

A Review of the Current Status of Running Buffalo Clover (*Trifolium stoloniferum*) in Ohio

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Abstract: Running Buffalo Clover, *Trifolium stoloniferum*, is a federally protected plant with two populations in Ohio. Although it had been generally accepted as extinct, populations were found in Ohio in 1985. Habitat losses, reduced soil disturbance, and invasive species have been major causes of declines in Running Buffalo Clover (RBC). This study investigated the status of RBC and provided additional evidence on several species impacting RBC's survival. Closely associated species are not well understood; however, endangered species of sage, bison, and elk are likely the most important associated species for RBC's survival, while white-tailed deer, rabbits, and cattle are detrimental. Several findings in this report support an important association with bison or elk. Root nodules are lacking in RBC, though they are present in closely related species of clover. Bison and elk manure is high in nitrogen, and RBC has low organic nitrogen-fixing abilities. There is an inverse relationship between available nitrogen and plants' nitrogen fixation capabilities. RBC requires a soil disturbance frequency that seems similar to bison migration frequencies. Recovery effort plans are now nine years old and current information about the plant's distribution and recovery is lacking. RBC still experiences declines in remaining areas and is in need of more robust management plans. Future investigations should focus on discovery of other suitable areas in Ohio for introduction, genetic analysis of RBC's ability to resist parasites, and the importance of bison and elk to RBC seed germination and survival.

Key Words: Running Buffalo Clover, *Trifolium stoloniferum*, endangered plant, Ohio

Introduction

Running Buffalo Clover (RBC), *Trifolium stoloniferum*, is an endangered plant found in a few fragmented locations in West Virginia, Indiana, Kentucky, and southwestern Ohio (US Fish and Wildlife Service, 2007). RBC is a perennial legume that produces runners with trifoliate leaves extending from one rooted crown system (Campbell et al., 1988). Its small white flowers appear in April and last until about June, resembling the common white clover (USFWS, 2007). RBC's natural habitat includes forest edges, clearings, and trails where there is some sun exposure (Bartgis, 1985; Cusick, 1989). RBC also requires elevated ground with layers of limestone underneath the soil (Andreas, Mack, and McCormac, 2004; Madarish and Schuler, 2002). Shortly after the arrival of European settlers in the Midwest, RBC began to decline due to habitat loss, elimination of disturbances, loss of associated species, invasive species, and possible diseases (Aldrich, Bacone, and Homoya, 1986; Brooks, 1983; Madarish and Schuler, 2002; USFWS, 2007). RBC was considered to be extinct, but in the mid-1980s, small populations were found in Ohio and in surrounding states (Bartgis, 1985). Unlike many legumes, RBC has low nitrogen-fixing capabilities and therefore grows in slightly acidic soil and may require large herbivores for effective seed germination (Morris et al., 2002). Disturbances produced by large herbivores, occasional mowing, logging, and fires are also important in sustaining the species as well as preventing invasive species or more competitive plants (Homoya, Aldrich, and Jacquart, 1989; Madarish and Schuler, 2002). Although Campbell et al. (1988) and Cusick (1989) claimed that RBC was reliant on American bison (*Bison bison*), there is little evidence to support this claim.

RBC is listed as endangered at federal and state levels and much attention has been focused on recovery efforts (Madarish and Schuler, 2002). RBC still requires continued management and monitoring (Scott et al., 2010) as evidenced by the Ohio Natural Heritage Program's (ONHP, 2007) finding that RBC populations have continued to decline in Ohio. With the USFWS (2007) recovery plan now nine years old, there is a need for current information on the status of RBC's population size, distribution, and the effectiveness of recovery plans for Ohio and other states within RBC's historic range. The purpose of this paper is to review the current status of RBC in Ohio and investigate important associated species.

Discussion

In Ohio, RBC was common in eight counties, but currently only two populations exist across three counties (ONHP 2007). RBC has the ability to penetrate the tough, compacted soil that characterizes much of Ohio and can convert the soil to a higher-quality soil (Madarish and Schuler, 2002). RBC also has the ability to withstand soil disturbances and droughts, making it an important part of pastures, where it can provide a food source for production animals (Morris et al. 2002; Taylor et al., 1994). RBC has a natural resistance to nematode infections that most cultivated clovers lack, making it agriculturally significant and an interesting subject for pathogen study (Quesenberry et al., 1997). RBC is an integral part of its ecosystem (Singha, Baker, and Bhatia, 1988) because of its important association with other plant species such as sedge, white snakeroot, and deer-tongue (Madarish and Schuler, 2002).

Current Threats. Habitat destruction began in the 1700–1800s, when settlers cleared almost all of RBC’s native range for agriculture and timber (Brooks, 1983; Campbell et al., 1988; USFWS, 2007). Cully, Cully, and Hiebert (2003) estimated that only 1% of the original North American prairie remains. This area was RBC’s historic range and also supported vast herds of bison and elk. Remaining habitat is highly fragmented and faces ongoing threats from continued development and the addition of invasive species (Allen, 1994; Burkhart, Rentch, and Schuler, 2013; Morris et al. 2002). This loss of key habitat has been cited as one of the main causes of decline in RBC and also played a role in the extirpation of other associated species (Campbell et al., 1988; Cusick, 1989; Ford et al., 2003; Morris et al., 2002; USFWS, 2007). Disease has been cited as a contributor to RBC’s decline (Bartgis, 1985), yet this seems speculative because no evidence of disease has been discovered in wild populations (USFWS, 2007). Mildew has been found growing on RBC, though it had little effect on survival (Campbell et al., 1988). Still, disease should not be excluded from the list of threats given the low genetic diversity of RBC populations (Hickey, Vincent, and Guttman, 1991; Kongkiatngam et al., 1995).

Regular habitat disturbances are vital for reducing RBC’s competitors, such as trees and other secondary growth (Burkhart, Rentch, and Schuler, 2013; Cully, Cully, and Hiebert, 2003; Madarish and Schuler, 2002). The frequency and intensity of these habitat disturbances are important, though the measures have not been firmly established. Timber forests, typically cleared about once every 10 years, were found to have several populations of RBC growing along the logging trails (Madarish and Schuler, 2002). Logging has been reduced in Ohio, leading to a decrease in disturbances and an increase in competitors (Burkhart, Rentch, and Schuler, 2013; Pavlovic, 1994). Following the reduction in logging, the Ohio Department of Natural Resources estimates that Ohio’s forest cover has improved from a low of 15% in 1940 to more than 30% by 1994 (ODNR, 1994). While the increase in forest cover may be hailed as an environmental success, it coincides with the recent declines in RBC populations. The loss of the disturbance events may also have left the soil less compacted, to the detriment of RBC, but allowing other species to dominate the area (Burkhart, Rentch, and Schuler, 2013).

Closely Associated Species. The relationships and importance of closely associated species and RBC are not well understood, though several are described. Madarish and Schuler (2002) found that sedge, white snakeroot, hog peanut, and deer-tongue were often found with RBC. According to the ODNR (2013a), more than 55 species of sedge and 30 species of grasses are in decline, which may contribute to RBC’s decline. Interestingly, Morris et al. (2002) reported that deer-tongue, which is not endangered, was always found among the areas with RBC. Herbivores play an integral role in RBC’s life cycle, but no actual evidence has been provided to support any type of special relationship with any particular herbivore (Campbell, 1988; Cusick, 1989). Establishing which species of herbivores are important associates has been difficult. Large herbivores, such as elk and bison, were thought to be important, but by 1850, both species had been extirpated throughout RBC’s range (Cox, 2011; Ford et al., 2003; ODNR, 2008). Research suggests that large herds of elk and bison grazing on RBC across large migratory routes likely provided ideal conditions with regular disturbances, effective seed dispersal, and manure that was high in nitrogen (Cox, 2011; Homoya, Aldrich, and Jacquart, 1989; Madarish and Schuler, 2002; Morris et al., 2002). Additional evidence comes from a comparison of RBC to other closely related species. Unlike closely related clovers, RBC has poor nitrogen-fixing abilities and lacks root nodules (Morris et al., 2002). In contrast, red clover (*Trifolium pratense*) has root nodules and is efficient at nitrogen fixation (Sturz et al., 1997). Large herbivores have shown a strong preference for RBC (Miller, Bratton, and Hadidian, 1992) and their manure is high in nitrogen, vital to RBC’s survival (Morris et al., 2002). Ledgard and Steele (1992) reported that plants’ nitrogen fixation ability decreases as the concentration of nitrogen in the soil increases. It is likely that RBC evolved reduced nitrogen-fixing abilities as large herbivores’ manure provided an abundant source of nitrogen.

With the extirpation of large herbivores, populations of smaller herbivores such as white-tailed deer and rabbits increased (Ford et al., 2003; Cox, 2011). Since 1970, the white-tailed deer population in Ohio has grown from 17,000 to 750,000 (ODNR, 2013b) and the population of Eastern cottontail rabbits is far larger now than before settlers arrived (ODNR, 2014). While elk and bison migrate across wide ranges, white-tailed deer, rabbits, and cattle remain in much smaller areas (Cox, 2011; Ford et al., 2003; USFWS, 2007). In contrast, longer migrations of elk and bison may have provided wide seed dispersal and the correct frequency of disturbances (Burkhart, Rentch, and Schuler, 2013; Ford et al., 2003; USFWS,

2007). Although white-tailed deer and rabbits have been proposed as replacements for large herbivores (Cusick, 1989), other evidence refutes this. Campbell (1988) found that RBC declined when rabbits and white-tailed were allowed to graze in the areas. RBC sites that were grazed by cattle also experienced declines, likely due to their intense and destructive grazing habits (Burkhart, Rentch, and Schuler, 2013; Pavlovic, 1994). The highly intense grazing habits of cattle, white-tailed deer, and rabbits may have selected for more competitive clovers or invasive species (Ford et al., 2003). Additionally, RBC seeds consumed by white-tailed deer showed decreased rates of germination and survival, indicating a specific vector for seed germination (Ford et al., 2003). RBC germination requires scarification of the outer seed coat (Campbell et al., 1988; Morris et al., 2002). It is possible that only the dentition of large herbivores or the more extensive digestive systems of bison and elk provides enough scarification along with adequate time in an acidic environment for RBC seed germination (Ford et al., 2003).

Recovery Efforts. Recovery efforts for existing populations are focused around the Cincinnati area and Wayne National Forest in southeast Ohio (USFWS, 2007). The former is protected and managed by the ODNR and Ohio Historical Society and the latter is managed by ODNR (USFWS, 2007). After successful reintroductions of RBC in the 1980s, a drastic decline occurred in 2005, possibly from an unknown disease and overgrazing (USFWS, 2007). In rare plant reintroduction efforts, initial successes followed by sudden collapses are common and the causes are usually unknown (Allen, 1994). Although disease cannot be completely ruled out, evidence to support the claim has not been found. The most plausible cause of recent RBC declines is overgrazing by white-tailed deer and rabbits. White-tailed deer and Eastern cottontail rabbits were most numerous in counties where RBC populations are found, and both were at record highs between 2003-2005 when the RBC populations declined (ODNR, 2011; 2014). Miller, Bratton, and Hadidian (1992) found that white-tailed deer showed a preference for RBC and quickly decimate populations. Given that the highest threat to survival seems to be from white-tailed deer and rabbits, recovery efforts must eliminate these herbivores or restrict their access to RBC locations.

Selecting additional recovery sites may improve the overall effectiveness of conservation efforts for RBC. Elevation and the presence of limestone formations are characteristic of the soil in areas around west and central Ohio (Ohio Division of Geological Survey, 2006). There may be several areas that could be ideal RBC habitat and support reintroductions. Another option is to reintroduce RBC to areas with existing herds of bison or elk. Cox (2011) referenced several isolated RBC individuals at elk reintroduction sites in Kentucky. Reintroductions at these sites could increase the number of individuals and eventually form another population.

Combining Efforts to Improve Conservation. While not all plants can produce roots from cut stems, RBC has shown the ability to produce new roots in artificial media (Singha, Baker, and Bhatia, 1988). This makes RBC an ideal candidate for research at the Cincinnati Zoo's Carl H. Lindner Jr. Family Center for Conservation and Research of Endangered Wildlife (CREW, n.d.). The facility is located in Cincinnati, Ohio, and specializes in rare species on the brink of extinction. Specimens at this facility are preserved in artificial media and kept in long-term storage. A closely related native species, *Trifolium calcaricum*, has had similar declines, yet no specific causes were determined (Collins and Wieboldt, 1992). Similarities exist between RBC and *T. calcaricum*, especially in terms of nitrogen fixation, and conservation efforts could be combined.

Understanding the importance of associated plant and animal species has been difficult because wild populations of RBC are often rare and literature is lacking. While there is no information to indicate that research is being done on RBC at sites where large herbivores have been reintroduced, it could be an opportunity to investigate and monitor them in their natural environment. Studies of elk reintroduced to abandoned strip mines in Kentucky (Cox, 2011) could be combined with RBC investigations because there is an important association between the two species. This area contains suitable habitat and isolated individuals have been observed (Cox, 2011; USFWS, 2007). The Wilds is a 10,000-acre park owned by the Columbus Zoo and Aquarium (2012), located in Cumberland, Ohio. The park has a herd of bison (Columbus Zoo and Aquarium, 2012) and contains areas that meet RBC's habitat requirements (Ohio Division of Geological Survey, 2006). The park may be able to support recovery efforts of RBC and provide an opportunity for additional research into the relationship of bison and RBC.

Future Investigation. All known populations of RBC exist in the southwest corner of Ohio and in the Wayne National Forest (USFWS, 2007). Future investigation should focus on finding new populations and new areas for reintroduction. Allen (1994) provided hopeful information about reintroduction through transplantation; however, there is no data to evaluate the long-term effectiveness of this method. USFWS (2007) also discussed reintroduction, but specific methods and successful long-term results were absent. Many of Ohio's counties have significant elevation and are rich in limestone (Ohio Division of Geological Survey, 2006), which may support unknown populations or could host reintroductions. Ohio has many abandoned coal mines in counties near existing populations of RBC (Harris, 2009). The existence of elk and bison in neighboring states creates an opportunity to evaluate the shared successes and investigate further reintroductions. A shared conservation project between these states could also provide an opportunity to investigate the importance of endangered species and closely associated

species. RBC is an ideal plant for researchers at the Cincinnati Zoo CREW facility, though no information could be obtained to determine if this has already been done. Further investigation is needed to understand the genetics and mechanisms of RBC's natural pest resistance. If this property could be better understood, it could lead to a reduction in the use of agricultural pesticides. The threat of disease must still be considered because remaining populations of RBC have low genetic variation and may have no resistance to current or future diseases (Hickey, Vincent, and Guttman, 1991; Kongkiatngam et al., 1995). The low genetic diversity of RBC identified by Kongkiatngam et al. (1995) will continue to be of concern and requires continued monitoring of remaining populations.

Conclusions

Running Buffalo Clover is an endangered plant that exists in fragmented populations across its former range. It is an important species for agriculture as well as a resource that can provide insight for agricultural pest management. Main threats include habitat loss, reduction of disturbances, and invasive species (Madarish and Schuler, 2002). Within Ohio, two areas are home to the remaining populations and are the focus of recovery efforts. Newer insight and compiled evidence supports that white-tailed deer and rabbits are a larger threat than previously thought. Current declines in remaining RBC populations are likely caused by the rapid growth of Ohio's white-tailed deer and rabbit populations. Several findings in this report support an important association with bison or elk. Root nodules are lacking in RBC, though they are present in closely related species of clover. Bison and elk manure is high in nitrogen and RBC has low organic nitrogen-fixing abilities. There is an inverse relationship between available nitrogen and plants' nitrogen fixation capabilities. RBC requires a specific soil disturbance frequency that could be provided by migratory herbivores such as bison and elk; however, intense, destructive grazing by white-tailed deer, rabbits, and cattle is detrimental. Sedge, deer-tongue, bison, and elk have important close associations with RBC and the effect of closely associated species is more critical to the survival of RBC than previously thought. The loss of these closely associated species has been the most significant factor in RBC's decline. Cattle, white-tailed deer, and rabbits should be restricted from all recovery sites. RBC's low genetic diversity is a concern, though disease still does not seem to pose an immediate threat. More work is needed in combining RBC conservation efforts with other endangered species. This would benefit multiple species and foster understanding of the nature and importance of closely associated species. Future investigations should seek to evaluate the status of reintroduction efforts, the frequency and intensity of disturbances, and the genetics and mechanisms of parasite resistance. After a thorough review of the literature, information on current distribution and status of recovery of RBC is lacking.

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