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EASTERN KENTUCKY UNIVERSITY

Stage Based Matrix Modeling of *Trifolium stoloniferum* Restoration Populations at Taylor Fork Ecological Area, Madison County, Kentucky

Honors Thesis

Submitted

in Partial Fulfillment

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By

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Faculty Mentor

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ABSTRACT

Stage Based Matrix Modeling of *Trifolium stoloniferum* Restoration Populations at Taylor Fork Ecological Area, Madison County, Kentucky

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Trifolium stoloniferum (running buffalo clover) is a federally endangered plant species that was once abundant from parts of the eastern United States like West Virginia and Kentucky and into parts of the west, such as Kansas, but was considered extinct for many years before the species was rediscovered. Although the species is recovering overall and is pending to be downlisted to threatened, this species, and many others, can benefit from more detailed population viability analyses such as the one conducted for the project. The objective of this research was to conduct a stage-based population viability analysis of restoration populations five and seven, located at the Taylor Fork Ecological Area in Madison County, Kentucky and to relate how this type of analyses can and ought to be used in the conservation of this species. In order to conduct the stage-based analyses, we first analyzed and found that the proposed life history stage classifications used are valid. Furthermore, the stage-based analyses conducted in this project has been compared to previous research done with the restoration populations at Taylor Fork Ecological Area with count-based population viability analyses to compare the value of stage-based modeling over the simpler count-based methods. Thus, even when data are limited, and even though stage-based modeling is more difficult and resource consuming to do, it is recommended to use it when assessing endangered plant species because of the critical

demographic information such as the dominant eigenvalue, the stable stage distribution, reproductive values, and the elasticity matrix that stage-based analyses provide.

Keywords and phrases: running buffalo clover, *Trifolium stoloniferum*, population viability analysis, stage-based, count-based, endangered species, restoration population, demographic

Table of Contents

List of Figures

List of Tables

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Introduction

Trifolium stoloniferum Muhl. ex A. Eaton (running buffalo clover or *T. stoloniferum*) is a federally declared endangered plant species that was once abundant from West Virginia to Kansas and Missouri (Campbell et al. 1988). After European settlers colonized and immigrated further inland, populations of *T. stoloniferum* experienced heavy declines and ultimately the species was considered extinct in 1983. However, later on in 1983 and in 1984, two sites were found in West Virginia along a forested road frequented by vehicles, renewing the search and monitoring of the species (Bartgis 1985). These sites were also coincidentally in the same region where the woodland bison (*Bison bison athabascae*) of the area were last seen before their extirpation from the region, thus reinforcing the plant's name sake and the common hypothesis of their downfall, which was the absence of the woodland bison (Campbell et al. 1988). The United States Fish and Wildlife Service then declared the species endangered in 1987 and in the following years more populations of *T. stoloniferum* have been discovered in multiple states such as Kentucky and Ohio (Jacobs 1987). With the discovery of yet more populations that were previously unknown, the species is on track to become downlisted from endangered to threatened (USFWS 2011). The only state that historically contained *T. stoloniferum* that no longer does is Illinois (Figure 1). The populations of *T. stoloniferum* that have been found in Kentucky occur in two different types of habitat. The first type occurs in periodically disturbed areas such as logging sites, cemeteries, and off hiking trails (Cusick 1989). However, the most significant amount of clover has been found in the second type of habitat, stream scoured areas next to streams on the Blue Grass Army Depot (USFWS 2007). The habitat in which *T. stoloniferum* is most commonly found on the depot can be further described as small bottomland areas with mature canopies that do not have a history of agricultural use. The sites tend to be between streams and distinct topographic features such as cliffs, and the canopy openness is about 40 to 60% (Koslow et al. 2018). The Blue Grass Army Depot had 50 known element occurrences of *T. stoloniferum* in the summer of 2017 (Koslow et al. 2017).

There are various hypotheses as to why *T. stoloniferum* has been reduced from a thriving and staple part of the ecosystem to its currently endangered state. The most prevalent explanation given is that the woodland bison that used to thrive in the same range as *T. stoloniferum* were vital to its success (Campbell et al. 1988). *Trifolium stoloniferum* is typically found in disturbed areas and woodland bison potentially provided an ideal amount of disturbance as well as the seed dispersal and fertilization that the clover needed. Although few plant species truly benefit from being disturbed and stressed by environmental factors, the stoloniferous habit of *T. stoloniferum* contributes to the plant's tolerance of disturbance, allowing it to survive through grazing that would kill other plants. This is because nodes are typically located in the stems, which in the case of *T. stoloniferum*, are close to the ground and often not eaten by grazers (unlike those of its upright competitors), while the leaves that grow further up are eaten. The persistence of the nodes after grazing is critical because the nodes are where primary growth occurs. This idea of tolerance to disturbance is strengthened by various other facts. Historically, *T. stoloniferum* populations were described as occurring very heavily along buffalo traces and on the edges of grazed fields where woodland bison once lived (Campbell et al. 1988). Native Americans are also believed to have assisted in the previous success of the species through the maintenance of trails and intentionally setting fires to manage the landscape for their needs (Burkhart 2010). Furthermore, the persistence of the species in heavily disturbed areas such as logging sites and cemeteries with frequent mowing further support the hypothesis that *T. stoloniferum*'s relative tolerance to disturbance is key to the persistence of its populations. A specific example of how logging sites seem to work as suitable habitat for *T. stoloniferum* is that there are significant populations that occur in the Fernow Experimental Forest in West Virginia near skid roads (Burkhart et al. 2013). An example of *T. stoloniferum* benefiting from a routine mowing schedule can be seen at Shawnee Lookout Park (Becus and Klein 2002). On the Blue Grass Army Depot, where most of the *T. stoloniferum* in Kentucky is found, the places the species is found in are periodically disturbed through stream scour, which may be a different disturbance than the species was previously known for, but disturbance, nonetheless. Despite the apparently obvious benefits of disturbance for *T. stoloniferum*, too much disturbance can be detrimental to the species because it has been found to prefer filtered light and too much disturbance can remove trees and other woody plants that provide shade (Hattenbach 1996). Another feature of *T. stoloniferum* that likely impacts the species ability to persist and outcompete other species is that they don't have nitrogen fixing abilities, unlike other clovers (Morris et al. 2002).

There are many other factors as to why the species has declined though. An example would be the introduction of *Trifolium repens*, commonly known as European white clover, into the similar habitat that *T. stoloniferum* has historically resided in. *Trifolium repens*, introduced by European settlers, is known to have introduced a virus that *T. stoloniferum* was susceptible to (Sehgal and Payne 1995). Furthermore, white clover contains cyanide in its leaves to deter the excess herbivory of small mammals and insects while *T. stoloniferum* has no such chemical defense against herbivory (Jacobs 1987). Additionally, the introduction of other invasive plant species such as *Microstegium vimineum* (Japanese stiltgrass), which has been observed to smother out *T. stoloniferum* patches previously found on the Blue Grass Army Depot, further threatens the species.

When dealing with the conservation of an endangered species, maintaining the genetic diversity of the species is critical to avoid inbreeding depression, which can often compound with other factors affecting a species, hastening its decline. Studies have been conducted in the past to assess the genetic diversity of *T. stoloniferum* populations and as expected, the larger populations had more genetic diversity than the smaller populations (Crawford and Windus 1995). Thus, the maintenance of the already larger populations could be seen as more beneficial than the management of smaller ones. Yet, not all larger populations are as stable as some of the smaller populations because of environmental factors and stochastic events. Thus, when encountered with limited time and resources while trying to conserve a species such as *T. stoloniferum*, some populations are bound to receive more attention than others and some populations need more attention than others. This creates a problem that can be solved by conducting population viability analyses to identify which populations of all sizes need more help, are relatively stable, and which ones are essentially bound to die out. Count-based population viability analyses are used as part of the restoration plan for *T. stoloniferum* (USFWS 2007) in order to get a better grasp of which populations are more relatively stable, identify when the species as a whole is more stable, and to identify which populations are more at risk.

The modeling of population growth for plants is often more complicated than the monitoring of animal species because plants species' life histories are often more complex. For

T. stoloniferum specifically, reproduction is possible through sexual reproduction, which produces new genets, and through asexual reproduction, which produces new ramets. The restoration populations at Taylor Fork Ecological Area (TFEA) only reproduce through asexual reproduction because the fruits that are produced from sexual reproduction are harvested in the field before they can drop off. The asexual reproduction is done through the production of stolons that move out horizontally above, and sometimes through, the soil until a new crown steam is created, roots into the ground, and splits off from the parent plant.

In order to conduct stage-based population viability analyses on the populations, detailed morphological data were collected on individual plants in previous monitoring efforts of *T. stoloniferum* and through current monitoring efforts. The morphological data could then be used to classify individuals into a life history stage. The proposed stage classification system for *T. stoloniferum* is based upon the total stolon length of a plant, the number of nodes on the stolons, the number of inflorescences made, and how many new rooting crowns were grown (Hickey 1995, Figure 4). The use of stages when modeling plant populations is common practice because the size of a plant, or certain features of a plant, often have great effect on reproduction. However, the assessment of any species' life history stages needs to be based on life history stages that aren't merely based upon necessity or convenience, but rather on actual indicators of better survival or reproduction (Pfister and Stevens 2003). Thus, the validity of proposed stage classifications was also analyzed for this project.

The importance of identifying critical life histories is well highlighted by the historic case of management of endangered Loggerhead Sea Turtles. Before stage-based population viability analyses were conducted on the species, it was believed that most important life history stage to protect and manage for were the eggs. Analyses showed that the eggs were actually the least responsive stage, and that for efficient management, the sexually mature adults should be the focus of conservation efforts (Crouse et al. 1987). For conservation biology, managing for the life history stages that have the greatest impact on population growth is essential, regardless if that species is an animal or plant (Schemske et al. 1994).

Past research on the restoration populations at TFEA included count-based population viability analyses (Brancheau and Koslow, unpublished, Table 1). Count-based analyses were also recently updated for the elemental occurrences of naturally occurring *T. stoloniferum* on the Blue Grass Army Depot (Koslow et al. 2018, Table 2). Count-based analyses have been conducted on the Blue Grass army Depot in the past (Dart-Padover et al. 2016).

Objectives

The objectives of this research were not only to continue the surveying and cataloging of data on the restoration populations at TFEA, but to also assess and ensure that our stage classifications are valid and to do a stage-based population viability analysis of restoration populations five and seven and to compare the results of the analysis to past count-based analyses of *T. stoloniferum*. Although count-based population viability analyses of clover populations are part of the species' recovery plan, stage-based analyses have not been conducted on the species and these analyses will produce new and significant insight into the management of the species (USFWS 2007). Thus, the ultimate goal of this research was to analyze the differences in results, highlight the unique insights that stage-based population viability analyses provide, and to provide both context and precedent for stage based population viability analyses of *T. stoloniferum* so that agencies such as the United States Fish and Wildlife Service will be more inclined to conduct the more accurate stage-based population viability analyses, instead of just the basic count-based population viability analyses, despite the extra time and resources it takes to conduct stage-based analyses (Menges 2000).

Methods

Study Species

Trifolium stoloniferum Muhl. ex Eaton (Fabaceae) can be identified by its paired set of three leaves below the inflorescences, the presence of a rooting crown stem, by the presence of stolons branching out horizontally along the ground away from the primary rooting crown, when stolons are present, and by the toothed edges around the leaflets (Figure 6, Burkhart 2010). A crown stem is defined by the United States Fish and Wildlife Service as a rosette that is rooted into the ground (USFWS 2007). Flowering can begin as early as mid-April and last through June, while fruiting can start at the end of June and will continue through July until all of the fruits fall off (USFWS 2007). *T. stoloniferum* can be differentiated from *T. repens*, which is the introduced and common European white clover, by the presence of stipules at the base of the leaves that *T. repens* lacks and by the lack of white chevrons on the leaves that are seen in *T. repens* (USFWS 2007). Lastly, *T. stoloniferum* lacks the nitrogen fixing habit that is commonly seen in other species of the Fabaceae family (Morris et al. 2002).

Field Study Area

All surveying of restoration populations took place at Taylor Fork Ecological Area (TFEA) in Madison County, Kentucky. TFEA is owned by Eastern Kentucky University, is located near the campus in Richmond, and is approximately 60 acres in size. The area is mostly old pastureland that has been managed as early successional habitat and has been the location for field experiments and on-site learning for students (Brown 2019).

A total of seven restoration populations exist at TFEA, but only six were surveyed for these analyses. Restoration population one was excluded because herbicide related experiments were conducted at that site in the past, which may have skewed the results of current analyses if included. Site number two is near a small stream that is shaded by trees, grasses, and other plants of intermediate size, and was planted in 2012. The site was grazed frequently by cattle, but cattle were removed in 2016, thus allowing for the surrounding vegetation to grow up to a degree in which the *T. stoloniferum* at the site experienced high mortality from being outcompeted and smothered starting in summer 2016. The cattle were reintroduced in the late spring of 2018 and have begun to graze and disturb the area once again, allowing for some recovery of *T. stoloniferum* at the site. The site also had a cattle gate marking off a square meter subplot, with clover inside and out of the gate. This square of cattle gates is the remnant from a previous experiment to assess the impact of cattle grazing. Site number three is located on a hill that is shaded by trees and was planted in 2014. The surrounding vegetation in this plot is limited, minimizing competition from the herbaceous layer. Cool season grasses dominate the hillside in the spring, but die off once summer sets in. Site number four occurs alongside a trail and is well shaded on one end, while the other end of the plot receives direct sunlight for extended periods of time. The prevalence of competing vegetation in this site varies. Site number five occurs within a high traffic area that is frequently used to set up mist nets. It is well shaded by trees and the high levels of disturbance have resulted in little competing vegetation. Site number six is the smallest site and occurs alongside a stream. The competing vegetation at site six is abundant and the clover at this site is limited in abundance. Site number seven occurs in a small clearing off one of the trails and is in a well shaded area with a small amount of vegetation shading out and competing with the clover. Sites number four through seven were planted in 2014. Across all surveyed sites, common herbaceous vegetation that grows around and above RBC includes *Viola* species, *Verbesina alternifolia* (wingstem), *Trifolium repens* (European white clover), and *Oxalis stricta* (common yellow woodsorrel), along with various grasses, sedges, and rushes. Trees and shrubs that are common around TFEA and around clover patches include *Lonicera maackii* (bush honeysuckle), *Juglans nigra* (black walnut), and *Gleditsia triacanthos* (honey locust).

Field Methods

In 2017, we conducted surveys every other week from 05/10/2017 to 06/26/2017 and every week from 07/05/2017 to 09/15/2017. In 2018, we conducted surveys every other week from 05/10/2018 to 05/31/2018 and every week from 06/07/2018 to 08/16/2018. Individual plants were marked with unique identifying tags with a four-digit number. The monitoring of each individual plant included counting the number of inflorescences, the number of stolons, the number of nodes on the stolons, and the number of rooting crowns still attached to the "parent" plant, as well as measuring the total length of all the stolons on a plant. As new plants arose through asexual reproduction, they were marked with individual tags. The parentage of every new plant was recorded with a confidence interval of one through three, three being the most confident. Threes were only assigned to plants that were visually confirmed to have been previously attached to the parent plant. Twos and ones are assigned to plants that appear to spring up from sexual reproduction or appear to be clones of a nearby parent plant, but confirmation was not possible. The total number of plants and inflorescences for each site on every visit were also be noted, and ultimately, a life history stage was assigned to each plant every time data was collected. The assigned life history stages range in value from 1 to 6, depending on the size of the plant and how reproductively successful it was (Figure 4, Hickey 1995). A stage one plant is only a small crown, which is indicative of either a seedling or a struggling plant. A stage two plant is a one with a full and healthy crown. A stage three plant is a crown with a total stolon(s) length of under 50 centimeters. A stage four plant has one to three flowers and/or a total stolon length between 50 and 100 centimeters. A stage five plant has four or more flowers and/or a total stolon length of between 100 and 150 centimeters. A stage six plant is one with a rooting crown stem on a stolon or one that possesses a total stolon(s) length over 150 centimeters. Under this classification system, it is worth noting that a plant with a total stolon length under 50 centimeters can be classified as a stage six, as long as it has a rooting crown along said stolon(s). It was decided to incorporate this into the assessment of stages in order to emphasize the importance of reproductive success in the assessment of stages, and new rooting crowns appear to be nearly guaranteed success, while reproduction through seeds is much more minimal and less guaranteed. These methods mirrored previously used methods to survey the restoration populations at TFEA.

Stage Validity Assessment

To assess whether or not the stage classification system used was valid, data from all six sites at TFEA in 2017 were fitted with two separate logistic regressions. The logistic regressions were fitted using a Poisson distribution with site as a random factor. The first regression compared total stolon length of individual plants to the number of offspring reproduced. The second regression compared number of nodes per rooting crown to the number of offspring reproduced. The two metrics of total stolon length and number of nodes per rooting crown were used, because those were the metrics historically proposed and used in the field to assess stages (Hickey 1995).

Stage-Based Population Viability Data Analysis

A stage-based population viability analysis was conducted using R (R Core Team 3.5.1) and RStudio (RStudio Team 1.1.456), using the packages popbio (Stubben and Milligan 2007), XLConnect (Mirai Solutions GmbH 2010), and lmtest (Zeileis and Hothorn 2002) while following the advice of Morris and Doak (2002).

The stage-based transition matrices were created from stage, survival, and fertility data from restoration sites five and seven from data collected in mid to late June from the years 2015 (Perkins 2015), 2016 (Goff and Kelly unpublished), 2017, and 2018. We chose this time period because the summer is both *T. stoloniferum's* growing season and the field season when monitoring is most easily done, plus, that period of summer is ideal for our analyses because *T. stoloniferum* is roughly at its peak in mid to late June, before new rooting crowns are produced from the stolons. Fertility was measured as the arithmetic mean number of offspring from all individuals in the same stage from the previous census (Morris and Doak 2002). Reproduction of new plants was used in the modeling regardless if the offspring survived to the next sampling period, as recommended for this type of modeling (Morris and Doak 2002).

For the purpose of the analyses conducted, the modeling for the populations were conducted based on three stages, stage two, stage three, and a single advanced stage comprised of all plants in stages four through six. The number of stages was condensed for the purpose of our analyses because stage one plants, seedlings, are not seen in the restoration populations because fruits are harvested before they drop off and because stage five and stage six plants are rarely seen in mid to late June, which was the time frame used for our analysis.

Results

Stage validity assessment of the relationship between the total length of stolons per plant and reproduction found a significant positive relationship ($p < 0.001$, Figure 2). Stage validity assessment of the relationship between the number of nodes per rooting crown and reproduction found a significant positive relationship ($p < 0.001$, Figure 3).

For restoration population five, a dominant eigenvalue of 3.04 was found. The stable stage distribution (Table 3), shows that over time, once the population becomes stabilized, stage two plants and stage three plants will each make up 43% of the population while advanced stages will make up the remaining 14%. The reproductive values of stage two plants, stage three plants, and advanced stage plants are 1.0, 16.4, and 14.56, respectively. The elasticity matrix for restoration population 5 values stage two plants staying a stage two from year to year at 0.001, stage two plants advancing to a stage three at 0.04, stage twos advancing to an advanced stage at 0.008, stage three plants regressing to stage two plants at 0.04, stage three plants staying stage threes at 0.56, stage three plants advancing to advanced stages at 0.14, advanced stages regressing to stage twos at 0.0, advanced stages regressing to stage threes at 0.14, and advanced stages staying advanced stages at 0.07 (Table 4).

For restoration population seven, a dominant eigenvalue of 1.35 was found. The stable stage distribution (Table 5), shows that over time, once the population becomes stabilized, stage two plants will make up 35% of the population, stage three plants will make up 41% of the population, and advanced stage plants will make up 24% of the population. The reproductive values of stage two plants, stage three plants, and advanced stage plants are 1.0, 5.34, and 3.31, respectively. The elasticity matrix for restoration population seven values stage two plants staying a stage two from year to year at 0.0, stage two plants advancing to a stage three at 0.05, stage two plants advancing to an advanced stage at 0.06, stage three plants regressing to a stage two at 0.12, stage three plants staying a stage three at 0.49, stage three plants advancing to an advanced stage at 0.06, advanced stage plants regressing to a stage two at 0.0, advanced stage plants regressing to a stage thee at 0.12, and advanced stage plants staying at an advanced stage at 0.12 (Table 6).

Discussion

The stage validity assessments show that because both the number of nodes per rooting crown and total stolon length are positively related to reproduction, that they qualify as criteria for classifying plants into distinct life history stages.

For restoration population five, the dominant eigenvalue, 3.04, indicates that over time, assuming no environmental stochasticity changes restoration population five, that the growth rate will eventually stabilize at 3.04% per year. For restoration population seven, this value indicates the population's growth rate will converge at 1.35% per year over time. For restoration population five, the stable stage distribution, again assuming that no environmental stochasticity changes the restoration population, indicates that as the population stabilizes over time, stage two and stage three plants will make up equal portions and most of the population, while there will be a smaller amount of advanced stages present. For restoration population seven, these values indicate that stage three plants will make up most of the population. The reproductive values for both restoration populations indicate that stage three plants overall contribute more to reproduction than stage two plants and advanced stage plants. Although per plant, advanced stages reproduce more, they are less common and thus appear to not contribute as much overall as stage three plants. The elasticity matrix indicates with a value of 0.56 for population five and a value of 0.49 for population seven that any change of stage three survival from year to year will have the greatest impact on the dominant eigenvalue of the population. It is important to note that although the stable stage distribution and the dominant eigenvalue given by these analyses are useful, as environmental stochasticity or other factors are added into the modeling, the distribution and growth rate values may change (Bierzychudek 1999).

When comparing the stage-based population viability analysis results to those of the count-based analysis, the dominant eigenvalue or growth rate for restoration population 5 is 3.04% while the count-based analysis has the growth rate at 1.57%. The main difference between the two analyses with respect to how the growth rates are calculated is that the count-based analyses include variance over the years sampled so that stochasticity is factored in, while this type of stage-based analysis assumes there isn't any environmental stochasticity. Additionally, the count-based analysis gives what the growth rate has been over the years sampled, while the stage-based analysis provides what the growth rate is projected to converge onto in the future. Thus, the count-based analysis provides a more accurate representation of what is currently occurring within the population while the stage-based analysis gives a better picture of where the population will head towards. However, because the stage-based analysis used does not account for environmental stochasticity and does not have a way knowing where to cap the population at, it is an overestimate of what the actual growth rate will be. More advanced stage-based analyses can better factor in stochasticity to give a more accurate growth rate, but the value of the stage-based analyses used is that it provides other important demographic information about what life history stage is most important for management.

When managing for endangered plant species, it is important for managers to be as efficient as possible because of the relatively limited funding that is available for endangered and threatened plant species, particularly considering how many plant species are in trouble. In 1990, endangered and threatened plant species under the endangered species act only received 8% of the recovery funds, even though they consisted of roughly half of the list in 1990 (Schemske et al. 1994). This disproportionately low funding for conservation of plant species has persisted and is still a problem managers must deal with to this day.

Population viability analyses are better than simply looking at basic population growth from year to year because population viability analyses provide the geometric growth rate, which is more accurate than the standard arithmetic growth rate, the variability from year to year, and ultimately provide a probability of quasi-extinction at some time period modeled out into the future. Although there are extreme confidence intervals within the count-based analyses (Tables 1 and 2), this is commonplace for these type of analyses and the data is still considered useful. Population viability analyses can additionally be used as a way to adaptively manage populations and species of conservation of concern because not only do the analyses provide information about the current statues of the assessed populations, they also provide information that can inform future management, such as which life history stages contribute most to reproduction (Boyce 1992). Adaptive management allows for simultaneously managing and learning about a species of conservation concern, which can more than pay for itself when the additional knowledge these approaches provide allow for more efficient management of the species in question (Williams 2003).

Potentially the greatest benefit of the stage-based analyses results is that it is now known that stage three plants provide the most reproduction. Although more research will be needed to confirm this, based on field observations, it appears that stage three plants tend to occur more frequently in mildly disturbed habitat while the larger, more advanced stages tend to occur more frequently in more heavily disturbed habitat. This means that a simple, occasional mowing treatment may be both relatively easy to do and provide better results than more extreme and/or repeated alterations to *T. stoloniferum* habitat.

Furthermore, although past genetic research has been conducted and indicated that larger populations had more genetic diversity than smaller populations, this research is over 20 years old (Crawford and Windus 1995). Modern genetic analyses of *T. stoloniferum* populations are critically needed as not only has genetic analyses advanced drastically over the past couple of decades, but genetic diversity in endangered and threatened species is critically important to know in order to mitigate the chance of genetic bottlenecking. This is of particular concern with *T. stoloniferum* because most of the species reproduction is done asexually.

Future demographic modeling of *T. stoloniferum* should include factors such as environmental stochasticity and density dependence because these factors are important in better determining population viability (Akcakaya 2000). For example, past research has shown that the weather over a specific growing season for *T. stoloniferum* can alter growth rates (Perkins 2015). However, although some future developments in the demographic modelling of *T. stoloniferum* should be done, it is important to note that some of the even more advanced modeling requires complicated data and modeling that is not worth many plant ecologists and conservationists efforts, or in other words, once modeling gets past the next few steps, there are diminishing returns in what data scientists get for their efforts (Crone et al. 2011). Although the analysis used does not consider the lifespan or age of the individuals in the population, because clonal species typically do not experience senescence and *T. stoloniferum* does not show any symptoms of senescence, the exclusion of this factor likely has little impact on the analysis (Tanner 2001). Yet, population viability analyses can only really be used for quantitative recovery criteria if more long-term data sets and more complex modeling are done, there is a fine line between the right amount of modeling and excessive (Zeigler et al. 2013).

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Figures

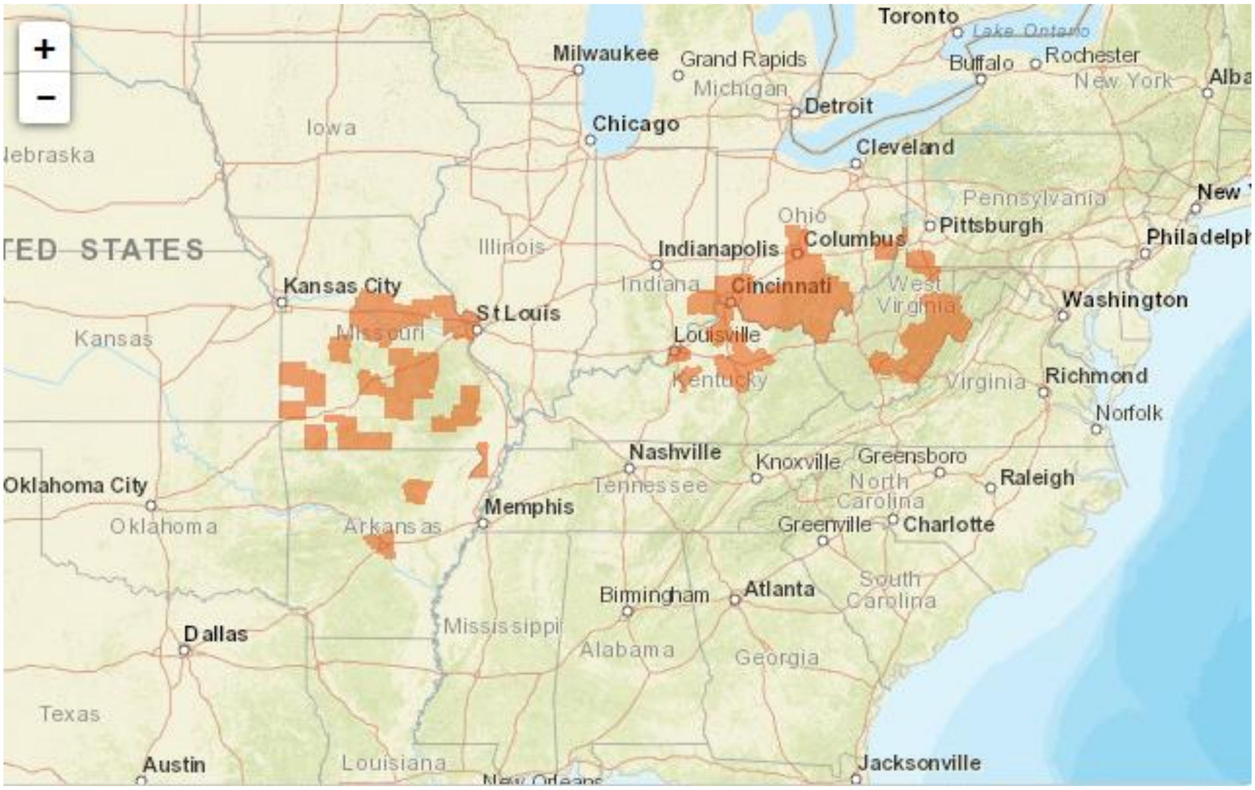

Figure 1. Current Range of *Trifolium stoloniferum* (USFWS 2019)

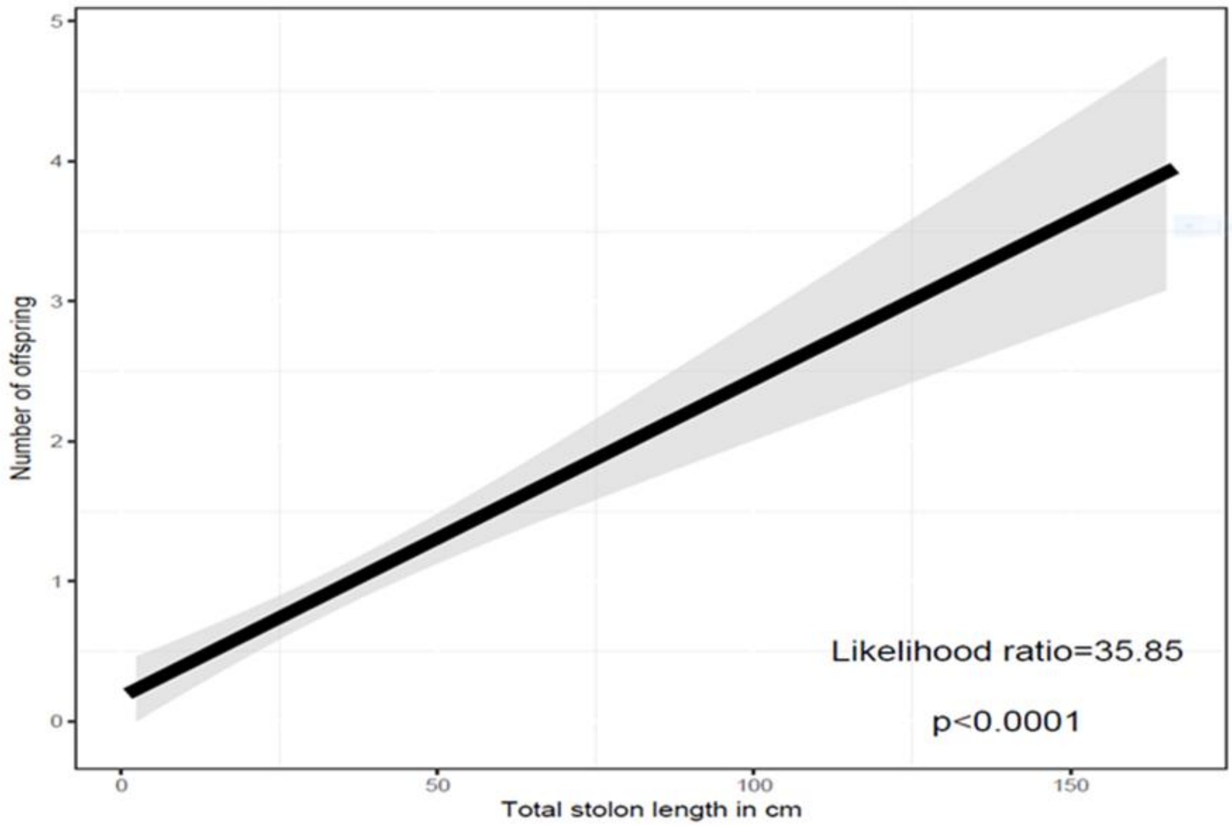

Figure 2. Total stolon length of *Trifolium stoloniferum* individuals within the restoration populations at Taylor Fork Ecological Area in Madison County, Kentucky in centimeters compared to the number of offspring reproduced

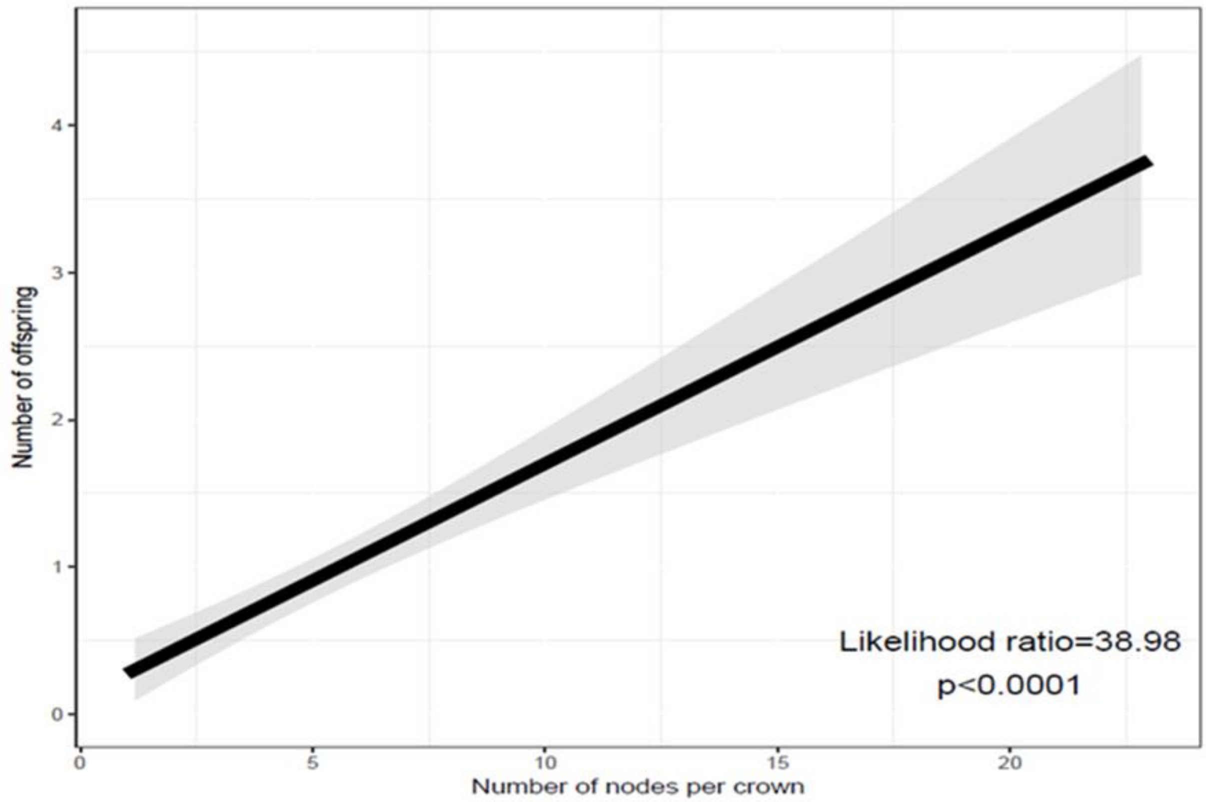

Figure 3. Total number of nodes per rooting crown of *Trifolium stoloniferum* individuals within the restoration populations at Taylor Fork Ecological Area in Madison County, Kentucky compared to the number of offspring reproduced

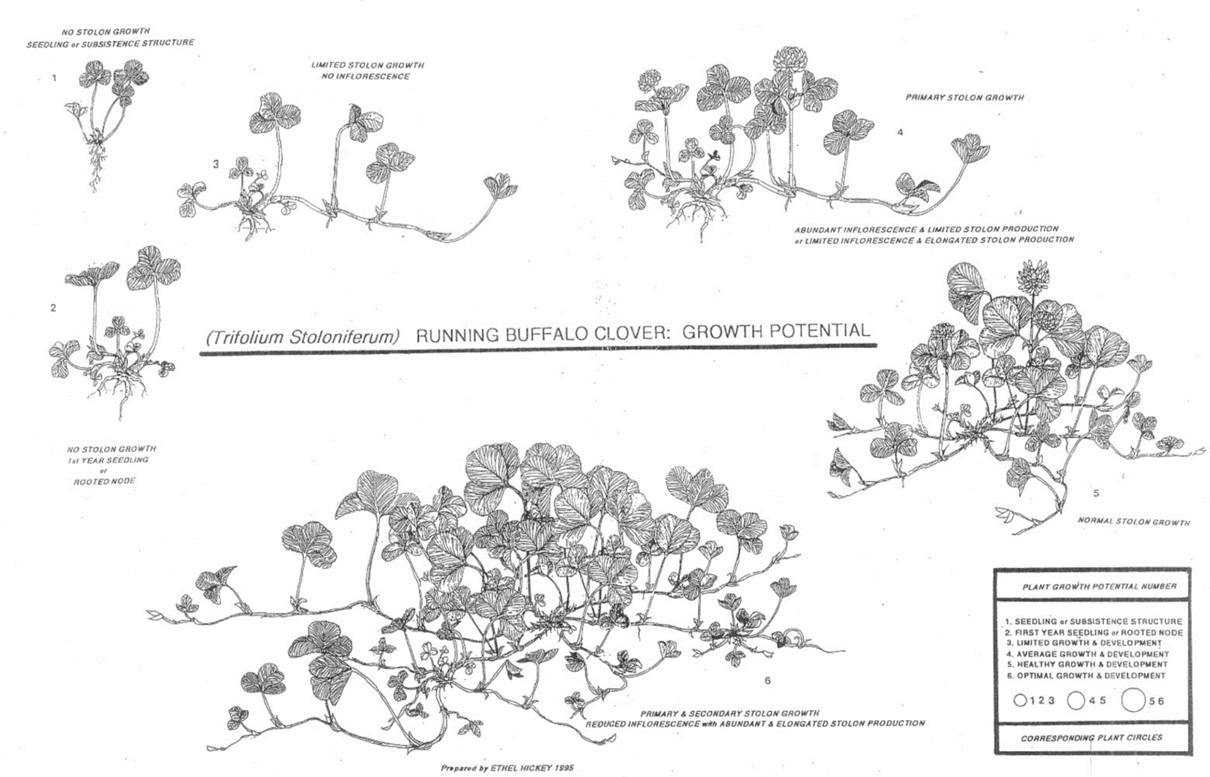

Figure 4. Life history stages of *Trifolium stoloniferum* as proposed by Ethel Hickey in 1995

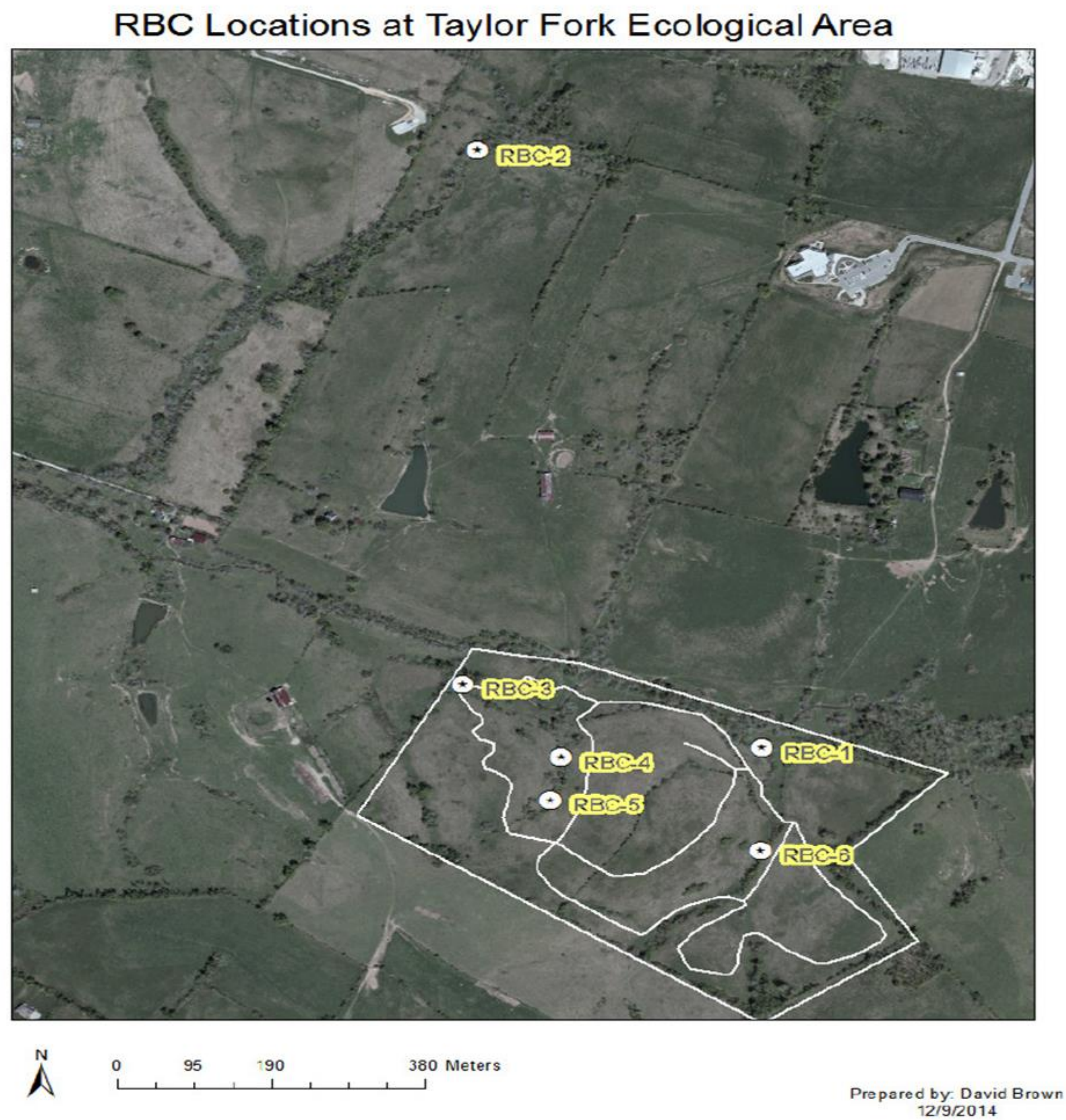

Figure 5. Map of *Trifolium stoloniferum* restoration population location at Taylor Fork Ecological Area in Madison County, Kentucky

Figure 6. Illustration of *Trifolium stoloniferum* depicting identifying characteristics (Burkhart 2010).

Tables

Site	μ	σ^2	Starting ramet count	Years	Probability of extinction (10 years)	Lower 95% CI	Upper 95% CI
2	0.17	1.947	10	3	0.817	0.03	1
3	.0575	0.124	101	3	0.001	0.01	0.39
4	0.402	0.405	45	4	0.061	0.01	0.99
5	0.195	0.32	24	4	0.104	0.01	0.99
6	0.096	0.777	4	3	0.408	0.01	1
7	0.337	0.054	11	3	0.001	0.01	0.18

Table 1. Count-based population viability analyses results for the restoration populations of *Trifolium stoloniferum* located at Taylor Fork Ecological Area, Madison County, Kentucky

E.O	μ	σ2	N_{2018}	Prob. Of Ext (50 yrs)	Lower 95% CI	Upper 95% CI	DW	Rank
46	0.191	0.213	1793	0.041	0	0.796	1.513	A
64	0.190	0.324	715	0.046	0	0.852	1.887	B
34	-0.066	0.479	524	0.181	0	0.994	1.508	B
59	0.139	0.377	239	0.103	0	0.983	1.945	B
51	0.060	0.183	234	0.057	0	0.952	2.264	B
56	0.015	0.455	202	0.108	0	0.967	1.532	B
40	0.000	0.486	120	0.230	0	0.986	1.091	B
52	-0.074	0.450	61	0.363	0	0.999	1.930	C
63	0.183	0.435	24	0.452	0	1.000	1.164	D
35	-0.010	0.325	12	0.266	0	0.998	0.962	D

Table 2. Count-based population viability analyses results for the elemental occurrences of *Trifolium stoloniferum* located at the Blue Grass Army Depot in Madison County, Kentucky

	Stable Stage Distribution	Reproductive Value
2	0.43	1.0
3	0.43	16.4
Advanced	0.14	14.5

Table 3. Stable stage distribution and reproductive values of *Trifolium stoloniferum* restoration population five at Taylor Fork Ecological Area, Madison County, Kentucky for stage 2, stage 3, and advanced stage plants

	2	3	Advanced
2	0.001	0.04	0.008
3	0.04	0.56	0.14
Advanced	0	0.14	0.07

Table 4. Elasticity matrix for restoration population five at Taylor Fork Ecological Area in Madison County, Kentucky

	Stable Stage Distribution	Reproductive Value
2	0.35	1.0
3	0.41	5.34
Advanced	0.24	3.31

Table 5. Stable stage distribution and reproductive values of *Trifolium stoloniferum* restoration population seven at Taylor Fork Ecological Area, Madison County, Kentucky for stage 2, stage 3, and advanced stage plants

	2	3	Advanced
2	0.0	0.046	0.059
3	0.105	0.494	0.059
Advanced	0.0	0.119	0.116

Table 6. Elasticity matrix for restoration population seven at Taylor Fork Ecological Area in Madison County, Kentucky