob. I studied the distribution of infected and uninfected trees to determine if patterns of disease distribution existed at the sample sites.

RESULTS

The disease percentage index scores for the South Satulah and Purchase Knob sites were comparable in their distribution. In both sites, around half of the surveyed trees showed signs of blight. Disease percentage index scores for each site showed similar patterns of distribution (Fig. 1, Fig. 2). The Purchase Knob site showed significantly lower levels of blight (Fig. 3).

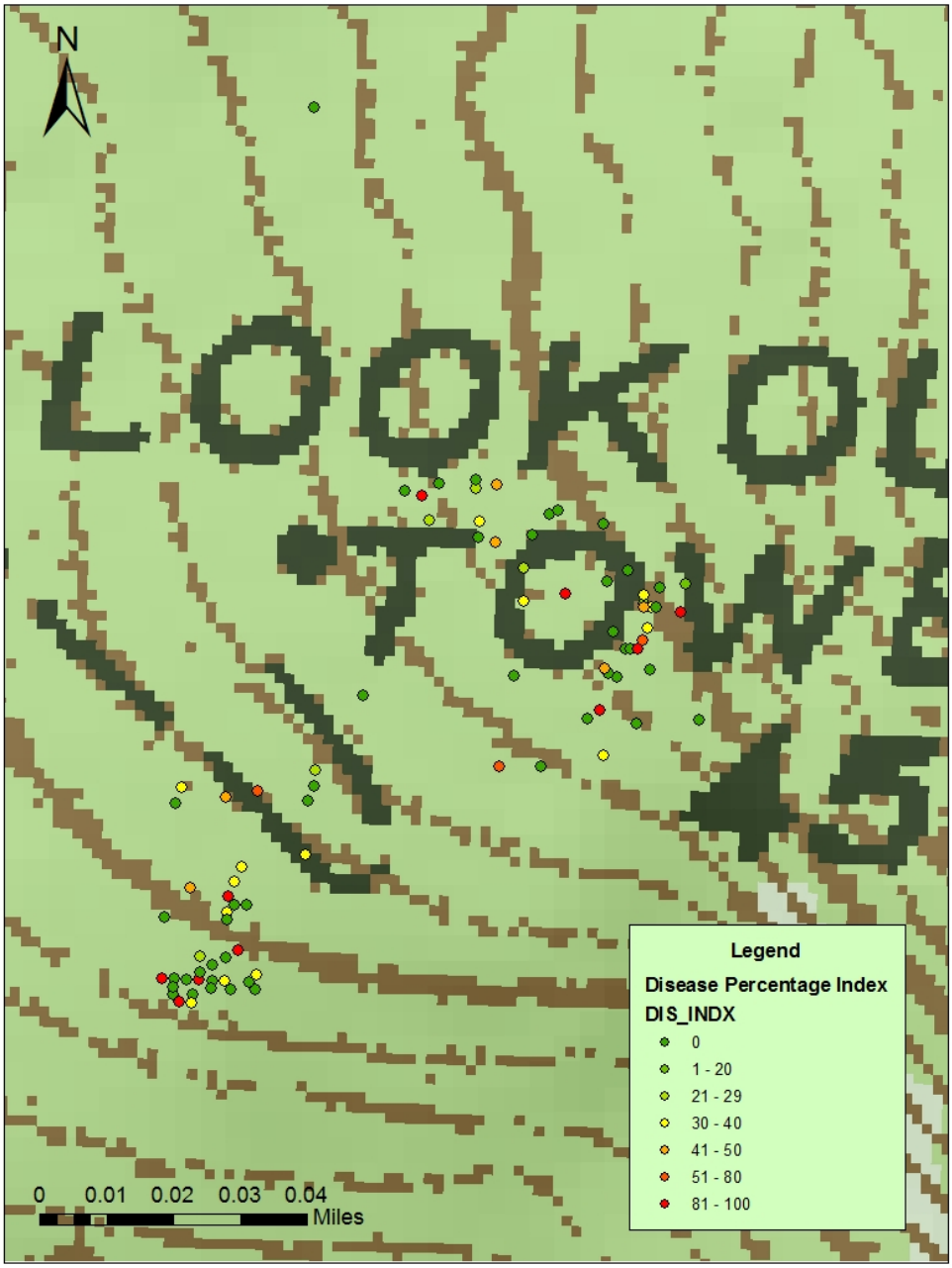

FIG. 1. Chestnut coppice distribution and disease percentage index data for the South Satulah site.

Distribution patterns were consistent between sites and largely followed the model of a clumped population distribution. Isolated coppices of American chestnut are also common, and are particularly notable at the Little Creek Road site (Fig. 2). Past research on chestnut seedling dispersal suggests that clumped distribution patterns are common for the species but that isolated stands are also known to occur (Heinrich 2014).

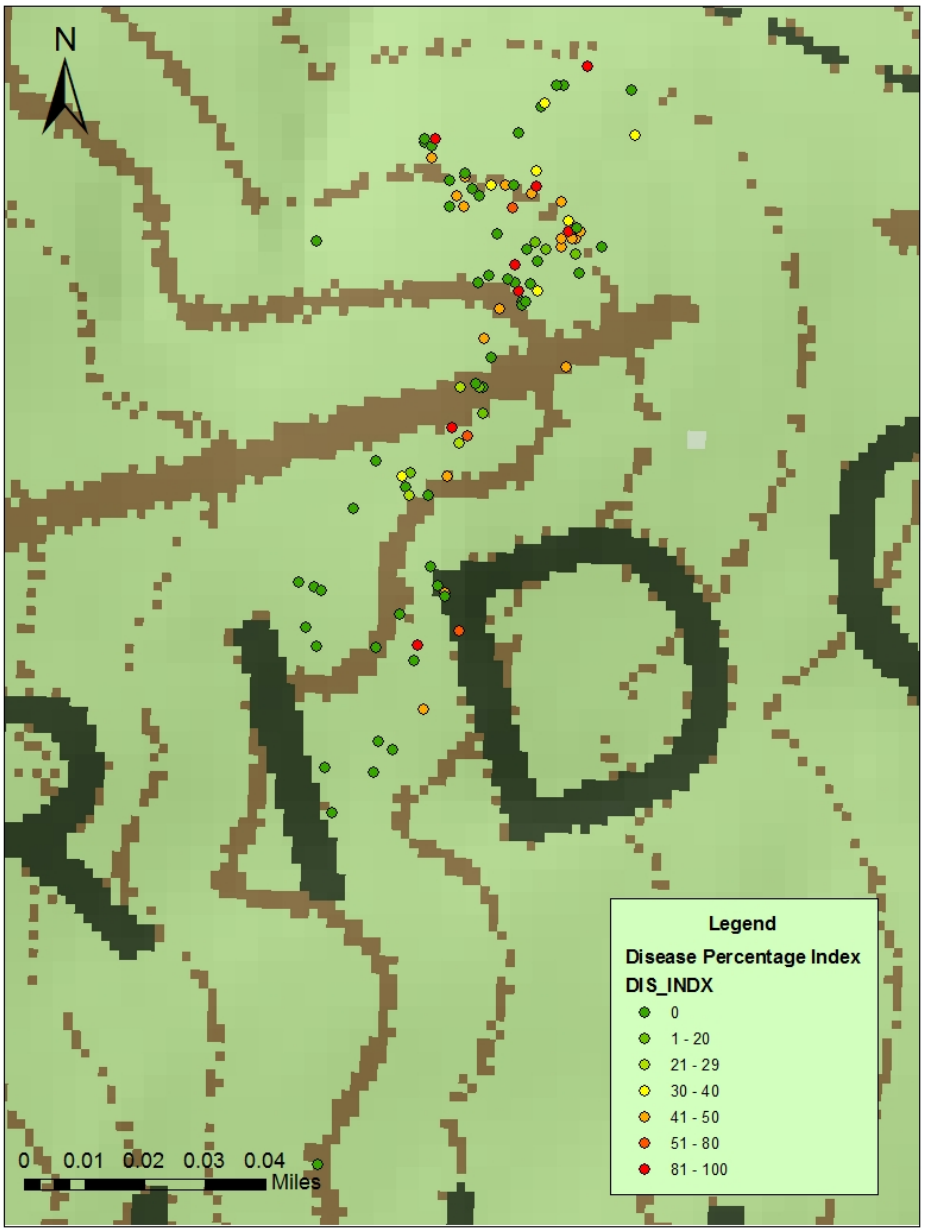

FIG. 2. Chestnut coppice distribution and disease percentage index data for the Little Creek Road site.

DISCUSSION

Several trends in American chestnut coppice distribution appeared with relative consistency throughout all three sample populations. With several exceptions, the majority of the coppices in each population showed evidence of a clumped population distribution. At the Purchase Knob and South Satulah sites, the clumps of chestnut coppices within the population also showed relative uniformity in size (Fig. 1, Fig. 2). The uniformity of the clumped distribution at Purchase Knob is particularly notable; clumps were of similar size and were spaced evenly throughout the site. The clumping pattern was less pronounced at the Little Creek

Road site, which was notable for its lower density of American chestnuts compared to other sites (Fig. 1).

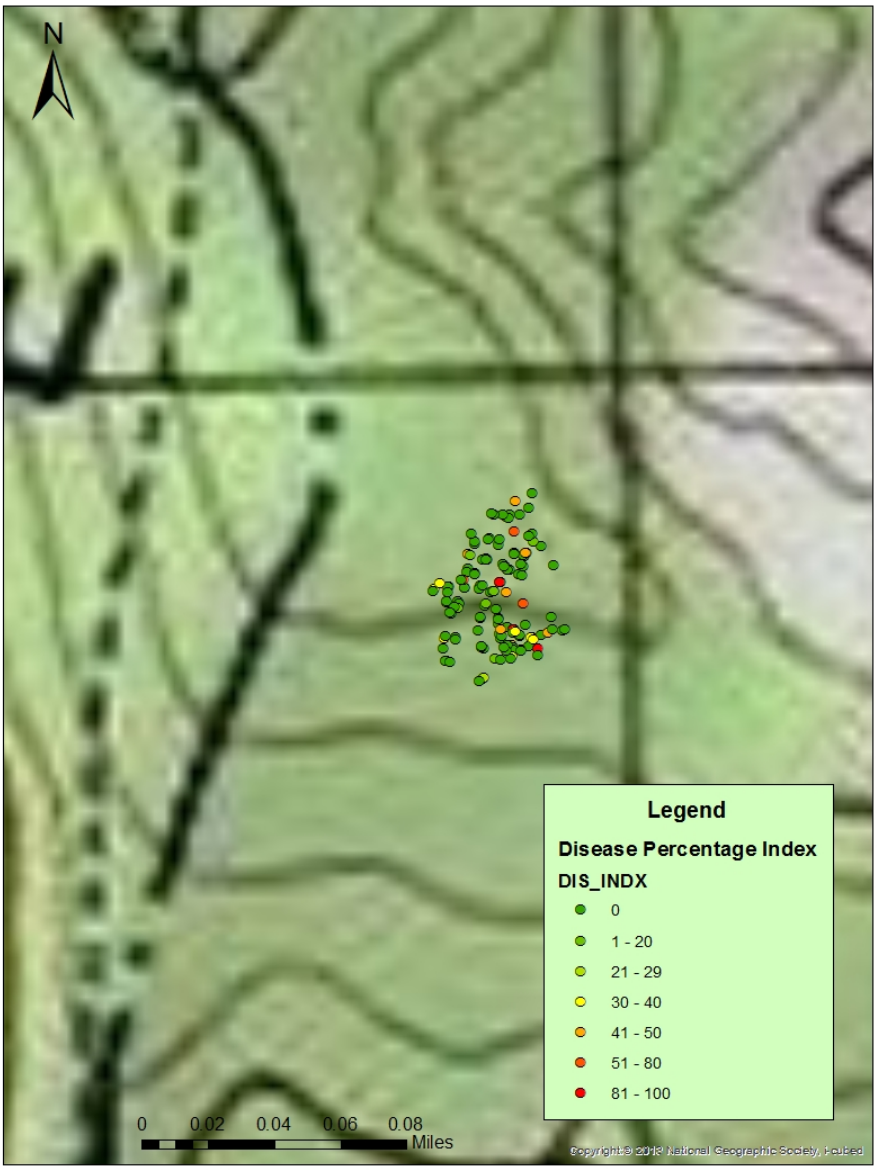

FIG. 3. Chestnut coppice locations and disease percentage index data for the Purchase Knob site.

The clumped distribution pattern of the coppices appears to be a contributing factor in the susceptibility of local populations to chestnut blight. In tightly grouped clusters of coppices, the presence or absence of chestnut blight appeared to be more locally influenced. When chestnut blight was present in a cluster, it affected most, but not all, trees within that cluster. In more isolated trees, the local presence or absence of disease appeared to have less influence on neighboring coppices. This pattern of disease transmission is consistent with known transmission patterns for *C. parasitica*, as its use of water as a vector would suggest that it can be highly virulent to nearby trees but less infectious over distance (Aganostakis 2001). The effects of windborne and insect-based transmission are still relatively unknown, but increased distance

would also reduce the likelihood of American chestnuts being infected by these vectors (Anagnostakis 2001).

Further examination of data from Little Creek Road and Purchase Knob shows that highly isolated coppices generally do not have blight and that infection rates in isolated coppices are less severe (Fig. 2, Fig. 3). While it is possible that these trees could eventually become infected, these trees tend to be healthier than those found in more densely populated areas, according to the disease percentage index. Trees further than 0.01 miles away from infected coppices rarely showed any signs of infection in this study.

CONCLUSION

The results of this research suggest that there is a correlation between coppice proximity to other American chestnuts and disease levels. Diseased trees tended to occur in clusters, perhaps indicating that the usual vectors of chestnut blight – water, insects, and possibly the wind – are spreading the fungus between neighboring trees. While not all isolated coppices were disease-free, those that were disease-free were located more than 0.01 miles from other American. Isolated coppices generally had lower disease percentage index scores than clumped coppices, indicating less severe infections. It is possible that infections in these isolated coppices may be treatable using currently known treatment methods, such as soil-packing and chemical fungicides (TACF 1992).

These findings have value when applied to potential reintroduction projects taking place throughout the southern Appalachians. The low disease ratings in isolated coppices suggest that there may be a minimum safe distance for the planting of new hybrids. Further study will be needed to determine the rate of disease transmission in hybrids, but this preliminary study indicated that isolation of hybrids will provide some protection from the spread of the fungus.

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The Highlands sites were both located on private property and were accessed with the owners' permission. The first site was located on the south side of Satulah Mountain and owned by Mr. Robert Haynes; the second site was located in the gated community of Ravenell on Little Creek Road.

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