January 2011

Edaphic, Microtopographic, and Light Characteristics Associated with the Endangered Running Buffalo Clover (Trifolium stoloniferm) at the Blue Grass Army Depot, Madison County, Kentucky

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By

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Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
August 2011

DEDICATION

This thesis is dedicated to my family

My wife, Mrs. Amanda Watt

And

My son, Mr. Logan Watt

who have been an incredible source of inspiration.

ACKNOWLEDGEMENTS

I am gratefully indebted to all who have assisted me during the preparation of my Master's thesis. Without their help, I could not have completed such a study. First, I would like to thank my major professor, Dr. Charles Elliott, for his guidance, suggestions, and most of all, patience. I would also like to thank the other committee members, Drs. Bill Staddon, Paul Cupp, and Carolyn Ivans for their comments and assistance. I would like to express my thanks to my wife, Amy, for her understanding, inspiration and incredible patience throughout this process. She encouraged me and made me stick with it during the difficult times for the betterment of both myself and my family. I also express my thanks to my son, Logan Watt, who has truly been an inspiration to achieve. I would like to extend special thanks to my mother, Dale Castle, and her husband Click Castle in Louisa, Kentucky, for all of her kind words and years of undying support. I would also like to extend thanks to my family in Lexington, Kentucky, including my father, Larry Watt, and his wife Linda Watt, and to my brother Lyle Watt, and his wife Bethany, and daughter Evelyn Watt, for being there by my side throughout my stay in Kentucky and always pushing the importance of education. Finally, I would like to extend thanks to my wife's parents, Lonnie and Debbie Nelson, in Vancouver, Washington, for all of their support in Washington and for believing in me. My sincere gratitude extends to the Blue Grass Army Depot for providing access to running buffalo clover populations, and logistical and financial support over the length of this study and for allowing me to collect soil and the valuable data contained within this project. Additionally, many thanks are extended to Dr. Frank Sikora, the University of Kentucky

Soil Testing Laboratories, Dr. Gary Janicke, Eastern Kentucky University Department of Agriculture, and Mr. Tim Weckman, Eastern Kentucky University Department of Biology, for the use of their laboratories, equipment, and for providing answers to all of my questions throughout the completion of this project. Finally, I would like to thank all of the graduate students who have assisted in the months and years of field and laboratory work throughout the completion of my thesis: Jodi Staci, Budd Veverka, Ryan Dunbar, Kristin Collins, Sarah Asher, and Matt Beckett. Many thanks as well to those who have provided the knowledge base before me, of whom I request permission to quote copyrighted material included in this document.

ABSTRACT

Soil, microtopographic, and light parameters associated with federally endangered running buffalo clover (*Trifolium stoloniferum*) plants on the Blue Grass Army Depot, Richmond, KY, were examined during the 2004 and 2005 growing season. Study samples or measurements were determined at 27 "long-term" sites (plots which have had clover present since 1992-1994), 23 "disappeared" sites (plots which had clover in 1992-1994, but did not have plants present at the time of this study) and 25 random/control sites (randomly selected 1 m^2 plots in areas in which running buffalo clover has never been reported).

Average precipitation on the Blue Grass Army Depot was significantly higher in May 2004 and May 2005, as well as June 2004 than in June 2005 (T-calculated= 3.37 and 2.38, respectively). Ambient temperatures at ground level were significantly lower in March 2005 than 2004 (T-calculated= 41.76) and significantly higher in June and July 2005 than 2004, respectively (T-calculated= 22.22 and 15.86, respectively). The average number of running buffalo clover rooted crowns and inflorescences per plot did not differ significantly between years.

Overall, there was no difference between long-term clover sites, disappeared clover sites, or random/control sites in terms of average soil nutrient values for NH_4, NO_3, Zn ,K, Mg, P, and pH. However, there were significant difference between years for some nutrients, i.e., average Mg (F= 8.77), pH (F= 14.04), and NO_3 levels (F= 115.06) were higher in 2004; while mean Ca (F= 11.22), NH_4 (F= 116.09), and P (F= 11.21) levels were higher in 2005. Only potassium showed a significant site-by-year interaction

(F =3.33), with clover sites having the most potassium for both years, random/control sites were intermediate, and disappeared clover sites had the lowest values.

Plots examined in this study occurred within eight soil texture types and 14 soil series. There were significantly more plots in the silt loam texture type (X^2 = 279.27). There was no significant difference in mean bulk density (F= 0.57), percent soil moisture (F= 0.22), soil water content (F= 0.07), volumetric water content (F= 0.09), or porosity (F= 0.56) between long-term clover sites, disappeared clover sites, or random/control sites. Between years, only bulk density (F=4.96) and porosity (F= 4.95) showed a significant difference. Bulk density readings were higher in 2005 than 2004; while porosity was higher in 2004 versus 2005.

There was significantly greater topographic variation of elevation within plots between long-term plots and disappeared clover plots when compared to random plots (F=11.13). When the overall shape of each running buffalo clover study plot was examined across the topographic spectrum, a trend was noted. Long-term clover plots occurred twice as often, compared to random, in a depression; long-term clover plots on mounds occurred three times more frequently than random. In plots in which running buffalo clover has been present since 1992-1994 (long-term sites), clover plants were not randomly distributed with regard to where they were encountered along a microtopographic transect ($\chi^2_{0.05,6}$ = 12.592) ; indicating microtopographic variation may be important for the establishment and maintenance of running buffalo clover plants. Although not statistically significant, sites with clover (long-term plots), on average, had lower maximum light values than their counterparts.

The information provided in this study can serve as base-line values with which future assessments of running buffalo clover sites on the Blue Grass Army Depot can be made; as well as provide data for comparative purposes at sites across the geographic range of *Trifolium stoloniferum*.

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CHAPTER I

INTRODUCTION

Running buffalo clover (*Trifolium stoloniferum* Muhl. ex. A. Eaton) is one of only two clovers native to Kentucky (Campbell et al. 1988). Running buffalo clover is a perennial herb, flowering in May and early June and fruiting through July (USFWS 2007). Running buffalo clover is unique among clovers because it does not contain root nodules to fix nitrogen back into the soil (Morris et al. 2002). The clover originates out of a central rosette then grows on stolons or "runners," giving running buffalo clover its characteristic name (Brooks 1983; USFWS 2007; for more detailed species ecology see Appendix I). Prior to 1985, experts believed the clover was extinct due to habitat destruction and lack of soil disturbance from trampling and grazing bison *(Bison bison;* Bartgis 1985; Campbell et al. 1988; Cusick 1989).

Historically, running buffalo clover was believed to have thrived throughout the mid-western United States from West Virginia through Kansas (USFWS 1989). Originally considered extirpated, it was rediscovered in 1985 in West Virginia (Bartgis 1985). The United States Fish and Wildlife Service (USFWS) recognized running buffalo clover as an endangered species in 1987 (USFWS 1987). Subsequent searches throughout the historical home-range yielded more populations in West Virginia (Brooks 1983; USFWS 2007), as well as in Kentucky (Campbell et al. 1988), Indiana (Homoya et al. 1989), Ohio (Cusick 1989) and Missouri (Taylor et al. 1994). Since its initial re-discovery, 101 populations have been located (USFWS 2007). According to the 2007 USFWS recovery plan, populations are divided into three geographical regions:

Appalachian (West Virginia and southeast Ohio), Bluegrass (southwest Ohio, Kentucky, and Indiana), and Ozarks (Missouri). The majority of populations occur within the Appalachian and Bluegrass regions, with the largest population in West Virginia, and the greatest number of populations in Kentucky (USFWS 2007). Reclassification of running buffalo clover to threatened status can be considered when its life history is better understood and 30 secure, self-sustaining populations are known to exist into perpetuity (USFWS 1989). The USFWS (2007) has assigned running buffalo clover a recovery priority number of 8 meaning the species has a moderate degree of threat and a high recovery potential.

Running buffalo clover occurs in shaded lawn habitats (cemeteries, parks, old home sites) and mesic forests (near streams and game trails). There is great speculation regarding the vegetation present within the historic range of running buffalo clover both during the time of Native American occupation and during European settlement (Harshberger 1922; Jakle 1968; Denevan 1992). Descriptions of Kentucky's Bluegrass region generally suggest an open-canopied savannah-like forest with extensive canebrakes having sparse tree cover; between the canebrakes there was open timber with grasses and legumes forming natural meadows (Wharton and Barbour 1991). Early descriptions often describe the savannah-like forests as "parks" or "grazing meadows (Campbell et al. 1988; Wharton and Barbour 1991)." In these descriptions, a white-flowered clover is mentioned as a major constituent of the herbaceous layer, a clover Campbell et al. (1988) strongly suggests was running buffalo clover.

The clover which was once common in Kentucky's bluegrass region was not only common in the expanses of bluegrass savannah but also was associated with Native

American paths, seasonally flooded stream terraces, and bison traces (Wharton and Barbour 1991; Fields and White 1996; USFWS 2007). Large herds of bison would create wide "traces" of trampled bare ground as they migrated between the numerous salt licks in the Ohio Valley and central Kentucky. These 'traces', sometimes referred to as bison roads, would later serve as conduits for travel for European explorers, and eventually become the blueprint for railroads and highways that exist today. At the time of European settlement, running buffalo clover is thought to have been dependent on large ungulates, such as bison (representing the species name sake), elk (*Cervus elaphus*) and deer (*Odocoileus virginianus*), for seed scarification and dispersal, soil enrichment, and for the maintenance of its moderately disturbed habitat along large game trails (Jacobs and Bartgis 1987; Campbell et al. 1988, Cusick 1989; Homoya et al. 1989; Tankersley 1992). Campbell (1985) noted a strong correlation between the appearance of cane and clover in early pioneer writings, with clover occupying disturbed areas in and around cane fields.

In Kentucky, running buffalo clover is broadly associated with the Inner and Outer Bluegrass physiographic regions (USFWS 2007). It is most often found in regions underlain with limestone or other calcareous bedrock, but not exclusively, with many occurrences in alluvial soils from calcareous parent material (Hattenbach 1996; USFWS 2007). Running buffalo clover occurs in mesic habitats with partial to filtered sunlight, where there is a prolonged pattern of moderate, periodic disturbance, such as mowing, trampling, or grazing (USFWS 1989; USWFS 2007). Characteristic habitat for running buffalo clover in Kentucky is mesic deciduous forest communities associated with occasionally flooded terraces of small to mid-sized streams (1^{st}-3^{rd} order; KSNPC 2001). However, running buffalo clover has been reported from a variety of habitats; including historic properties, mesic woodlands, savannahs, floodplains, stream banks, sandbars (especially where old trails cross or parallel intermittent streams), grazed woodlots, mowed paths (cemeteries, lawns, and parks), old logging roads, jeep trails, skidder trails, mowed wildlife openings within mature forests, and steep ravines (USFWS 1989; Bloom et al. 1995; KSNPC 2001; Becus and Klein 2002; Madarish and Schuler 2002; USFWS 2007). Over two-thirds of the present populations in Kentucky are associated with mixed mesophytic forested riparian corridors, of which a majority is open forests with filtered light and alluvial soils (Campbell et al. 1988; Cusick 1989; Homoya et al.1989; Bloom et al. 1995; Fields and White 1996; Madarish and Schuler 2002; USFWS 2007). Critical habitat is not currently designated for running buffalo clover (USFWS 2007).

In 1992, running buffalo clover was discovered on the Blue Grass Army Depot (BGAD), in Madison County, Kentucky, and has been monitored for persistence intermittently since then (Bloom et al. 1995; Fields and White 1996; White et al. 1999; Elliott 2003). The Blue Grass Army Depot contains the largest aggregate population of running buffalo clover plots in the state (n=145: Bloom et al. 1995; Fields and White 1996). Bloom et al. (1995) defined a plot as "one or more clustered running buffalo clover plants at least 7.5 meters from any other running buffalo clover plants". Bloom et al. (1995) reported that if nearby plots were aggregated, running buffalo clover plots on the Blue Grass Army Depot were confined to 38 separate areas (or populations). The majority of running buffalo clover plots were discovered on stream terraces and forested riparian floodplains, which researchers attribute to past agricultural land uses (Bloom et al. 1995; Fields and White 1996; USFWS 2006). Since its discovery in 1992, almost half of the original Blue Grass Army Depot plots no longer contained running buffalo clover

(49%; USFWS 2006); of the remaining plots, many have reduced numbers (White et al. 1999; Elliott 2003).

In 1996, a portion of the Blue Grass Army Depot was portioned off as a wildlife management zone, while the rest remained a grazing/agricultural management zone (Fields and White 1996). Cattle (*Bos taurus*) are excluded by fence from the wildlife zone allowing forested riparian corridors to return to a natural succession scheme. Since the 1950s agricultural management at the Blue Grass Army Depot has included heavy cattle grazing (6000 head of cattle) and hay production (USFWS 2006). In the 2000's, cattle grazing pressure on the Blue Grass Army Depot was cut in half.

Campbell et al. (1988) and Cusick (1989) reviewed the historical distribution and decline of running buffalo clover in Kentucky and Ohio, respectively, and noted the apparent relationship between the plant's presence and patterns of long-term soil disturbance. One thought concerning declining numbers of clover is that extra competition from succession impedes clover growth. The USFWS (2007) indicates the most critical biological constraint for the recovery of running buffalo clover is the plant's dependence on disturbance; which limits competition from other plants and prevents successional changes in the landscape. Any recovery strategy for running buffalo clover must include a habitat management component designed to ensure the long-term viability of the species.

Running buffalo clover displays a cyclic nature of appearance and disappearance, with a high probability of small populations experiencing localized extinctions; making detection of small populations difficult. Due to the clonal nature of running buffalo clover, genetic variation within populations is low; however genetic variation between populations is higher (Crawford et al. 1995; Vincent and Hickey 1996; Hickey et al. 1991). Protection of several small populations across the landscape could help ensure viability of the species range-wide. In many plants, factors influencing the number of populations may be as important as within-population dynamics in determining whether a species persists or becomes extinct (Schemske et al. 1994). Schemske et al. (1994) report metapopulation dynamic processes have implications not only for survival of local populations but also for survival of the species; the metapopulation affects within-population dynamics and hence population persistence through dispersal. For a species to spread or persist, plants must colonize unoccupied plots at least as frequently as populations become extinct. Only by identifying the life history stages that have the greatest impact on population growth can the conservation biologist begin to design efficient recovery efforts (Schemske et al. 1994).

The running buffalo clover recovery plan encourages new management techniques, conducted by professionals, which emphasize "increasing both the clover's vigor and abundance (USFWS 1989)." Pavlovic (1994) noted that the effects of disturbance on disturbance-dependent rare plants can be viewed according to their realized niche spaces; which are maintained by the natural disturbance regimes. Novel disturbances which mimic natural disturbance regimes may expand or replace realized niche spaces for disturbance dependent plant species (Pavlovich 1994). In Ohio regular mowing regimes have maintained running buffalo clover populations in lawn-type habitats, such as parks and cemeteries (Becus and Klein 2002; USFWS 2004). Maneuvering mowers into riparian forest as part of running buffalo clover management may be physically impossible, and hand-picking competing plants is economically unrealistic for such habitats (USFWS 2004). Madarish and Schuler (2002) discovered that controlling the intensity of surface disturbance will help sustain populations of running buffalo clover in forests managed for silviculture, with moderate disturbances from logging machinery being most beneficial over the long term.

Bloom et al. (1995) found four types of disturbance at running buffalo clover plots on the Blue Grass Army Depot: cattle grazing, trampling, flood scour, and suspected deer grazing. White et al. (1999) reported that simulated trampling with cleats, continuous grazing (at heavy levels), and herbicide applications were all detrimental to running buffalo clover. Ford et al. (2003) postulated that white-tailed deer may not significantly contribute to running buffalo clover recovery because of their limited ability to create disturbance and distribute seeds. However, cattle may provide moderate surface disturbance similar to that created by logging machinery in a silvicultural harvest which proved beneficial (Madarish and Schuler 2002). Prescribed burning in forested riparian zones is generally ineffective as a running buffalo clover management tool (USFWS 2006).

The USFWS (1989) recovery plan for running buffalo clover noted the need for baseline data concerning running buffalo clover soil and microhabitat requirements. Past studies of soil characteristics on the Blue Grass Army Depot suggest central Kentucky may contain unique habitat conditions within running buffalo clover's current range (Hattenbach 1996). My objective was to determine what physical and chemical soil composition, soil water content, overstory and understory cover, and microtopography are associated with running buffalo clover plots on the Blue Grass Army Depot in Madison County, Kentucky.

CHAPTER II

STUDY AREA

The study was conducted on the Blue Grass Army Depot in Madison County, Kentucky. The Blue Grass Army Depot is a chemical and ammunition storage facility for the U.S. Army (Thompson 1992). The property lies approximately 5.63 km southeast of Richmond, KY, and encompasses about 5907 ha (Thomas 1994; USFWS 2006). Storage facilities are grouped in different regions of the Blue Grass Army Depot's Restricted Area (area inside the inner security fence into which there is restricted public access). The storage regions are designated by letters (e.g., Area A, Area B, etc.). Between the Blue Grass Army Depot's outer and inner security fences lies the Perimeter Area. For management purposes, the Perimeter Area is divided into tracts which are identified based on the gate which allows access to the tract. The Blue Grass Army Depot is predominantly pasture dominated by fescue and is maintained by cattle grazing and mowing, with some scattered early successional woody vegetation generally restricted to the riparian zones; these forests probably started growing in the early 1940's after completion of the facility (USFWS 2006). Thomas (1994) reported the vegetative communities on the Blue Grass Army Depot fall primarily into three broad categories: pasture (74%; 2,568 ha), upland forest (14%; 506 ha), and bottomland forest 12%; 404 ha). Additional descriptions of the flora of the Blue Grass Army Depot can be found in Thomas (1994) and Jones (2000).

The Blue Grass Army Depot is broadly located in the Kentucky River drainage basin; several smaller drainages, including Muddy Creek, Silver Creek, and Otter Creek, all drain toward the Kentucky River to the north (USFWS 2006). The majority of the Blue Grass Army Depot is drained by the Muddy Creek basin, which includes the Muddy Creek mainstem, Viny Fork, Little Muddy Creek, and smaller unnamed tributaries. Otter Creek drains the northwest corner of the Blue Grass Army Depot, running toward Lake Reba near the city of Richmond, KY.

The Blue Grass Army Depot is located in the Outer Bluegrass Subsection within the Bluegrass Region of the Interior Lowland Plateau physiographic region (Jones 2005). Gentle rolling slopes are typical although moderately steep slopes do occur in areas of stream dissection (Wharton and Barbour 1991). The majority of the Blue Grass Army Depot is underlain by the Upper Ordovician Drakes Formation consisting of dolomite, shale, and limestone (Fields and White 1996).

The following four soil associations occur at the Blue Grass Army Depot; the Lowell-Faywood-Cynthiana group, the Shelbyville-Mercer-Nicholson group, the Beaseley-Brassfield-Otway group, and the Lawrence-Mercer-Robertsville group (Newton et al. 1973). The extreme northwest and southwest portions belong to the Lowell-Faywood-Cynthiana Rock outcrop association, characterized as deep, well-drained, gently sloping soils on fairly wide ridge tops and moderately deep and shallow, sloping to steep soils on side slopes. The Shelbyville-Mercer-Nicholson association occurs in the western portion of the Blue Grass Army Depot and are characterized as deep, well-drained to moderately well-drained, level to gently sloping soils on wide ridge tops and deep, well-drained to moderately well-drained sloping soils along drainage ways. The Beaseley-Brassfield-Otway association occurs in the east and southeast part of the Blue Grass Army Depot, characterized by deep, well-drained, gently sloping to sloping soils on narrow ridge tops and moderately deep, well-drained, strongly sloping to steep soils on side slopes. The Lawrence-Mercer-Robertsville association occurs in the north and northeast areas of the Blue Grass Army Depot and is characterized as somewhat poorly drained and poorly drained soils, on broad flats; moderately well-drained, level to gently sloping soils on wide ridge tops; and moderately well-drained, sloping soils along drainage ways. Further descriptions of each soil association can be found in Newton et al. (1973). On the Blue Grass Army Depot, running buffalo clover occurs on all soil associations except the Lawrence-Mercer-Robertsville association (Fields and White 1996).

Braun (1950) places Kentucky's Outer Bluegrass section in the Western Mesophytic Forest region. The western mesophytic region contains a wide variety of vegetation types, including mixed mesophytic, mixed hardwoods, oak-hickory, cedar glades, and swamp forest. Wharton and Barbour (1991) estimate that natural plant communities in central Kentucky cover approximately 4 % of their historic area. Bloom et al. (1995) noted the presence of the rare Bluegrass Mesophytic Cane Forest and Bluegrass savanna-woodlot on the Blue Grass Army Depot.

Wharton and Barbour (1991) described the climate within the region encompassing the Blue Grass Army Depot as continental, with extremes and changeable weather in all seasons. The average annual precipitation and temperature place the area in the warm temperate, humid category (Bryant et al. 1980). The average annual precipitation of 112 cm is distributed throughout the year with September and October having the least and March through July having the most. Droughts sometimes occur, the effects of which are intensified by the limestone karst (Henderson 1998). The minimum length of the growing season is 181 days (Wharton and Barbour 1991). The average annual temperature is 12^{o} C, with high temperatures in the summer around 27^{o} C and lows in the winter in around -7^{o} C (Newton et al. 1973).

CHAPTER III

MATERIALS AND METHODS

Bloom et al. (1995) recorded 145 specific locations of running buffalo clover plots on the Blue Grass Army Depot using a Geographical Positioning System (GPS). Bloom et al. (1995) defined a plot as "a group of one or more plants which occur in isolation within an area, or which appear to be separated physically from other groups of running buffalo clover within the immediate area." A majority of the plots had less than 20 plants with low numbers of flowers. Using a Garmin GPS12 unit (Garmin, Kansas City) and local site descriptions, Bloom et al.'s (1995) plot sites were relocated in the flowering period for running buffalo clover (May-June) 2004 and 2005 and plots established which encompassed the original area covered by each running buffalo clover plot. The number of rooted plants and flowering heads within each plot were counted. A rooted plant was defined as any vertical projection of the clover on the stolon under which a root is located, such that if the stolon were severed, the clover could survive independently. Bloom et al. (1995) found 38 populations on the Blue Grass Army Depot; the USFWS (2006) currently notes 150 plots representing 16 populations.

Three running buffalo clover habitat categories on the Blue Grass Army Depot were compared: long-term sites, disappeared sites, and control sites. "Long-term" sites were running buffalo clover plots which have had clover present since the 1992-1994 survey; this was considered good habitat since plants still persist; this also includes sites which had been classified as disappeared, but clover was discovered on them during survey efforts. "Disappeared" sites were running buffalo clover plots which had clover in

1992-1994, but did not have plants present when site selection for this project began in 2004; these plots had favorable growing conditions at one time. "Random or control" sites were randomly selected 1 m^2 plots within the forested floodplain that have never historically contained clover. Twenty-five running buffalo clover plots or random plots were chosen per habitat category (N=75). While conducting the surveys, clover was rediscovered in two of the disappeared sites (#429 and #634, respectively), increasing the number of sites with clover to 27 and decreasing the number of disappeared sites to 23.

Locations chosen as long-term running buffalo clover sampling sites were selected using a repeated measures ANOVA to compare the number of rooted plants at each plot; the plot was the repeated variable run over the previous three years sampling numbers. The 25 plots supporting the highest mean number of running buffalo clover plants were designated as long-term sites. Plots must have had three years prior presence to be included. Disappeared sites were randomly chosen from a list of all plots in which running buffalo clover was no longer present. Random/control sites were selected by choosing a pre-existing plot (with clover or disappeared clover) and walking a random distance in meters along a random compass azimuth. The endpoint of the random transect served as the center of a 1m^2 plot.

Microtopography was measured at all plots using a modified pin frame (Harper et al. 1965) with 10 pins spaced at 11 cm intervals; pins were calibrated in 1 cm intervals, starting with 0 at the top and increasing incrementally down the pin. Since running buffalo clover plots and control plots were rectangular or square in shape, pin frame transects bisected the plots (and 1m beyond the boundaries of the plot) in a north-south and east-west direction. For each transect, the pin frame was leveled and each pin was lowered until it reached the ground. The distance from where a pin hit the ground to where it intersected the top of the frame was determined. If the bottom of a pin hit rocks or woody debris, an "X" was recorded on the data sheet. For analysis, "X" topography values were an average of the two points on either side. If a pin hit a running buffalo clover plant, this was also noted on the data sheet. For purposes of interpreting the pin frame data, the following criteria were used: (a) Level areas had no topographical change; (b) slope areas were situated on an inclined plane; (c) depressions were areas where topography decreased on one reading and increased on a later reading, creating a trough or bowl; (d) mounds were areas where topography increased on one reading and decreased on a following reading, creating a hill shape; (e) base of a hill was defined as an area which was level, then immediately followed by an uphill slope; (f) a plateau was defined as a level area followed by a downward slope; (g) a terraced slope was a slope interrupted by a level area, and then continued as a slope.

Soil samples were obtained from all plots to determine soil matrix and nutrient properties. The soil type at each sample location was determined by entering the GPS coordinates of the sample location into a GIS soil layer. Representative soil matrix samples (within each plot) were collected from May through August 2004/2005 during the flowering/growing season using a volumetric soil sampler. Soil matrix variables including texture, bulk density, porosity, soil water content, and volumetric water content, were determined by Eastern Kentucky University's Department of Agriculture using standard laboratory techniques.

Using a 60 cm soil corer, a soil moisture sample was collected from each plot on the same day each month (15 April - 15 August, 2004 and 2005). Each 60 cm core sample was subdivided into three subsamples based on depth, 0-20 cm, 20-40 cm, and 40-60 cm. For each sampling day, subsamples from the same depth were pooled, placed in plastic bags and stored on ice in a cooler until the sample could be returned to the laboratory and placed in a cold room. Soil moisture was determined by weighing the wet soil sample, drying the sample for 24 hrs at 105° C, and reweighing the sample. This difference in wet versus dry weight was then divided by the dry weight and multiplied by 100 to determine percent soil moisture.

Samples for soil nutrient analysis were obtained using a soil corer. Five soil nutrient cores were randomly obtained from all plots within a dry two day period near 1 June of each year to ensure homogeneity of samples. Sites with running buffalo clover present had at least one core obtained within 5cm of clover plants. Soil cores from each plot were pooled into one sample. Approximately 15g were taken from each pooled sample and mixed with a pre-measured 2 molar potassium chloride (KCl) mixture to immobilize NO_3 and NH_4 and prevent leaching losses. All soil samples were stored in the field on ice and transferred the day of collection to the University of Kentucky Soils Laboratory. Samples were analyzed to determine levels of nitrate (NO_3), ammonium (NH_4), calcium (Ca), magnesium (Mg), zinc (Zn), potassium (K), phosphorus (P), and pH. Mehlich III solution was used to extract P, K, Ca, Mg, and Zn; and soil pH was determined using Sikora buffer (University of Kentucky 2008a).

A sub-population of running buffalo clover plots (11 long-term clover sites, 9 disappeared clover sites, 10 random sites) were monitored from May through August 2005 to determine maximum and minimum light availability; in April, due to accessibility issues to area A, fewer plots were sampled (7 long-term clover sites, 9 disappeared clover sites, 10 random clover sites). All light readings obtained for plots were obtained before 1200 hours. An Extech Instruments Easyview light meter (Extech Instrument Corporation, Waltham, MA) was held at or near ground level in the center of the plot. If running buffalo clover was present, the meter was held directly next to the largest number of clover plants. All light readings were obtained before 1200 hours. All values are in lux. The maximum range for the light meter was 107640 lux. Values that maxed out the light meter were recorded as 107640 lux, although the readings could have been higher. Meteorological data (ambient temperature, precipitation) were obtained from the Blue Grass Army Depot weather station.

Multivariate analysis of variance (MANOVA) was used to determine if a significant difference ($P<0.05$) existed between soil nutrients and soil structure for long-term, disappeared, and random/control sites, as well as by year. If a significant difference was detected, discriminant analysis was used to determine which soil and habitat parameters best discerned a difference between long-term sites vs. control sites, long-term sites vs. disappeared sites, disappeared vs. control sites, as well as by year.

Microtopography data within plot boundaries and plot plus an assessment line 1m beyond the plot in each cardinal direction were analyzed separately by calculating a running mean and determining the standard deviation of the running mean similar to Grant et al. (1990). Large standard deviations indicated uneven topography and greater elevation differences; while small deviations indicated more level terrain. An ANOVA was utilized to determine if there was a difference in the overall topography between sites with clover, disappeared sites, and random sites. On sites with clover, actual clover plants were noted on the survey form when they were encountered on the transect; a Chisquared goodness of fit test was run on topographical position of running buffalo clover plants when they were encountered within the plot on the survey (level, sloped, depression, mound, base of a hill, plateau, and terraced hill).

A student's t-test and a Rank-sum test were run on weather data (ground temperature and precipitation) to test for deviation from average conditions. All ANOVA and MANOVA analyses were conducted using the Statistical Analysis System (SAS Institute 1999).

CHAPTER IV

RESULTS

Precipitation on the Blue Grass Army Depot was significantly higher in May 2004 and May 2005 (Table 6; Student's T-test, T-calculated= 3.375; T-critical= 2.0235), as well as June 2004 than in June 2005 (Table 6; Student's T-test, T-calculated= 2.387; T-critical= 2.042). Ambient temperatures at ground level were significantly lower in March 2005 than 2004 (Table 6; Student's T-test, T-calculated= 41.765; T-critical= 2.000) and significantly higher in June and July 2005 than 2004, respectively (Table 6; [June] Student's T-test, T-calculated=22.22; T-critical=2.002; [July] Student's T-test, T-calculated=15.8688; T-critical=2.032). The average number of rooted crowns (MANOVA, F=0.73; Pr>F = 0.3927) and inflorescences (MANOVA, F=2.57; Pr>F=0.1099) per plot did not differ significantly between years (Table 1).

Overall, there was no difference between long-term clover sites, disappeared clover sites, or random sites in terms of average soil nutrient values for NH_4 (Table 2; MANOVA, F= 0.63; Pr>F=0.5372), NO_3 (MANOVA, F= 0.04; Pr>F= 0.9597), Zn (MANOVA, F= 0.29; Pr>F= 0.7514), K (MANOVA, F= 1.71; Pr>F= 0.1884), Mg (MANOVA, F= 0.32; Pr>F= 0.7296), Ca (MANOVA, F= 1.90; Pr>F= 0.1567), P (MANOVA, F= 0.08; Pr>F= 0.0197), and pH (MANOVA, F= 2.80; Pr>F= 0.0673). However, there were significant difference between years for some nutrients, i.e., average Mg (Table 2, MANOVA, F= 8.77; pr>F= 0.0042), pH (MANOVA, F= 14.04; pr>F=<0.0001), and NO_3 levels (MANOVA, F= 115.06; pr>F= <0.0001) were higher in 2004; while mean Ca (MANOVA, F= 11.22; pr>F= 0.0013), NH_4 (MANOVA, F=116.09; pr>F= <0.0001), and P (MANOVA, F= 11.21; pr>F= <0.0001), levels were higher in 2005. Only potassium showed a significant site-by-year interaction (MANOVA, F=3.33; Pr>F= 0.0416), with clover sites having the most potassium for both years, random sites were intermediate, and disappeared clover sites had the lowest values (Table 2).

Plots examined in this study occurred within eight soil texture types (Table 3) and 14 soil series (Table 4). There were significantly more plots in the silt loam texture type (X^2 = 279.27, P< 0.05). There was no significant difference in mean bulk density (MANOVA, F= 0.57; Pr>F=0.5668), percent soil moisture (MANOVA, F= 0.22; Pr>F=0.8040), soil water content MANOVA, F= 0.07; Pr>F=0.9311), volumetric water content (MANOVA, F= 0.09; Pr>F=0.9122), or porosity (MANOVA, F= 0.56; Pr>F=0.5729) between long-term clover sites, disappeared clover sites, or random sites (Table 5). Between years, only bulk density (MANOVA, F=4.96; Pr>F= 0.0276) and porosity (MANOVA, F= 4.95; Pr>F= 0.0278) showed a significant difference. Bulk density readings were higher in 2005 than 2004; while porosity was higher in 2004 versus 2005.

There was significantly greater topographic variation of elevation within plots between long-term plots (9.72± 5.25) and disappeared clover plots (8.524± 5.32) when compared to random plots [(2.45± 1.28) (Table 7; ANOVA, F=11.13; Pr>F <0.0001)]. This trend continues when assessment lines were included with plot measurements (Table 7; ANOVA, F=9.69; Pr>F=0.0002). Long-term plots (13.63± 7.13) and disappeared clover plots (12.951± 9.85) were both significantly higher in terms of microtopographic elevation than random plots (5.172± 1.96).

When the overall shape of each running buffalo clover study plot was examined across the topographic spectrum (Table 8), a trend was noted. On Long-term clover sites, plots that were deemed to be a depression occurred twice as often as random while plots on mounds occurred three times more frequently than random. In plots in which running buffalo clover has been present since 1992-1994 (Long-term sites), clover plants were not randomly distributed with regard to where they were encountered along a microtopographic transect (Table 9, $\chi^2_{0.05,6} = 12.592$; $\chi^2_{calculated} = 22.712$).

Maximum light values approached significance for site (ANOVA; F=3.12; Pr>F=0.0599) and showed a significant difference for month (ANOVA; 18.60; Pr>F=<0001). Minimum light values were significantly different for both site (ANOVA; 3.51; Pr>F = 0.0439) and month (ANOVA; 10.00; Pr>F = <0.0001). There were no significant statistical interactions between plots or site by month. Although not statistically significant, sites with clover (Long-term plots), on average, had lower maximum light values than their counterparts (Table 10). A trend for light values by month was detected, with light levels decreasing as leaf set occurred.

CHAPTER V

DISCUSSION

Hattenbach (1996) analyzed soil samples from running buffalo clover sites in Ohio, West Virginia and Kentucky (i.e., the Blue Grass Army Depot), and indicated running buffalo clover was not limited to a particular soil type and exhibited a high variability in soil characteristics among sites. This trend was also noted in this study.

Many factors contribute to a plant's micro-environment, but plant associations and soil factors are the most important (McKinney 1997). Running buffalo clover at the Blue Grass Army Depot occurs primarily in forested riparian areas (White 1998; USFWS 2006). Riparian areas reduce water velocity, thus allowing deposition of suspended nutrient rich soil particles (Cooper et al. 1987; Naiman and Decamps 1997). Groffman and Tiedje (1989) speculated that riparian areas receive nutrient inputs in surface and subsurface flow from larger upland areas nearby. Flooding has been reported to result in greater N and P enrichment and led to C, N, and P accumulations in low lying soils (Pinay et al. 1992; Sibbesen and Sharpley 1997). Lowrance et al. (1984) concluded that the riparian forest was a sink for calcium, magnesium, potassium sulfate, and phosphorus, with mean seasonal concentrations of NO_3, NH_4, total N, Ca, Mg, and K exhibiting an increasing trend from April through June and decreasing concentrations from July through September. Riparian areas had higher means of NH_4, Ca, Mg, and K during the summer months, possibly due to higher rates of mineralization of forest litter (Lowrance et al. 1984). Thom (1990) states that soil testing indicates the residual soil fertility of a soil, which is influenced by "residual pools" of nutrients in the organic matter, exchangeable nutrients, slowly soluble chemical compounds, and nutrients in the soil mineral fraction. Plant availability of residual fertility is affected by the release of plant nutrients from the soils mineral and organic fractions by dissolution and decomposition, past fertilization practices, and past cropping history (Thom 1990).

CHEMICAL SOIL ANALYSIS

Percent Hydrogen (pH):

"Percent Hydrogen" (pH) is a measure of the percentage of hydrogen ions in an aqueous solution and shows how acidic or basic an aqueous solution is; e.g. pH values between 0 and 7 are considered acidic and those from 7-14 are considered basic (Foth 1990). Soil pH is largely governed by the geology of the parent material, climate, and weathering (Foth 1990; USDA 1998). Management of soils often alters the natural pH because of acid-forming nitrogen fertilizers, or removal of bases (potassium, calcium, and magnesium; USDA 1998). The pH of the soil solution is important to plants because the solution carries vital nutrients required for plant growth and maintenance (Foth 1990). Soil pH also affects soil micro-organisms responsible for breaking down organic matter as well as most chemical transformations in the soil (USDA 1998). If a soil is too acidic, plants cannot absorb the nutrients they need; instead picking up toxic heavy metals which can lead to plant toxicity poisoning and eventual death. Concerning major nutrients, if the pH of a soil solution is above 5.5, nitrate (nitrogen) is made available to plants. Phosphorus is most available when soil pH is between 6 and 7 (Foth 1990; USDA 1998).

The University of Kentucky Cooperative Extension Service (2008b) reported average soil pH for Madison County to be 6.4 in 2004 and 6.5 in 2005; a soil pH of 6.1-6.5 is considered slightly acidic and values between 6.6-7.3 are considered neutral (USDA 1998). Hattenbach (1996) found pH to be higher near running buffalo clover rooting zones compared to samples taken more than 2 meters away, a trend also noted in this study. Soil pH ranges determined for long-term and disappeared running buffalo clover plots in this study (7.02-7.17 and 7.14-7.30, respectively) were generally comparable to pH values reported previously for running buffalo clover on the BGAD [6.12-7.73; Hattenbach (1996)], and in southwest Ohio [6.48-7.76 (Becus 1995); 4.85-7.31 (Hattenbach (1996)] and West Virginia [5.68-6.01 (Becus 1995); 4.8-7.4 (Mitchell and Harmon (1995); 4.88-5.72 (Hattenbach 1996)].

Phosphorus (P):

Phosphorus is a naturally occurring element that exists in minerals, soil, living organisms and water (Sharpley and Rekolainen 1997). Plant growth and development requires phosphorus in large amounts and is considered a macronutrient (Hodges 1996). Phosphorus is involved in photosynthesis (as part of phosphates attached to sugars), energy transfer (as part of adenosine tri-phosphate, ATP, a high energy molecule), and as part of nucleic acids (Foth 1998; Hodges 1996). Phosphorus is essential for early root development and hastens plant maturity (Hodges 1996). Phosphorus is most available when soil pH is between 6 and 7 (USDA 1998).

The soils of the Bluegrass Region of Kentucky have naturally high levels of phosphorus through rock weathering (Rasnake et al. 2001), with the calcareous soils contributing higher unit area loads of phosphorus (Sonzogni et al. 1980). The mobility of phosphorus in soil is low compared with other plant nutrients because of the generally low solubility of phosphate compounds and strong phosphorus-binding capacity of soil material (Pierzynski 2000). Riparian zones are important phosphorus sinks where phosphorus adsorbed to clay particles is deposited during high flow or trapped in runoff from surrounding uplands allowing time for plant uptake or microbial use, although it is unknown if phosphorus is a limiting nutrient in riparian zones (Cooper et al. 1987; Green and Kauffman 1989). Often 90% of the phosphorus uptake originates from residual phosphorus in the soil (Sibbesen and Sharpley 1997).

The efficiency of phosphorus uptake by plants increases as soil temperature, moisture, aeration, and nutrient status increase (Sharpley and Rekolainen 1997). Research has found a higher frequency of flooding may result in greater nitrogen and phosphorus enrichment and led to carbon, nitrogen, and phosphorus accumulations in lower lying soils (Pinay et al. 1992; Sibbesen and Sharpley 1997). Kozlowski (2002) noted, however, that plants in the floodplain have reduced absorption of phosphorus during times of high soil moisture or inundation. Thus, with a spring dry down, transported phosphorus-rich organic material appears to add to decomposition-based phosphorus enrichment and result in much higher phosphorus concentrations in low areas versus slopes (Stoeckel and Miller-Goodman 2001). While the topographic position of soil samples collected in this study was not noted, it may have had an impact on phosphorus levels detected and should be considered in future studies.

Soil phosphorus ranges determined in this study for 2004 were generally comparable to those for Madison County [73.99-85.19 kg/ha (66-76 lbs/acre); University of Kentucky (2008b)], while those for 2005 were considerably higher (Table 2). Since phosphorus is a slow moving ion, it is possible that the higher than average precipitation at the Blue Grass Army Depot in 2004 increased rock weathering, thus raising levels of phosphorus in the soil in 2005. Phosphorus is frequently applied as fertilizer in agricultural practices. The widespread use of phosphorus containing fertilizers in the 20th century in agriculture has increased soil-phosphorus status of agricultural lands from traditionally very low levels to medium and high levels (Thom 1990; Pierzynski and Logan 1993; Whalen and Chang 2001). During this study, no agricultural fertilizers were known to be used on the Blue Grass Army Depot; aside from excretia from cattle that naturally occurs with grazing. Phosphorus from manure has a high concentration of soluble phosphorus, and high rates of manure application can cause a significant buildup of P, especially the first few weeks after manure application (Eghball et al. 1996; Wiederholt and Johnson 2005). During this study soil samples were not obtained from surrounding farmland; hence external sources of fertilizers (washed downstream from agricultural lands outside the Blue Grass Army Depot) could have also contributed to the elevated phosphorus levels detected in 2005.

Average soil phosphorus levels for long-term and disappeared running buffalo clover sites examined in this study in 2004 were much higher (52.88-57.13 kg/ha, Table 2) than the mean reported for running buffalo clover locations in West Virginia [20.18 kg/ha (18 lb/acre); Mitchell and Harmon (1995)]; however levels fell within the upper limit detected [70.62 kg/ha (63 lb/acre); Mitchell and Harmon (1995)]. Mean soil phosphorus levels detected at the Blue Grass Army Depot in 2005 (102.84-110.69 kg/ha, Table 2) were much higher than levels reported from West Virginia [7.85-70.62 kg/ha (7-63 lb/acre); Mitchell and Harmon (1995)]. Values reported by Hattenbach (ppm; 1996) were converted to units reported in this study (kg/ha) using standard procedures (Hodges 1996; University of New Mexico 2009). A comparison of bio-available phosphorus levels with Hattenbach's (1996) study [4.44 kg/ha (3.96 lb/acre) for running buffalo clover sites at the Blue Grass Army Depot is difficult. Bray-1 phosphorus tests in the Midwest commonly produce erroneously low results for calcareous soils (Sawyer and Mallarino 1999); Mehlich 3 tests, as used in my study, are more reliable estimators of available phosphorus in calcareous soils and thus provided much higher readings (Mallarino 1995). The results from the Mehlich 3 tests in my study represent plant extractable phosphorus and were much lower than the total phosphorus [2141 kg/ha (1910 lb/acre)]; which represents both bio-available phosphorus and inorganic phosphorus compounds) reported by Hattenbach (1996). Inorganic phosphorus in the soil is typically between 50-70% of the total phosphorus, although this fraction can vary anywhere between 10-90% (Sharpley and Rekolainen 1997). Soil phosphorus readings of 34.75-7.26 kg/ha (31-60 lb/acre) are considered medium, 68.38-89.68 kg/ha (61-80 lb/acre) are the low end of high, and >89.68 kg/ha (80 lb/acre) considered the medium range of high (University of Kentucky 2008a).

Potassium (K):

Potassium is an essential macronutrient required by plants in approximately the same or slightly larger amounts as nitrogen (Hodges 1996). Most of the functions of potassium in the plant are indirect in that potassium is necessary for other chemical reactions to operate properly. Some 60 enzymes require the presence of potassium, with high concentrations of potassium found in the meristem and immature seeds (Hodges 1996). Plants use potassium in photosynthesis, carbohydrate transport, water regulation, and protein synthesis (Hodges 1996).

Weathering of rocks in Kentucky can provide a valuable source of potassium (Rasnake et al. 2001). Potassium is relatively immobile (Murdock and Wells 1973), and as such, movement of potassium in soils depends on soil texture. As clay content increases, potassium movement decreases as the negatively charged clay holds on to the potassium cation, making it unavailable to roots (Foth 1990). Average potassium levels for soil samples collected in this study were within the medium range [238.77-302.67 kg/ha (213-270 lbs/acre); Table 2] recommended for plant growth by the University of Kentucky (2008a), and generally comparable to average potassium levels reported for Madison County [302.67-303.79 kg/ha (270-271 lbs/acre); University of Kentucky (2008b)]; indicating potassium is likely not limiting the growth of running buffalo clover on the Blue Grass Army Depot. Mean potassium levels for long-term and disappeared running buffalo clover sites examined in this study were nearly twice as high as corresponding information reported from running buffalo clover sites in West Virginia [146.85 kg/ha (131 lb/acre); Mitchell and Harmon (1995)]. The average values reported in this study were much lower than mean potassium levels noted for running buffalo clover sites on previous studies at the Blue Grass Army Depot [1477.48 kg/ha (1318 lb/acre); Hattenbach (1996)], even falling well below the lowest value in the range [585.83 kg/ha (522.6 lb/acre)]. Values for Hattenbach (me/100g; 1996) were converted to kg/ha using standard measures (Hodges 1996).

Magnesium (Mg):

Magnesium is a secondary element in plants that appears to activate a number of enzymes and plays a role in protein synthesis and phosphorus reactions (Shure and Gottschalk 1985; Hodges 1996). About 15-20% of the plant's magnesium is contained in chlorophyll, without which the plant could not capture energy from the sun for growth and development (Foth 1990).

Magnesium deficiency in plants within Kentucky is rare (Thom et al. 2000) and the mean levels detected in this study (Table 2) would be considered "high" [values between 560.6-1121 kg/ha (500-1000 lb/acre); University of Kentucky 2008a]. The difference in magnesium levels between years in this study may be a reflection of the higher rainfall in 2004 and the subsequent runoff from the abundant dolomite containing soil. Many of the sites that were higher for magnesium were on bedrock which contained dolomite, a mineral which contains magnesium (McFarlan 1943).

Mean magnesium levels for long-term and disappeared running buffalo clover sites examined in this study (Table 2) were much higher than those observed at running buffalo clover sites in West Virginia [77.35-229.80 kg/ha (69-205 lb/acre); Mitchell and Harmon 1995] and Madison County [491 kg/ha (438 lb/acre) in 2004; 258.95 kg/ha (231 lb/acre) in 2005; University of Kentucky 2008b); but were comparable to magnesium levels previously reported for running buffalo clover sites on the Blue Grass Army Depot [672.6-1937.09 kg/ha; Hattenbach (1996)]. Values for Hattenbach (me/100g; 1996) were converted to kg/ha using standard measures (Hodges 1996).

Calcium (Ca):

Calcium, a structural component of plant cell walls, is most abundant in plant leaves (Hodges 1996). It is involved in cell growth, both at the plant terminal and at the root tips, and apparently enhances uptake of nitrate-N (Foth 1990). Calcium is not translocated within the plant, so an adequate supply throughout the season is important for sustained terminal and root growth (Hodges 1996). Calcium is a slow moving nutrient, accumulating over time. Shure and Gottschalk (1985) found nutrient return (Calcium > Nitrogen > Potassium >Magnesium) in leaf fall was relatively high, particularly for the less mobile elements (Calcium, Magnesium). Because of the limestone rich soils prevalent within central Kentucky, calcium deficiency has not been observed in field crops (Thom et al. 2000). From an agricultural standpoint, the mean levels of calcium detected in this study (Table 2) would be considered "medium-high" [values between 4484-8968 kg/ha (4000-8000 lb/acre); University of Kentucky 2008a]. The higher levels of calcium detected in soil samples collected during this study in 2005 were surprising. If running buffalo clover is dependent on limestone and calcium, as has been suggested (USFWS 2007), perhaps an influx of calcium helped account for the higher number of rooted crowns detected in 2005 (Table 1). Mean calcium levels for long-term and disappeared running buffalo clover sites examined in this study (Table 2) were nearly twice that reported for running buffalo clover sites in West Virginia [2811.47

kg/ha (2508 lb/acre); Mitchell and Harmon 1995] and generally higher than other soil samples collected elsewhere in Madison County [3977-3993 kg/ha (3548-3562 lb/acre); University of Kentucky 2008b)]; but were comparable to calcium levels previously reported for running buffalo clover sites on the Blue Grass Army Depot (5291-8564 kg/ha; Hattenbach 1996). Values for Hattenbach (1996) were converted to kg/ha using standard measures (Hodges 1996).

Zinc (Zn):

Zinc is important in plant nutrition and is involved in a number of plant processes, e.g., enzyme formation and function, stability of cytoplasmic ribosomes, oxidation processes, transformation of carbohydrates and synthesis of auxin indole acetic acid (Foth 1990). Because plants vary in their requirement for zinc, even among cultivars, it is difficult to establish a single critical value. Micronutrient deficiencies are often induced because of interactions with other nutrients, e.g., zinc uptake is often depressed in the presence of excess phosphorus (Hodges 1996). From an agricultural standpoint, the average levels of zinc detected in this study (Table 2) would be considered “medium” (University of Kentucky 2008a; [values between 1.121-6.726 kg/ha (1-6 lbs/acre)]; but were much higher than levels reported from sites elsewhere in Madison County [3.03-3.363 kg/ha (2.7-3.0 lb/acre); University of Kentucky 2008b]. Mean zinc levels for long-term and disappeared running buffalo clover sites examined in this study (Table 2) were generally higher than levels reported previously for running buffalo clover locations on the Blue Grass Army Depot (0.94 – 3.68 kg/ha; Hattenbach 1996). Values for Hattenbach (ppm;1996) were converted to kg/ha using standard measures (Hodges 1996; University of New Mexico 2009).

Nitrogen (NO_3, NH_4):

From the standpoint of plant nutrition, NO_3- and NH_4+ are considered macronutrients and are the primary sources of nitrogen for plant species; nitrogen is the fourth highest chemical absorbed by plants behind carbon, hydrogen, and oxygen (Foth 1990). These two ionic forms react differently in soil. Ammonium (NH_4+) is a positively charged ion and is attracted to the negatively charged sites on soil particles, particularly clay, and is relatively immobile. In this position, it is available to plants but held tightly enough to prevent leaching. Aerobic bacteria are responsible for the nitrification processes (conversion of ammonium to nitrate; Foth 1990); e.g., clovers from the genus *Trifolium* are typically associated with nitrogen fixing bacteria within the genus *Rhizobia* (Ryle et al. 1979; Frame et al. 1998a,b). However, no nitrogen fixing bacteria are currently known associated with *Trifolium stoloniferum* (USFWS 2007).

Nitrate ions react much less with the soil than do ammonium ions, and most nitrate remains in the soil solution, moving with the soil water (Hodges 1996). The proportion of nitrate and ammonium absorbed by vegetation varies depending on the species of plant and environmental conditions. Most of the nitrate absorbed is reduced to ammonium in roots and/or leaves and enters pathways leading to the synthesis of amino acids and proteins (Novoa and Loomis 1981; Dunlop and Hart 1987). The main pathway of nitrogen entry into food chains is by absorption of nitrate by roots of terrestrial plants (Foth 1990). Nitrate is often a limiting factor in plant growth.

The amount of N available in the soil solution for uptake by plants is highly variable and represents the balance between the incorporation of N in microbial bodies (immobilization) and the release of N resulting from decomposition of soil organic matter (mineralization; Hobbs 1996). Ammonium ions normally constitute only a very small portion of the mineral nitrogen content of the soil in temperate regions since the nitrifying micro-organisms oxidize them to nitrates (Foth 1990). In temperate zones, an exclusively ammonia-based nutrition is very often unfavorable for the growth and metabolism of plants (Bollard and Butler 1966; Chapin 1980). Ammonium-nitrogen is immobilized into a variety of compounds in the biomass of nitrogen fixers or their hosts. Upon organic matter decomposition, nitrogen is released in this form. Under anaerobic conditions ammonium may become locally abundant and plants can take up nitrogen in this form (Green and Kauffman 1989). When anaerobic soil layers are present, ammonium can diffuse into an aerobic horizon where it is rapidly converted to nitrate-nitrogen by the bacterially mediated processes of nitrification (Green and Kauffman 1989). Any factor that decreases water flow in a soil has been postulated to increase denitrification (Sextone et al. 1985). Soils close to the edge of streams have been found to exhibit higher rates of denitrification than other sites; thus decreasing the absorption of N by floodplain plants (Green and Kauffman 1989; Kozlowski 2002).

Leaching losses of soil N occur because much of the N in soil is partially, or soon ends up as, nitrate which largely remains in solution in the soil water (Wells et al. 1997). Because ammonium has a strong affinity for the negatively charged sites in clay soils, it is a relatively immobile ion and is not as susceptible to leaching or denitrification as is nitrate. During periods of excess rainfall, particularly winter and early spring, nitrate can leach below the root zone of plants; during dry periods, nitrate may move up and accumulate at the soil surface (Gambrell et al. 1975; Davidson and Swank 1987). During the late spring, summer and fall months, transpiration of moisture by the growing crop and evaporation at the soil surface removes moisture rapidly. With rapid removal of soil water during the growing season, precipitation is seldom great enough for water to move through the soil profile beyond the plant root depth, except in well drained sandy soils (Gambrell et al. 1975). In most years leaching of N during the growing season is negligible on silt loam and finer textured soils in Kentucky (Wells et al. 1997).

The reason(s) for the differences observed in this study between years for mean NO_3 and NH_4 levels (NO_3 and NH_4 significantly higher in 2004 and 2005, respectively) are unclear. One would expect that NH_4 levels should have been higher in 2004 since high amounts of precipitation, as observed during the 2004 field season, can create conditions of poor aeration in which anaerobic bacteria in the soil reduce NO_3 to volatile forms of N via denitrification (Wells et al. 1997); yet just the reverse was observed. Thom et al. (2000) noted that neither the amount of organic matter nor the amount of nitrate has proven to be a reliable indicator of available nitrogen for crops grown in Kentucky. Sampling one time during the growing season, as was done in this study, may not have provided an accurate representation of a highly mobile soil element such as nitrogen.

Hattenbach (1996) reported the mean total nitrogen at 11 running buffalo clover locations on the Blue Grass Army Depot to be 2701 ppm (range 720-3895 ppm). Comparisons of nitrogen levels determined in this study for long term and disappeared running buffalo clover sites at the BGAD with Hattenbach's (1996) values is difficult since this study assessed only nitrate and ammonium independently. The values reported for nitrate and ammonium in this study (Table 2) were markedly lower than the total N reported by Hattenbach (1996). Because of the importance of nitrogen in terms of a plant's overall survival and reproductive output, the nitrogen requirements of running buffalo clover throughout its range warrant further investigation.

PHYSICAL SOIL ANALYSIS

Soil texture, type, & matrix:

Although in this study there were significantly more clover-related plots (long-term and disappeared plots) and random/control plots occurring on silt loam, this was not unexpected since silt loams are common in floodplains (Newton et al. 1973) where most of the Blue Grass Army Depot's clover-related plots are located (Fields and White 1996). The richness of nutrients in silt loams makes them very productive ecosystems and attractive farmland. Sibbesen and Sharpley (1997) found that low areas were more frequently flooded and had finer soil textures. The higher frequency of flooding resulted in greater nitrogen and phosphorus enrichment and led to carbon, nitrogen, and phosphorus accumulations in lower lying soils. With spring dry down, transported phosphorus-rich organic material appeared to add to decomposition-based phosphorus enrichment and resulted in much higher phosphorus concentrations in low areas versus slopes (Stoeckel and Miller-Goodman 2001).

Running buffalo clover on the Blue Grass Army Depot does not appear to be limited to one specific soil series or soil texture. Plots currently supporting running buffalo clover plants (long-term plots) and sites in which running buffalo clover occurred historically (disappeared plots) were represented by a variety of soil textures (6 vs 5, respectively, with 4 common to both; Table 3) and soil series (11 vs 7, respectively, with 5 common to both; Table 4). Such adaptability might be expected in what is considered a disturbance-dependent plant (USFWS 2007). Disturbance in the environment would likely be unpredictable and occur across multiple soil textures and series, thus requiring running buffalo clover to respond in turn. This corresponds with Hattenbach (1996), who stated that running buffalo clover was not limited to a particular soil type and exhibited high variability in soil characteristics.

As a soil becomes more compacted, the porosity decreases, thus increasing the bulk density (Rieu and Sposito 1991). The significantly higher porosity noted in this study in 2004, and correspondingly higher bulk density in 2005, is probably a reflection of the greater amount of precipitation recorded on the site in 2004. A lower bulk density suggests that the roots for running buffalo clover would have an easier time penetrating the soil, however, it appears that a higher bulk density in 2005 led to a higher yield of rooted running buffalo clover plants. While cattle and deer are the likely the ultimate causes of soil compaction at the Blue Grass Army Depot, the magnitude of recovery from soil compaction (as measured through bulk density) is more related to soil texture than length of time from compaction (Orr 1960).

LIGHT

When compared to sites in which running buffalo clover was present in 1992-1994 (long-term plots) but not present in 2004 (disappeared plots), and random/control plots in which running buffalo clover had never been reported; long-term plots on the Blue Grass Army Depot tended to exhibit lower mean maximum and minimum light levels, providing support for the proposal that running buffalo clover survives best in filtered light settings (USFWS 2007). Dennis and Woledge (1983) found white clover (*Trifolium repens*) leaves have a high photosynthetic capacity as long as they emerge in bright light and will maintain that photosynthetic capacity throughout the growing season, even if placed under more dense shade at a later point; such a development scheme may exist with running buffalo clover, attempting to emerge before leaf set on the trees. In a study on red clover (*Trifolium pretense*), Bowley et al. (1987) found that the critical day length to induce stem elongation may be shorter than the critical day length for flower induction; to my knowledge, no study has been conducted on day length for physiological processes for running buffalo clover. Madarish and Schuler (2002) found running buffalo clover sites had greater gap areas and lower leaf area indexes than average for the surrounding forest. Thus controlling the intensity of surface disturbance, combined with the reduction in canopy density associated with uneven-aged silviculture, could possibly help sustain populations of running buffalo clover in managed forests. No studies have been conducted concerning running buffalo clover and light levels necessary for survival; more work needs to be done regarding the light requirements of *Trifolium stoloniferum*.

MICROTOPOGRAPHY

Bratton (1976) noted that herbaceous species respond strongly to differences in microtopography and that these differences tend to be related to species responses along major gradients. Although light and other environmental factors may be important in the distribution of understory species, microtopography is probably the major influence on the occurrence of clumps of the dominant herbaceous species (Bratton 1976, McKinney 1997). Microtopography and seasonal change are responsible for much of the niche differentiation between herbs in the understory (Bratton 1976).

To my knowledge, there are no published accounts of studies which examined the topography or soil roughness associated with running buffalo clover plants. Microtopographic variability, appears to be important to running buffalo clover. This is evident through both the higher soil roughness values on long-term and disappeared plots, as well as the higher than expected occurrence of running buffalo clover plants on either mounds or depressions. Plots on the Blue Grass Army Depot in which running buffalo clover was present (long-term plots), or had been present in the past (disappeared plots), exhibited greater microtopographical variability than plots in which running buffalo clover had never been reported (random/control plots), which suggests the presence of safe sites may be important for the establishment and maintenance of running buffalo clover plants (Harper 1977), although possibly for different reasons. For a clonal herb, like running buffalo clover, which may share nutrient resources via stolons (Rathcke and Lacey 1985; Hay and Newton 1996), the ability of this plant to exploit a variety of resources may both benefit and explain the apparent dependence on topographic variability.

In floodplains, elevated sites generally have a higher number of surviving seedlings compared to lower elevation microsites (Huenneke and Sharitz 1986; Battaglia et al. 2000). Nutrient content in a forest is often extremely variable from one square meter to the next due to microtopography (Boerner and Koslowsky 1989; Smith 1998). Concave slopes, on the lowest and wettest plots (depressions), suffered the greatest leaching losses while having better nutrient content, while the driest slopes on convex slopes (mounds) had the smallest losses but also the poorest nutrient content; balances of nitrogen and phosphorus were most negative for the plots in depressions (concave slopes) and most favorable on convex slopes (Beatty 1984; Brouwer and Powell 1998).

As plants are largely sedentary and dependent on the land and environment surrounding them for survival, morphologically distinct microsites are different in physical characteristics, a difference often reflected by the resulting plant growth (Hardin and Wistendahl 1983; Huenneke and Sharitz 1986, McKinney 1997). The microsites caused by relief in the soil's surface can affect individual plants by affecting light levels, water and nutrient availability, aeration of the soil, and temperature (Currence and Lovely 1970; Harper 1977). McKinney (1997) notes microtopography generally affects a site by determining the plant species composition and spatial distribution, which may, in turn, affect plant communities by creating differing opportunities for fine root development, germination, survival, and growth for different species (Harper et al. 1965; Harper 1977; Davis 1994; Ehrenfeld 1995; Jones et al. 1996; Smith 1998).

Soil roughness can be influenced by many factors. In addition to wind, water, and the natural cycles of wetting/drying and freezing/thawing playing a role in weathering the landscape, animals may also play a part in shaping microtopography (Huang 1998). Trimble and Mendel (1995) found the direct force (impact/shear) from an animal's hoof on the soil increases meso-micro relief, thus enhancing surface roughness. This in turn leads to a more heterogeneous soil surface, which may affect solar radiation reflection and infiltration and evaporation rates (Saleh 1993, Darboux and Huang 2005). Based on personal observations, I believe that all of the major forces influencing topography at the fine-grain level on the Blue Grass Army Depot, i.e., flooding, white-tail deer and cattle, are all important to creating the soil roughness patterns that emerged.

CHAPTER VI

CONCLUSIONS

The fact that running buffalo clover was rediscovered at the Blue Grass Army Depot in 1993 by the KSNPC indicates that some combination of factors has allowed it to survive. However, upon observing the numbers of rooted crowns and patches decline over the past decade, it becomes apparent that the conditions needed to maintain running buffalo clover are also declining.

The interaction of soil type, general climate, and physiography combine to determine the microclimate of a specific location which in turn determines plant community composition and structure (Hutchins et al. 1976). Schemske et al. (1994) notes at some spatial scale, “plant species are patchily distributed due to their sedentary habit and the spatial heterogeneity of the environment, a point only exacerbated by anthropogenic habitat fragmentation and destruction.” Populations of running buffalo clover at the Blue Grass Army Depot were not evenly distributed throughout the available landscape.

While some studies on edaphic factors have been conducted throughout the known range for running buffalo clover, with the wide range of geologic and soil conditions over the range of the species, comparisons between studies are problematic and difficult. While some soil nutrient tests have been conducted at the Blue Grass Army Depot, given the wide variety of soil tests available, comparison between tests are not as definitive as desired. For many of the variables I tested in this study, little or nothing is specifically known (quantifiably) regarding the importance of the nutrient to running buffalo clover. While filtered light has been presumed to be preferable, it had not been tested to this point. No studies concerning microtopography or general topographic requirements for running buffalo clover are known to have been conducted. The information provided in this study can serve as base-line values with which future assessments of running buffalo clover sites on the Blue Grass Army Depot can be made; as well as provide data for comparative purposes at sites across the geographic distribution of *Trifolium stoloniferum*.

CHAPTER VII

FUTURE STUDIES

The U.S. Fish and Wildlife Service (2007) indicates that perhaps the most critical biological constraint and need for the recovery of running buffalo clover is its dependence on disturbance. When disturbance regimes eliminate populations and the species can disperse only in space, restoration of the landscape scale disturbance regime will be necessary to maintain viable populations (Pavlovic 1994). If disturbance patches are smaller than the population scale or occur at the microsite level, then population persistence requires only maintenance of appropriate disturbance regime at the population level (Pavlovic 1994). Open canopied forests are indicative of some sort of moderate disturbance, such as flooding, silvicultural thinning, fire, grazing and/or trampling that prevents the canopy from closing (Pickett and White 1985; Homoya et al. 1989; Lorimer and Frelich 1989). This disturbance regime is characteristic of activities at the Blue Grass Army Depot; but the intensity and frequency of many of the disturbance sources appears to be much less today than it was in the past, e.g., grazing and/or trampling by livestock and deer. The impact of livestock grazing, fire, timber harvest, invasive species, and deer density on running buffalo clover assemblages on the Blue Grass Army Depot should be more thoroughly researched.

Management strategies for running buffalo clover should consider the role of these factors in maintaining favorable habitat. Whigham and Chapa (1999) suggest that the timing and amount of herbivory clearly influences current and future growth and reproduction of clonal woodland herbs, with responses to different amounts of herbivory appearing to be small or non-existent later in the growing season. Whigham and Chapa (1999) found that 50% and 100% leaf removal applied shortly after leaves had expanded resulted in decreased numbers of ramets produced and ramet root biomass; early herbivory also shortened rhizome, and presumably stolon length (Hay and Newton 1996). Herbivory after two months of shoot development had no noticeable influence on the number of new ramets or root biomass, agreeing with Stevenson and Laidlaw (1985) who noted that after six weeks, ramets could survive independently. Responses to herbivory vary among species depending on phenology and life-history attributes. Few clover species are likely to survive frequent herbivory no matter what level of herbivory occurs (Hay and Newton 1996).

While grazing may help maintain suitable running buffalo clover habitat, overgrazing and trampling may be detrimental. Any changes in grazing regime should be carefully reviewed for possible impact to running buffalo clover populations. Kinch (1989) states that sometimes the exclusion of livestock grazing may be the most logical and responsible course of action, at least for a time sufficient to achieve a level of recovery for running buffalo clover, and stability which can support grazing in the context of management objectives for cattle. While grazing pressures were not examined for this project, it could be an inexpensive and valuable management tool for running buffalo clover at the Blue Grass Army Depot. If cattle grazing is to be used as a management tool at the Blue Grass Army Depot for running buffalo clover, Rasnake et al. (2001) suggest testing the animal waste for future nutrient management.

Naiman and Decamps (1997) state the characteristics of life-history strategies for riparian plants include tradeoffs between sexual and asexual reproduction, which may also be seen in running buffalo clover. The modular nature of running buffalo clover growth may permit each ramet to behave as an independent physiological unit, controlling its own resource assimilation and allocation (Rathcke and Lacey 1985; Hay and Newton 1996). The connectivity of the clones may also allow for sharing of water and nutrients. However, as plants age, roots become more porous, thus losing more nutrients; if a parent plant were cut off from the daughter plants, it may have a greater chance of death (Dunlop and Hart 1987; Thomas 1987). Die off of parent clovers without recruitment of new plants from buried stolon nodes may look like periodic die-off ("blinking").

Currently on the blue Grass Army Depot presence/absence surveys are conducted yearly during the running buffalo clover flowering season for ease of locating individual ramets. Although the information may provide yearly population estimates and allow for between year comparisons; it provides little insight into why the population may fluctuate. Additionally, the procedure gives a biased population census because it does not take into account any vegetative spread of the species after flowering. The life history theory of clonal organisms predicts a trade-off between allocation to reproductive output and vegetative growth because resources invested in offspring are not available for future survivorship, or growth (Hay and Newton 1996). Surveys on the BGAD throughout the year may allow for a comparison of running buffalo clover populations within a growing season as well as between growing seasons.

The length of the growing season for running buffalo clover on the Blue Grass Army Depot should be determined in order to define a "normal" growing season. From this, one may examine how weather affects running buffalo clover numbers year-to-year, and potentially address if global climate change is affecting RBC. If there were a longer growing season, running buffalo clover may respond by creating more rooted crowns as stolons would have longer to grow and extend, thus exploiting new resources. For red clover, spring is an important time for stolon growth, and thus vegetative expansion (Montpetit and Coulman 1991). For red and white clovers, many stolons are buried throughout the course of the growing season due to various factors, e.g., earthworm activity, grazing, flooding. It is these buried stolons, with their associated root, that create new rosettes the following year (Hay 1983). If each buried stolon were to develop into a new plant, it would appear that the population was increasing, although many plants would be genetically identical to their neighbors (Thomas 1987). It is reasonable to assume running buffalo clover could have some physiological responses similar to white/red clover as they are in the same genus. In white clover, more clover recruitment may come from stolons regenerating as a result of flooding or grazing pressures versus seed germination (Hay 1983, 1985).

Monitoring nutrient levels of both the soil and running buffalo clover itself should be conducted (provided proper permissions are given from the USFWS for “take” on running buffalo clover). While Morris et al. (2002) determined that nitrogen fixation did not contribute a significant portion of the plant’s nitrogen demand in West Virginia, this information at the Blue Grass Army Depot is non-existent. Conducting nutrient tests more frequently (i.e. before growing season, during growing season, and after growing season) would help determine more accurately levels of mobile elements like nitrogen, which is thought to be important to running buffalo clover since it does not have roots that fix nitrogen (USFWS 2007).

Soil moisture resources available to running buffalo clover on the BGAD should be examined, as water resources are not distributed evenly throughout a riparian system (Davidson and Swank 1987; Bellows 2003); where most of the running buffalo clover plants on the BGAD are located. Belaygue et al. (1996) discovered that for white clover, drought conditions affect numbers and lengths of stolons as well as resulting in a smaller leaf area. Dawson and Ehleringer (1991) discovered that trees growing alongside streams may pull more water from groundwater sources versus stream sources (Dawson and Ehleringer 1991). Groundwater as a water source is thought to be more stable, verses the flashiness of certain streams or drought-prone karst soils; thus allowing deep-rooted plants to survive droughts, and in turn leave upper level soil moisture for more shallow rooted herbs (like RBC) (Naiman and Decamps 1997). Roots will leak with age as their water-proof sheaths begin to fail or erode (Crozier and Boerner 1984).

CHAPTER VIII

MANAGEMENT RECOMMENDATIONS

Since there appears to be nothing unusual about the geology or physiography of the Blue Grass Army Depot (McFarlan 1943), when compared to neighboring lands, it is likely that land-use history provides an explanation of the clover's occurrence at the Depot (Appendix B). Specifically, the seclusion from agricultural and population induced land use pressures, both historic and present, that occurred in Madison County and the rest of the state, probably provided an island refuge for the clover.

The largest running buffalo clover populations occur exclusively on floodplains at the Blue Grass Army Depot, outside of areas of major construction efforts, indicating that these kinds of activities should be avoided in the vicinity of known running buffalo clover populations. To aid possible future expansion of clover populations, efforts should be made to preserve potential habitat surrounding the running buffalo clover plant assemblages. Large blocks of connected habitat would help protect running buffalo clover populations and encourage gene flow between populations (Hickey et al. 1991, Taylor et al. 1994).

Homoya et al. (1989) state that the removal or suppression of vegetation by bison may have created the open understory and light gaps necessary for running buffalo clover. Cattle grazing, deer trails, and periodic scouring flooding have probably provided the moderate disturbance needed to maintain running buffalo clover habitat at the Blue Grass Army Depot. Management strategies for running buffalo clover should consider the role of these factors in maintaining favorable habitat.

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Appendix A: Tables

Table 1: Mean (±SD) number of running buffalo clover rooted plants and inflorescences present in plots surveyed on the Blue Grass Army Depot, Madison County, KY, 2004 and 2005.

	Rooted Plants			Inflorescences		
	Total	Average	Standard Deviation	Total	Average	Standard Deviation
2004	5500	31.6	79.1	1413	8.1	18.1
2005	7578	43.3	138.5	899	5.1	14.4

Table 2. Mean (±SD) percent hydrogen (pH), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), ammonium (NH_4), and nitrate (NO_3) levels associated with plots containing running buffalo clover (c = Long-term sites), plots from which running buffalo clover has disappeared (o = Disappeared sites) and random/control plots (r) on the Blue Grass Army Depot, Madison County, KY, 2004 (year = 4) and 2005 (year = 5).

			pH		P		K	
site	year	N	Mean	SD	Mean[2]	SD	Mean[2]	SD
c[1]	4	27	7.17	0.82	57.13	53.99	282.95	102.20
c	5	27	7.02	0.65	110.69	86.36	303.75	105.10
o	4	23	7.30	0.63	52.88	36.28	246.77	78.09
o	5	23	7.14	0.51	102.84	63.81	239.70	74.46
r	4	25	6.98	0.70	52.10	35.28	286.93	96.73
r	5	25	6.59	0.78	120.22	73.57	269.26	95.13

			Ca		Mg		Zn	
site	year	N	Mean[2]	SD	Mean[2]	SD	Mean[2]	SD
c	4	27	5737.16	2165.95	1062.92	585.01	6.32	2.66
c	5	27	6702.80	1569.32	996.20	446.92	6.87	4.32
o	4	23	5897.92	1139.03	1061.15	505.11	6.29	3.09
o	5	23	6635.98	1218.84	962.55	428.74	5.86	2.97
r	4	25	5339.10	2066.29	1006.97	567.26	6.77	3.61
r	5	25	5769.56	1780.70	850.43	470.27	6.76	2.79

			NO_3		NH_4	
site	year	N	Mean[3]	SD	Mean[3]	SD
c	4	27	0.57	0.37	0.51	0.20
c	5	27	0.15	0.06	0.81	0.13
o	4	23	0.57	0.27	0.48	0.29
o	5	23	0.13	0.07	0.85	0.16
r	4	25	0.55	0.31	0.47	0.13
r	5	25	0.14	0.07	0.80	0.16

[1] c = sites in which running buffalo clover has been present since 1992-1994; o = sites in which running buffalo clover was present in 1992-1994 but not present in 2004; r = random/control sites which have had no record of running buffalo clover presence.
[2] units are in kilograms/hectare
[3] units are in milligrams/liter

Table 3. Soil textures associated with plots containing running buffalo clover (c = Long-term sites), plots from which running buffalo clover has disappeared (o = Disappeared sites) and random/control plots (r) on the Blue Grass Army Depot, Madison County, KY, 2004. Numerical values indicate sample size.

	Plot Types		
Soil Texture	c[1]	o[1]	r[1]
Silt Loam	18	19	20
Silt	3	1	0
Sandy Clay Loam	1	0	0
Sandy Loam	3	1	2
Loam	1	1	2
Clay Loam	1	0	0
Loamy Sand	0	1	0
Silty Clay loam	0	0	1

[1]c = sites in which running buffalo clover has been present since 1992-1994; o = sites in which running buffalo clover was present in 1992-1994 but not present in 2004; r = random/control sites which have had no record of running buffalo clover presence.

Table 4. Soil series associated with plots containing running buffalo clover (c = Long-term sites), plots from which running buffalo clover has disappeared (o = Disappeared sites) and random/control plots (r) on the Blue Grass Army Depot, Madison County, KY, 2004. Numerical values indicate sample size.

	Plot Types		
Soil Series	c[2]	o[2]	r[2]
BcC3[1]	3	0	0
Ne[1]	4	5	5
NhC[1]	2	0	0
BrE[1]	5	3	4
Ld[1]	3	5	5
BaC[1]	1	0	0
OtE[1]	4	5	4
MuB[1]	2	0	0
MuC[1]	1	0	1
Du[1]	1	2	1
Eg[1]	0	2	0
ShC[1]	0	1	2
LwD[1]	0	0	2
NhB[1]	1	0	1

[1] BcC3 = Beasley silty clay loam, 6-12% slopes, severely eroded; Ne = Newark silt loam; NhC = Nicholson silt loam, 6-12% slopes; BrE = Brassfield silt loam, 12-30% slopes; Ld = Lindside silt loams; BaC = Beasley silt loam, 6-12% slopes; OtE = Otway silty clay, 6-12% slopes; MuB = Mercer silt loam, 2-6% slopes; MuC = Mercer silt loam, 6-12% slopes; Du = Dunning silty clay loam; Eg = Egam silty clay loam; ShC = Shelbyville silt loam, 6-12% slopes; LwD = Lowell silt loam, 12-20% slopes; NhB = Nicholson silt loam, 2-6% slopes (Newton et al. 1973).

[2] c = sites in which running buffalo clover has been present since 1992-1994; o = sites in which running buffalo clover was present in 1992-1994 but not present in 2004; r = random/control sites which have had no record of running buffalo clover presence.

Table 5. Mean (±SD) bulk density, porosity, (%) soil moisture, soil water content (swc), and volumetric water content (vwc) associated with plots containing running buffalo clover (c = Long-term sites), plots from which running buffalo clover has disappeared (o = Disappeared sites) and random/control plots (r) on the Blue Grass Army Depot, Madison County, KY, 2004 (year = 4) and 2005 (year = 5).

			-----bulk density-----		-------porosity--------		----soil moisture-----	
site[1]	year	N	Mean	SD	Mean	SD	Mean	SD
c	4	19	1.41	0.11	46.74	4.02	21.97	4.19
c	5	25	1.44	0.16	45.79	5.89	20.45	4.35
o	4	20	1.43	0.15	46.24	5.67	21.18	7.06
o	5	25	1.49	0.19	43.88	7.26	19.72	6.29
r	4	18	1.39	0.09	47.37	3.20	21.45	4.67
r	5	25	1.49	0.19	43.75	6.98	19.85	5.98

			----------swc----------		----------vwc-----------	
site	year	N	Mean	SD	Mean	SD
c	4	19	0.28	0.07	0.40	0.08
c	5	25	0.26	0.07	0.37	0.07
o	4	20	0.28	0.12	0.38	0.12
o	5	25	0.25	0.10	0.36	0.11
r	4	18	0.28	0.07	0.38	0.08
r	5	25	0.26	0.10	0.37	0.11

[1] c = sites in which running buffalo clover has been present since 1992-1994; o = sites in which running buffalo clover was present in 1992-1994 but not present in 2004; r = random/control sites which have had no record of running buffalo clover presence.

Table 6. Mean (±SD) ambient temperature at ground level (^oC) and precipitation (mm) at the Blue Grass Army Depot, Madison County, KY, March-July, 2004 and 2005, and long-term mean ambient temperature and precipitation for Madison Co., KY.

	Ground temperature	Total precipitation
2004		
March	12.24	101.3
April	17.56	123.9
May	23.99	231.3
June	26.62	160.2
July	27.92	191.4
2005		
	Ground temperature	Total precipitation
March	7.65	102.7
April	16.21	125.4
May	26.57	76.83
June	42.62	11.1
July	49.93	99.0
	Average Temperature[1]	Average Precipitation[1]
March	12.78	124.46
April	19.44	101.6
May	24.44	99.06
June	28.89	121.92
July	30.00	129.54

[1]*Source:* Newton, J. H., H.P. McDonald, D.W. Preston, A.J. Richardson, and R.P. Sims. 1973. Soil survey of Madison County, Kentucky. Soil Conservation Survey, U.S. Department of Agriculture, Washington, DC, and Kentucky Agricultural Experiment Station, Lexington.

Table 7. Mean (±SD) microtopographic variation (cm) associated with plots containing running buffalo clover (c = Long-term sites), plots from which running buffalo clover has disappeared (o = Disappeared sites) and random/control plots (r) on the Blue Grass Army Depot, Madison County, KY, 2005.

	plot[2]			plot + assessment[2]	
site[1]	N	Mean	SD	Mean	SD
c	27	9.7	5.3	13.6	7.1
o	23	8.5	5.3	13.0	9.8
r	25	2.5	1.3	5.2	2.0

[1] c = sites in which running buffalo clover has been present since 1992-1994; o = sites in which running buffalo clover was present in 1992-1994 but not present in 2004; r = random/control sites which have had no record of running buffalo clover presence.
[2] plot = average of both north/south and east/west transect lines within a plot; plot + assessment = average of both north/south and east/west transect lines within a plot and extending 1m beyond the plot (assessment lines).

Table 8. Topographic positions associated with plots containing running buffalo clover (c = Long-term sites), plots from which running buffalo clover has disappeared (o = Disappeared sites) and random/control plots (r) on the Blue Grass Army Depot, Madison County, KY, 2004.

	Number of occurrences		
Position	c[1]	o[1]	r[1]
Level	13	25	12
Slope	10	4	11
Depression	12	9	6
Mound	15	9	5
Base of hill	3	2	5
Plateau	1	0	3
Terraced slope	0	1	7
Total	54	46	50

[1] c = sites in which running buffalo clover has been present since 1992-1994; o = sites in which running buffalo clover was present in 1992-1994 but not present in 2004; r = random/control sites which have had no record of running buffalo clover presence.

Table 9. Topographic positions of running buffalo clover plants encountered on plots containing running buffalo clover (Long-term sites) on the Blue Grass Army Depot, Madison County, KY, 2004.

Position	Number of occurrences
Level	8
Slope	28
Depression	25
Mound	21
Base of hill	15
Plateau	13
Terraced slope	8

Table 10. Mean (±SD) maximum (max) and minimum (min) light levels [lux] for running buffalo clover (RBC) Long-term, Disappeared, and Random/control plots, on the Blue Grass Army Depot, Madison County, KY, April-August, 2005.

	April	May	June	July	August
Long-term Plots [a]					
Max	30968± 9020[d]	35952± 38740[e]	13035± 20452[e]	5059± 5027[e]	4790± 2282[e]
Min	6017± 990[d]	8008± 11808[e]	1184± 1098[e]	1238± 797[e]	979± 700[e]
Disappeared Plots [b]					
Max	74756± 40064[f]	33767± 42227[f]	7384± 6910[f]	7976± 10990[f]	11324± 8891[f]
Min	14338± 16372[f]	10484± 13057[f]	2476± 3326[f]	2099± 2551[f]	1668± 1249[f]
Control Plots [c]					
Max	65198± 37491[f]	53960± 47501[g]	22486± 31366[g]	8321± 10064[g]	10592± 6889[g]
Min	17556± 14413[f]	16878± 18449[g]	1410± 1593[g]	1206± 1259[g]	1625± 1894[g]

[a] Plots in which RBC has been present since 1992-1994.
[b] Plots in which RBC was present in 1992-1994 but not present in 2005.
[c] Plots which had no record of RBC ever being present.
[d] n = 7
[e] n = 11
[f] n = 9
[g] n = 10

Appendix B: Aspects of running buffalo clover ecology

Species Description:

Running buffalo clover usually acts as a perennial species, forming long stolons that root at the nodes (USFWS 2007). Plants produce erect flowering stems, 10-40 cm tall that send out stolons (Vincent 2001). The leaves of the runners have 1-2 cm long ovate-lanceolate stipules, whose tips gradually narrow to a distinctive point. Erect stems arise from nodes along the stolon, with 2 large rounded trifoliate leaves at their summit, their obovate leaflets 2-3 cm long and wide (USFWS 2007). Stipules on the stolon are distinctive, being leaf-like, ovate-oblong, and slightly serrated. The prostrate stem has few branches, and is glabrous or nearly so. The inflorescence is 15-30 mm wide, umbellate, spherical, with up to 40 small white flowers; the inflorescence is on a peduncle above the uppermost branching leaflets (Vincent 2001). Running buffalo clover flowers from mid-April to June; fruiting occurs from May to July (Brooks 1983).

Because of its stoloniferous growth form, individual running buffalo clover plants can be difficult to distinguish. Because vegetative reproduction appears to predominate in wild populations, the number of genets represented is presumably few (USFWS 1989). A rooted crown is a rosette that is rooted into the ground. Rooted crowns may occur alone or be connected to other rooted crowns by stolons (USFWS 2007).

Running buffalo clover stolon growth is most vigorous after flowering in the spring (USFWS 1989). Campbell et al. (1988) found stolon growth to be vigorous during flowering in April to June, and slows during warmer, drier weather. Stolons may confer competitive advantages for colonizing sites that have compacted soil conditions, little surface organic material, or depositional overburden (Madarish and Schuler 2002).

Becus and Klein (2002) began a mowing regime that included disturbance twice before flowering (mid-April), and then a third time in late June after the running buffalo clover plants in the study had fruited. The reduced competition from grasses during mowing resulted in a significant population increase. If clipping, either by mowing or grazing, occurs after seed is set, running buffalo clover may benefit from increased seed dispersal; this may also encourage continued vegetative growth.

Chemical scarification of running buffalo clover seeds by the digestive system of herbivores, historically believed to be bison, deer, elk, or small herbivores such as rabbits or groundhogs (*Marmota monax*), was likely a major factor influencing past running buffalo clover distribution in natural populations (Cusick 1989; USFWS 2007). Pickering (1989) speculates that in post-settlement times, cattle may have functionally replaced the bison as a major disperser of running buffalo clover seeds; however there is disagreement among researchers (USFWS 2007) The USFWS (2007) notes that cattle historically were typically confined in pastures and not migratory as were bison. The scarification process is believed to be important for running buffalo clover germination and as a means of seed dispersal (Cusick 1989; USFWS 2007). Mechanical scouring through trampling by ungulates or scouring by rivers may have occurred but was probably infrequent (USFWS 2007). Cusick (1989) noted that running buffalo clover plants frequently occur in small clumps, prompting the speculation that running buffalo clover seeds are deposited in deer feces. Although deer are viable vectors for running buffalo clover seeds, the survival and germination rates of ingested seeds are low (Ford et al. 2003). Ford et al. (2003) speculated that dispersal and establishment of new populations of running buffalo clover by white-tailed deer may not be significant. Comparatively fewer populations of running buffalo clover have survived relative to presumably larger pre-settlement numbers of deer (Cusick 1989).

Spring temperature fluctuations appear to be a major dormancy breaker in natural populations of running buffalo clover (USFWS 2007). Seeds typically germinate during early spring (mid-March through early April) when temperatures are between 15-20° C during the day and 5-10° C at night (USFWS 2007). Hattenbach (1996) found that scarification accelerates the germination process; whereas natural germination may occur over time if the right temperature fluctuations occur. The relationship between dispersal, scarification, and subsequent germination remains unclear.

The plant structure of running buffalo clover usually includes rooted crowns, or rooted rosettes, and stolons, or above-ground creeping stems connecting several rooted or un-rooted crowns that eventually separate to leave “daughter” plants (USFWS 2007). At an introduced running buffalo clover population, most first-year seedlings displayed little or no stolon development. However, some individual seedlings developed stolons with rooted crowns and remained connected to the “parent” plant until the following spring. In the second or third year, the “parent” plant separated from the “daughter” plant and both produced stolons (USFWS 2007). Developmental variation has been observed throughout the growing season. For example, between May and June, running buffalo clover plants flower and produce stolons with associated unrooted daughter crowns. By July, the daughter crowns begin to root but remain connected by stolons to the parent plant. Starting in September stolons senesce and parent and daughter crowns are no longer connected, a time of high mortality for the parent plant (USFWS 2007).

Long term monitoring data suggest that running buffalo clover populations often display widely fluctuating population sizes, including variation within a given growing season (USFWS 2007). The timing of life history events relative to competitive and environmental interactions needs more investigation. The cause for changes in population size may be due to disturbance, weather patterns, management strategy, natural succession, or other unknown factors; for example, timing of disturbance may be more important than intensity of disturbance if disturbance creates potential rooting sites that coincide with peak stolon growth (USFWS 1989).

Taylor et al. (1994) found that running buffalo clover was visited by bees and is cross-pollinated in the wild. They also suggest that running buffalo clover sets fewer seeds by self-pollination than by out-crossing, but that selfed seed set may be adequate to maintain the species in the wild. While there has been speculation about the effects of inbreeding depression on the decline of running buffalo clover (Hickey et al. 1991; Taylor et al 1994); selfed seeds have shown high germination rates and develop into vigorous plants (USFWS 2007).

Unlike all other species of *Trifolium*, running buffalo clover lacks a rhizobial associate (Campbell et al. 1988; USFWS 1989; 2007). Because of the lack of nitrogen (N) fixing capabilities, researchers speculated that running buffalo clover could not compete when habitat conditions changed across the eastern United States (Campbell et al. 1988; Cusick 1989). Oddly, running buffalo clover appears robust and healthy in many situations even without such an associate, for example in drought and mowing regimes (Morris et al. 2002; USFWS 2007). Morris et al. (2002) suggest that running buffalo clover may have lost biological nitrogen fixing capabilities due to its close associations with bison, elk, deer, and other ruminant animals. As running buffalo clover plants and seeds were consumed by animals and viable seeds excreted in the manure; Morris et al. (2002) postulated there was a high enough N level in manure to support clover establishment and growth. Morris et al. (2002) speculated that running buffalo clover may have a low nitrogen requirement and may have never developed the need for a rhizobial associate. Established forested areas would have been expected to have organic matter in the surface soil (via leaf litter and other debris decomposition) that could have provided N and other essential nutrients for running buffalo clover growth (Prichett and Fisher 1987). During the 1700's and 1800's large areas of land were cleared for crop and cattle production, and clovers capable of fixing high rates of N were introduced into the running buffalo clover's habitat. With high or moderate available soil N levels, N fixation may be inhibited. Ultimately, the rhizobial association may not have been necessary for running buffalo clover establishment, resulting in selection against biological nitrogen fixation capability.

Genetic variation:

The chromosome number (2n=16) of running buffalo clover is the same as other native clovers found in the eastern United States (Campbell et al. 1988). Hickey et al. (1991) found running buffalo clover to have low genetic diversity, which may be explained by the clonal nature of the species. However, given that there is genetic diversity between populations, it suggests that the original gene pool was initially quite diverse (Hickey et al. 1991). Hickey et al. (1991) reported that small running buffalo clover populations have lower levels of diversity than large ones and that the majority of the diversity occurs within populations. There were also significant differences in average similarities between and within patches at the population level, suggesting that there is sub-structuring within populations. In addition, gene flow between populations was limited, even between populations separated by short distances (Hickey et al.1991). Crawford et al. (1995), using more advanced RAPD technology, discovered levels of diversity in smaller populations to be equal to that in larger ones. No allozyme variation was detected in half of the populations sampled and smaller populations were often monomorphic. As such, to conserve maximum levels of diversity in running buffalo clover as many populations as possible should be preserved across its range; because much of the total diversity resides among individual populations. Small populations of running buffalo clover contribute as much genetic diversity as large populations and exhibit unique banding patterns, which is important for the species adaptability and genetic stability.

In theory, reduced heterozygosity can result in decreased population growth due to inbreeding depression (Schemske et al. 1994). Allele richness could contribute to population growth through its effect on evolutionary potential, or the ability of a species to respond to changes in its selective environment (Schemske et al. 1994). In spite of theoretical relationships between genetic diversity and species persistence, no empirical evidence exists that directly links the genetic composition of plant populations to their growth rate or survival (Schemske et al. 1994). Even though the Blue Grass Army Depot populations exhibit low genetic variability (97.5% similarity), they serve as a valuable reservoir for running buffalo clover since there are large numbers of populations and they occur on federally protected land (Vincent and Hickey 1996).

Disease is not thought to play a major role in the decline of running buffalo clover. Sehgal and Payne (1995) found a cucumovirus (type of peanut stunt) and a comovirus (peavine mottle) afflicting running buffalo clover in lab-grown plants; however, the effects of the viruses do not seem to be region-wide nor severely limiting.

Appendix C: Land use history as related to running buffalo clover

In landscapes with long histories of human activities, whether from pre-European settlement or post-European settlement, past land uses exert strong and enduring impacts on vegetation patterns and ecosystem processes (Gerhardt and Foster 2002). Pioneer descriptions of the Bluegrass in the 1770's depict dense canebrakes with scattered trees, meadowlands, and open forests of oak (*Quercus* sp.), ash (*Fraxinus* sp.), walnut (*Juglans* sp.), cherry (*Prunus* sp.), sugar maple (*Acer saccharum*), with an abundance of grasses (including cane and wild rye), legumes, and other herbaceous plants flourishing beneath as ample light reached the ground; dense, closed forests likely existed near rivers and creeks (Bryant et al 1980; Campbell 1989; Wharton and Barbour 1991).

The vegetation European settlers saw when they arrived in the Bluegrass region was mistaken as pristine wilderness versus actually being 200 years of re-growth in a formerly extensively modified Indian environment; prior to European arrival, the Ohio River valley was populated by small groups of Native Americans (Denevan 1992; Henderson 1998). In the Bluegrass Region, agriculture was utilized on a tribal subsistence level, although lands also served as hunting grounds, with bison, elk, and deer providing sources of game (Jakle 1968). There was an extensive network of Native American and bison traces in the region connecting villages, salt licks and foraging grounds throughout the Bluegrass; with salt licks generally located in the Outer Bluegrass (Jakle 1968; Campbell 1985; Fields and White 1996; Henderson 1998). Short of the Kentucky River Palisades, travel between sources of water, salt, and food resources in both the Inner and Outer Bluegrass regions was unencumbered (Henderson 1998). Speculation exists regarding the creation and maintenance of the fertile, park-like savannahs described by early Kentucky settlers; were they relic communities from thousands of years earlier, perpetuated by herds of large herbivores and possibly periodic burning by Native Americans as suggested by Bryant et al. (1980) and Wharton and Barbour (1991). McEwan and McCarthy (2008) stated that human history is an important driver of ecological dynamics within historic eastern North American forests. In Kentucky, McEwan and McCarthy (2008) note three land-use “eras”: Native American habitation/utilization (<1670); land abandonment (1670-1780); Euro-American land clearance (>1780). They speculate that the creation of the savannahs coincides with increased light striking the forest floor around the time of Euro-American settlement (McEwan and McCarthy 2008). The trees representing pre-settlement Bluegrass savannah have wide canopies of low hanging branches, indicating they were well spaced as saplings and did not develop in a dense forest (Wharton and Barbour 1991). However, the ground layer in the savannah-woodland is not thought to be true prairie, although some prairie species undoubtedly existed (Bryant et al. 1980). Braun (1950) noted that the canelands and wild rye dominated lands were not treeless and must have strongly contrasted with the true prairies to the west.

Likely what early Kentucky pioneers described as savannah-woodland had several underlying environmental, climatic, geologic, and cultural factors affecting it. Probably the most potent factor in the continuance of savannah-like areas of vegetation within Kentucky was the activity of large herbivores; i.e. great herds of bison trampling down tree seedlings as they grazed on cane and other herbage, and numerous deer and elk browsing tree seedlings. The Bluegrass region was well suited for large herbivores with its rolling topography, a soil high in calcium and phosphorus to provide nutritious forage, and numerous salt licks (Wharton and Barbour 1991; Tankersley 1992). The large bison herds would create wide trails or "roads" while navigating between salt licks, feeding grounds, and springs, trampling everything in their path (Jakle 1968; Tankersley 1992). When the herds came upon the shoals of streams, they would wallow, creating "stamping grounds" that could cover many acres (Jakle 1968; Tankersley 1992). Wharton and Barbour (1991) stated "While these herds of herbivores may not have converted forests to pasture, they could prevent the pasture from maturing into a dense forest." Others (Tankersley 1992, Henderson 1998) question Wharton and Barbour's (1991) conclusions, noting that bison entered the Bluegrass region around 1650. While bison trampling may have encouraged savannah-like woodlots, it would have occurred only around salt licks and along migration routes, not across the whole Inner Bluegrass (Tankersley 1992). In addition, the bison's postulated impact on the environment encountered by the first European settlers would have taken place for only 100 years (Henderson 1998).

Mesic grasslands are prone to shrub and tree invasion. Once cessation of the conditions which allowed grasslands to thrive in lands east of the Mississippi River occurred, namely prescribed burning by Native Americans and subsequent trampling by herds of bison, shrubs and trees quickly moved in, covering old buffalo traces (Campbell 1989; Wharton and Barbour 1991). Campbell (1989) notes that many of the trees mentioned by settlers are indicative of early succession, and thus disturbance (or drought); however, when grazing/agriculture uses cease, mesic species invade as opposed to the drier oak-ash savannah.

Canebrakes are dense stands of *Arundinaria gigantea* that covered large areas of the southeastern North America (Campbell 1985). Canebrakes were most common on alluvial floodplain terraces where they occurred beneath sparse forest canopies as cane is shade-intolerant (Campbell 1985; Dattilo and Rhoades 2005; Wharton and Barbour 1991). Cane can tolerate inundation, but not prolonged submergence; hence, canebrakes were restricted to the first ridge or natural levee (Platt and Brantley 1997). At the Blue Grass Army Depot, canebrakes occur near streams which are bordered by scattered trees.

Alluvial soils are subject to periodic flooding and nutrient deposition, such that early settlers considered canebrakes as good indicators of fertile soils for farming; just as the Native Americans had done (Naiman and Decamps 1997; Platt and Brantley 1997). With agricultural development, canebrakes were quickly converted to crop and pastureland and now occur only in small, isolated patches (Campbell 1985; Dattilo and Rhoades 2005). Owing to extensive loss, canebrakes have been designated an endangered ecosystem (Noss et al. 1995) and thus are afforded priority for conservation and restoration.

Most vegetation in the Bluegrass is not suited to fire management, as are tall-grass prairies and canebrakes. Campbell (1985) suggested canebrakes developed under regimes of moderately intense biotic or human disturbance. Cane can be flammable in the right season and recovers quickly from fire (Fields and White 1996). Seven to ten year burning cycles are commonly mentioned as a method used by Native Americans to maintain cane and grasslands (Williams 1989; Platt and Brantley 1997). Significant rhizome elongation appears necessary before cane culms increase in height (Platt and Brantley 1997). Frequent fires deplete stored resources in the rhizome and kill young plants lacking a developed rhizome. Campbell (1985) postulated that by burning cane at the proper time, Native Americans could have maintained and even expanded canebrakes. Bryant et al.

(1980) differs from Campbell (1985), stating that none of the cane present in early Kentucky occurred in remnant savannah-woodlands.

Bison and other ungulates preferentially feed on recently burned grasslands because of the rapidly growing, succulent, nutritive young shoots (Hughs 1951). Periodic burning by Native Americans to encourage game has been suggested as a contributing factor in the historical maintenance of prairies (Campbell 1985). Fire was undoubtedly a tool available to ancient Native American cultures, with evidence of fire use in Kentucky dating back as far as 3000 years ago (Delcourt and Delcourt 1997) and could have been used by earlier Indian cultures (Fort Ancient: 1000-1700 A.D.; Henderson 1998). To what extent fire was used by Native Americans to promote the grasslands of the Bluegrass Region is unclear (Fields and White 1996). Early explorers gave no indication of burning in the Bluegrass region (Bryant et al. 1980). While bur oak (*Quercus macrocarpa*), a common species in the Bluegrass savannah, is described as fire-resistant, other tree species noted as common in early land surveys, such as sugar maple (*Acer saccharum*), are fire-sensitive (Campbell 1988). The prevalence of cane observed by early settlers could be attributed to land abandonment by Native Americans due to devastating pandemics followed by 200 years of successive regrowth (Henderson 1998; McEwan and McCarthy 2008).

Platt and Brantley (1997) note many historic accounts mentioning bison in association with cane. Cane was an important forage to settlers in Kentucky because it provided grazing and shelter throughout the winter [see historical accounts in Platt and Brantley (1997)]. Cane is particularly sensitive to overgrazing, especially during the growing season, and continuous grazing leads to rapid decline. Canebrakes quickly disappeared in Kentucky following European settlement largely due to overgrazing by cattle, lack of cyclic burning regimes and land clearing for agriculture (Platt and Brantley 1997). When settlers burned the cane once, it encouraged new shoots which the cattle ate, however repeated burns would kill canebrakes (Wharton and Barbour 1991).

Kentucky's loss of canebrakes and native grasslands was progressive following the removal of Native American tribes, increasing rapidly following the westward expansion by European settlers. By 1758, the Shawnee had abandoned all permanent towns in the Bluegrass region; by 1800, the Cherokee and Iroquois has been pushed west of the Mississippi (Fields and White 1996). By 1792, when Kentucky officially became a state, almost all of the land in the Bluegrass region had been claimed and settled (Wharton and Barbour 1991). By 1775, there was a noticeable reduction in game, prompting Daniel Boone and other early settlers to create game laws to warrant off the wanton killing and protect the resources needed to sustain them (Belue 1996). By 1800, all bison had been eliminated from Kentucky, with elk and deer becoming increasingly rare (Fields and White 1996). By 1790, settlers were already sowing European white clover (*Trifolium repens*) as well as timothy grass (*Phleum pretense*) and bluegrass (*Poa pratensis*) for a burgeoning cattle industry; settlers quickly realized that the rich lands that supported vast game herds would also support herds of domestic livestock. While the park-like woodland pastures continued to be a substantial part of the rural landscape throughout the 1800's, the ecosystem lacked the ability to regenerate and thus diminished in size over time (Bryant et al. 1980). As more settlers arrived into the Bluegrass region, there was increased clearing of land for agricultural purposes. The groundcovers have become less diverse and mowing had replaced grazing, limiting the ability of tree seedlings to grow. By the 1830's, nearly all of the original vegetation had been removed to clear farm land, leaving only strips and pockets of forest vegetation (Fields and White 1996). Large game animals, such as bison and elk, were driven from the Bluegrass Region and eventually from the state altogether. The giant canebrakes and savannahs of Madison County, KY, were decimated, being relegated to small, scattered pockets (Fields and White 1996). Around this same time, reports of running buffalo clover became scarce as well (Campbell 1985). It stands to reason that the only clover habitat remaining was alluvial forested stream corridors and other moderately disturbed fringe habitats in the remaining dwindling forests of the Bluegrass.

Early settlers were primarily subsistence farmers, however Fields and White (1996) note that at least one plantation was known to exist in the area that would eventually become the Blue Grass Army Depot. The majority of farms in Madison County tended to be land occupied by tenant farmers and share croppers, a trend that increased dramatically after the Civil War (Fields and White 1996). After the war, livestock increased in agricultural importance, becoming Madison County's main agricultural resource (Trimble and Mendel 1995; Fields and White 1996).

Agricultural practices on lands encompassed by the Blue Grass Army Depot were suspended with the acquisition of land and the construction of the Blue Grass Army Depot in the early 1940's. In the early 1950's the Blue Grass Army Depot began to lease land to local farmers for grazing. Although a grazing history (areas leased, number of farmers participating, etc.) cannot be reconstructed, it is probable that all of the Blue Grass Army was subject to grazing. Grazing practices at the Blue Grass Army Depot were modified in the 1990's (USFWS 2007); and by 1996, only half of the Blue Grass

Army Depot was open for grazing (Fields and White 1996) and hay production (USFWS 2007). About the time that the Blue Grass Army Depot lands were being acquired and construction of facilities initiated, mechanized agriculture was changing the method and intensity with which Madison County and the rest of the Bluegrass region was being farmed. In general, Blue Grass Army Depot lands have not been utilized with the same intensity as surrounding county farmland (Fields and White 1996).