EXAMINATION OF MORPHOLOGICAL AND HABITAT VARIATION WITHIN *STENANTHIUM GRAMINEUM* (EASTERN FEATHERBELLS, MELANTHIACEAE)

A thesis submitted to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Biology

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June 2020

ACKNOWLEDGEMENTS

I would like to thank my advisors for their help and guidance over the past two years. In particular, I'd like to thank Dr. Mathews for her careful guidance and patience, and Dr. Collins for her advice and wisdom. I'd also like to thank Dr. Sharma for her help and encouragement, and Dr. Thomas Martin for the time and patience he put into aiding me with the statistics for this project. I'd like to extend my thanks to my lab mate Austin Brenek, who helped me tirelessly and optimistically throughout my fieldwork, and to Elizabeth Joslin, whose assistance in collecting morphological measurements from herbarium specimens was invaluable. Finally, I'd like to thank my mom, Myra Cook, for her unwavering belief in me, as well as her endless support and love.

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ABSTRACT

Stenanthium gramineum (Ker. Gawler) Morong (Melanthiaceae), has historically been an understudied species. This species is generally considered to consist of two varieties: var. *gramineum*, a habitat generalist, occurring on grassy balds, rock outcrops, and in dry and mesic woodlands, and var. *robustum*, a habitat specialist, occurring in mountain bogs and wet meadows. A third variety, var. *micranthum* is not formally recognized, but was described on the basis of its small stature and unique granitic dome habitat. However, many taxonomists do not recognize any of the varieties, suggesting that they are indistinct and sympatric. The purpose of this study was to determine if the three varieties of *Stenanthium gramineum* should be recognized as distinct entities, and at what taxonomic rank each should be recognized. I performed morphological and ecological analyses of the three varieties of S. *gramineum*, including taking macro and micro morphological measurements from the field and from herbarium specimens, as well as measuring ecological characters of the field sites I visited. I then ran univariate and multivariate statistical analyses on the data collected to aid in clarifying the taxonomy of *S. gramineum* varieties. Results suggest that var. *robustum* should be elevated to species level, based on morphological separation, while var. *micranthum* should be recognized as

a taxon discrete from the typical var. *gramineum*. The findings in this study emphasize the need for conservation of all varieties, as anthropogenically caused changes threaten their habitats. This is especially critical in the case of var. *robustum*, a mountain bog specialist of the Appalachians.

CHAPTER ONE: INTRODUCTION

Background

Stenanthium gramineum (Ker. Gawler) Morong (Melanthiaceae), commonly known as "Eastern Featherbells" is a perennial herb that occurs in the Southern Appalachians and more broadly throughout the eastern U.S. (USDA, NRCS 2019; Weakley 2015). Melanthiaceae, commonly known as the bunchflower family (Weakley 2008, 2015), is comprised of perennial, monocotyledonous herbs commonly found within woodland and/or alpine habitats throughout the Northern Hemisphere, ranging from temperate to artic zones (Zomlefer et al. 2006). The Melanthiaceae family likely arose during the Cretaceous period, ca. 46-62 million years ago (Zomlefer et al. 2006). Melanthiaceae was first described by Batsch in 1793 (Weakley 2008, 2015) and segregated from Liliaceae in 1802, due to the apically diverted carpels (Zomlefer 1997). It consists of five tribes, of which *Stenanthium* belongs to tribe Melanthieae (Kim et al. 2016).

The genus *Stenanthium* currently contains six species, distributed within eastern North America (Weakley 2008, 2015) after having undergone many taxonomic rearrangements until finally being segregated from other genera, including *Helonias* and *Xerophyllum* (Heikens et al. 2002). Overall, *Stenanthium* is characterized by a terminal, paniculate inflorescence with white to yellow/green flowers. Plants have a basal rosette and are slender or bulbous at the base, with fibrous remnants of prior leaf bases (Gleason 1952). *Stenanthium diffusum* Wofford, described in 2006, is most morphologically similar to *S. gramineum*. *Stenanthium diffusum* is endemic to rockhouses of the northern Cumberland plateau of Tennessee.

Taxonomic splitting and additional new species recognition has recently occurred in *Stenanthium* based on careful analyses of morphology, phenology, habitat and geographic range

(Carter et al. 2009; Morris 2012; Sorrie and Weakley 2017). High variation in habitat and morphology among *S. gramineum* varieties suggests that there may be more species than currently recognized. (Weakley, A. pers.comm.; Wofford 2006).

Currently, two varieties of *S. gramineum* are recognized, distinguished in part by habitat differences. *Stenanthium gramineum* var. *gramineum* is considered a habitat generalist, occurring on grassy balds, rock outcrops, and in dry and mesic woodlands (Weakley 2015). *Stenanthium gramineum* var. *robustum* (S. Watson) Fernald occurs in bogs and wet meadows; it is endangered and threatened throughout its native range, causing need for special attention (Weakley 2015; USDA, NRCS 2019). A third variety, *S. gramineum* var. *micranthum* Fernald, is not recognized currently, but was described on the basis of its unique granitic dome rock outcrop habitat and its small size (Fernald 1950).

Various authors have distinguished the *S. gramineum* varieties by several morphological characters (Table 1; Fernald 1946, 1950, Small 1933, Weakley 2015), while others claim they are indistinct and sympatric (Gates 1918, Johnson 1969, Utech 2002). Based on the characters emphasized in the literature, it appears that traits vary the greatest between var. *gramineum/micranthum* and var. *robustum*, with the main differences being in plant height, leaf distribution and texture, fruit characteristics, and tepal length (Table 1).

Table 1. Variation in morphological characteristics among *S. gramineum* varieties emphasized in the literature (Fernald 1946, 1950; Small 1933; Weakley 2015).

	Plant Height (m)	Leaf Distribution	Leaf Texture	Tepal Length (mm)	Style Beak Curvature on Capsule	Capsule Shape	Capsule Length (mm)	Seed Length (mm)
Var. *gramineum*	0.5-1.9	Crowded below, diminishing below panicle	Firm – coriaceous, opaque, surface corrugated	3-8 (-10)	Deflexed	Ovoid-urceolate	6-9	5-5.5
Var. *robustum*	Up to 1.8	Crowded and numerous nearly up to panicle	Thin, membranous, translucent, surface smooth	5-10	Erect	Oblong-subcylindric	9-10	5-8
Var. *micranthum*	0.25-1.0	Crowded below, diminishing below panicle	Firm – coriaceous, opaque, surface corrugated	3-4.5 (-5)	Unknown	Unknown	Unknown	Unknown

In sum, each of these varieties may have unique morphological traits, and each may occupy a unique, sensitive habitat. They potentially could be recognized as separate species if discrete differences in morphological characteristics vary significantly. The goal of my research was to investigate macro- and micromorphological, phenological, ecological and habitat characteristics of the three *S. gramineum* varieties in order to clarify their taxonomy and provide information for conservation.

Key Question

Should the three varieties of *Stenanthium gramineum* (var. *gramineum*, var. *robustum* and var. *micranthum*) each be recognized as distinct entities on the basis of their morphological and ecological characteristics, and at what taxonomic rank?

Significance

This exploration of three varieties of *S. gramineum* will increase descriptive knowledge of these attractive native plants, as well as our knowledge of the differences among the varieties within the species. This study will not only add to our knowledge of *Stenanthium* morphologic and geographic variation, but will also increase awareness and interest in conservation of these delicate plants. Analyses of morphology and ecological measurements of each variety's habitat will provide insight into whether morphology is correlated with habitat conditions. This study should provide new knowledge that will aid in conservation, which is crucial for all varieties as they face loss of their unique and uncommon habitat types.

Species Concepts

In order to determine if any or all of the named varieties of *S. gramineum* should be elevated to species level, a species definition must be recognized. Though many valid species concepts are recognized, there is no one concept heralded as the universal standard, though some are more popular than others, particularly the biological species concept (BSC) (Lucklow 1995; McDade 1995; DeQueiroz 2007). The BSC recognizes that the most imperative characteristic that separates species is the inability to interbreed. This is the fundamental concept that separates the BSC from other species concepts (Lucklow 1995).

Many taxonomic studies that use morphological, phenological, habitat and distribution data follow the BSC, though not always directly stated. Instead, these studies generally use indirect evidence to support that interbreeding has not occurred, or that it likely could not occur. Wofford (2006) uses indirect evidence for the BSC by providing support for the circumscription of a new species, *Stenanthium tenneeseense,* by a combination of characteristics. Though S. *tenneeseense* appears to be morphologically similar to *S. gramineum*, they are easily distinguishable by distinct differences in morphology, phenology, geography and habitat. Particularly, the phenological difference, (as this species has a much later and shorter bloom time than S. gramineum), and habitat/ geographical difference (occurs only on rockhouses along the northern portion of the Cumberland Plateau in Tennessee), make it apparent that interbreeding between this species and *S. gramineum* would likely be impossible, as they are phenologically and geographically separated, and do not occur in the same habitat type. Wofford's characterization and justification of this species clearly lies within the assumption that they are fundamentally unable interbreed.

Knapp and Naczi's delimitation of *Juncus longii* (2008) also relies on the BSC without it explicitly being stated. The authors draw evidence for this taxon to be recognized at the species level through univariate and multivariate analysis of morphology versus environmental conditions to show differentiation is not caused by environment (showing a distinction among/between morphology that must have a genetic basis) and noted that *J. longii* was found in the field alongside *J. marginatus* without any intermediates present, showing that there was no inbreeding. Habitat and distributional differences were another key indicator of different species, providing support for the BSC. *Juncus marginatus* and *J. biflorus* are more of habitat generalist and exhibit a more extensive distribution than *J. longii*, which is endemic to the southeastern United States.

Janovec & Harrison (2002) provide yet another example of the use of the BSC without explicit statement. *Compsoneura mexicana* was raised to species level using a combination study of morphological analyses, biogeography, and ecology. Furthermore, though not explicitly stated, authors claimed that the Andes mountain chain serves as geomorphic barrier between *C. mexicana* and *C. sprucei* which prevents cross-fertilization, dispersal, and gene flow. This indicates that the BSC was used, as the authors based their claim that speciation had occurred on the presence of geographic boundaries that prevented interbreeding.

In order to determine the taxonomic classifications of *S. gramineum's* varieties assuming the BSC, I determined 1) if varieties are morphologically different from one another, 2) whether morphological variation is correlated to environmental conditions of their habitats, 3) if varieties occur together in the field, and/or if any intermediates are present in my samples, and 4) if phenology and range/distribution differ significantly. Compiling and analyzing these data through the use of multivariate and univariate analyses helped determine the taxonomic classification for these varieties according to the BSC.

CHAPTER TWO: METHODS

Morphological Data Collection

I used the morphological descriptions in Identification and Reidentification of North American Plants (Fernald 1946), Gray's manual of Botany 8th Edition (Fernald 1950), Manual of Southeastern Flora (Small 1933), and Flora of the Carolinas, Virginia, Georgia, northern Florida, and surrounding areas (Weakley 2015) to compile a list of 28 potentially diagnostic characters for the varieties of S. *gramineum*. I analyzed 24 herbarium specimens from Western Carolina University Herbarium (WCUH), North Carolina State University Herbarium (NCSC), University of North Carolina at Chapel Hill Herbarium (NCU) and the University of Georgia Herbarium (GA) to discern additional potentially diagnostic characteristics among varieties, for a total of 35 characteristics (Appendix A).

I analyzed 58 specimens total from the Carnegie Museum (CM), NCU, and WCUH to collect morphological data among all varieties across *S. gramineum*'s range (Appendix B). I recorded vegetative and reproductive traits as well as phenology and geographic location (Appendix B).

All herbarium specimens used were identified to variety based on comparison to digital images of lectotypes of each variety, available online through Harvard University Herbaria's online database. Specimens used for data collection were chosen based on location and completeness of specimen. For location, I selected specimens from a wide geographic range, focusing on areas of potential overlap as well as range edges. This allowed me to look for possible intermediates and any intermediate morphology throughout ranges. For *S. gramineum* var. *gramineum*, which can occur in several different habitats, I used specimens collected from various habitat types in order to determine if there were intermediates among the various habitats

(Culley 2013). Since each variety encompasses a large range (Fig. 1-3), the herbarium specimens were useful in extending the distribution of plants I was able to sample compared to my field sampling.

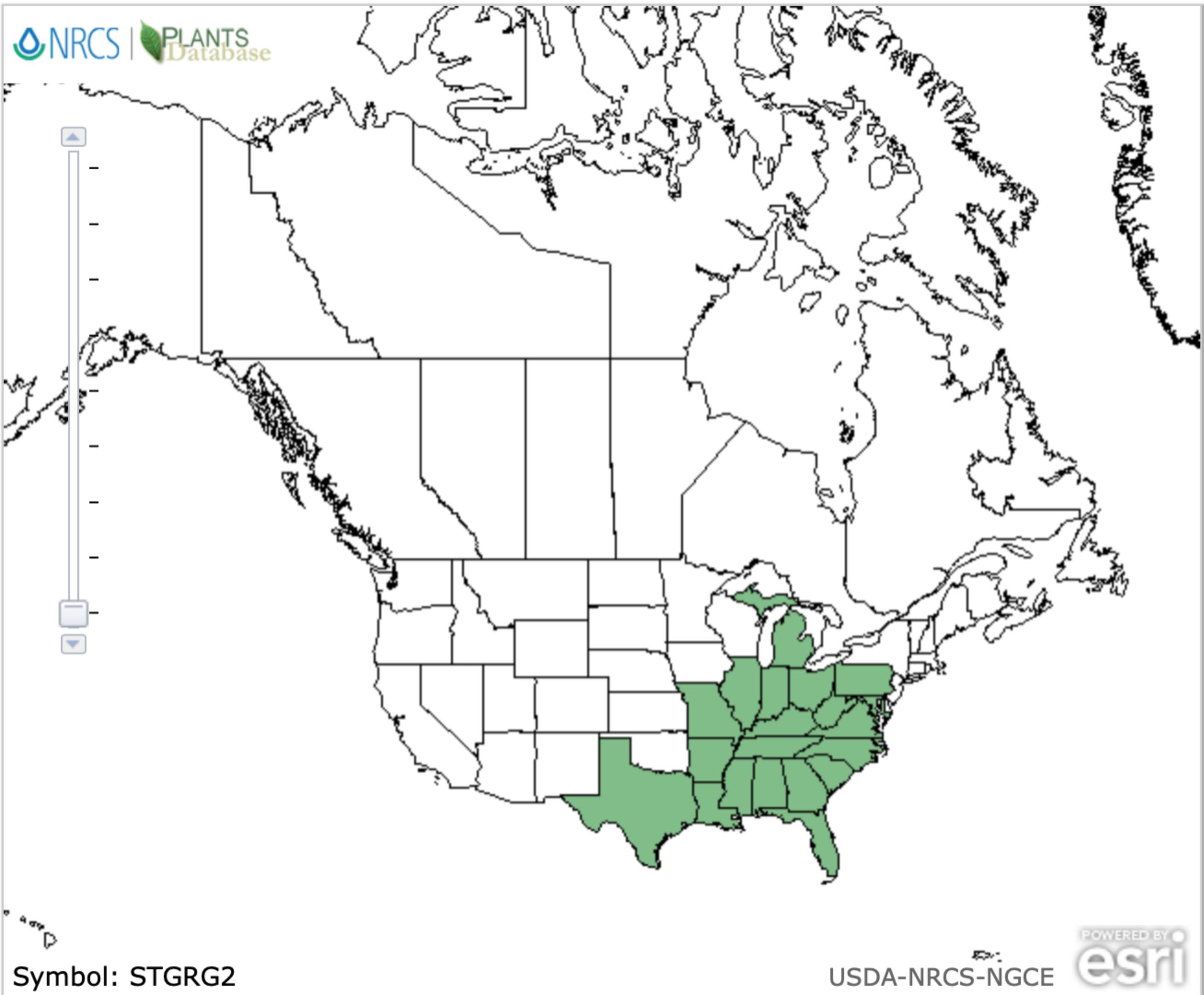

Fig. 1. State-level distribution of *Stenanthium gramineum* var. *gramineum* (USDA, NRCS. 2020. The PLANTS Database (http://plants.usda.gov, 8 June 2020). National Plant Data Team, Greensboro, NC 27401-4901 USA.)

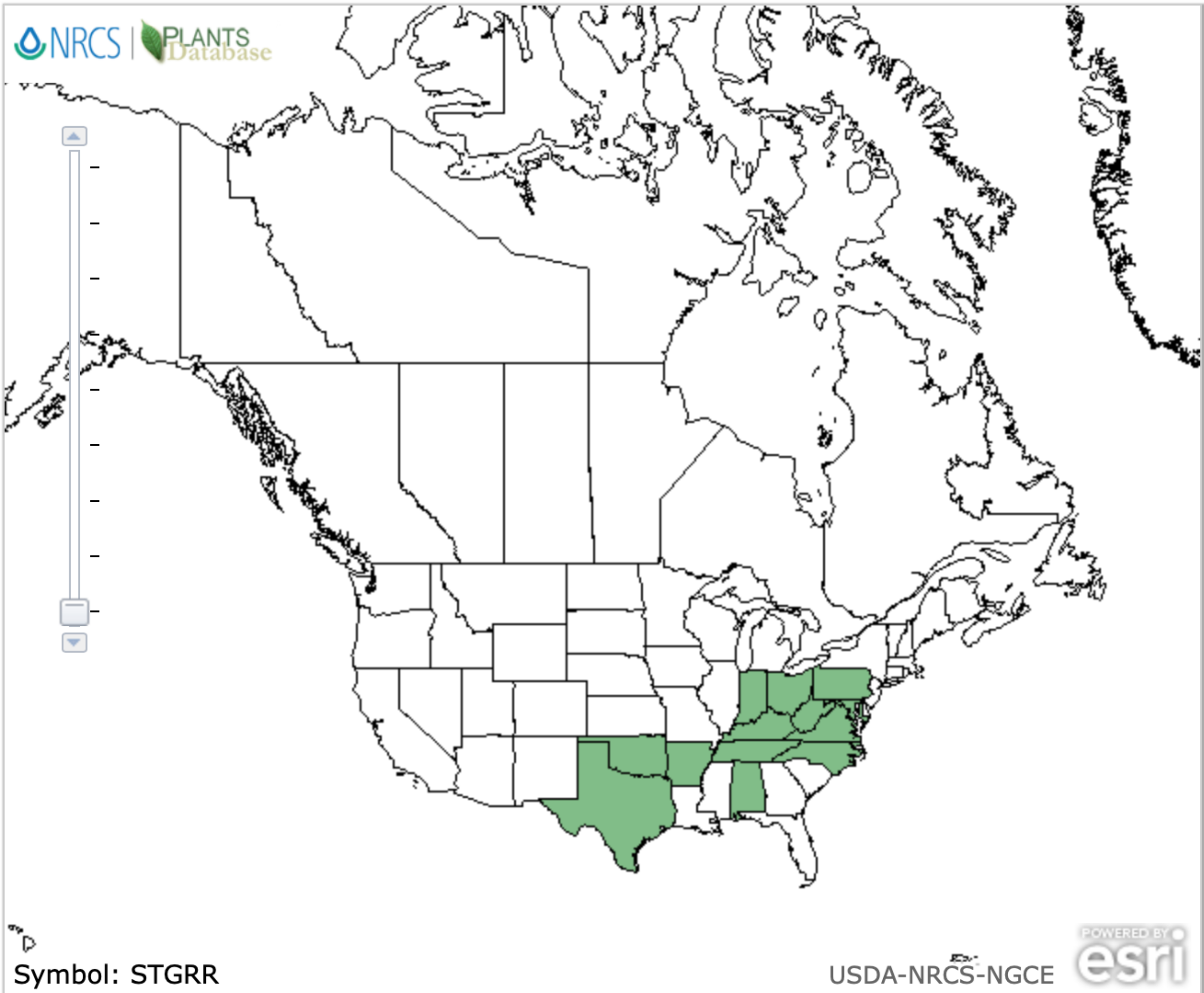

Fig. 2. State-level distribution of *Stenanthium gramineum* var. *robustum* (USDA, NRCS. 2020. The PLANTS Database (http://plants.usda.gov, 8 June 2020). National Plant Data Team, Greensboro, NC 27401-4901 USA.)

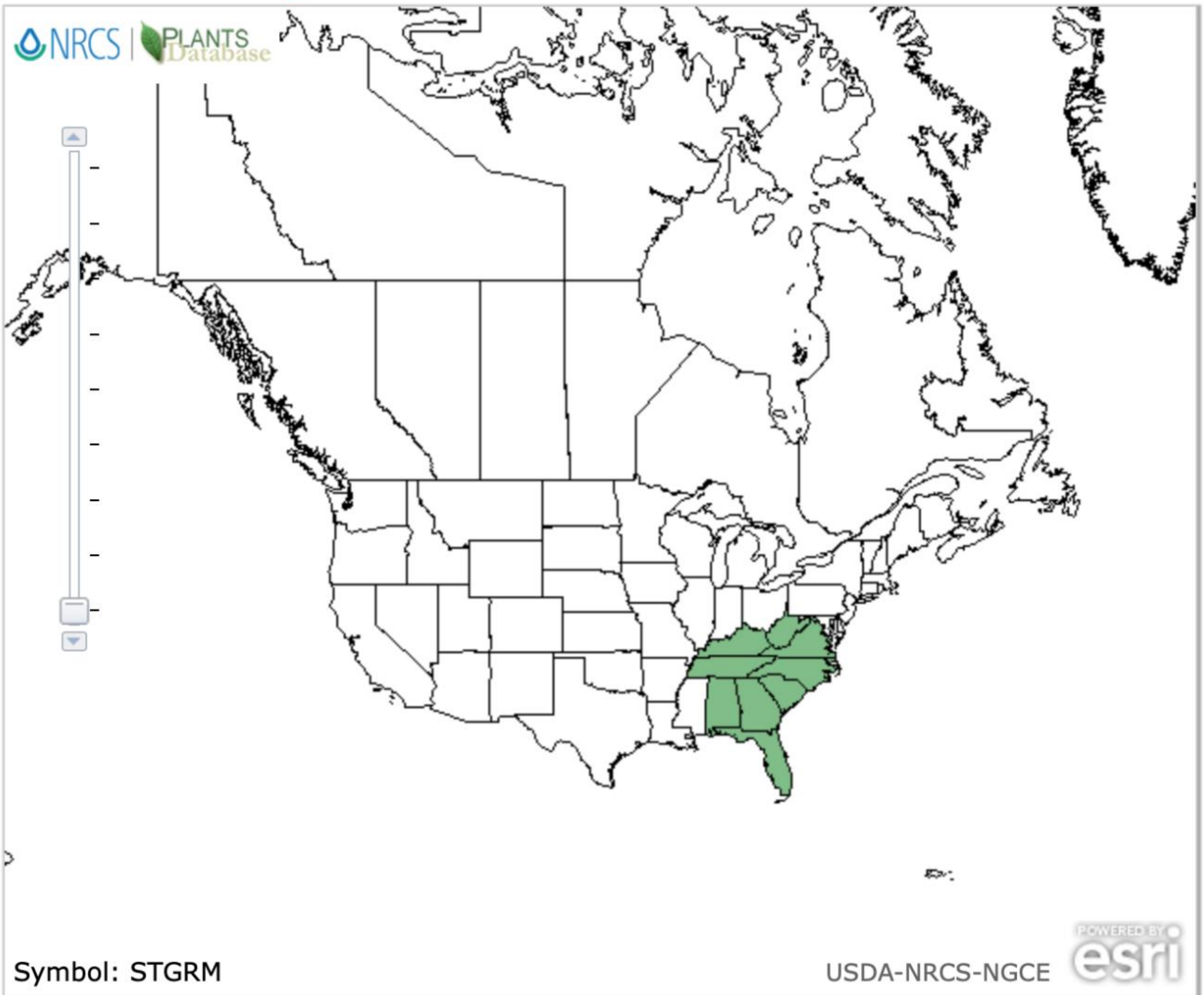

Fig. 3. State-level distribution of *Stenanthium gramineum* var. *micranthum* (USDA, NRCS. 2020. The PLANTS Database (http://plants.usda.gov, 8 June 2020). National Plant Data Team, Greensboro, NC 27401-4901 USA.)

To locate living populations of each variety, I obtained occurrence data from herbarium specimens that were gathered within the last eight years. I also obtained occurrence records for var. *robustum* from the North Carolina Natural Heritage program, Kentucky Natural Heritage Program, iNaturalist.org, and word-of-mouth (T. Govus, pers. comm.). I obtained permits for collection for Blue Ridge Parkway National Park (BLRI-2019-SCI-0013), Great Smoky Mountains National Park (GRSM-2019-SCI-2468), Nantahala and Pisgah National Forests

(2600), NC Plant Conservation Program (710), Cumberland State Park, KY, and private landowners.

I located about fifteen documented field sites during June - August of 2019 using a handheld GPS unit. However, among these sites, I only located six flowering populations total (Table 2; Fig. 19). Measurements taken in the field include: plant height, panicle height, peduncle length, first internode, second internode, average length of two bottom branches, average width of two most basal leaves, average width of two midstem leaves, longest tepal average, seed length average, seed width average, capsule length, capsule width, pollen length, pollen width, stomatal density and leaf texture (Appendix A). Individuals that had been measured in the field were flagged and their number and code were included, as they were given unique identifiers such as an abbreviation for the site and a number. From late August to September, six sites were revisited in order to collect fruit from flagged specimens. Note that site GSM (Table 2) was not used in data analyses, as it could not be accessed a second time to collect fruit, but a voucher specimen was collected. Environmental information to determine habitat characteristics was collected at each population visited. The information collected includes: habitat type, ratio grazed, percentage of sun exposure, elevation, average soil depth, soil pH, Munsell Chart soil score (Appendix A).

Table 2: Field sites where plant material was collected.

Collection Site	Site Abbreviation ID	County	Latitude/Longitude	Var.
Roadside mountain bog	ROB	Avery Co., NC	Protected	*robustum*
Roadside mountain bog	HWY	Alleghany Co., NC	Protected	*robustum*
Buck Creek Serpentine Barrens	BUCR	Clay Co., NC	35.083871; -83.615503	*unknown*
Andrew's Bald, grassy bald	ABD	Swain Co., NC	35.53909, -83.49364	*gramineum*
High elevation mixed oak forest in Great Smoky Mountains National Park	GSM	Haywood Co., NC	35.56618; -83.10338	*gramineum*
Low elevation mixed oak forest	GVS	Gilmer Co., GA	Private	*gramineum*

Collection of plant material in field included: Up to 10 basal rosette leaves per population of each variety– one leaf from up to 10 plants, dermal peels taken from bottom of 1 basal rosette leaf for up to 10 plants per population, up to 5 soil samples per population: 2.54cm diameter x up to depth of plant roots (no more than 25.4cm), 1 flowering lateral branch from 5 individuals from each population, 1 fruiting branch from 5 individuals from each population. In populations of more than 10 plants, up to 1 plant per population for each variety was collected for documentation as a voucher specimen and deposited at WCUH (Appendix B).

Leaves collected were stored in small, airtight bags with silica gel. In the lab, each sample was transferred into 50mL centrifuge tubes and labeled with their collection date and location and stored in a -80℃ freezer for potential future genetic analysis. Soil samples were scored against a set of Munsell Soil Color Charts (2000) for color and stored in paper bags and labeled with permanent marker. pH of each soil sample was taken using a Fieldscout SoilStik Meter. Flowering branches and fruiting branches were stored on ice within labeled air tight bags in the field and immediately taken to the lab and stored in FAA fixative (formaldehyde 10%: 95% ethanol 50%: glacial acetic acid 5%: water 35%). After at least 48 hours, they were transferred to a 75% ethanol solution within a sealed test tube.

Macromorphological characters, (see Appendix A), were measured with a metric ruler either with or without the use of a dissecting microscope. Micromorphological characters (pollen and stomata dimensions) were measured using a compound microscope with an eyepiece graticule calibrated to micrometers for each level of magnification (40x, 100x, 400x). Pollen was taken from both field-collected samples and voucher specimens, while stomatal measurements were only taken from field-collected samples, as leaves had to be fresh in order to be analyzed. Pollen was analyzed from each specimen gathered in the field and from four herbarium specimens of each variety.

Pollen was extracted by removing a single flower from each specimen and placing it under a binocular dissection microscope. All anthers were then removed with forceps and a probe and placed on a glass slide. A glycerol drop was added atop anthers on the slide and anthers were scraped using a probe to liberate pollen. The anthers were then removed and a glass coverslip was placed on the slide. Slides were then viewed at 400x total magnification using a dissection scope. Pollen length, width and total 2D area, (Area of an ellipses= a x b x π), were measured using an eyepiece graticule calibrated for micrometers. Three randomly selected grains of pollen from each sample were measured and averaged. Pollen shape and color were classified using a rubric as well. Pollen shape was recorded as “round” or “oblong.” Color was standardized as “light yellow,” “dark yellow,” or “brown.”

I used the dermal peel technique (Dunlap and Stettler 2001) to gather impressions of stomata in the field (Heatherington 2003). I covered 2.54cm - 12.7cm of the underside of one basal rosette leaf for up to 10 plants per population from all populations located in the field in clear nail polish. After drying, the painted area was peeled away in order to gather a sample of the epidermis of the underside of the leaf. This was then placed onto a microscope slide and taken to the lab to be viewed under a compound microscope and measured against an eyepiece graticule calibrated to micrometers at 400x total magnification. Stomata were counted within a standard area of $819.96 \mu m^2$ to determine density, and three individual stoma were measured within each sample to determine length, width, and area in micrometers. Measurements were then averaged. Stomatal shape was also recorded as either “round” or “oblong.”

Eighteen capsules were dissected using forceps and a probe under a dissection microscope to liberate seeds: two capsules per each variety from herbarium sheets (6 total), and 2 per field sample, except for buck creek and the GVS site, (private property site with var. *gramineum*), in which 3 were taken (12 total). Seeds were measured to the nearest millimeter using a ruler. Length, width, area, shape, color and texture were recorded.

Data Analyses

I used a combination of univariate and multivariate analyses to test for differences in morphological measurements among the varieties and to determine if there is a strong correlation between each variety and its habitat.

Principal component analysis (PCA) is commonly used with morphological data to determine patterns of variation. It allows researchers to more easily visualize multivariate data to identify groups, and therefore aids in the delimitation of species within a complex (e.g., Ellison et al. 2004; Janovec and Harrison 2002; Knapp and Naczi 2008). In my study, I performed PCAs before other statistical tests because classes are not pre-defined, as it is a type of unsupervised machine learning. Therefore, I used this test to help identify groupings in my multivariate data. I performed three separate PCAs: one for field measurements, one for environmental characteristics at field sites, and one for morphological variation among herbarium specimens. These analyses allowed me to determine if varieties were morphologically unique and if habitats were unique, based on groupings. The PCA analyses were also useful in determining which morphological characteristics accounted for the largest amount of variation among groups.

Analysis of variance is a commonly used technique in morphological studies to test for significant discontinuities among taxonomic entities (e.g., Knapp 2014). I used multivariate analysis of variance (MANOVA) to determine if there was a significant difference in morphology among all three *Stenanthium* varieties, and then between individual varieties. I performed this test for both herbarium specimens and field-gathered measurements. This allowed me to test the null hypotheses that 1) there is no morphological variation among *S. gramineum* varieties and 2) there is no morphological variation between each individual variety.

Linear discriminate analysis (LDA), is a test type often used in conjunction with PCA (e.g., Lumley and Sperling 2010). In an LDA, classes are pre-assigned, unlike in a PCA. The goal of an LDA is to maximize the separation among multiple classes by maximizing the component axes for class separation. In my study, an LDA was used to identify the morphological measures that best separated the varieties when used as pre-assigned categories.

Canonical correlation analysis (CCoA) is commonly used to determine the strength of the relationship between ecological variables and an organism's (e.g., Miles and Ricklefs 1984; Moran 1986; Kores et al. 1993; Pélissier et al. 2001). In my study of the varieties of *S. gramineum*, a CCoA was used to determine the amount of correlation between the field-collected morphological measurements and environmental measures from their habitat. This analysis can suggest whether or not morphological differences are environmentally driven. Non-significant correlations corroborate the hypothesis that phenotypic differences are due to genotypic differences rather than morphological plasticity. However, if there is a significant relationship between morphology and environmental factors, the information gained is less straightforward. This may suggest that the varieties are ecotypes of the same species, or that local adaptation is taking place, but to what extent? In this study the CCoA was performed on a reduced dataset of eight morphological characters identified in the PCA as describing the greatest variation among groups and environmental measures.

Data were compiled into three Excel spreadsheets (Microsoft Corporation 2018): data collected for herbarium specimens, data collected for specimens measured in the field, and ecological data gathered for each field site. All statistical analyses were performed using R (R Core Team 2016). The missMDA package (Josse & Husson 2016) was used to impute missing values for herbarium specimen data. Imputations were performed individually for all three varieties.

Principal Component Analyses (PCA) were performed on all three data sets with the FactomineR package (Le et al. 2008) using the PCA function. Scatterplots were produced from the PCA. I then overlaid 95% confidence ellipses for the mean on the scatterplots. Visualizations were built using the factoextra package (Kassambara & Mundt 2020). Variable vectors were produced to show which characters accounted for the highest correlations with the PCA axes and were overlaid onto biplots. Eigenvalues and dimensions were analyzed to further investigate which characters helped describe the greatest multivariate variance. The three most negatively correlated morphological characteristics and the three most positively correlated morphological characteristics for the first three dimensions were plotted against one another on scatterplots and ellipses with 95% confidence intervals for the mean were used for each characteristic.

A permutation Multivariate Analysis of Variance (MANOVA) was performed on the herbarium specimens dataset and on the field specimens dataset. A permutation MANOVA was used since it does not require the assumption of normality. This test was run using the adonis2 function using the vegan package (Oksanen et al. 2019). The purpose of this test was to determine if the variation seen in morphology among the varieties in the PCA was significant. I compared all morphological data gathered for each variety in the field for one set, and for another set, I compared all morphological data gathered from herbarium specimens.

Linear Discriminate Analyses (LDA) were run on the herbarium specimens dataset and the field specimens dataset with samples pre-assigned to one of three varieties. LDA was performed using the following packages: factomineR, factoextra, MASS (Venables & Ripley 2002). A training run was performed on all data in order to allow the algorithm to determine the best predictors for each class defined by the user- in this case, each variety. Once a training set was ran, the full dataset was used and scatterplots and histograms were produced from this data.

A Canonical Correlation Analysis was executed on the top eight morphological characteristics, (those that showed the highest variation in PCA results), for field-collected specimen dataset against the ecological characteristics field dataset (Appendix A). I used the CCA package (Gonzalez & Dejean 2012) to run the Canonical Correlation Analysis and the CCP Package (Menzel 2012) to estimate the P-values of the CCoA results.

Phenology was analyzed by compiling the bloom dates from my field-collected data and the borrowed voucher specimens. Individuals that were at least 50% in bloom were used in this study. These specimens were then sorted into varieties and their date of collection was recorded. The earliest and last bloom dates were recorded as the range. The mean bloom date was also recorded. The mean was taken by averaging all bloom date for each variety.

Geographic range of field-collected data and voucher specimens were recorded onto a Google map using the pin drop function. I recorded all at county level, as most voucher specimens did not include GPS coordinates, and because var. *robustum* sites are protected.

CHAPTER THREE: RESULTS

Field Results

During my field season, I traveled to about fifteen field sites, only locating six populations. I found two high-elevation mountain bog habitats containing var. *robustum* and three habitats with occurrences of var. *gramineum*. No populations corresponding to *S. gramineum* var. *micranthum* were found. The mountain bog habitats were wet with deep mud and full sun exposure. They were at elevations of 1012m and 892m. Some associated plant species with *S. gramineum* var. *robustum* were *Solidago* spp., *Eutrochium* spp., and Poaceae spp. The three var. *gramineum* sites I located were found in two forests and a grassy bald. The forested habitats differed greatly in elevation (644m and 1672m). In the lower elevation forest, *S. gramineum* var. *gramineum* was accompanied by *Quercus alba*, *Acer rubrum*, *Carya* spp., and *Calycanthus floridus*. The ground was dense with leaf litter, and the soil was loamy and moist. In the high-elevation forest (1672m), *S. gramineum* var. *gramineum* was accompanied by *Quercus* spp., *Cornus florida*, *Acer pensylvanicum*, and *Thelypteris noveboracensis*. There was less leaf litter at this site than the lower-elevation forest. The third site I located with a population of var. *gramineum*, was a high-elevation grassy bald (1759m), with full sun exposure. Associated species included Poaceae spp., *Solidago* spp., Apiaceae spp., and *Vaccinium* spp.

One population of *S. gramineum* found at Buck Creek Serpentine Barren in Clay Co., NC, did not appear to fit the description for any known varieties, as it was shorter in stature and smaller in general than var. *robustum* but had much longer tepals that var. *robustum*. There was also color variation present in this population, as some individuals had pink venation in the tepals as opposed to the tepals being all white. This population was at an elevation of 1015m. Some plants were found in a forested area and some in an open, grassy field surrounded by prairie grasses including *Andropogon gerardii*, *Schizachyrium scoparium*, *Panicum virgatum*, *Sorghastrum nutans*, *Sporobolus heterolepis*, and interspersed with *Vaccinium* spp. In the forested area, *S. graminuem* was associated with *Quercus alba* and *Acer rubrum*.

Morphological and Ecological Results

PCA Herbarium Morphology Results

Results indicate morphological distinction among the varieties. Since it is considered general practice to include all dimensions until the cumulative variance percentage is at least 60%, I visualized results on the top three dimensions for the herbarium specimen data, since the top three accounted for 68% of the total variation (Tables 3 & 4).

Table 3. PCA of herbarium specimen data showing eigenvalues and percent of variance per dimension, as well as cumulative variance for the data. The first three dimensions account for 68% of the variation.

	eigenvalue	variance.percent	cumulative.variance.percent
Dim.1	9.240	41.999	42.00
Dim.2	3.150	14.317	56.32
Dim.3	2.650	12.047	68.36
Dim.4	1.540	7.000	75.36
Dim.5	1.009	4.587	79.95
Dim.6	0.792	3.602	83.55
Dim.7	0.768	3.492	87.04
Dim.8	0.569	2.586	89.63
Dim.9	0.459	2.088	91.72
Dim.10	0.399	1.812	93.53
Dim.11	0.373	1.696	95.23
Dim.12	0.291	1.322	96.55
Dim.13	0.155	0.704	97.25
Dim.14	0.143	0.650	97.90
Dim.15	0.128	0.581	98.48
Dim.16	0.096	0.437	98.92
Dim.17	0.077	0.349	99.27
Dim.18	0.056	0.256	99.52
Dim.19	0.038	0.172	99.70
Dim.20	0.033	0.151	99.85
Dim.21	0.020	0.090	99.94
Dim.22	0.014	0.063	100.00

Table 4. PCA loadings on the first 3 dimensions for herbarium specimen data. Loadings >0.6 are in bold. (See Appendix A for key for abbreviations).

	Dim.1	Dim.2	Dim.3
PAN.HEIGHT	0.406	**0.610**	0.157
PED.LENGTH	-0.079	**0.739**	-0.109
FIRST.INT	0.146	**0.660**	0.147
SEC.INT	0.231	**0.634**	0.056
L.BB	**0.621**	0.543	-0.013
L.SB.FIRST	0.591	-0.014	**0.720**
L.SEC.BRAN	**0.829**	0.286	-0.046
L.SB.SEC	**0.642**	0.054	**0.716**
DIST.PAN.FLRS	-0.194	0.568	-0.116
DIS.BRANCH.FLRS	0.112	0.185	-0.282
W.BAS.L	**0.884**	-0.164	0.136
W.MID.L	**0.781**	-0.298	-0.292
MSW	**0.851**	0.053	0.359
BSW	**0.765**	0.068	0.459
TLP	**0.668**	-0.417	0.174
TLB	**0.784**	-0.211	0.041
PC.L	**0.868**	-0.044	-0.374
PC.W	**0.823**	-0.103	-0.488
SEED.L	**0.777**	-0.339	-0.224
SEED.W	**0.712**	0.071	-0.577
POLLEN.L	0.404	0.424	-0.439
POLLEN.W	**-0.802**	0.068	0.180

I first ran a PCA that included all 22 morphological characters from herbarium specimens and visualized it as a scatterplot of dimension 1 x dimension 2, with 95% confidence ellipses surrounding the mean of each cluster. The scatterplot showed clustering of representatives of each *S. gramineum* variety and separation among all varieties for the most part, indicating that all three varieties show distinct morphology (Fig. 4). Only three individuals of var. *gramineum* did not fall within the 95% confidence ellipse for that grouping.

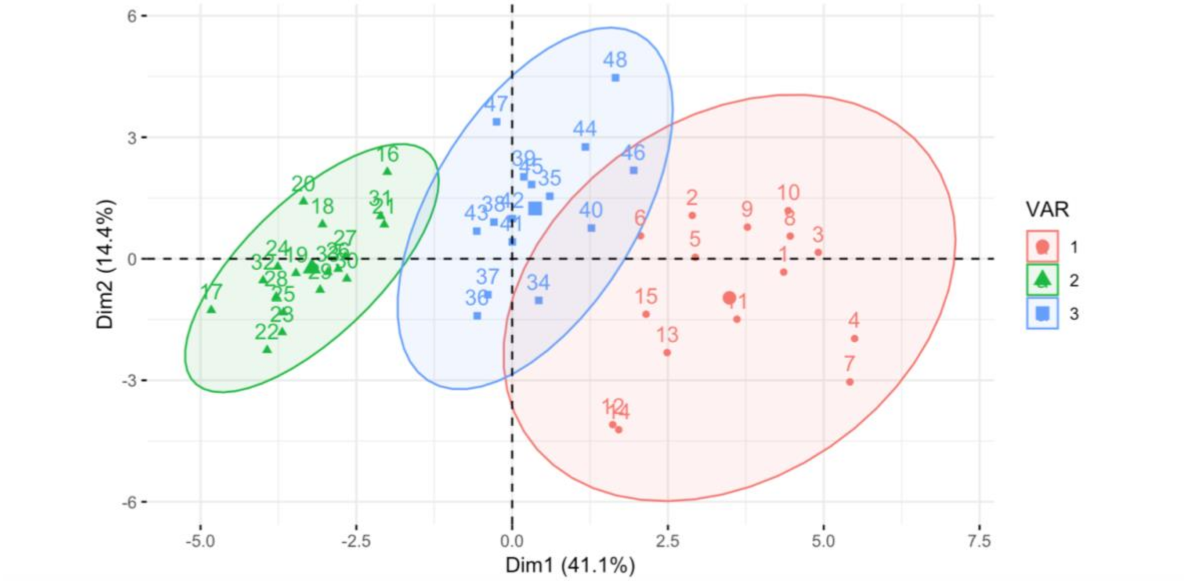

Fig. 4. PCA scatterplot of herbarium specimen data: Individual sample scores plotted against the first two principal components, which accounted for 56% of the variation in the data. Each variety is identified by color and symbol shape: red circle is var. *robustum*, green triangle is var. *micranthum*, and blue square is var. *gramineum*. The ellipses represent 95% confidence intervals drawn around each cluster. The centroid of the group is represented by a larger symbol.

Understanding which morphological characters had the greatest effect on the PCA allowed me to determine which ones are most important in identifying each variety in the field. Nineteen morphological characters had the highest loadings on the first three dimensions (greater than 0.6 and less than -0.6), including both vegetative and reproductive traits (Table 4). Vegetative characteristics included peduncle length, second internode length, midstem width, distance between panicle flowers, width of basal leaves, and width of mid-stem leaves. Reproductive characteristics included pollen width, panicle capsule length, tepal length of panicle flowers, and seed length.

I then ran a PCA with just the 19 characters with top loadings to see if these traits provided any unique grouping patterns and visualized the first three dimensions in scatterplots (Figs. 5 and 6).

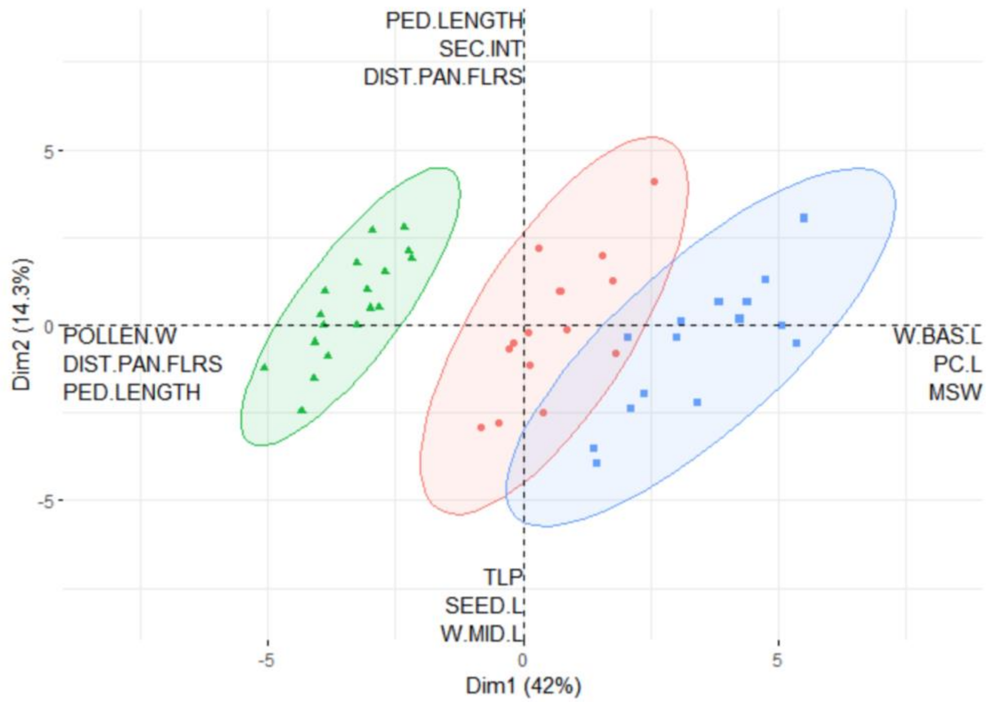

Fig. 5. PCA scatterplot for herbarium specimen morphological characters with the top three loadings on dimensions 1 and 2 in the full dataset PCA. (See Appendix A for key for abbreviations). Each variety grouping is represented by an ellipsis color: red is var. *robustum*, green is var. *micranthum*, and blue is var. *gramineum*.

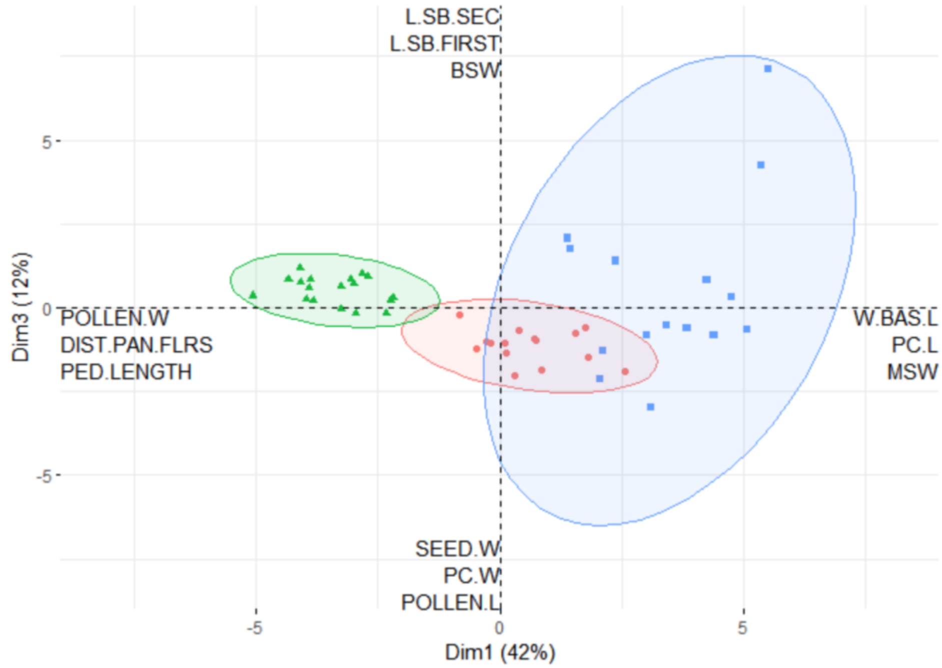

Fig. 6. PCA scatterplot for herbarium specimen morphological characters with the top three loadings on dimensions 1 and 3 in the full dataset PCA. (See Appendix A for key for abbreviations). Each variety grouping is represented by an ellipsis color: red is var. *robustum*, green is var. *micranthum*, and blue is var. *gramineum*.

To further examine the strength of the top morphological characters (the strength being the weight that particular character holds as far as separating the three groups) and to compare the strengths of those top variables in each of the first three dimensions, I overlaid the PCA scatterplots with vectors of the variables with the three highest positive and the three most negative loadings for each dimension. Vegetative characteristics included peduncle length, second internode length, midstem width, distance between panicle flowers, width of basal leaves, and width of mid-stem leaves. Reproductive characteristics included pollen width, panicle capsule length, tepal length of panicle flowers, and seed length (Fig. 7 & 8).

Based on the relative length of the arrows, pollen width and width of basal leaves may be two of the most useful morphological characteristics in separating the varieties on dimension 1, while length of the bottom branch may be one of the most useful in dimension 2 (Fig. 7). Length of the subtending bract of the first branch is most useful on dimension 3 (Fig. 8).

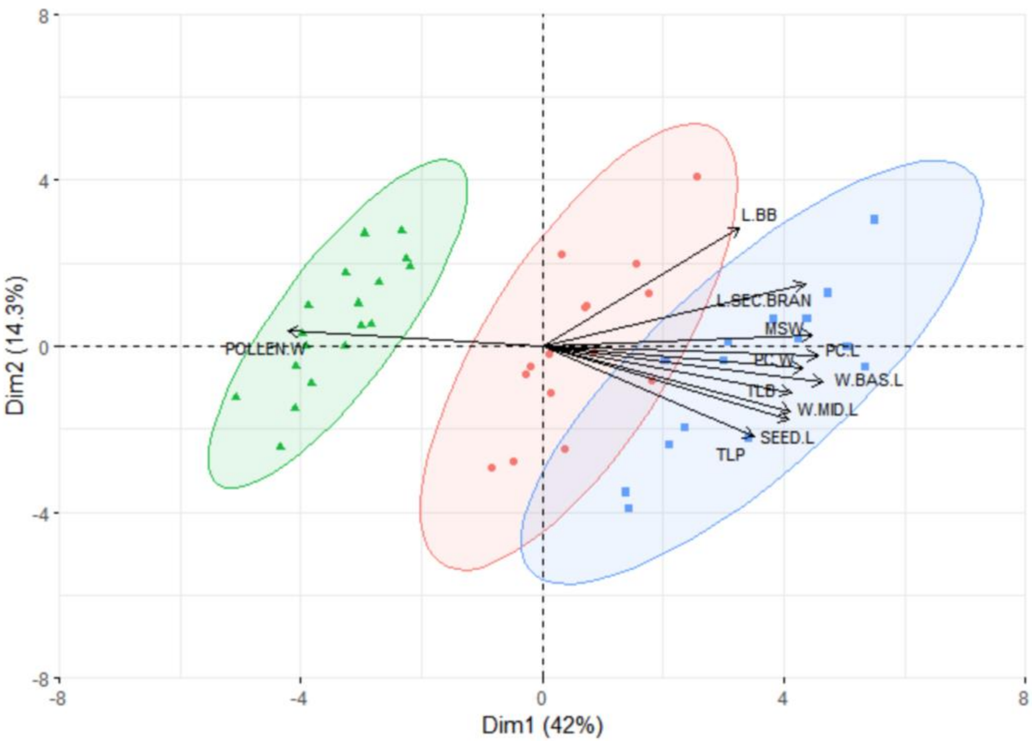

Fig. 7. PCA Scatterplot for dimension 1 x dimension 2 for herbarium specimen data, with vectors showing which characters accounted for the highest percentages of variation (See Appendix A for key for abbreviations). Each variety grouping is represented by an ellipsis color: red is var. *robustum*, green is var. *micranthum*, and blue is var. *gramineum*.

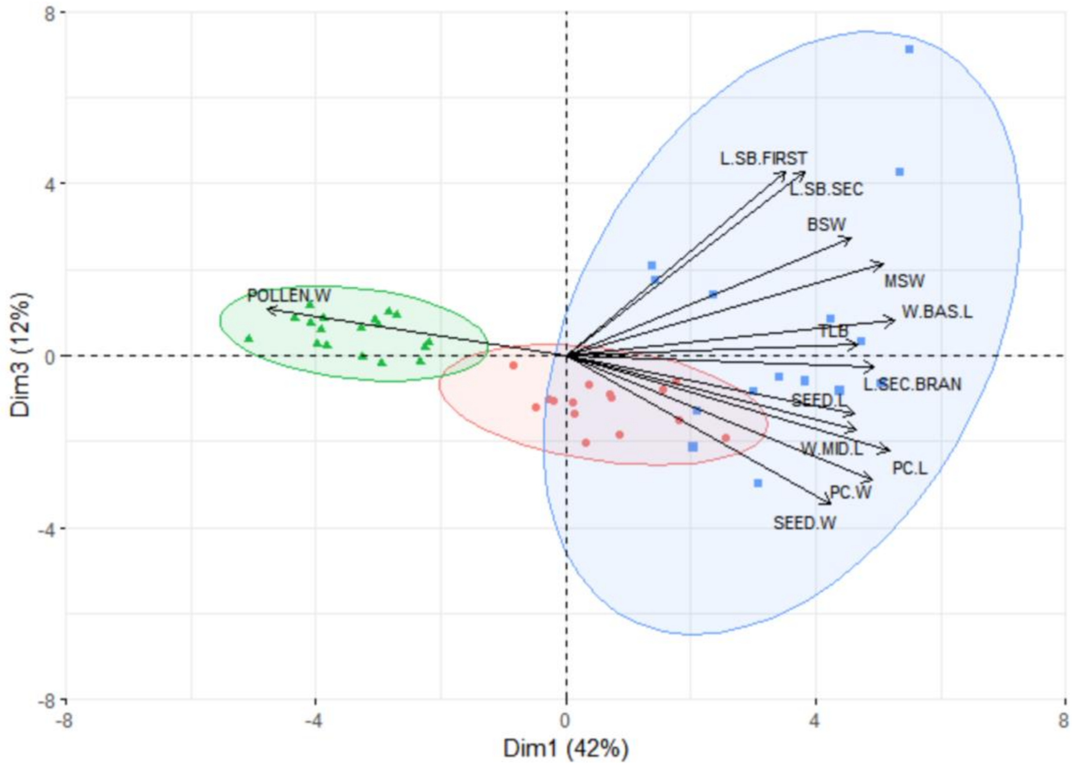

Fig 8. PCA Scatterplot for herbarium specimen data: Dimension 1 x Dimension 3. (See Appendix A for key for abbreviations). Each variety grouping is represented by an ellipsis color: red is var. *robustum*, green is var. *micranthum*, and blue is var. *gramineum*.

PCA Field Morphology Results

PCA analyses were performed on the character measurements of live plants taken during my field season. As stated above, I located two field sites with occurrences of var. *robustum*, three of var. *gramineum*, none of var. *micranthum*, and one of a unique morphotype (Buck Creek). However, only two populations of var. *gramineum* had plants in fruiting condition, so I included only these two var. *gramineum* sites in my field data analysis. These were the Andrew's Bald and the GVS sites. The first two dimensions accounted for 63.71 percent of the variation together (Table 5) and were used to visualize the PCA results.

Table 5. Eigenvalue and cumulative variance of the first three dimensions for field-collected data.

	eigenvalue	variance.percent	cumulative.variance.percent
Dim.1	6.321	39.504	39.50
Dim.2	3.874	24.210	63.71
Dim.3	1.661	10.380	74.09

Three distinct groupings were found based on field morphology: var. *robustum*, var. *gramineum* and the Buck Creek population (Fig. 9). Buck Creek plants make a unique cluster, and points within this cluster are closer together than the points within the other two groupings, however, this grouping shows the most variation on dimension two, which carries less weight than dimension one. This means that the grouping shown may not be as strong of a group as it appears.

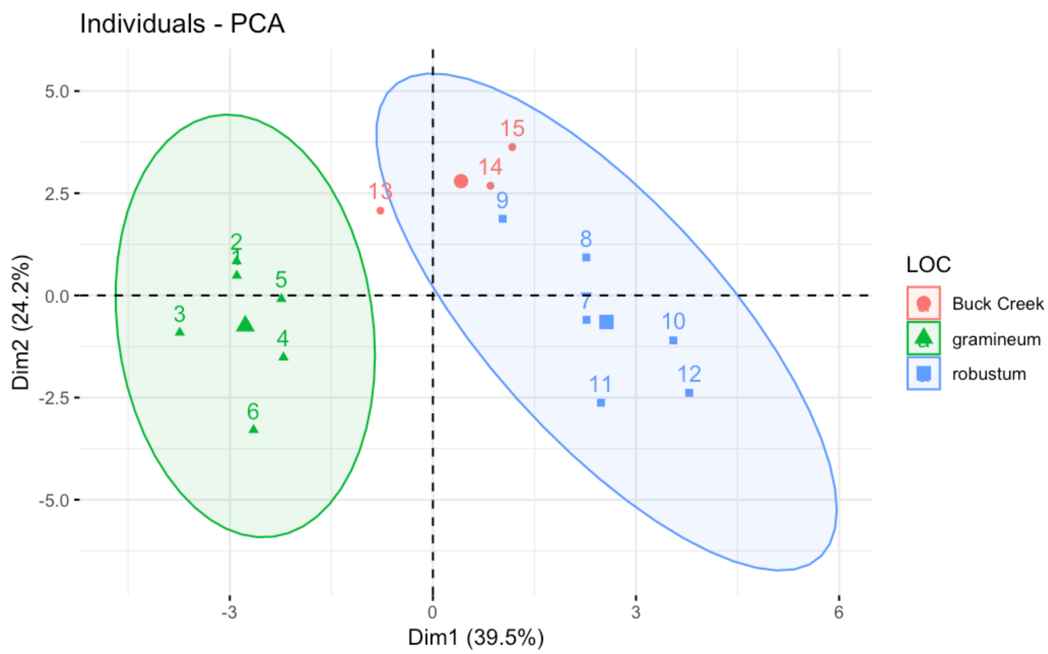

Fig. 9. Scatterplot of field-collected data for Dimension 1 x Dimension 2 with 95% confidence ellipses (not enough points for ellipsis to be added to Buck Creek points). Red: Buck Creek population, Green: var. *gramineum*, and Blue: represents var. *gramineum*

I then determined which morphological characteristics showed the highest variation among the groupings based on the PCA by examining the loadings on the first two dimensions (Table 6). On dimension one, the average width of the two most basal leaves has the highest loading, followed by average longest tepal length.

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Table 6. PCA loadings of field-collected data show correlations between original morphological measures and the principal components. Correlations greater than 0.6 are indicated with double asterisks. (See Appendix A for key for abbreviations).

	Dim.1	Dim.2	Dim.3
plant.height.cm	**0.858**	0.242	-0.265
panicle.height.cm	0.314	**0.643**	-0.127
peduncle.length.cm	-0.432	**0.830**	-0.194
first.internode.cm	-0.525	**0.614**	-0.122
second.internode.cm	-0.271	**0.860**	0.194
average.length.bottom.two.branches.cm	0.210	**0.786**	-0.392
average.width.of.2.most.basal.leaves.mm	**0.900**	-0.145	-0.311
average.width.of.two.midstem.leaves.mm	**0.747**	-0.134	-0.506
Longest.Tepal.average.mm	**0.870**	0.271	-0.059
Seed.Length.average.mm	**0.615**	-0.050	0.380
seed.width.average.mm	**0.815**	-0.186	0.034
capsule.length.1.mm	**0.867**	0.266	0.210
Capsule.width.1.mm	**0.858**	0.289	0.276
Pollen.length.1.um	0.296	**0.678**	0.480
pollen.width.1.um	-0.380	0.458	-0.130
Stomatal.density.um	0.136	0.074	**0.679**

For this same dataset, vectors are overlaid onto scatterplots of dimensions 1 x 2 (Fig. 10) and dimensions 1 x 3 (Fig. 11). These vectors show the direction and magnitude of the morphological traits with loadings over 0.6, which reveals which traits show the highest variation for the dimensions represented. These are average width of two most basal leaves, longest tepal average, and capsule width.

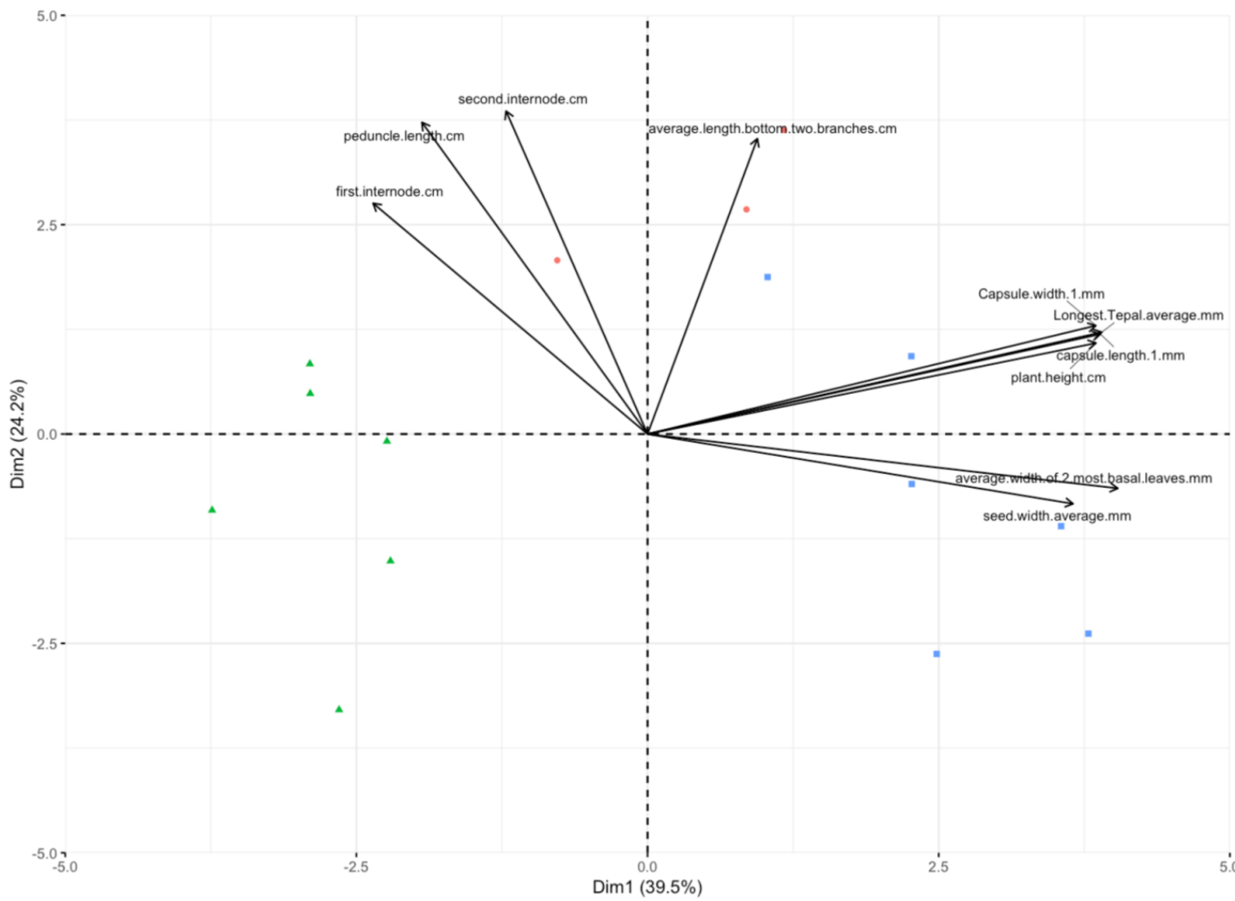

Fig. 10. PCA Scatterplot of field-collected data of dimension 1 x dimension 2 with vectors showing the direction and magnitude of each morphological character's variation among each variety. (See Appendix A for key for abbreviations). Red: Buck Creek population, Green: var. *gramineum*, and Blue: represents var. *gramineum*.

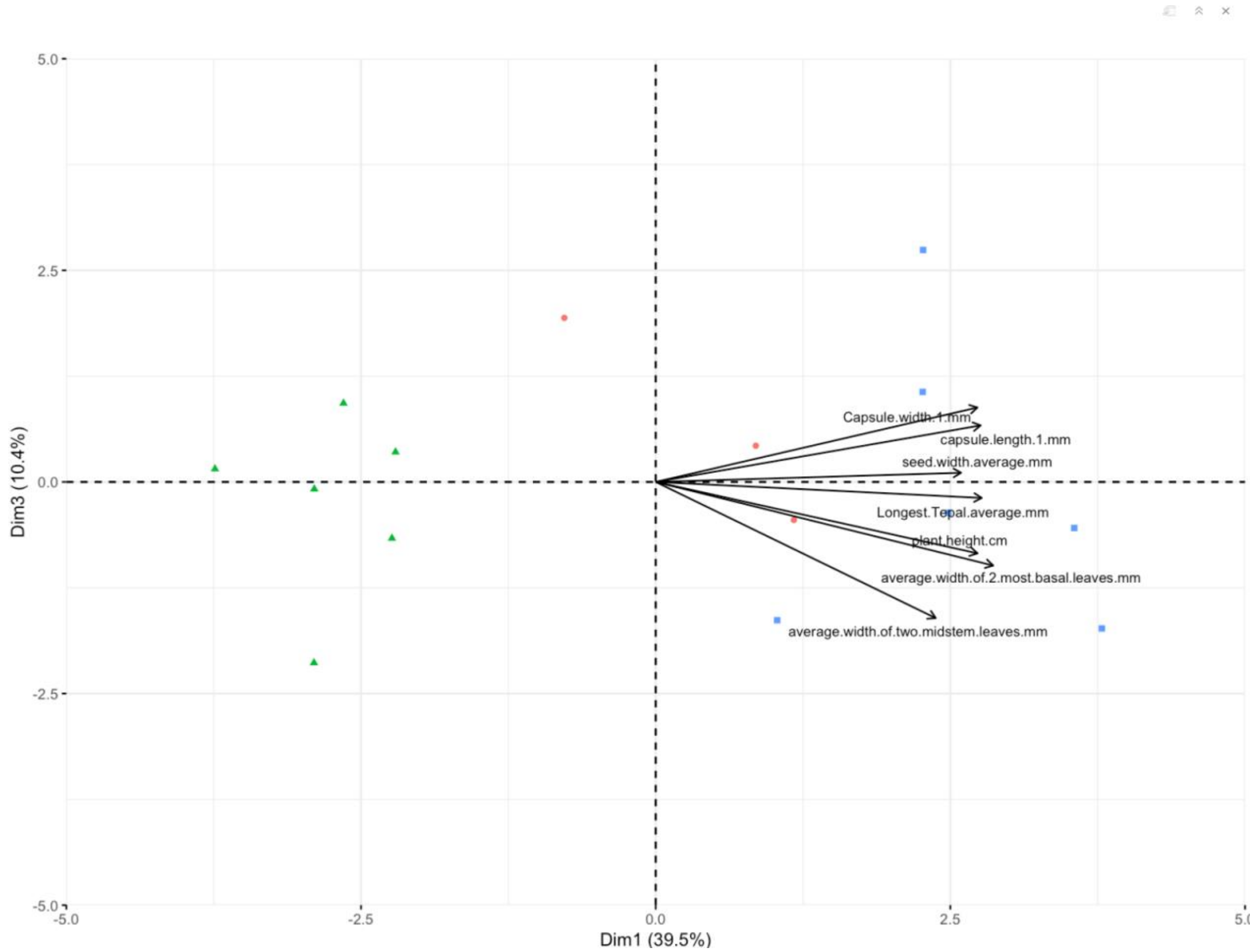

Fig. 11. PCA Scatterplot of field collected data of dimension 1 x dimension 3 with vectors shows the direction and magnitude of each morphological character's variation among each variety. (See Appendix A for key for abbreviations). Red: Buck Creek population, Green: var. *gramineum*, and Blue: represents var. *gramineum*.

<u>PCA Ecological Measurements Results</u>

A PCA analysis was performed on ecological measurements taken at each field site I found with populations of *S. gramineum* (five sites total). These measurements were elevation, average soil depth, ratio grazed, soil pH and sun exposure. The results were visualized on dimension 1x2 because the top two dimensions accounted for 70.7% of the variation among the field sites.

The two var. *robustum* field sites (HWY and ROB) were most similar because they clustered closest together (Fig. 12). Both of these are mountain bog habitats. The two habitats in which I found var. *gramineum* (ABD and GVS) separate noticeably on the scatterplot. The ABD site was a grassy bald, while the GVS site was forested. The BUC site, which is a serpentine barren, also separated distinctly from the other sites based on the ecological measurements taken.

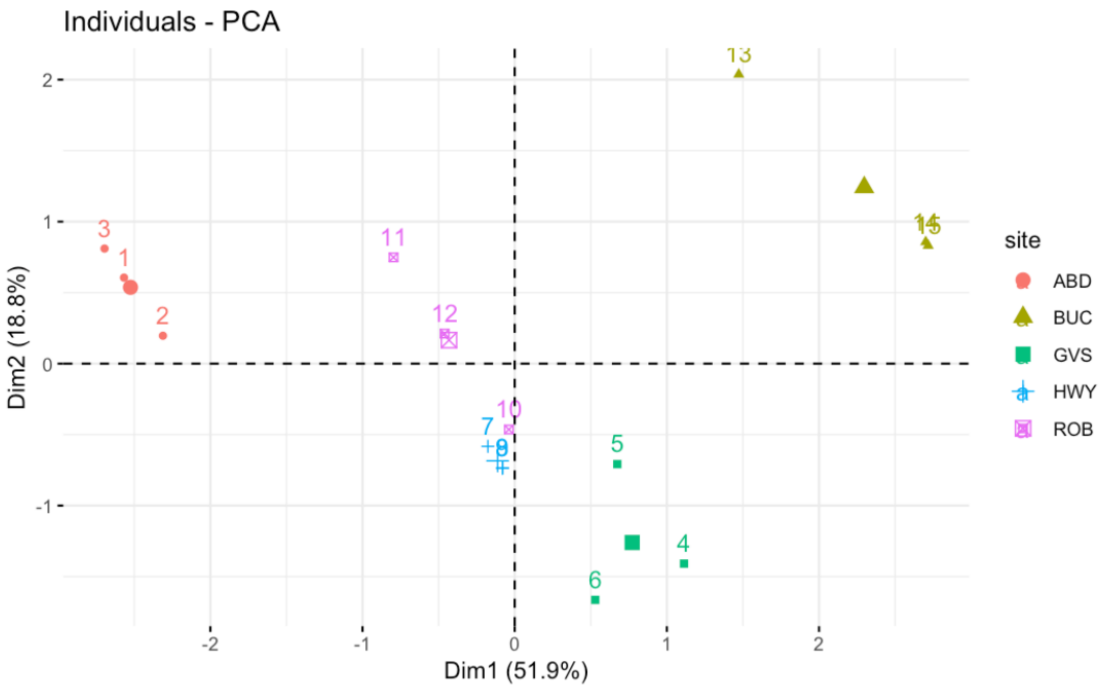

Fig. 12. PCA scatterplot for ecological data color-coded for location for dimension 1 x dimension 2. ABD: Andrew's Bald (var. *gramineum*), BUC: Buck Creek (Buck Creek Population) GVS: private property (var. *gramineum*) HWY: protected location (var. *robustum*) ROB: protected location (var. *robustum*).

The most positive and most negative loadings on dimensions 1 and 2 are those that made the biggest impact on the groupings of habitats based on ecological measurements (Table 7). Ratio grazed had the highest loading on dimension one, therefore impacting the groupings the most. However, several negative and positive loadings are above 0.6, the level of highest impact, in dimension 1 & 2.

Table 7. PCA loadings for ecological data on dimensions 1-5.

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
ratio.grazed	0.801	0.440	-0.263	0.169	-0.259
sun.exposure	0.758	-0.162	0.472	0.401	0.126
elevation.m	-0.713	0.533	-0.281	0.319	0.162
avg.soil.depth.cm	-0.483	0.466	0.729	-0.073	-0.114
soil.pH	0.797	0.471	0.005	-0.310	0.216

Permutation Multivariate Analysis of Variance (MANOVA(s))

Omnibus MANOVA results on herbarium specimens are given in Table 8. With a significance value of 0.05, there is a significant difference among varieties.

Table 8. Omnibus permutation MANOVA test of herbarium specimen data among all three varieties.

	df	SS	R^2	F	P-value
var	2	0.1652	0.3482	12.02	0.0001
Residual	45	0.3092	0.6518		
Total	47	0.4743	1.0000		

Pairwise MANOVA tests (Tables 9-11) were used to compare between-variety level of variation, with a significance level of <0.0167. There is a significant variation between var. *robustum* from the other two, however, var. *micranthum* and var. *gramineum* were not found to be significantly morphologically different.

Table 9. Pairwise permutation MANOVA test of herbarium specimen data between var. *micranthum* and var. *gramineum*. A P-value less than 0.0167 is considered significantly different than expected to protect the family-wise error rate of 0.05.

	df	SS	R^2	F	P-value
var	1	0.0147	0.0544	1.784	0.2835
Residual	31	0.2559	0.9456		
Total	32	0.2706	1.0000		

Table 10. Pairwise permutation MANOVA test of herbarium specimen data between var. *robustum* and var. *gramineum.* A P-value less than 0.0167 is considered significantly different than expected.

	df	SS	R^2	F	P-value
var	1	0.0543	0.3647	16.07	0.0001
Residual	28	0.0945	0.6353		
Total	29	0.1488	1.0000		

Table 11. Pairwise MANOVA test of herbarium specimen data between var. *robustum* and var. *micranthum.* A P-value less than 0.0167 is considered significantly different than expected.

	df	SS	R^2	F	P-value
var	1	0.1762	0.3968	20.39	0.0001
Residual	31	0.2679	0.6032		
Total	32	0.4441	1.0000		

In the field-collected data, there is no significant difference among the MANOVAs (Tables 12-14).

Table 12. Omnibus MANOVA test of field-collected data among all three “varieties.”

```
--------------------------------------------------------
             df        SS    $R^2$       F   P-value
-------------- ---- --------- -------- ------- ---------
**LOC**           2   0.02176   0.2242   1.734    0.2785

**Residual**     12   0.07530   0.7758

**Total**        14   0.09706   1.0000
--------------------------------------------------------
```

Table 13. Permutation MANOVA test of field-collected data between Buck Creek population and Andrew’s Bald site (which has var. *gramineum*).

	df	SS	R^2	F	P-value
LOC	1	-0.00351	-0.161	-0.5547	0.9
Residual	4	0.02531	1.161		
Total	5	0.02180	1.000		

Table 14. Permutation MANOVA test of field-collected data between Buck Creek population and a protected site (which has var. *robustum*).

	df	SS	R^2	F	P-value
LOC	1	-0.003254	-0.07674	-0.2851	0.7
Residual	4	0.045655	1.07674		
Total	5	0.042401	1.00000		

Linear Discriminant Analysis (LDA)

Since results of the PCAs confirmed clustering of members within each of the three varieties and results of MANOVAs confirmed distinctiveness between at least one of the varieties and the other two, LDAs were performed with predefined categories shown in the PCAs. LDAs were performed as a way to maximize the separation among the groupings. Table 15 shows the coefficients of linear discriminants for herbarium specimen data for LD 1 x LD 2, in which LD1 accounts for about 88% of the variation.

Table 15. The coefficients of linear discriminants of herbarium specimen data of LD 1 x LD 2, in which LD1 accounts for about 88% of the variation.

	LD1	LD2
PAN.HEIGHT	0.01450922	0.009886146
PED.LENGTH	0.17005731	0.133278044
FIRST.INT	0.10570748	0.048898852
SEC.INT	0.17094435	-0.126745396
L.BB	-0.23366605	0.080509462
L.SB.FIRST	0.11429509	0.105896850
L.SEC.BRAN	-0.13387182	0.057664730
L.SB.SEC	-0.48548548	0.179599665
DIST.PAN.FLRS	0.37768409	-0.018250489
DIS.BRANCH.FLRS	-0.09604050	-0.300949642
W.BAS.L	-0.28749950	0.165421418
W.MID.L	0.01932993	0.025422940
MSW	0.37909044	-0.168659157
BSW	0.12034805	0.076221647
TLP	-0.63802018	0.428095981
TLB	0.39949418	0.227480879
PC.L	-0.94799407	-2.430613807
PC.W	-1.81810440	5.493179000
SEED.L	-1.57934739	-1.135353273
SEED.W	-0.14764289	1.240825814
POLLEN.L	-0.46812977	-1.047795404
POLLEN.W	0.35141993	-0.018099237

Proportion of trace:

LD1	LD2
0.8811	0.1189

For herbarium specimen data, all three varieties were predefined as var. *gramineum*, var. *robustum*, or var. *micranthum*. Fig. 13 and 14 display stacked histograms of discriminant function values of herbarium specimen data to provide a visual representation of the separation among varieties in linear discriminant 1 and 2.

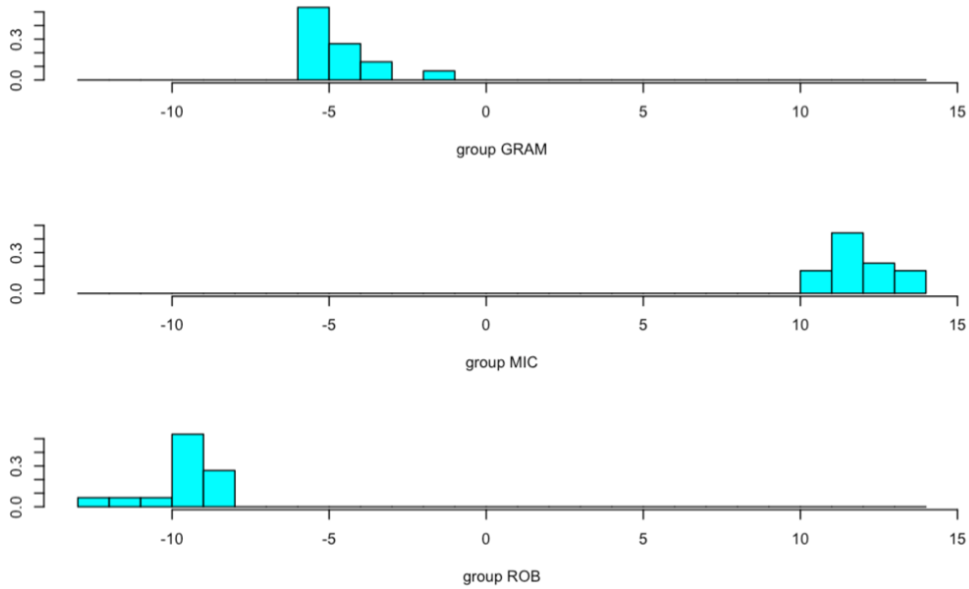

Fig. 13. Stacked histograms of dimension one of herbarium specimen data shows separation among varieties. This dimension accounts for 88.11% of the among-variety variation.

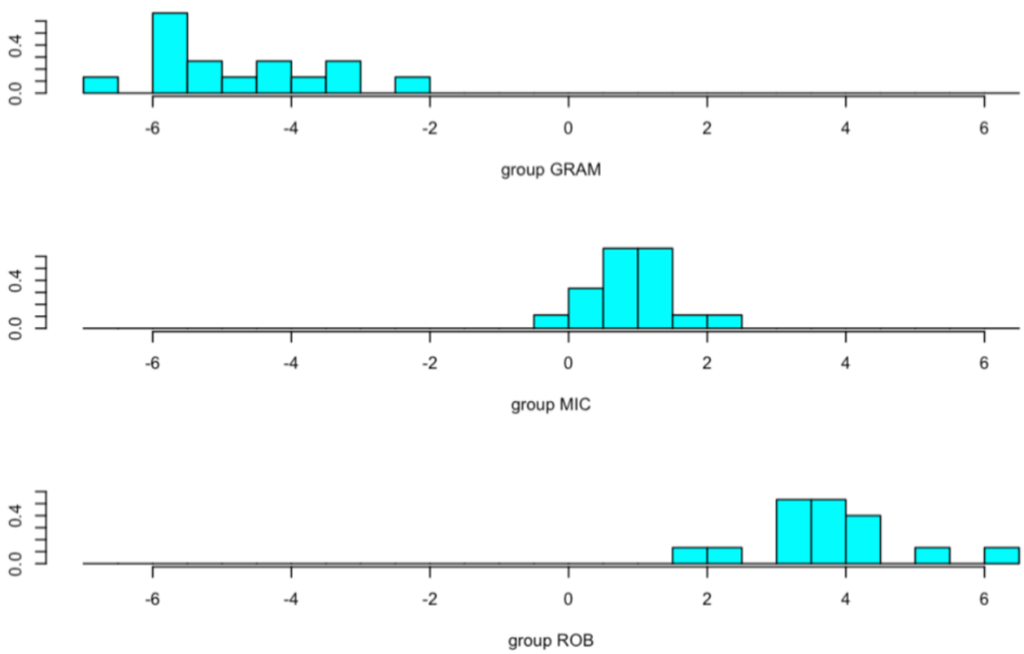

Fig. 14. Stacked histograms of dimension two of herbarium specimen data shows separation among varieties. This dimension accounts for 11.89% of the among-variety variation.

For field-collected data, var. *gramineum*, var. *robustum*, and Buck Creek were used as predefined categories. Table 16 shows the coefficients of linear discriminants for field-collected data for LD 1 x LD 2, in which LD1 accounts for about 91% of the variation.

Table 16. The coefficients of linear discriminants of field collected data of LD 1 x LD 2, in which LD1 accounts for about 91% of the variation.

Coefficients of linear discriminants:

	LD1	LD2
plant.height.cm	-0.0101954458	0.005953190
panicle.height.cm	0.0008538064	-0.004460784
peduncle.length.cm	0.0211945392	0.002725474
first.internode.cm	0.0061101620	-0.055734440
second.internode.cm	-0.0133405892	-0.049341200
average.length.bottom.two.branches.cm	0.0408880752	0.037139056
average.width.of.2.most.basal.leaves.mm	0.0004229982	0.043620294
average.width.of.two.midstem.leaves.mm	0.0153625631	0.053329211
Longest.Tepal.average.mm	-0.1569200831	0.002656689
Seed.Length.average.mm	0.1860227500	-0.162289293
seed.width.average.mm	-0.0943296594	0.200492855
capsule.length.1.mm	-0.3435222127	0.064958351
Capsule.width.1.mm	-0.5884763899	-0.246692101
Pollen.length.1.um	-0.0316203394	-0.108828285
pollen.width.1.um	0.0003945541	0.008807310
Stomatal.density.um	-0.0007462273	-0.002895876

Proportion of trace:

LD1	LD2
0.9138	0.0862

Stacked histograms of discriminant function values of field-collected data were produced to provide a visual representation of the separation among varieties in linear discriminant 1 and 2 (Fig. 15 & 16).

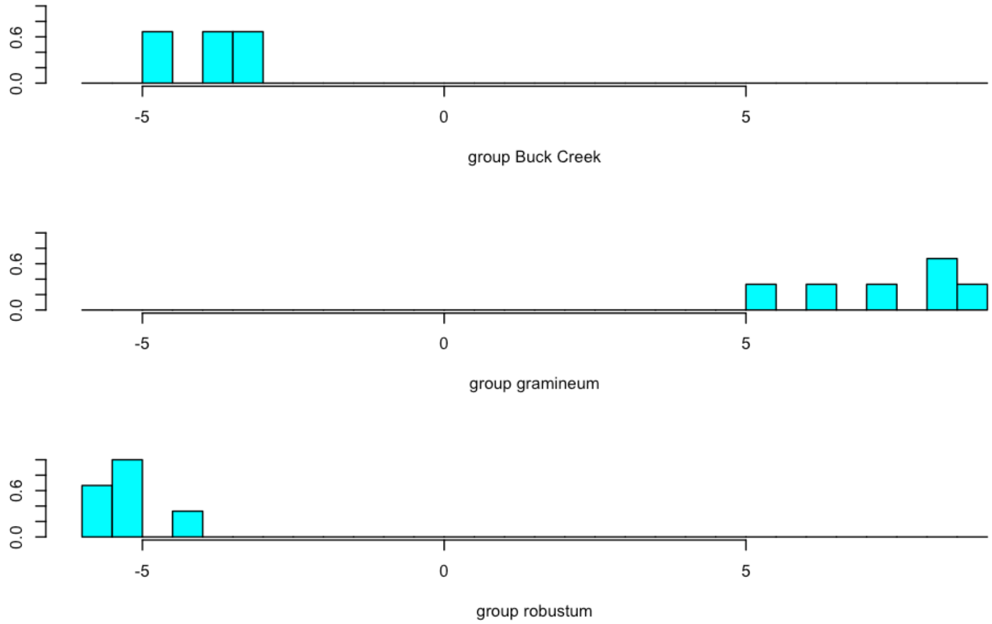

Fig. 15. Stacked histograms of dimension one of field-collected data shows separation among varieties. Dimension 1 accounts for 91% of the among-variety variation.

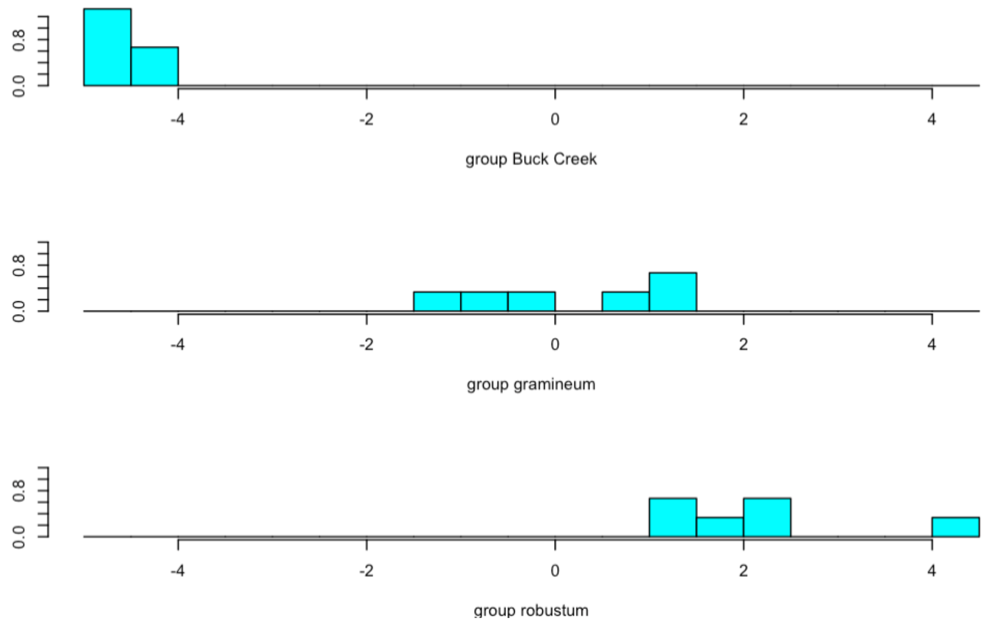

Fig. 16. Stacked histograms of dimension two of field-collected data shows separation among varieties. Dimension 2 accounts for about 9% of the among-variety variation.

Scatterplots of LDA results of herbarium specimen data (Fig. 17) and for field-collected data (Fig. 18) color-coded for varieties show separation among varieties and reduced variation within varieties to better delimit the varieties.

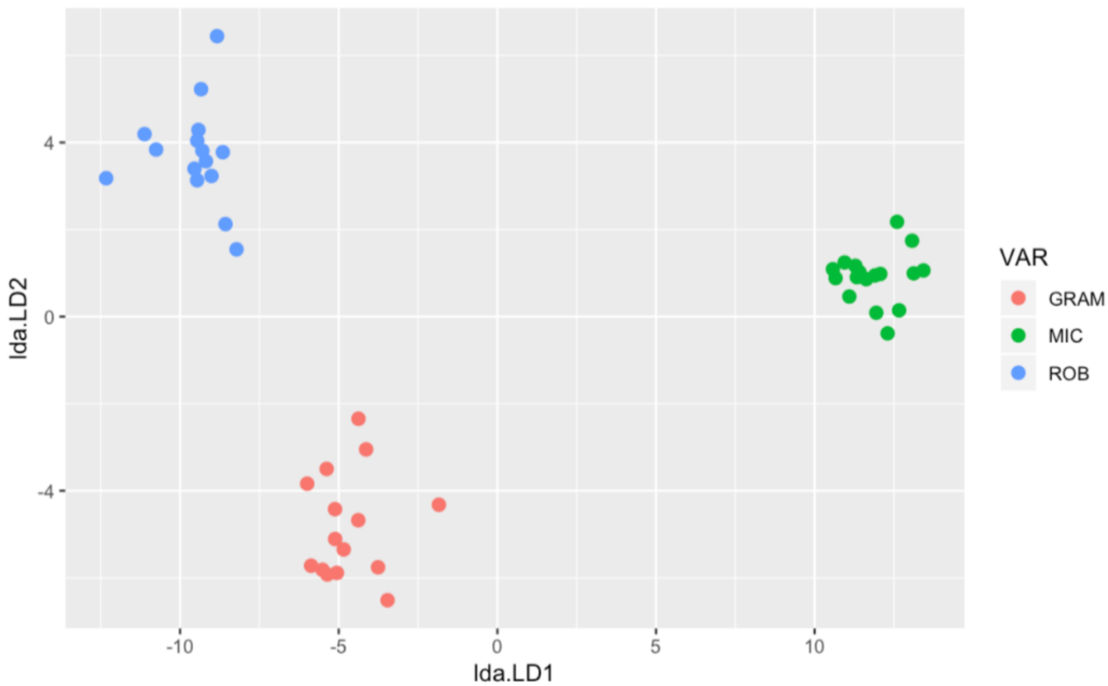

Fig. 17. LDA results of herbarium specimen data color-coded for varieties. Red: var. *gramineum*; Green: var. *micranthum*; Blue: var. *robustum*

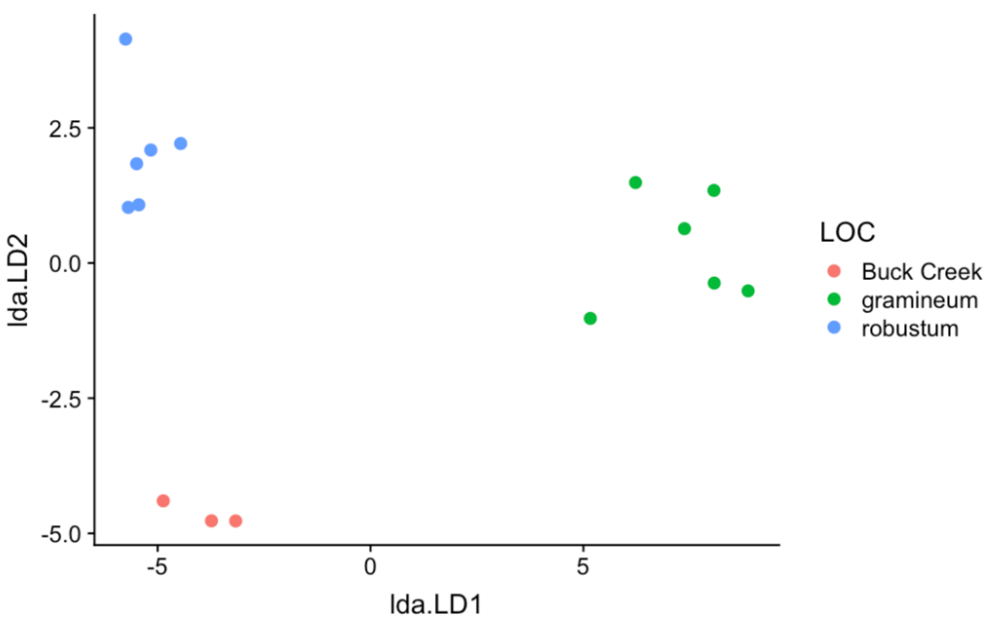

Fig. 18. LDA results of field-collected data color-coded for varieties or population.

Canonical Correlation Analysis (CCoA)

Canonical correlations and tests of ecological data using the Pillai-Bartlett Trace were used to determine the canonical variates and the p-value for each (Table 17). Canonical variates 1, 2, and 3 showed a significant correlation between environmental and morphological characters.

Table 17. Canonical Correlations and tests of ecological data using Pillai-Bartlett Trace.

Canonical Variate	Canonical Correlation	Pillai Trace	F approx	num df	den df	P value
1	0.996	4.04	3.17	40	30	0.0008
2	0.980	3.05	2.24	28	40	0.0097
3	0.970	2.09	2.00	18	50	0.0281
4	0.790	1.15	1.79	10	60	0.0811
5	0.726	0.53	2.06	4	70	0.0951

Standard canonical coefficients are displayed in Table 18 & 19 for ecological and morphological characters for the significant canonical variates. Plant height is negatively correlated to environment in variate two (Table 18).

Table 18. Standardized correlation coefficients of morphological variables from field-collected data on environmental variates 1-3.

	Variate1	Variate2	Variate3
plant.height.cm	0.05331	-0.007102	-1.0965
average.width.of.2.most.basal.leaves.mm	-1.12842	-0.236701	-0.2916
average.width.of.two.midstem.leaves.mm	0.35193	0.239716	0.3855
Longest.Tepal.average.mm	-0.11931	0.056034	1.1504
Seed.Length.average.mm	-0.48017	-0.434537	-0.7973
seed.width.average.mm	-0.64019	-0.117569	1.0065
capsule.length.1.mm	1.10722	-0.706176	-0.3310
Capsule.width.1.mm	0.52310	0.131618	0.1343

Elevation, sun exposure, and soil pH are negatively correlated to morphology in variate one, while average soil depth and ratio grazed are positively correlated. Ratio grazed has the highest positive correlation, while sun exposure has the most negative correlation. On variate two, ratio grazed, sun exposure, elevation, and average soil depth are positively correlated, while soil pH is negatively correlated. The highest positive correlation is elevation (Table 19).

Table 19. Standardized correlation coefficients of ecological variables on morphological variates 1-3.

	Variate1	Variate2	Variate3
ratio.grazed	1.42158	0.24205	-0.7653
sun.exposure	-0.53663	0.71895	0.5928
elevation.m	-0.03465	0.76290	0.7609
avg.soil.depth.cm	0.52673	0.03355	0.3118
soil.pH	-0.41333	-0.88533	1.0863

Distribution and Phenology

Distribution overlaps among each variety, but each has a unique pattern (Fig. 19, USDA, NRCS 2020). Variety *robustum* mainly occurs along the Appalachian Mountains from PA to NC, while var. *micranthum* favors the southern portion of *S. gramineum's* range, from the Blue Ridge east to the NC coastal plain and south to the GA piedmont. Variety *gramineum* does not extend further east than the Appalachian Mountains, but extends as far west as KY, into the Cumberland plateau. I found that this distribution coincides with the USDA NRCS maps of the ranges of all three varieties (Fig. 1-3). However, I did find a more specialized geographic pattern for var. *robustum*. Each variety was found, through field-collected data and voucher specimens, to occur in the habitat type that the literature claimed. However, var. *micranthum* was found to occur in additional habitat types other than just granitic domes, such as woodlands.

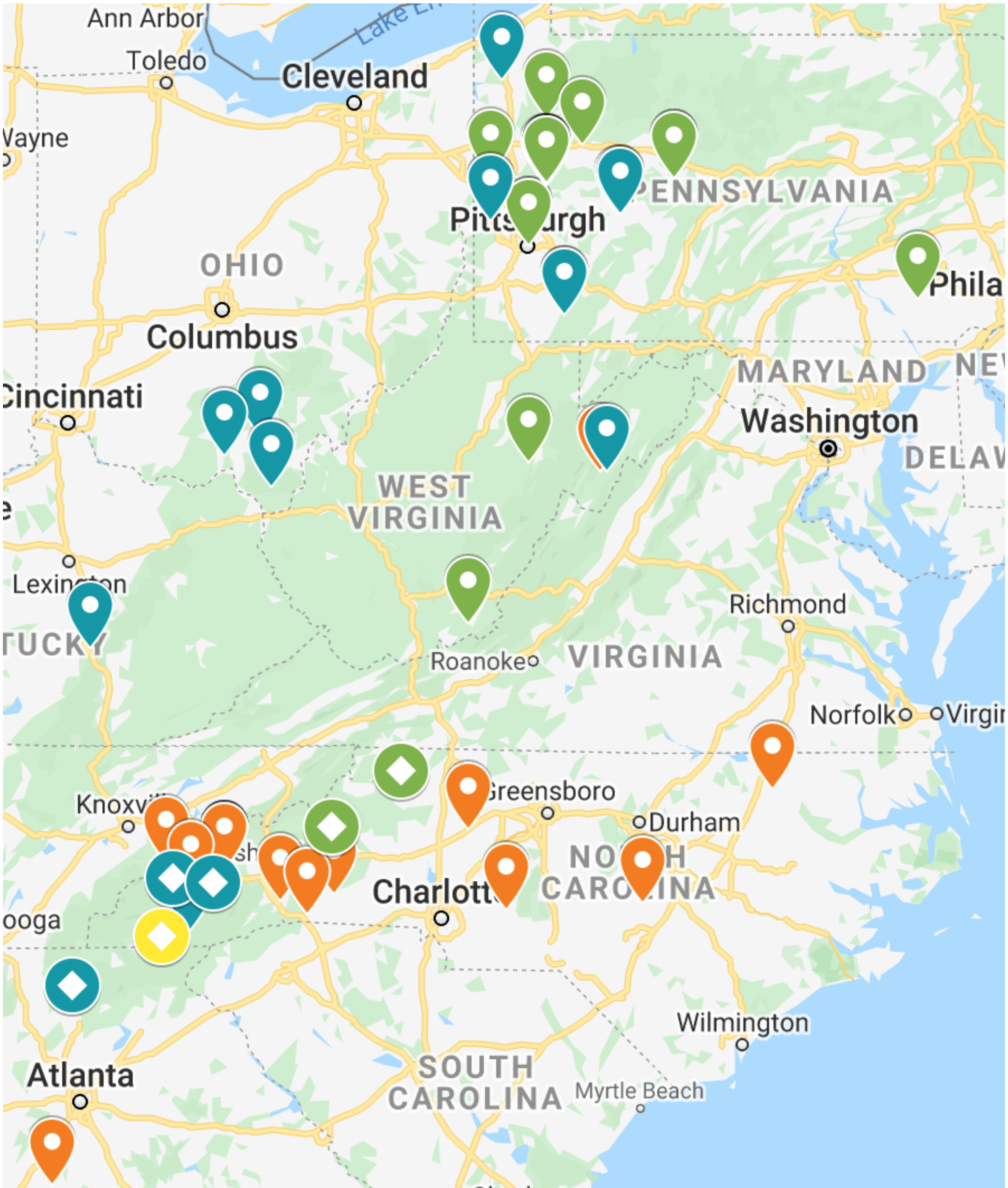

Fig. 19. Distribution of S. *gramineum* varieties from field collection sites and voucher specimens. Varieties are pinned within their counties of occurrence and not on exact GPS locations, due to the plants' conservation status. Green Pin: var. *robustum*, Blue Pin: var. *gramineum*, Orange Pin: var. *micranthum*, Yellow Pin: Buck Creek population. Populations that I located in the field are marked by a diamond shape (googlemaps.com, 8 June 2020).

Mean bloom date differs but bloom period does overlap among the varieties. The phenological data given in table 20 below is based on herbarium specimens and personal field observations. I was unable to determine flowering date range for the Buck Creek population, since I only collected on one date and did not find any herbarium specimens from this location. I collected my sample at this location on July 24th, 2019. At this time, the flowers were in late anthesis.

Table 20. Flowering date range and mean flowering date for all three varieties and the Buck Creek population

	var. *gramineum*	**var. *micranthum***	**Buck Creek population**	**var. *robustum***
Flowering Date Range	7/20-8/31	5/6-10/17	Unknown	7/25-9/11
Mean Flowering Date	8/11	8/3	NA	18/19

My data analyses showed that key distinguishing morphological and phenological features, (loadings higher than 0.6 for variation on dimension 1), were much more abundant than what is provided in the literature (Table 21). I have also included the Buck Creek population in this table. The Buck Creek population has a similar height of var. *gramineum*, but has extremely long tepals, surpassing the average length of those of var. *robustum*.

Table 21. Morphological variables with highest loadings on PCA dimension 1 for both the herbarium and field data analyses among the *S. gramineum* varieties, as well as the Buck Creek population. Legal status source: *USDA*, *NRCS;* Habitat type sources: Fernald 1946, 1950; Weakley 2015; USDA, NRCS 2019

Character State	**var. *gramineum***	**var. *micranthum***	**Buck Creek population**	**var. *robustum***
Mean Width of Most Basal Leaf (mm)	9.16	5.75	10	15.61
Mean Panicle Capsule Length (mm)	10.45	6.21	13.33	10.52
Mean Midstem Width (mm)	3.03	1.72	4	5.53
Mean Length of Second Branch from Peduncle (cm)	5.92	3.58	14	7.92
Mean Panicle Capsule Width (mm)	5.01	3.62	6.67	5.6
Mean Tepal Length of Branch Flowers (mm)	5.9	4.39	9	8.08
Mean Width of Midstem Leaves (mm)	5.7	2.84	9.17	8.92
Mean Seed Length (mm)	7.38	3.87	6.45	7.25
Mean Basal Stem Width (mm)	6	3.92	4.5	9.93
Mean Seed Width (mm)	1.53	1.19	1.53	1.55

Mean Tepal Length of Panicle Flowers (mm)	6.32	4.25	10.83	8
Mean Length of Subtending Bract to Second Branch from Peduncle (cm)	3.19	2.46	9.75	10.19
Mean Length of Bottom Branch from Peduncle (cm)	6.3	3.31	18.5	6.74
Habitat	forests, meadows, rock outcrops (and currently considered to occur in serpentine barrens)	granitic dome rock outcrops and woodlands	Serpentine Barrens	mountain bogs & wet meadows
Legal Status	threatened and endangered in parts of range	endangered in parts of range	possibly endemic with small population	threatened and endangered throughout entire range

Comparative Morphology

Images taken of plant tissue samples provide a visual for distinguishing among varieties. Variety micranthum is not present in this sample, because morphological comparison images were only done on field-collected plant tissue. Each variety represented differs in average tepal length, capsule size and shape, and seed size and shape (Fig. 20-22).

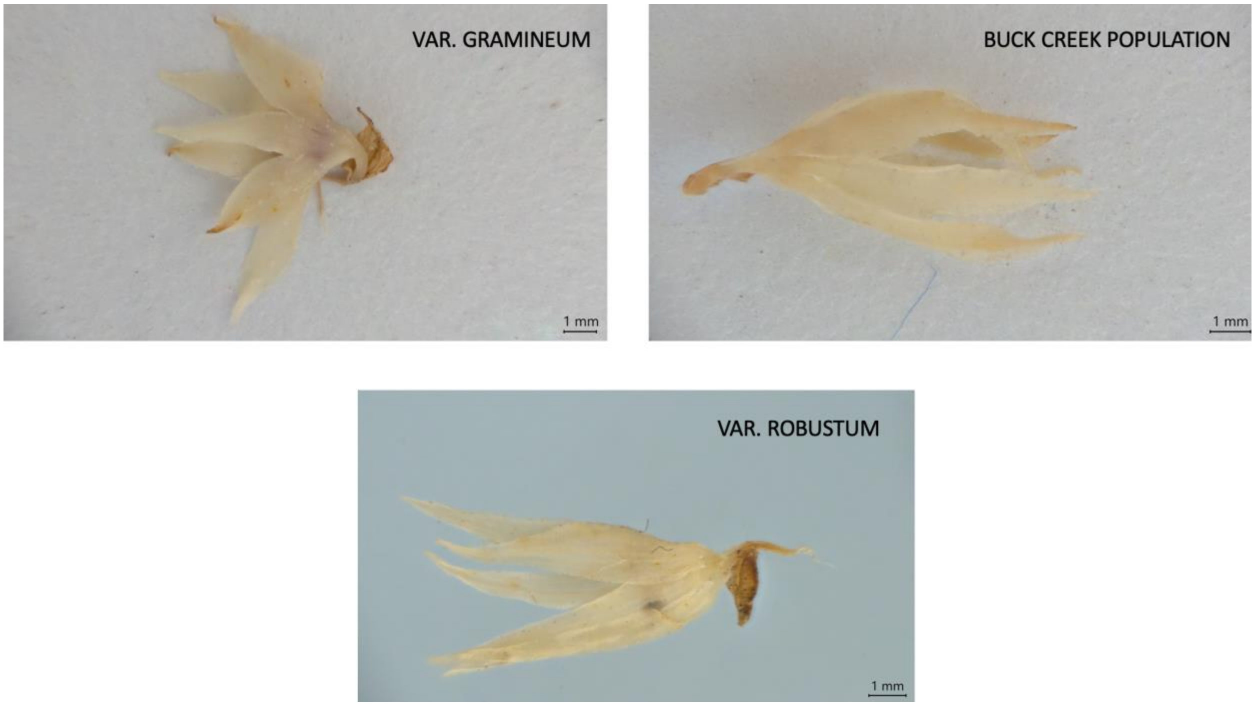

Fig. 20. Morphological variation among the flowers of var. *robustum*, var. *gramineum*, and the Buck Creek population. Tepal length varies among these varieties, with the shortest being those of var. *gramineum* and the longest being those of the Buck Creek population.

Fig. 21. Morphological variation among the capsules of var. *robustum*, var. *gramineum*, and the Buck Creek population. Size and shape vary among varieties, while style beak angle differs upon singular capsules.

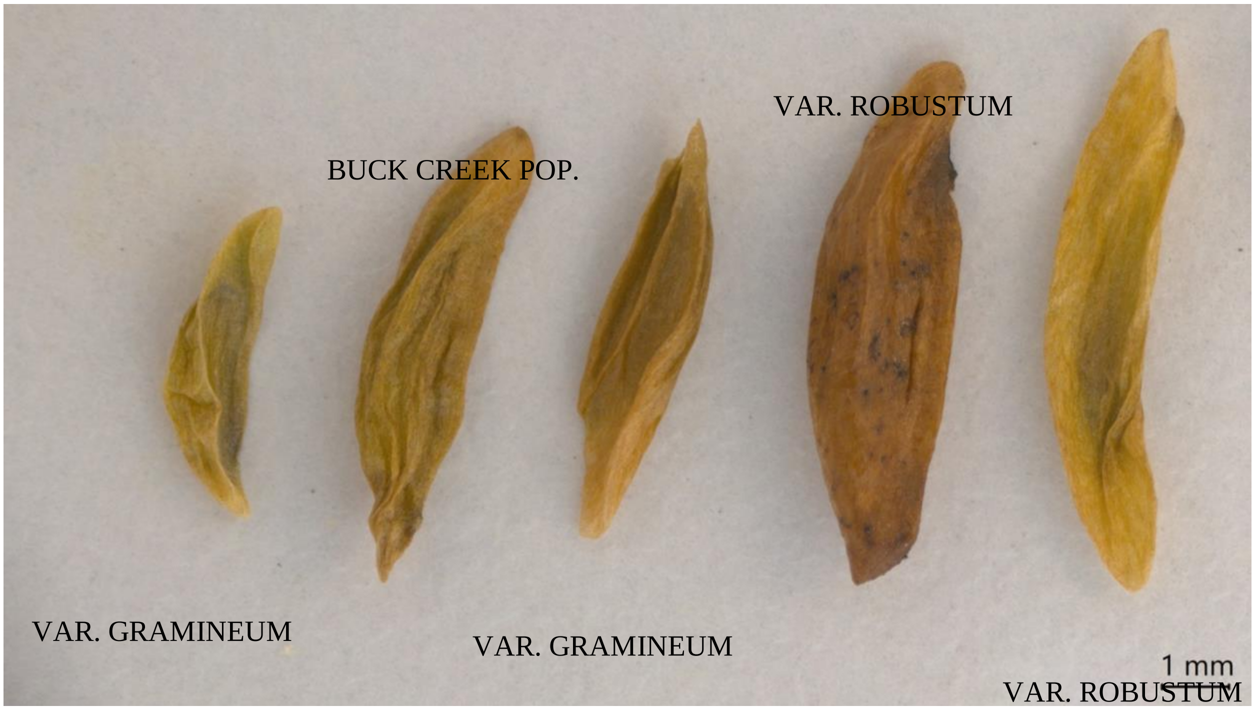

Fig. 22. Var. *robustum* tends to have a constant seed size, while those of var. *gramineum* vary among populations. Though the seed pictured here representing the Buck Creek population is larger than those of var. *gramineum,* they are on average smaller than those of var. *gramineum*.

CHAPTER FOUR: DISCUSSION & CONCLUSIONS

The goal of this study was to clarify the taxonomy of *Stenanthium gramineum*, based on the biological species concept, which defines species as interbreeding groups that do not interbreed with other such groups. Evidence that implies the inability to interbreed and produce viable offspring among different groups would support separate species status for those groups. Data analysis provides evidence for the delimitation of *S. gramineum* varieties at the variety or species levels (Table 20 & 21).

Morphological, habitat, and distribution data support elevating var. *robustum* to species level. This is further supported by the lack of the appearance of intermediates, even in its area of overlap with var. *gramineum*. Key characteristics that may be used in the field to identify var. *robustum* include its large size, full basal rosette, large basal stem width, and long tepals. A full list of characteristics is provided in Table 21. The PCA scatterplots showed separation of this taxon from other varieties (Fig. 4 & 9), while the pairwise MANOVA of voucher specimens indicated that this variety is significantly different morphologically from other varieties (Table 10 & 11). Though the omnibus and the pairwise MANOVAs for field-collected data (Table 12 & 14) showed no significant variation among any of the varieties, this may have been caused by the extremely small sample size, and therefore the MANOVAs of field gathered data are inconclusive.

Similarly, the CCoA, although showing a significant correlation between my ecological variables and morphology for canonical variates 1-3 (Table 17), was also based on too small of a sample size for this test to give reliable results, therefore making the CCoA results inconclusive. However, even if morphological traits are correlated to their habitats, this does not necessarily mean these populations are not independent species. Although environmental factors may be driving forces for some vegetative differences, reproductive characteristics are much less likely to be influenced by these environmental factors (Murrell 2010).

Variety *robustum*'s distinct range along the Blue Ridge mountain chain also supports its species level status. Also, it is only found in mountain bogs and wet meadows, as found in the literature, and supported by my findings in the field and by herbarium specimen labels. These findings reveal a constrained pattern for the occurrence of this species, in high-elevation mountain bogs and wet meadows along the Blue Ridge. Measurements taken in bog habitats during my field season revealed that these bogs had the highest average soil depth out of all habitat types sampled, direct sun exposure, and the lowest amount of grazing. The average pH for the two var. *robustum* habitats I sampled was 5.24, which was higher than all other sites except for Buck Creek. Range distinction and habitat specialization provide support for reproductive isolation (Moyle et al. 2004). I found no intermediates among herbarium specimens from several counties in which var. *gramineum* and var. *robustum* co-occur. Therefore, elevating var. *robustum* to species level is supported by the biological species concept.

Stenanthium gramineum var. *gramineum* and *S. gramineum* var. *micranthum* should be recognized as distinct varieties. In PCA analyses of herbarium specimen data, these varieties separate out as unique groupings (Fig. 4), although in the MANOVA pairwise test on herbarium data, they were not found to be significantly morphologically different (Table 9).

Var. *gramineum* is not found to favor a geographic area within its large distribution. Furthermore, it is a habitat generalist. In my study sites, I found it on a grassy bald and in mixed pine/oak forests. Though I was unable to locate var. *micranthum* in the field, herbarium specimens revealed that it was found in forested areas and on granitic domes. However, the literature claims that it is unique to granitic domes (Fernald 1946). Upon the creation of the map of all voucher and field-collected samples (Fig. 19), I found that it was not restricted to a certain geographic pattern within its distribution. Voucher specimens indicate that it can also be found in forested areas, as well as in the piedmont and coastal plains of NC.

Varieties of the same species may interbreed and produce viable offspring. The general understanding of the definition of the rank of variety is that varieties may be recognized by noticeable morphological differentiation and occupy different parts of the species' range. Because of these key factors, var. *micranthum* and var. *gramineum* should continue to be recognized as varieties. Additionally, evidence from the statistical analyses does refute the claim made by some (e.g., Utech 2002) that they are "indistinct and sympatric." Each of the three varieties should very well be recognized, as they display noticeable morphological differences and somewhat distinct distributions. These distinctions clearly support that they are within the parameters of the distinction of variety.

The population at Buck Creek Serpentine Barren in Clay Co., NC, does not fit the morphological description for any variety of *S. gramineum* and may represent an endemic species. This special habitat is known to harbor many endemic plant species (e.g., Kauffman et al. 2004; Boufford et al. 2014). This population of *S. gramineum* has larger flowers than those of var. *robustum*, with a tepal length average of 10.8 cm (Table 20). This population also displayed tepal color variation not seen in other varieties, as there was pink venation in some tepals (pers. obs.). In the PCA analysis of field-collected data (Fig. 9), the Buck Creek population showed a unique cluster, not grouping with another variety (Fig. 12). However, since I provided only four samples per population visited, and the Buck Creek population did not group with any other variety, there were too few data points to produce a 95% confidence interval around the Buck Creek data points on the scatterplot.

As previously stated, the MANOVAs run for field-collected data did not show varieties as significantly different (Table 11). However, this test was not reliable due to the small sample size, which renders its results inconclusive. A larger sample size of data for key morphological features for this population should be compared to the other varieties in a PCA and in pairwise MANOVAs to clarify whether this population is significantly morphologically distinct from the other varieties. It is clear that it occupies an extremely specialized habitat as well as restricted range. This means it could potentially have limited gene flow with other varieties, as it grows within a serpentine barren that may be large enough minimize the contact this population has with outside populations. The PCA of ecological data showed that the Buck Creek serpentine barrens habitat is extremely ecologically different from all other habitats sampled. One of these key differences is the soil pH, which is much higher than that of other locations sampled (6.37). Therefore, further research must be done to determine if there is significant morphological distinction of this population before a taxonomic conclusion can be reached. Future studies may include a common garden experiment (e.g. Baskins et al. 1993), or genetic analyses (e.g. Weins & Penkrot 2002).

Revisiting Table 1, which holds all variety information found in the literature, I was able to fill in all missing data, and correct information that was inaccurate (Table 22). I added in missing information for var. *micranthum*, based on findings in my research. I also found that the style beak curvature on capsules was not consistent, even on a single capsule of any variety. Therefore, I removed the suggested curvature for each variety that was in my original table, as curvature of style beak varies heavily. All updates from the original table are in red within Table 22.

Table 22. Variation in morphological characters, as stated in original literature, updated based on my research.

	Plant Height (m)	**Leaf Distribution**	**Leaf Texture**	**Tepal Length (mm)**	**Style Beak Curvature on Capsule**	**Capsule Shape**	**Capsule Length (mm)**	**Seed Length (mm)**
Var. *gramineum*	0.5-1.9	Crowded below, diminishing below panicle	Firm – coriaceous, opaque, surface corrugated	3-8 (-10)	Varies	Ovoid-urceolate	6-9	5-5.5
Var. *robustum*	Up to 1.8	Crowded and numerous nearly up to panicle	Thin, membranous, translucent, surface smooth	5-10	Varies	Oblong-subcylindric	9-10	5-8
Var. *micranthum*	0.25-1.0	Crowded below, diminishing below panicle	Firm – coriaceous, opaque, surface corrugated	3-4.5 (-5)	Varies	Ovoid-urceolate	5-7	3-5

A complication to this study was the inconsistent bloom years of *S. gramineum*, which made it difficult to locate previously documented populations. Although I traveled to many documented field sites, I was only able to find six flowering populations. Though I could not find anything about it in the literature, I spoke with several people who had experience with this plant and was informed that it does not come up every year (Tom Govus et al., pers. comm.).

Variety *robustum* is an endangered taxon within an imperiled habitat and should have a higher level of protection. Though var. *robustum* sites seemed to suffer less grazing overall, these sites were the most influenced by anthropogenic impact. Because of land use practices, mountain bog habitats have become increasingly rare. The two mountain bog habitats I located in the field had not been well protected. One had been mowed, and had a road running through the center of it. What was left of the bog was being treated as roadside ditches. The other site that I located in the field was also mostly destroyed, as roads ran on either side of the bog. What was left was a small bog habitat between two roads. Though var. *robustum* is legally protected, this protection does not seem to be recognized. Greater attention must be paid to the protection of mountain bog habitats in order to protect this endangered taxon.

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APPENDICES

Appendix A. Morphological characters measured on herbarium specimens, in the field, and ecological characters measured in the field.

Those in red were not used in statistical analyses and therefore do not have abbreviations

Morphological Characters Measured on Herbarium Specimens	Abbreviation
percent bud	
percent bloom	
percent fruit	
panicle height (cm)	PAN.HEIGHT
peduncle length (cm)	PED.LENGTH
length of first internode (cm)	FIRST.INT
length of second internode (cm)	SEC.INT
length of bottom branch (cm)	L.BB
length of subtending bract to bottom branch (cm)	L.SB.FIRST
length of second branch (cm)	L.SEC.BRAN
length of subtending bract to second branch (cm)	L.SB.SEC
distance between five panicle flowers (mm)	DIST.PAN.FLRS
distance between five branch flowers (mm)	DIS.BRANCH.FLRS
width of basal leaf one (mm)	W.BAS.L
width of basal leaf two (mm)	
width of mid stem leaf one (mm)	W.MID.L
width of mid stem leaf two (mm)	
Mid stem width (mm)	MSW
basal stem width (mm)	BSW
average of longest tepal length of three panicle flowers (mm)	TLP

average width of longest tepals of three panicle flowers (mm)	
average of longest tepal length of three branch flowers (mm)	TLB
average width of longest tepals of three branch flowers (mm)	
average seed capsule length(mm) of three panicle capsules	PC.L
average seed capsule width (mm) of three panicle capsules	PC.W
capsule beak shape (deflexed, erect, etc).	
seed shape (based off of seeds in one capsule)	
Seed color (based off of seeds in one capsule)	
Seed texture (based off of seeds in one capsule)	
average seed length of seeds in one panicle capsule (mm)	SEED.L
average seed width of seeds in one panicle capsule (mm)	SEED.W
average length of three pollen grains in one flower (um)	POLLEN.L
average width of three pollen grains in one flower (um)	POLLEN.W
pollen shape	
pollen color	

Morphological Characters Measured in Field	Abbreviation
plant height (cm)	plant.height.cm
panicle height (cm)	panicle.height.cm
peduncle length (cm)	peduncle.length.cm
first internode (cm)	first.internode.cm
second internode (cm)	second.internode.cm
average length bottom two branches (cm)	average.length.bottom.two.branches.cm
average width of two most basal leaves (mm)	average.width.of.2.most.basal.leaves.mm
average width of two midstem leaves (mm)	average.width.of.two.midstem.leaves.mm
Longest Tepal average (mm)	Longest.Tepal.average.mm
Seed Length average (mm)	Seed.Length.average.mm
seed width average (mm)	seed.width.average.mm
capsule length one (mm)	capsule.length.1.mm
Capsule width one (mm)	Capsule.width.1.mm
Pollen length one (um)	Pollen.length.1.um
pollen width one (um)	pollen.width.1.um
Stomatal density (um)	Stomatal.density.um
leaf texture	

Ecological Characters Measured in Field	Abbreviation
habitat type	
ratio grazed	ratio.grazed
sun exposure percentage	sun.exposure
elevation (m)	elevation.m
average soil depth of four soil measurements (cm)	avg.soil.depth.cm
Soil pH	soil.pH
Munsell Chart Score of soil	

Appendix B. Representative Specimens Examined

1. ***Stenanthium gramineum*** (Ker-Gawler) Morong var. ***gramineum*** Fernald.

Variety	State	County	Collector's Description	Day	Month	Year	Collector/Collector's #	Herbarium	Barcode
gramineum	IN	Kosciusko	Black, sandy loam soil on low black-white oak ridge	5	6	1935	C.C. Deam	CM	426473
gramineum	IN	Kosciusko	Black, sandy loam soil on low black-white oak ridge	5	6	1935	C.C. Deam	CM	426473
gramineum	KY	Rockcastle	Low, moist sandy soil near railroad bank	3	8	1943	F.T. McFarland	CM	184383
gramineum	NC	Macon	open woods around HIghland	18	8	1882	John Donnell Smith	CM	350823
gramineum	NC	Haywood	Forested former pastureland	27	7	2019	Hannah Cook #2209	WCU	
gramineum	NC	Swain	Grassy Bald	29	7	2019	Hannah Cook #2209	WCU	
gramineum	OH	Jackson	under powerline on top of NW facing sandstone cliff	31	8	1992	Allison W. Cusick #30,484	CM	375130
gramineum	OH	Scioto	on damp bank, full of sun	25	7	1976	John C. Bryant #510	CM	386257
gramineum	OH	Lawerence	full sun by powerline	13	8	1986	Allison W. Cusick #25.679	CM	326516
gramineum	PA	Butler				1946	LeRoy Black	CM	29988
gramineum	PA	Butler	Field along creek	23	7	1944	O.E. Jennings	CM	30032
gramineum	PA	Butler	Field along creek	17	9	1944	O.E. Jennings	CM	capsule suppliment from 30032
gramineum	PA	Butler	Valley	23	7	1939	O.E. Jennings	CM	29989
gramineum	PA	Crawford	swamp			1890	Jennie E. Whiteside	CM	29994
gramineum	PA	Venango	cobble bar on medium-grad ient stream	15	8	2008	Steven P. Grund 4648	CM	524301
gramineum	PA	Crawford	swamp			1890	Jennie E. Whiteside	CM	29994
gramineum	PA	Venango	cobble bar on medium-grad ient stream	15	8	2008	Steven P. Grund 4648	CM	524301
gramineum	PA	Fayette	Ohiopyle "borough"	16	8	1924	John Bright	CM	29995
gramineum	PA	Indiana	grassy road/ opening in immature forrest	15	8	2007	Loree Speedy 07-1098	CM	515322
gramineum	PA	Indiana	open moist old field	14	8	2008	Loree Speedy 08-1010	CM	468693
gramineum	PA	Beaver		1	8	1939	Andrew Lester	CM	30047
gramineum	PA	Clearfield	Chest Creek above Newburg Bell Twp.	15	5	2003	Jessica McPherson #1276	CM	532851
gramineum	WV	Pendleton	Rocky Roadbank; George Washington National Forest	20	7	1985	Allison W. Cusick #24549	CM	326542
gramineum		Indiana	Recovering woodland with moderatley dense tree canopy	15	8	2007	Loree Speedy 07-1114	CM	515323

2. ***Stenanthium gramineum*** (Ker-Gawler) Morong var. ***micranthum*** Fernald.

Variety	State	County	Collector's Description	Day	Month	Year	Collector/Collector's #	Herbarium	Barcode
micranthum	GA	Meriwether	rocky soil along Pine Mountain trail	6	9	1982	Mark A. Garland #94	CM	318973
micranthum	NC	Johnston	Mixed disiduos forest, Near moccasin Creek	19	6	1957	A. E. Radford #25276	CM	426476
micranthum	NC	Haywood	Pisgah Mt.	7	8	1926	W.C. Coker	NCU	75708
micranthum	NC	Henderson	open woods near bent creek	2	8	1957	O.M. Freeman - #57642	NCU	144950
micranthum	NC	Haywood	SE facing upper slopw of fork mountain, near fire Scald Ridge. PLot 18m downslope from fork mountain trail. Shining rock wilderness.	9	8	1993	Claire L. Newell #SRW2004	NCU	574178
micranthum	NC	Haywood	transition zone, Mt. Pisgah	13	7	1957	George S. Ramsuer #3561	NCU	137912
micranthum	NC	Haywood	transition zone, Mt. Pisgah	13	7	1957	George S. Ramsuer #3561	NCU	137912
micranthum	NC	Harnett	crest of mesic ravine-like slope of small blackwater river	6	5	2005	B. A. Sorrie #11614	NCU	581599
micranthum	NC	Halifax	rich mesic hardwood slope, 1.55 miles E of US 301-NC 561 junction- colony of 15 plants, only one in flower	18	6	1979	J. M. Lynch	NCU	499369
micranthum	NC	Cherokee			8	1890	Unspecified	NCU	75703
micranthum	NC	Davie	Mixed deciduous forest, Yadkin River	3	8	1956	A. E. Radford #14824	NCU	144948
micranthum	NC	NA				N/A	N/A	NCU	75705
micranthum	NC	Rutherford	One mile SE of Poor's ford bridge	21	8	1959	O.M. Freeman - #59119	NCU	177535
micranthum	NC	Polk	Dry woods	11	8	1921	Donald C. Pattie #1183	NCU	75707
micranthum	NC	Stanly	morrow mountain state park:unnamed tributary of Mountain creek, west of morrow mountain	30	5	2009	B.A. Sorrie #12340	NCU	593392
micranthum (Lectotype)	SC	Greenville	Ceasar's Head, open woods		7	1881	John Donnell Smith	HUH (viewed online)	96772
micranthum	TN	Sevier	Fighting Creek Gap GSMNP	17	10	1946	F.H. Beer	CM	426478
micranthum	WV	Pendleton	Rocky Roadbank; George Washington National Forest	20	7	1985	Allison W. Cusick	CM	326542

3. ***Stenanthium gramineum*** (Ker-Gawler) Morong var. ***robustum*** (S. Watson) Fernald.

Variety	State	County	Collector's Description	Day	Month	Year	Collector/Collector's #	Herbarium	Barcode
robustum	NC	Avery	Mountain Bog/ Highway Ditch	4	8	2019	Hannah Cook #2209	WCU	
robustum	NC	Alleghany	Ditch near Highway-Mowed	2	9	2019	Hannah Cook #2209	WCU	
robustum	PA	Butler	tributary of Glade Runs	5	9	1946	L.K. Henery	CM	30036
robustum	PA	Allegheny	Wildwood	6	9	1891	Joseph Kereeig	CM	30004
robustum	PA	Allegheny	Wildwood	30	8	1913	John Bright	CM	30000
robustum	PA	Venango		21	8	1954	W.E. Buker	CM	30027
robustum	PA	Beaver		25	7	1898	Mrs. Manfield	CM	30045
robustum	PA	Fayette	Mouth of Cucumber Run, Ohio Pyle	19	8	1918	E.M. Gress	CM	30051-2
robustum	PA	Lawerence	Floodplain N. side of Slippery Rock Creek	16	8	1942	O.E. Jennings	CM	30018-461833
robustum	PA	Washington	Near McDonald Reservoir	18	7	1946	Ellen Mason	CM	29998
robustum	PA	Clarion	Roadside Field	10	8	1946	L.K. Henry	CM	30049 & 461825
robustum	PA	Lancaster	Pleasant Grove	19	8	1886	J.J. Carter	CM	30017
robustum	PA	Clearfield		12	7	1908	O.E. Jennings	CM	30050
robustum	PA	Butler	woods	11	9	1969	L.K. Henry	CM	30009-3008(Panicle 1; Left)
robustum	PA	Butler	woods	11	9	1969	L.K. Henry	CM	30010-11(Panicle 2; Right)
robustum	PA	Venango	along sandy cr	9	8	1968	L.K. Henry	CM	29986
robustum	PA	Washinton	near reservoir	18	7	1946	Ellen Mason	CM	29998
robustum	PA	Clearfield		12	7	1908	Otto E. Jennings	CM	30050
robustum (Lectotype)	PA	Clarion	Sligo Furnace		8	1859	J.R. Lorrie	HUH (viewed online)	260645
robustum	WV	Monroe	Peter's Mountain	7	8	1924	Fred W. Gray	CM	426481
robustum	WV	Randolph	in wet field	31	8	1946	Mr. & Mrs. H. A. Davis	CM	132957

4. Buck Creek Population (Unspecified)

Variety	State	County	Collector's Description	Day	Month	Year	Collector/Collector's #	Herbarium	Barcode
unknown	NC	Clay	Sepentine Barren	24	7	2019	Hannah Cook #2209	WCU	