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Running Buffalo Clover (*Trifolium stoloniferum*) Recovery Plan: First Revision

June 2007

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Department of the Interior
U. S. Fish and Wildlife Service
Great Lakes-Big Rivers Region (Region 3)
Fort Snelling, MN

Running Buffalo Clover (*Trifolium stoloniferum*) Recovery Plan: First Revision

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Literature Citation

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Availability

Recovery Plans can be downloaded from the U.S. Fish and Wildlife Service website: http://www.fws.gov/endangered/recovery/index.html

Cover photo of running buffalo clover by Sarena M. Selbo, USFWS.

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EXECUTIVE SUMMARY
Running Buffalo Clover Recovery Plan: First Revision

Current Species Status: Running buffalo clover (*Trifolium stoloniferum*) occurs in 101 populations in three geographical regions: Appalachian (West Virginia and southeastern Ohio), Bluegrass (southwestern Ohio, central Kentucky and Indiana), and the Ozarks (Missouri). The majority of populations occur within the Appalachian and Bluegrass regions, with the largest population in West Virginia and the most populations in Kentucky. Running buffalo clover was listed as endangered in 1987. At the time of listing only one population was known; in 1989 when the original recovery plan was completed, running buffalo clover was known from 13 populations. This is the first recovery plan revision.

Habitat Requirements and Limiting Factors: Running buffalo clover occurs in mesic habitats of partial to filtered sunlight, where there is a prolonged pattern of moderate periodic disturbance, such as mowing, trampling, or grazing. It is most often found in regions underlain with limestone or other calcareous bedrock. The primary threat to running buffalo clover is habitat alteration. Factors that contribute to this threat include natural forest succession, and subsequent canopy closure, competition by invasive plant species, permanent habitat loss through development or road construction, and may include the elimination of bison and other large herbivores.

Recovery Strategy: Running buffalo clover was listed under the Endangered Species Act (ESA) because the few known populations were threatened by habitat alteration. Current threats to the species include habitat destruction, habitat succession, and invasive plant competition. In addition to these threats, inherent biological vulnerabilities for this species include its reliance on pollinators, seed scarification, and dispersal mechanisms as well as a dependence on disturbance.

Since its listing in 1987, several positive outcomes have been realized due to recovery implementation: 1) more information is available regarding the species biology; and 2) the known number of populations has dramatically increased as survey efforts have expanded throughout the historic range. Although many of the threats to running buffalo clover populations still exist, some initially identified potential threats do not appear to be a risk to the species.

Recovery of running buffalo clover will be achieved by implementing actions which address the species distribution, numbers, and threats. Given the known threats and constraints, this recovery effort focuses primarily on increasing the number of protected and managed populations, determining the viability of existing populations, and research into the species ecological requirements. Key to this strategy is the protection and ecological management of various-sized populations of running buffalo clover throughout its geographic range. The recovery criteria and subsequent recovery actions rely heavily on retaining and managing the habitats on which running buffalo clover needs to maintain viability. In addition, the recovery strategy relies on a greater understanding of the biotic and abiotic needs of running buffalo clover. Numerous scientific studies have started to shed light on the ecological requirements of

running buffalo clover, but more information is needed to understand the level of periodic disturbance required to maintain the species.

In order to reclassify and eventually delist running buffalo clover, adequate numbers and sizes of populations need to be monitored, protected, and managed and the ecological factors that regulate the populations need to be further defined. Additionally, until these population regulation factors are better understood, the genetic diversity of known populations of all sizes should be conserved.

Recovery Goal and Objectives: The ultimate goal of this recovery program is to remove running buffalo clover from the Federal List of Threatened and Endangered Plants (50 CFR 17.12), with an intermediate goal of reclassification to Threatened. To merit delisting, a minimum number of viable populations should be protected and managed throughout a majority of the species geographic range. Populations are considered protected when there are permanent assurances that the habitat will be managed. Management objectives for running buffalo clover include 1) controlling invasive species, 2) reducing habitat succession, and 3) defining population regulation factors. Additional recovery objectives include 1) ensuring viability of protected populations, 2) maintaining genetic diversity and germplasm, and 3) promoting public understanding of the species.

Recovery Criteria: Running buffalo clover may be reclassified from endangered to threatened when the following criteria are met. Numerical goals are based on most recently available scientific information and are subject to revision as new information becomes available.

1. Seventeen populations, in total, are distributed as follows: 1 A-ranked, 3 B-ranked, 3 C-ranked, and 10 D-ranked populations across at least 2 of the 3 regions in which running buffalo clover currently occurs (Appalachian, Bluegrass, and Ozark). The number of populations required in each rank is based on what would be necessary to achieve a 95% probability of persistence within the next 20 years based on population viability analysis (see Appendix 5). Rankings refer to the Element Occurrence (EO) ranking categories (Table 1).

2. For each A-ranked and B-ranked population described in #1, population viability analysis indicates a 95% probability of persistence within the next 20 years, OR for any population that does not meet the 95% persistence standard, the population meets the definition of viable. For downlisting purposes, viability is defined as follows: A) seed production is occurring; B) the population is stable or increasing, based on at least five years of censusing; and C) appropriate management techniques are in place.

3. The land on which each of the populations described in #1 occurs is owned by a government agency or private conservation organization that identifies maintenance of the species as one of the primary conservation objectives for the site, OR the population is protected by a conservation agreement that commits the private landowner to habitat management for the species. Natural Resource Management Plans on Federal lands may be suitable for meeting this criterion. This criterion will ensure that habitat-based threats for the species are addressed (see Appendix 6).

Running buffalo clover may be removed from the List of Endangered and Threatened Plants (50 CFR 17.12) when the following have been met:

1. Thirty-four populations, in total, are distributed as follows: 2 A-ranked, 6 B-ranked, 6 C-ranked, and 20 D-ranked populations across at least 2 of the 3 regions in which running buffalo clover occurs (Appalachian, Bluegrass, and Ozark). The number of populations in each rank is based on what would be required to achieve a 95% probability of persistence within the next 20 years; this number was doubled to ensure biological redundancy across the range of the species. Rankings refer to the Element Occurrence (EO) ranking categories (Table 1).

2. For each A-ranked and B-ranked population described in #1, population viability analysis indicates 95% probability of persistence within the next 20 years, OR for any population that does not meet the 95% persistence standard, the population meets the definition of viable.[1] For delisting purposes, viability is defined as follows: A) seed production is occurring; B) the population is stable or increasing, based on at least 10 years of censusing; and C) appropriate management techniques are in place.

3. Downlisting criterion #3 is met for all populations described in delisting criterion #1.

Actions Needed:

1. Conserve and manage running buffalo clover populations and the habitat on which they depend.
2. Define population regulation factors.
3. Conserve germplasm and genetic diversity.
4. Promote public understanding.
5. Review and track recovery progress.

Estimated Cost of Recovery (in $1,000's) for first five years:

Year	Action 1	Action 2	Action 3	Action 4	Action 5	**Total**
1	91	40	1	8	0	140
2	91	40	1	5	5	142
3	106	35	1	5	0	147
4	106	35	1	5	5	152
5	106	0	1	5	0	112
Total	500	150	5	28	10	**693**

Date of Recovery: Recovery could occur by 2020 if recovery criteria are met and with adequate funding.

[1] C-ranked and D-ranked populations are not included for the purposes of viability in recovery criteria # 2 due to their inherently small population sizes and marginal habitat quality. Due to the cyclic nature of running buffalo clover and the high probability of small populations blinking in and out, maintaining viability for a specific C-ranked or D-ranked population at a given time may not be possible. Regardless, small populations have displayed high levels of genetic diversity that is important for survival of the species as a whole and thus are included in the recovery criteria referring to protection and management of sites.

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PART I. INTRODUCTION

Running buffalo clover (*Trifolium stoloniferum* Muhl. ex A. Eaton), a member of the Fabaceae (pea family) was formerly known, based upon herbarium records, historical accounts, and scientific literature, from West Virginia to Kansas. It is currently extant in limited portions of Indiana, Kentucky, Ohio, Missouri, and West Virginia (Figure 2). In Ohio, Kentucky and Indiana, populations are centered around the limestone-underlain area in the Bluegrass region. In West Virginia, most populations have been found in regions of limestone-underlain substrate of the east-central part of the state. Some of Missouri's populations are also underlain with limestone (Ozark Dome).

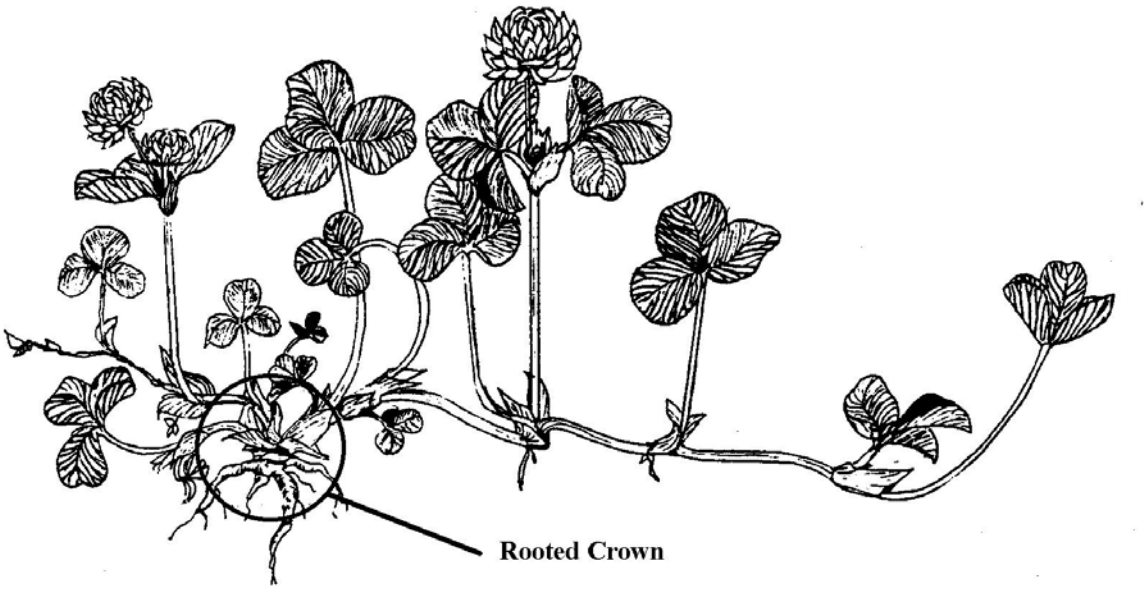
The United States Fish and Wildlife Service (USFWS) designated running buffalo clover as an endangered species on July 6, 1987 (50 FR 21478-21480) (USFWS 1987). The Running Buffalo Clover Recovery Plan was approved on June 8, 1989 (USFWS 1989). This first Revision of the Recovery Plan provides updated information on the status and biology of the species and guides the recovery of running buffalo clover throughout its range. The Recovery Priority Number for this species is 8, which means this species has a moderate degree of threat and a high recovery potential.

SPECIES DESCRIPTION

Running buffalo clover usually acts as a perennial species, forming long stolons that root at the nodes (Figure 1). Plants produce erect flowering stems, 10-30 cm tall that send out long basal runners (stolons). The leaves of the runners have 1-2 cm long ovate-lanceolate stipules, whose tips gradually narrow to a distinctive point (attenuate tip). Erect stems arise from nodes along the stolon, with 2 large trifoliolate leaves at their summit, their obovate leaflets 2-3 cm long and wide (Gleason and Cronquist 1991). Flowering stalks (peduncles) originate from the upper axils, producing 9-12 mm round (sub-globose) flower heads with the corolla white, tinged with purple and exceeding the calyx (Gleason and Cronquist 1991). Running buffalo clover flowers from mid-April to June; fruiting occurs from May to July (Brooks 1983). Brooks (1983) provides a discussion of morphological and distinguishing features for this and related clover species. The chromosome number (2n=16) was found to be the same as that of other clovers native to the eastern United States (Campbell et al. 1988).

Because of the soloniferous growth form, individual plants can be difficult to distinguish. The Running Buffalo Clover Recovery Team has defined an individual plant as a rooted crown. A rooted crown is a rosette that is rooted into the ground (Figure 1). Rooted crowns may occur alone or be connected to other rooted crowns by stolons (or runners). Appendix 1 describes the population monitoring protocol that has been developed utilizing rooted crowns as the basis for censusing.

Figure 1. Illustration of running buffalo clover with stolon growth and flowering stems (Ethel Hickey; reprinted with permission)



POPULATION TRENDS AND DISTRIBUTION

Running buffalo clover has been collected historically from Arkansas, Illinois, Indiana, Kansas, Kentucky, Missouri, Ohio, and West Virginia. There were very few reports rangewide between 1910 and 1983. Prior to 1983, the most recent collection had been made in 1940 in Webster County, West Virginia (Brooks 1983). Although thought to be extinct, running buffalo clover was rediscovered in 1983 in West Virginia (Brooks 1983). At the time of listing only one population was known to exist. Soon after being listed in 1987, several additional populations were discovered in Indiana, Ohio, Kentucky, and West Virginia. Populations were not rediscovered in the wild in Missouri until 1994.

Extant populations of running buffalo clover are known from 101 populations in three eco-regions: Hot Continental, Hot Continental Mountainous, and Prairie Division (Bailey 1998). For recovery purposes, the populations are divided into three regions based on proximity to each other and overall habitat similarities. These regions are Appalachian (West Virginia, and southeastern Ohio), Bluegrass (southwestern Ohio, central Kentucky and Indiana), and Ozark (Missouri). The majority of populations occur within the Appalachian and Bluegrass regions (Figure 2).

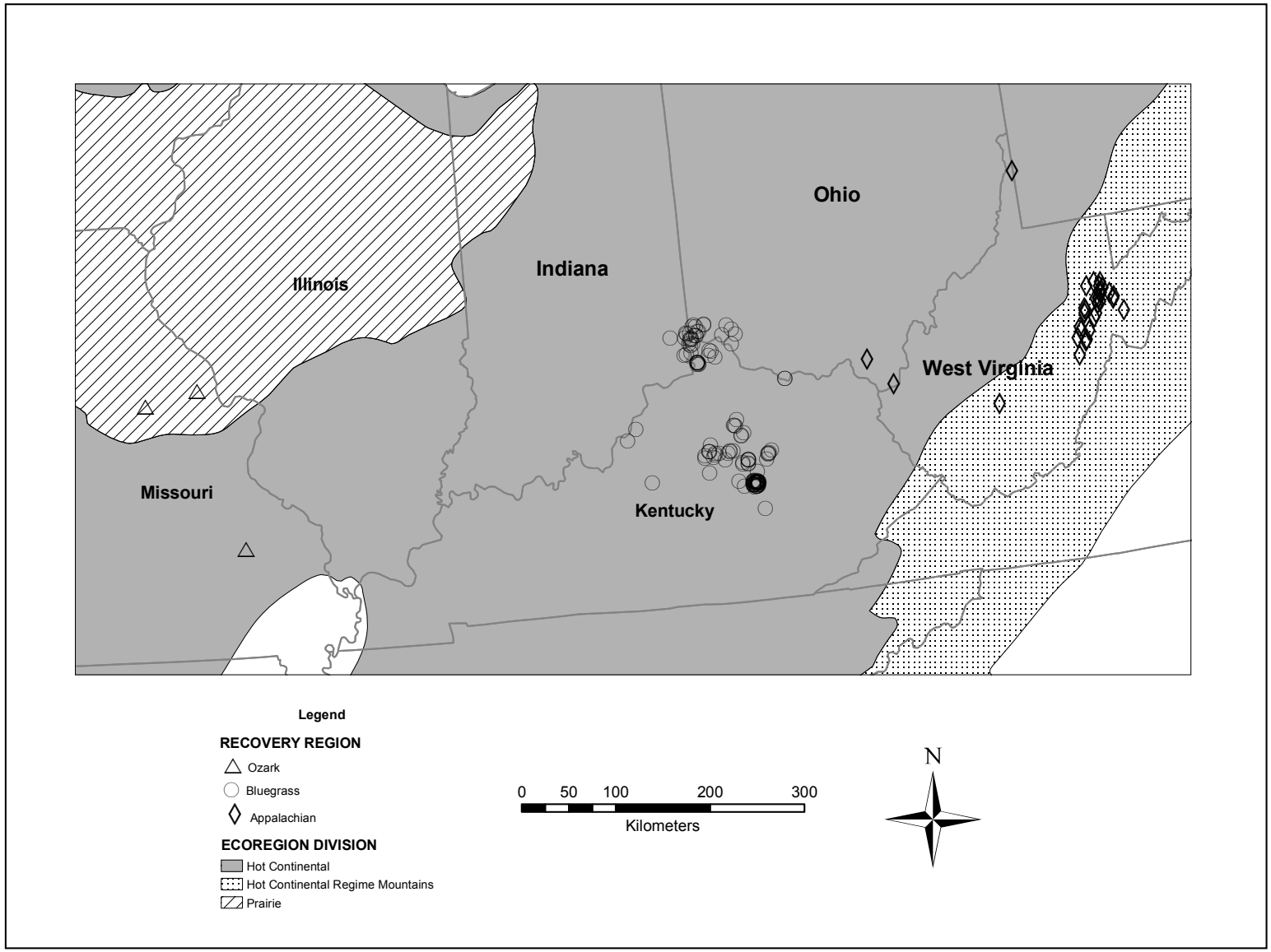
Element occurrence rankings (EOs), which integrate population size and habitat integrity, indicate that known populations fall into all ranking categories (A-D). Table 1 provides an explanation of the specifications used to rank running buffalo clover populations. Ranking criteria were developed by the Recovery Team based on NatureServe's element occurrence specifications criteria (http://www.natureserve.org/prodServices/eodata.jsp). Most of the A-

ranked EOs have been found on the Monogahela National Forest in West Virginia, while the majority of D-ranked EOs are located at the Bluegrass Army Depot in Kentucky. In 2005, the total number of ranked populations included: 10 A-ranked, 25 B-ranked, 27 C-ranked, and 38 D-ranked. Appendix 2 lists all known populations, the state and region in which they occur, EO rank, general habitat, and protection status. For purposes of this Recovery Plan, population and elemental occurrence are one in the same.

Table 1. Elemental Occurrence Ranking Categories

Rank	
A	Population has 1,000 or more naturally occurring rooted crowns. Plants occur in natural suitable habitat (mesic woodland or river terraces) where the disturbance regime is maintained by natural processes (such as large mammal trampling, canopy gap creation, stream scouring); **OR** in somewhat suitable habitat maintained by anthropogenic activities (old roads, jeep trails, "skidder" trails) where disturbance for a prolonged period (such as grazing, trampling, light logging traffic) is mild to moderate.
B	Population has between 100 and 999 naturally occurring rooted crowns. Plants occur in suitable habitat (mesic woodland, river terraces, or partially shaded lawn) where the disturbance regime is maintained by natural processes (such as large mammal trampling, canopy gap creation, stream scouring); **OR** in somewhat suitable habitat maintained by anthropogenic activities (old roads, jeep trails, "skidder" trails, old cemeteries, savannah-like lawns at old home sites) where disturbance for a prolonged period (such as mowing, grazing, trampling, or logging) is mild to moderate.
C	Population has between 30 and 99 naturally occurring rooted crowns. Plants occur in suitable habitat (mesic woodland, river terraces, or partially shaded lawn) where the disturbance regime is maintained by natural processes (such as large mammal trampling, canopy gap creation, stream scouring); **OR** in somewhat suitable habitat maintained by anthropogenic activities (old roads, jeep trails, "skidder" trails, old cemeteries, savannah-like lawns at old home sites) where disturbance for a prolonged period (such as mowing, grazing, trampling, or logging) is curtailed or limited.
D	Population has between 1 and 29 naturally occurring rooted crowns. Plants occur in suitable habitat (mesic woodland, river terraces, or partially shaded lawn) where the disturbance regime is maintained by natural processes (such as large mammal trampling, canopy gap creation, stream scouring); **OR** in somewhat suitable habitat maintained by anthropogenic activities (old roads, jeep trails, "skidder" trails, old cemeteries, savannah-like lawns at old home sites) where disturbance for a prolonged period (such as mowing, grazing, trampling, or logging) is curtailed or limited.

Figure 2. Map of Running Buffalo Clover Occurrences



Missouri

The first survey for running buffalo clover in Missouri was conducted in 1988 based on suitable habitats in areas near historical collection sites. No populations were located. In 1990, a few plants were discovered which had sprouted from seed in topsoil delivered to a home landscape in St. Louis. Subsequent searches of the Meramec River basin, where the topsoil originated, did not yield any populations.

In May 1994, a naturally occurring population was discovered on private land in eastern Missouri's Madison County. This population is in a mesic forest with a logging and grazing history, but with a rich mesic ground flora. Although plants are still present there, the population has declined since 1994 from 199 to 9 plants. A 1994 survey for additional populations in this area and along the Meramec River Basin was unsuccessful.

In 1998, a small population of 10 plants was discovered in Maries County at a river access along the Gasconade River. By 2001, no plants remained at the site, despite the fact that land managers protected the population and the habitat appeared unchanged. Another population consisting of seven plants was discovered during a survey of suitable habitat along the Gasconade River in 1999. These plants were near the edge of a parking area and had apparently been sprayed with herbicide earlier in the year. By the next year, no plants were present at the site. Additional searches in the Gasconade River Basin in 2000 and 2001 yielded no new populations.

The largest known Missouri population was found in 2003 at Graham Cave State Park in east-central Missouri's Montgomery County. It consisted of 139 plants in 2003 in the Loutre River valley. Another population of 112 plants on State Park property was discovered in 2005 at Cuivre River State Park in Lincoln County. Park personnel have been made aware of the plant's significance and have been cooperative in protecting it at both locations.

In 1995, the Missouri Department of Conservation and Missouri Botanical Gardens established 24 reintroduced populations throughout Missouri. The running buffalo clover reintroductions have been relatively unsuccessful, but four populations have persisted. Twenty populations are considered extirpated. Of the persisting populations, the largest consisted of 35 plants in 2002. Thus, Missouri has three naturally occurring populations and four reintroductions.

Indiana

Although a few pre-1900 collections of running buffalo clover are known for Indiana, it was not rediscovered until 1987. Surveys during that year yielded two occurrences in Ohio County. One of the Ohio County populations thought to be extirpated, apparently due to successional habitat changes, was relocated in 2005. In the 1990s, additional populations were found in nearby Dearborn County. The Hidden Valley population, discovered in 1994, was estimated to contain approximately 2,500 plants. Like most of Indiana's occurrences, this population is on private property. One of the owners of some of this population have registered it in the Indiana Natural Areas Registry under a non-binding protection agreement between the private property owner

and the Department of Natural Resources. Two other populations in Dearborn County on private property are comparatively small, one containing 15 plants and the other 20 plants. In 2005, a population of 40 plants was discovered on Dearborn County property (the first in Indiana in public ownership). The site is managed as a working farm and forest. Thus, Indiana has six extant populations.

Most of the historic populations, and all of the extant populations, occur in the southeastern corner of the state. The newly discovered population is the furthest west occurrence in the state. The quantity of appropriate habitat for running buffalo clover in Indiana is tremendous. Although surveys have been conducted, only a small percentage of suitable habitat has been thoroughly inventoried.

Kentucky

Although there were very early general reports and collections of running buffalo clover in Kentucky from the 1800's, extant populations were re-found in Kentucky in 1987. Since that time, numerous directed surveys for this species have resulted in the discovery of 74 populations (46 currently extant) in 13 counties. Most populations have been found on alluvial terraces, possibly because these are the most undisturbed forests in a region (Bluegrass) that has been heavily cleared for agriculture and other land uses. There are a few populations persisting on lawns of large historic homes and a few on upland sites. Light disturbance such as trail use, periodic grazing, mowing, or stream scour is commonly associated with populations in Kentucky.

A large group of populations (ca. 28) are known from the Bluegrass Army Depot, a 15,000-acre U.S. Army munitions storage facility in Madison County. Although these populations have declined from the time of original discovery (presumably because of land use changes such as differences in cattle grazing and/or a subsequent increase in weedy and woody plants), data over the last couple years have shown the average number of plants to be stable or increasing (Elliot 2003-2006). In 2006 a plan was developed to specifically address running buffalo clover recovery and management at this installation (Floyd 2006).

In addition to the Federal facility, running buffalo clover occurs on two state properties (a park and a wildlife management area) and a property owned by a land trust. Recovery efforts are being implemented on two private historic sites and in the Howard's Creek watershed, private land adjacent to a state nature preserve where running buffalo clover has not been relocated.

Experimental populations of running buffalo are being established at Griffith Woods, a preserve in Harrison County managed by the University of Kentucky and The Nature Conservancy. Under the guidance of the Kentucky State Nature Preserves Commission, seeds will be either directly planted at the site or grown in pots in an effort to investigate natural seed establishment. Once established, the experimental populations would be used for various habitat management studies.

Ohio

Running buffalo clover was rediscovered in Ohio in 1988 when eight populations were found during intensive surveying. As of 2005, 18 extant populations were known from Ohio plus an additional eight extirpated populations. Populations have been found primarily in mesic forest and lawn habitats in Hamilton, Clermont, Brown, and Lawrence counties. An estimated 3,138 plants were documented in Ohio during 2005.

Ohio has been annually censusing rooted crowns and flowering stems since the late 1980s. Some demographic monitoring has also been conducted at a few populations to document the status of rooted crowns over time. Surveys for new populations were mainly conducted in the late 1980s, but these are still being conducted, resulting in several new populations in recent years.

Most of the known populations are located on county park lands and have been managed to protect and encourage running buffalo clover. No formal protection agreement is in place for these populations. The two A-ranked occurrences now occur on Hamilton County Park District lands, Shawnee Lookout and Mitchell Memorial Parks. Two populations, both currently B-ranked, are formally protected in Ohio. Congress Green Cemetery, an Ohio Historical Society site, has a memorandum of understanding in place which provides for running buffalo clover protection, management, and monitoring. Warder-Perkins, a dedicated State Nature Preserve, is owned by the Audubon Society and managed by the Ohio Department of Natural Resources. Many of the plants at this site have been transplanted from a nearby privately owned population (Niehaus), part of which is currently being developed for residences. The Niehaus population had been an A-ranked occurrence until 1999 when it declined dramatically, possibly due to shading and lack of disturbance. In 2005, only 75 plants were located at this site. The first population on Federal land in Ohio was located in 2005 on the Wayne National Forest.

West Virginia

Bartgis (1985) rediscovered running buffalo clover in West Virginia in 1983 and 1984 in Webster and Fayette counties. Both of these populations occupy old river terraces of the New River and Back Fork of the Elk River, on a dirt road and at the edge of a lawn beside a gravel road, respectively. New interest in the status of this species developed among researchers, and in 1989, they acquired search images of the species by visiting populations in Kentucky and Ohio. Subsequent surveys on river terraces, at old historical home sites, and in cemeteries proved fruitless in West Virginia.

A small clump of plants was then discovered along an unpaved road on a mountain ridge in Randolph County. The soil at the population location was derived from limestone substrate. Surveys were launched throughout the mountainous portions of the state resulting in 29 documented populations. The West Virginia Natural Heritage Program has been monitoring running buffalo clover through an annual census of rooted crowns and flowering stems since 1989.

In West Virginia, running buffalo clover seems to occur more frequently on old logging roads, off-road vehicle (ORV) trails, hawthorne thickets, grazed woodlands, jeep trails, railroad grades, game trails, and old fields succeeding to mesic woodlands. The larger occurrences exist within a matrix of mesophytic deciduous forest. All populations are associated with light to moderate disturbance such as occasional ORV traffic, stream scour, grazing, or foot-traffic. Plants occur primarily in regions underlain by limestone. To date, extant populations are located in or near the Allegheny Mountains of central to eastern West Virginia: Barbour, Fayette, Pendleton, Preston, Pocahontas, Randolph, Tucker, and Webster counties. One additional population has been documented from Brooke County in the Central Low Plateau of the Northern Panhandle.

An estimated 76,000 plants were seen in West Virginia in 2003, down from an estimated 77,800 seen in 1996. Among all populations in West Virginia in 2003, four appear to be increasing and six appear to be dramatically declining, whereas the rest have been fluctuating in numbers of rooted crowns over an eight to ten year period of monitoring. At this time it is unknown if these trends are meaningful or if this pattern is due to normal population fluctuations.

Other States

In addition to the extant range, specimen-documented records of running buffalo clover exist in Arkansas, Kansas, and Illinois (Brooks 1983). Surveys to relocate the species were conducted in Illinois in 1988, but no plants were found. Although multiple locations were surveyed along the eastern edge of Kansas in 1989, no running buffalo clover was found in that state. The historical record in Arkansas occurred along a railroad track and is thought to represent an accidental introduction (Brook 1983).

SPECIES BIOLOGY

Dispersal and Germination

Scarification of seeds by the digestive system of herbivores, historically believed to be bison, deer, elk, or small herbivores such as rabbits or groundhogs, was likely a major event in natural populations (Thurman 1988, Cusick 1989). It has been hypothesized that in post-settlement times, cattle may have functionally replaced the bison (Pickering 1989). However, some researchers disagree, feeling that bison and cattle are not ecologically equivalent (Recovery Team, personal communication, 2002). Seeds may pass through the digestive system of cattle, but cattle are confined, not migratory as bison historically were. Research on this theory is needed.

The scarification process is believed to be important for germination and as a means of seed dispersal. Cusick (1989) observed that plants are frequently found in clumps of four to five individuals and speculated that deposition of seeds occurs in deer feces. Although deer are viable vectors for running buffalo clover seeds, the survival and germination rates of ingested seeds are low. Of 300 running buffalo clover seeds fed to white-tailed deer, only 80 were recovered intact, and only seven of the remaining seeds germinated (Ford *et al.* 2003). Dispersal

and establishment of new populations of running buffalo clover by white-tailed deer herbivory may not be significant (Ford *et al.* 2003).

Mechanical scarification through trampling by ungulates or scouring action of rivers may also have occurred but was probably infrequent. Baskin and Baskin (University of Kentucky, personal communication, 2004.) suggested that spring temperature fluctuations appear to be a major dormancy breaker in natural populations of running buffalo clover. Seeds possess a specific site on the seed coat that becomes permeable to water during certain temperature regimes. Seeds typically germinate during early spring (mid-March to early April) when temperatures are between 15 and 20 degrees Celsius (°C) during the day and 5 to 10°C at night. A long-term study has shown that roughly 60% of the seeds that were initially planted have germinated over a span of three years (C. Baskin, University of Kentucky, personal communication, 2004).

Scarification may aid in the germination of running buffalo clover seeds. Little or no germination was observed in unscarified seeds, whereas 90%-100% germination was noted for scarified seeds (Campbell *et al.* 1988). In a subsequent study, seed germination and soils characterization revealed that germination was low when seeds were mechanically scarified; only 4.3% germination after 60 days (Hattenbach 1996). However, immersion in sulfuric acid scarified the seeds sufficiently after 40 minutes exposure to get 90% germination after only two days (Hattenbach 1996). It appears 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.

Life Stages and Population Structure

Substantial variability in the growth and development of running buffalo clover has been documented in both introduced and wild populations. 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. At an introduced population on the Mark Twain National Forest in Missouri, 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 (Hickey 1994, see also Figure 1).

In Ohio, developmental variation has been observed throughout the growing season. For example, between May and June, plants flower and produce stolons with associated un-rooted “daughter” crowns. By July, the “daughter” crowns begin to root but remain connected by stolons to the “parent” plant. Seedlings (first or second year plants) are often present at this time. Starting in September, stolons senesce and “parent” and “daughter” crowns are no longer connected. This is a time of high mortality for “parent” plants (Becus 1993, Cochrane *et al*. 1994).

Long-term monitoring data suggest that running buffalo clover populations often display widely fluctuating population sizes. The cause for changes in population size may be due to disturbance, weather patterns, management strategy, or other unknown factors. Ohio's population data (over 15 years of data for some sites) indicate that the numbers of rooted crowns in a given sub-population may vary widely over time, including variation within a given growing season (Becus 1993). One population in Ohio had 235 rooted crowns in 1992 and then disappeared for the next 3 years; in 2003, this same population had 1,157 plants. Similarly, a West Virginia sub-population consisting of 31 rooted crowns in 1990 and 1991, disappeared in 1992, and returned the next year. Running buffalo clover has not been observed at this location since 1993 and is now considered extirpated at this site.

Reproduction

Running buffalo clover is reported to be visited by bees (*Apis* spp. and *Bombus* spp.) and is cross- pollinated under field conditions (Taylor *et al.* 1994). Taylor *et al.* (1994) suggested that running buffalo clover sets fewer seeds by self-pollination than by outcrossing, but that selfed seed set may be adequate to maintain the species in the wild. Franklin (1998) documented that although running buffalo clover is genetically self-compatible, it cannot automatically self-pollinate. Although pollen needs to be transferred by an outside agent (pollinator) in order for seeds to set successfully, the pollen can fertilize ovules of flowers on the same plant. Self-compatibility provides plants reproductive assurance when outcrossing opportunities are limited (such as in small populations). Although researchers have speculated that inbreeding depression may have contributed to the decline of running buffalo clover (Hickey *et al.* 1991, Taylor *et al.* 1994), selfed seeds have been shown to germinate well and develop into vigorous plants (Franklin 1998).

In cultivation, Campbell *et al.* (1988) reported that a flower head with 20-40 florets typically produced a minimum of 10-20 seeds. In one Kentucky accession grown from a single clone, plants averaged 11, 6, and 14 seeds per head in different years (Taylor *et al.* 1994). In Kentucky, plants in a small wild population averaged 10 seeds per head, while two large populations averaged 35 and 28, respectively (Taylor *et al.* 1994). Ohio data range from 4.3 to 68.6 seeds per flowering head (Franklin 1998).

Franklin (1998) compared fruit and seed production of running buffalo clover in small and large populations. Although the smaller populations produced more flowers with more seeds per head than the larger populations, this result may have more to do with the open habitat of the smaller populations. Franklin (1998) proposes that higher light availability in more open habitats attracts more pollinators, which increases pollination success and higher seed production (1998).

Nitrogen Fixation

Running buffalo clover, like other perennial *Trifolium* species, lacks a rhizobial associate. Populations in Kentucky, West Virginia, and Indiana have been examined for rhizobial nodules, but none have been found (Campbell *et al.*1988, Morris *et al.* 2002). Small nodules, uninfected

by *Rhizobium*, were reported in the original recovery plan for running buffalo clover (USFWS 1989) as having been observed in cultivation, suggesting past associations. However, to date no directed research supports this claim in wild populations. In addition to examining running buffalo clover for root nodules, Morris *et al.* (2002) conducted isotope dilution studies to calculate quantities of nitrogen fixation, and found no evidence that running buffalo clover plants were fixing nitrogen. Research suggests that running buffalo clover may have a low nitrogen requirement and may, therefore, never have developed the need for a rhizobial associate (Morris *et al.* 2002). In fact, running buffalo clover plants appear robust and healthy in many situations even without such an associate. Even after periodic drought and a 3-inch clipping regime (to simulate grazing/mowing), running buffalo clover appeared to persist much better than other associated plant species (Morris *et al.* 2002).

Genetic Variation

Genetic studies of running buffalo clover have been conducted using allozymes for Ohio, West Virginia, Indiana, and Kentucky populations. Results of these early studies suggested low genetic diversity for this species (Hickey *et al.* 1991, Hickey and Vincent 1992). Among other things, this may reflect the clonal nature of the species. Hickey and Vincent (1992) also indicated that smaller populations had lower levels of diversity than larger ones and that the majority of the diversity occurred among populations. In addition, gene flow between populations was limited, even between populations separated by short distances (Hickey and Vincent 1992).

Using random amplified polymorphic DNA markers (RAPDs), Crawford *et al.* (1998) examined genetic variation within and among populations of running buffalo clover throughout its known geographic range. Unlike allozymes, RAPDs can presumably provide an unlimited number of markers throughout the genome (Whitkus *et al.* 1994), producing estimates of the levels of genetic diversity within the species (Stewart and Porter 1995). Twenty-one populations of varying sizes were sampled in Ohio, Kentucky, West Virginia, Indiana, and Missouri from 1994-1996. The average within-population RAPD banding similarity values were high, ranging from 0.902 to 0.984 (mean = 0.952). The mean banding similarities for comparisons between-populations ranged from 0.856 to 0.902 (mean = 0.884). The average within-population similarity values are always higher than any of the between-population comparisons, implying that much of the diversity resides among populations in this species. There were also significant differences in average similarities between and within patches at the population level. These differences suggest that there is sub-structuring within populations, which is consistent with the clonal nature of running buffalo clover.

The results from allozyme electrophoresis (Hickey *et al.* 1991) and the RAPDs show relatively low levels of diversity and low levels of gene flow between populations, even between those separated by short distances. In contrast, the results from the two techniques differ in that RAPD marker variation was detected in all populations sampled, with levels of diversity in several smaller populations equal to that in larger ones. No allozyme variation was detected in half of the populations sampled, and smaller populations were often monomorphic. The RAPD study suggested that 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 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.

HABITAT CHARACTERISTICS

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. It is most often found in regions underlain with limestone or other calcareous bedrock, but not exclusively. It has been reported from a variety of habitats, including mesic woodlands, savannahs, floodplains, stream banks, sandbars (especially where old trails cross or parallel intermittent streams), grazed woodlots, mowed paths (e.g. in cemeteries, parks, and lawns), old logging roads, jeep trails, ATV trails, skid trails, mowed wildlife openings within mature forest, and steep ravines.

It has been suggested that the original habitat may have been open woods or savannah (S. Packard, The Nature Conservancy, personal communication 1988). At the time of European settlement of North America, running buffalo clover is thought to have been dependent on the once-common bison, or other large mammals, such as elk and deer, for seed scarification and dispersal, and for the maintenance of its moderately disturbed habitat along large game trails (Campbell *et al.* 1988, Cusick 1989).

Associate species of running buffalo clover vary across its range with some similarities indicated in Table 2 (for a list of associate species by state, see Appendix 3).

Table 2. Species commonly associated with running buffalo clover

SCIENTIFIC NAME	COMMON NAME
Overstory Species	
Acer negundo	Box elder
Acer saccharum	Sugar maple
Fraxinus americana	White ash
Juglans nigra	Black walnut
Ulmus americana	American elm
Herbaceous Species	
Amphicarpa bracteata	Hog peanut
Carex spp.	Sedges
Cryptotaenia canadensis	Honewort
Eupatorium rugosum	White snakeroot
Galium spp.	Bedstraw
Glechoma hederacea	Ground ivy
Oxalis spp.	Wood-sorrel
Pilea pumila	Clearweed
Poa spp.	Grasses
Stellaria media	Chickweed
Trifolium repens	White clover
Verbesina alternifolia	Wingstem
Viola spp.	Violet

CRITICAL HABITAT

Critical habitat is not currently designated for the running buffalo clover. If following completion of this Plan, the USFWS finds that it is prudent and determinable to designate critical habitat for this species, the USFWS will prepare a critical habitat proposal at such time as our available resources and other listing priorities under the ESA allow. This proposal will be based on essential physical and biological features needed to ensure the conservation of this species, many of which have been documented in the above Habitat Characteristics section of the Recovery Plan.

REASONS FOR LISTING AND ONGOING THREATS

The original Running Buffalo Clover Recovery Plan (USFWS 1989) identified the threats to the survival of running buffalo clover as habitat destruction, competition from invasive species, lack of a rhizobial associate, small population sizes, herbivores, and pathogens. Specific threats identified by the Running Buffalo Clover Recovery Team in 1995 were: 1) any irreversible, permanent habitat loss, such as road construction that completely destroys the habitat and/or kills

all plants and seeds within the path of the disturbance; 2) the closing of forest canopies through succession to the point of severe shading, leading to reduced flower and fruit production; 3) the elimination of bison leading to reduced seed dispersal and release of competing vegetation; 4) small population size and associated fragility and susceptibility to catastrophe; 5) excessive herbivory; 6) viral and fungal diseases; 7) reduction in pollinators; and 8) competition from non-native, invasive plant species.

With the exception of viral and fungal diseases, excessive herbivory, and lack of a rhizobial associate, the threats identified in both 1989 and 1995 are still affecting the species. The most significant threats rangewide are habitat destruction, habitat succession, and invasive plant competition. The following analysis details past and continuing threats to this species as they relate to the five listing factors outlined in section 4(a)(1) of the Act.

Habitat Loss, Alteration, and Degradation

Threats to running buffalo clover's habitat are largely due to direct and indirect human impacts that have lead to habitat loss, alternation, and significant degradation. Homoya *et al.* (1989) stated that the removal or suppression of vegetation by bison may have created the open understory and light gaps necessary for this species. Jacobs and Bartgis (1987) suggested that bison may have provided the right balance of periodic disturbance, soil enrichment, seed dispersal, and seed scarification necessary to maintain running buffalo clover. According to Homoya *et al.* (1989), the removal of bison does not completely explain the range-wide depletion of this species; they suggested that there was not a sufficient time interval between the loss of bison and the introduction of cattle to account for the rarity now present in the species because cattle should have satisfied the same biological necessities as bison. However, unlike bison, cattle are not migratory and may provide long-term grazing pressures to running buffalo clover populations. According to many researchers the ecological equivalency of bison and other ungulates is also uncertain. Investigations into the influences of white-tailed deer on running buffalo clover germination have shown that although deer are viable vectors for running buffalo clover seed, the rates of germination of ingested seeds are low (Ford *et al*. 2002).

In some populations it appears that both overgrazing and no grazing at all are threats to running buffalo clover. In Kentucky, overgrazing poses threats to running buffalo clover, but removal of cattle from clover populations has resulted in overshading and competition from other vegetation (White *et al.* 1999). Periodic grazing at the Bluegrass Army Depot has probably provided the moderate disturbance needed to maintain running buffalo clover (Fields and White 1996). Without some level of disturbance, a population will become too shaded to provide enough sunlight for the species (Cusick 1989, Homoya *et al.* 1989). A greater understanding is needed concerning the level of disturbance required by this species.

Various researchers have supported the hypothesis that during pre-settlement time running buffalo clover habitat was likely produced through canopy gaps created by the felling of large old-growth trees (Madarish and Schuler 2002). Current logging practices may also benefit running buffalo clover. At the Fernow Experimental Forest in north-central West Virginia, running buffalo clover is most often associated with skid roads in uneven-aged silvicultural areas

(Madarish and Schuler 2002). A study examining running buffalo clover abundance before and after logging suggests that populations may initially decrease after disturbance, but then rebound to higher than pre-disturbance levels (Madarish and Schuler 2002).

Land development and the consequential loss of habitat is also a serious threat to running buffalo clover. Cusick (1989) notes that running buffalo clover was formerly relatively frequent in central and southwestern Ohio, particularly in the vicinity of Cincinnati prior to urban sprawl. Remnant populations have become even more isolated, persisting in areas maintained by appropriate disturbance.

Jacobs and Bartgis (1987) suggested that along with the destruction of habitat, the introduction of non-native species may have contributed to the decline of running buffalo clover. Non-native white clover (*Trifolium repens*) may have invaded the habitat of running buffalo clover, out-competing it for available resources (Jacobs and Bartgis 1987). Other invasive plants that compete with running buffalo clover include Japanese stiltgrass (*Microstegium vimineum*), garlic mustard (*Alliaria petiolata*), Japanese honeysuckle (*Lonicera japonica*), Amur honeysuckle (*Lonicera maackii*), wintercreeper (*Euonymus fortunei*), and periwinkle (*Vinca minor*).

Overutilization for Commercial, Recreational, Scientific, or Educational Purposes

Running buffalo clover is not known to be used for any commercial or recreational purpose. When originally listed (USFWS 1987), overutilization for scientific or educational purposes was clearly a threat given the fact that only one population consisting of four individuals was known. Today collection for scientific or educational purposes is limited and spread out among many populations.

Disease or Predation

Although at the time of listing, disease was predicted to threaten running buffalo clover, recent studies indicate that disease and predation are not major threats. Jacobs and Bartgis (1987) suggested that the decline of this species may have partially centered on a pathogen introduced from the exotic white clover, but no specific disease has been identified. A number of viral and fungal diseases are reported to have attacked the species in greenhouses at the Missouri Botanical Garden, including cucumber mosaic virus and the comovirus (Sehgal and Payne 1995). No evidence has been gathered showing these viruses' impact on running buffalo clover decline in the wild.

Parasitism by root-knot nematodes (*Meloidogyne* spp.) is common in clovers and often limits productivity in cultivated clovers used as forage crops (Quesenberry *et al.* 1997). Investigations have been conducted on the effects of root-knot nematodes on native North American clovers, including running buffalo clover. After inoculation of the parasite, running buffalo clover displayed high resistance to three of the four nematode species analyzed, and only an intermediate response to the fourth species of nematode (Quesenberry *et al.* 1997). Thus, the threat from this parasite is not considered significant.

Although, herbivory by a variety of species has been reported for running buffalo clover, it is not considered a primary threat. In Missouri, running buffalo clover plants are repeatedly grazed by rabbits, rodents, and slugs (Pickering 1989). Similar observations have been made in Kentucky (Davis 1987) and West Virginia (P.J. Harmon, West Virginia Natural Heritage Program, personal communication, 2003). The Fayette County, West Virginia population was eaten to the ground by a ground hog (*Marmota monax*), but more than a dozen rooted crowns were observed at the population the following year. White-tailed deer can also consume large amounts of running buffalo clover (Miller *et al.* 1992). It should be noted that herbivores are also the potential dispersers of seeds for this species, so palatable greens may be an evolutionary advantage for the species as a whole (M. Vincent, Miami University, personal communication, 2004). In sum, although a population may be entirely consumed during a growing season, plants may return again the next year. If herbivory occurs after seed is set, the species may benefit from increased seed dispersal.

Inadequacy of Existing Regulatory Mechanisms

With the exception to the protection that the ESA provides listed plants on Federal lands, current state and Federal laws provide little or no protection to plants listed under the ESA. Plants are viewed as property of the landowner and in most cases landowners need not provide protection to these populations under the law. Several states provide protection against commercial taking and subsequent trade or sale of endangered plants, as described in the following paragraph. Regardless of the lack of existing protections, commercial taking does not appear to be a threat to running buffalo clover, because it is not known to be used for any commercial or recreational purpose.

As well as being federally listed, running buffalo clover is state listed as endangered in Missouri, Indiana, Ohio, Kentucky, and West Virginia. The degree of provided protection varies among the states (Table 3). Ohio and Missouri have similar laws prohibiting commercial taking of plants. Kentucky has a Rare Plant Recognition Act, but provides no protection to those species listed under this law. Indiana has a non-rule policy, where the Natural Resources Commission takes listed plants into consideration if a project over which they have jurisdiction contains those listed plants. West Virginia has been unsuccessful in passing an endangered species law, but state agencies are recommended to consult with the Natural Heritage Database for known locations of running buffalo clover on proposed project sites.

Table 3. Legal protection for running buffalo clover by State.

State	Protection Act	Level of Protection
Missouri	Endangered Species Law	Prohibits exportation, transportation, or sale of endangered plants. Prohibits removal of plants without landowner permission. Requires consultation between Department of Conservation and state and local agencies authorizing or funding actions impacting listed plants.
Indiana	Non-rule policy	Indiana Natural Resource Commission may consider protection of listed plants for projects they have jurisdiction over.
Kentucky	Rare Plant Recognition Act	Kentucky State Nature Preserves Commission maintains an endangered species list.
Ohio	Endangered Plant Law	Prohibits taking of plants for commercial purposes. Prohibits the take, possession, or transport for botanical, educational, or scientific purpose without obtaining a permit from the Division of Natural Areas and Preserves. No destruction or removal of plants located within a designated State Nature Preserve. Requires state and local agencies authorizing or funding actions impacting listed plants to consult the Heritage Database.
West Virginia	No law	Maintains a list of the federally endangered species list, but no state legislation has been enacted to protect state listed species. Consideration of listed plants on state property is conducted through consultation with the Heritage Database.

Other Natural or Manmade Factors

Additional factors that may threaten running buffalo clover include small population sizes, inadequate seed dispersal, and poor seed quality. It has been suggested that running buffalo clover has a limited seed dispersal mechanism (Cusick 1989). Deforestation, farming, and other human activities created many new habitats for the species, but with the loss of large herbivores after European settlement, Cusick (1989) suggested that there were no effective means of dispersal remaining for the species. White-tailed deer and bison were effectively eliminated from the landscape due to over-hunting. Only recently have deer returned to pre-settlement numbers. According to this theory, habitat in which running buffalo clover formerly occurred gradually closed due to the absence of disturbance. Although a presumed primary disperser (deer) is again present, the rate of seed germination from seeds ingested by deer is low, and relatively few populations of running buffalo clover have survived as compared to presumably larger pre-settlement populations (Cusick 1989).

Although researchers have speculated that inbreeding depression may have contributed to the decline of running buffalo clover (Hickey *et al.* 1991, Taylor *et al.* 1994), selfed seeds have been shown to germinate well and develop into vigorous plants (Franklin 1998). However, temporal

variations in seed quality have been reported. Seed quality may be correlated with rainfall; quality decreases in years with unusually high rainfall (Franklin 1998).

Long-term monitoring data suggest that running buffalo clover populations often display widely fluctuating population size. The cause for changes in population size may be due to disturbance, weather patterns, management strategy, natural succession, or other unknown factors. The cyclic nature of running buffalo clover and the high probability of small populations blinking in and out, may lead to difficulty in protecting small populations. Regardless, small populations have displayed high levels of genetic diversity that is important for survival of the species as a whole. Protection of several small populations across the landscape will help ensure viability of the species range-wide.

CONSERVATION MEASURES

Running buffalo clover was listed as an endangered species under the ESA on July 6, 1987 (USFWS 1987). Conservation measures provided for running buffalo clover include Federal Regulatory Protection, state protection, surveys and population monitoring, conservation plans and agreements, habitat management and invasive species control, and education and outreach. Recognition through listing encourages and results in conservation actions by Federal, state, local municipalities and private agencies, groups, and individuals. Recovery actions completed to date or ongoing include: inventorying known populations, surveying for additional populations, investigating a rhizobial associate, maintaining current reintroductions, storing seed and existing lines, invasive plant control, and providing public information about running buffalo clover.

Federal Regulatory Protection

Section 7(a)(2) of the ESA requires Federal agencies to consult with the USFWS prior to authorizing, funding, or carrying out activities that may affect Federally listed species. Section 7(a)(1) also requires that these agencies use their authorities to further the conservation of Federally listed species. Section 7 obligations relative to running buffalo clover have resulted in a number of consultations for projects such as timber harvest, land management activities, and road building, administrated by Federal agencies including the U.S. Forest Service, U.S. Department of the Army, the Federal Energy Regulatory Commission, and the Federal Highway Administration. Federal actions have resulted in some habitat loss for the species, but section 7 consultations have added to increased survey efforts and research and monitoring.

Sections 9 and 10 of the ESA and the corresponding implementing regulations found in 50 CFR 17.61, 17.62 and 17.63 set forth a series of prohibitions and exceptions that apply to all federally endangered plants. These prohibitions, in part, make the following activities illegal for any person subject to the jurisdiction of the United States: import or export; transport in interstate or foreign commerce; sell or offer for sale this species in interstate or foreign commerce; remove and reduce to possession this species from areas under Federal jurisdiction; and maliciously damage or destroy this species on any other area in knowing violation of any state law or

regulation or in the course of any violation of a state criminal trespass law. These regulations apply to any part of the plant, including seeds, roots, and other parts. Certain exceptions apply to agents of the Service and state conservation agencies. The ESA provides for the issuance of permits for scientific purposes or for the enhancement of propagation and survival of the endangered species.

State Protection

Conservation measures at the state level are often voluntary and limited. Existing regulatory measures vary by state, but may provide some protection to running buffalo clover (see Table 3). States such as Ohio and Missouri that do provide endangered species regulation have limited effects on projects that occur on private land. However, voluntary conservation measures that emphasize habitat management have been successful if consistently implemented.

Surveys and Population Monitoring

Survey efforts for running buffalo clover were widespread after the species was rediscovered in 1987. Several states including West Virginia, Ohio, and Missouri provided information to assist local botanists in locating and identify new running buffalo clover occurrences. Since 1990, many new populations have been found in West Virginia, Kentucky, and Missouri, particularly in areas with soils derived from limestone. Currently surveys are typically project-driven under the auspices of section 7 consultation. In 2004, USFWS provided funding for population monitoring and for additional survey efforts across the range of the species.

Population monitoring can be a very useful tool for gaining information on the structure of populations through time. According to historical journals, a white clover presumed to be running buffalo clover was frequent in pioneer days (Campbell *et al.* 1988; Cusick 1989); however, many of the historical populations have disappeared. Trend data from annual census monitoring are currently available from West Virginia and Ohio for over ten years at some populations. Monitoring protocols have been developed, and if adhered to range-wide, will provide consistent data on population trends (see Appendix 1). Actual or estimated population sizes are expressed in the form of numbers of rooted crowns.

Conservation Plans/Agreements

Conservation plans or agreements to protect running buffalo clover exist where the plant occurs on Federal lands in West Virginia, Ohio and Kentucky and for two state managed sites in Ohio. No known running buffalo clover populations occur on Federal lands in Indiana or Missouri. A 2004 amendment to the Monongahela National Forest Land Management Plan (West Virginia) indicates that surveys will be conducted in broken-canopied forest or non-forest areas to be affected by land transfer, repeated vehicular use, or earth-disturbing activities; any known running buffalo clover populations will be conserved. The Wayne National Forest Revised Forest Land Management Plan includes protective standards and guidelines for all activities

occurring in and near running buffalo clover sites. The Bluegrass Army Depot in Kentucky protects and manages running buffalo clover under an Endangered Species Management Plan included as part of their Integrated Natural Resource Management Plan (INRMP). The 2006 revision of this plan includes habitat management techniques for running buffalo clover (Floyd 2006). Both of Ohio's state managed populations are protected. A memorandum of understanding between the Ohio Historical Society, Division of Natural Areas and Preserves, and the U.S. Fish and Wildlife Service provides for running buffalo clover protection, management, monitoring, and visitor education. Formal dedication of Warder-Perkins State Nature Preserve includes a management plan for the protection and management of running buffalo clover.

Habitat Management and Invasive Species Control

Ongoing management of running buffalo clover habitats is critical for maintaining populations of this species. Running buffalo clover occurs in two fairly distinct habitat types (shaded lawn and mesic forest) thus, management recommendations are required for the clover in both habitats. Lawn populations include cemeteries, parks, and old home sites. Mesic forest populations are often associated with streams and trails. Forested populations require open areas where the clover is exposed to indirect sunlight. Controlling invasive species such as Japanese stiltgrass, garlic mustard, Japanese honeysuckle, Amur honeysuckle, wintercreeper, and periwinkle is critical in both lawn and forested populations. Use of herbicide is not currently recommended near running buffalo plants in the growing season, but manual methods may be effective. Manual pulling of invasives such as Japanese honeysuckle and perwinkle have reduced these species while maintaining populations of running buffalo clover at Congress Green Cemetery in Ohio. A grass-specific herbicide has been used experimentally on Japanese stiltgrass at the Bluegrass Army Depot populations in Kentucky. The effects of this treatment were somewhat inconclusive, with running buffalo clover populations decreasing one year and increasing two years later. Additional research on the effects of herbicide to non-target species, such as running buffalo clover would be valuable.

Minimal data exists on the effectiveness of various management techniques for running buffalo clover. One exception is the mowing regime used for lawn populations in Ohio. Mowing is allowed early in the growing season and then again after running buffalo clover has set seeds. This technique has been implemented for several years with positive results. Although these populations are frequently mowed, if seasonal restrictions are followed, the clover appears to thrive under these conditions (Becus and Klein 2003).

Agricultural out-leasing (cattle grazing, hay production) has used been used as a land management tool by Bluegrass Army Depot to maintain habitat for running buffalo. Grazing schemes were modified in the late 1990s, resulting in the suspension of grazing at some sites and continued grazing (at varying levels of intensity) at other sites. A review of running buffalo clover monitoring data from 2003 to 2005 revealed preliminary trends regarding grazing and the total number of rooted crowns recorded from each patch (Elliot 2003-2005). The average number of rooted crowns was highest in patches that had been excluded from grazing, and the second highest average was recorded for patches that had been subjected to grazing for only a partial year. These results suggest that intensive grazing and associated trampling has a

detrimental effect on running buffalo clover patch size. Because many areas excluded from grazing since the late 1990s have displayed an increase in rooted crowns from 2003 to 2005, it appears that grazing (i.e., disturbance) by cattle is required at less frequency and intensity than was commonly believed necessary to provide the appropriate disturbance regime for this species (Floyd 2006). In accordance with the Bluegrass Army Depot's INRMP, annual monitoring of these populations will continue into 2010 and will provide valuable long term trend data.

Managing habitat succession by opening up the forest canopy has been conducted in both Ohio and West Virginia. At the Fernow Experimental Forest in north-central West Virginia, running buffalo clover is most often associated with skid roads in uneven-aged silvicultural areas (Madarish and Schuler 2002). A study examining running buffalo clover abundance before and after logging suggests that populations may initially decrease after disturbance, but then rebound to higher than pre-disturbance levels (Madarish and Schuler 2002). Although light is thought to be important for running buffalo clover plants to flower, the amount of light needed is unknown.

In cooperation with various agencies, researchers, and botanists, the USFWS has developed management recommendations for running buffalo clover in Ohio (Appendix 4). The recommendations are used to guide property owners and land managers in the management of running buffalo clover habitats. If implemented consistently, the recommendations can be a useful tool to protect and recover this endangered plant species. Because running buffalo clover exists over a wide range, habitat recommendations at one site may not be suitable for another. It is critical to document the types of management that are occurring on a site specific basis to gain a greater understanding of the species habitat needs throughout the range.

Education and Outreach

Since rediscovery in the mid-1980s, numerous education and outreach activities have occurred across the range of running buffalo clover. These activities have focused on plant identification, population status, habitat management, and natural heritage awareness.

Several states including Missouri, Ohio, West Virginia, and Kentucky have produced brochures to help distinguish running buffalo clover from other more common species. Other printed materials include a poster summarizing running buffalo clover distribution, life history and recovery efforts in Kentucky, Ohio's Habitat Management Recommendations for Running Buffalo Clover (Appendix 4), and the Center for Plant Conservation's, "I brake for running buffalo clover" bumper sticker.

In Ohio and West Virginia, staff with the Wayne and Monongahela National Forests have been trained to identify running buffalo clover and their associated habitats. District conservationists from Kentucky's Natural Resource Conservation Service office have also been trained in identification of running buffalo clover and can direct landowners where to get more information on the species. Missouri has produced a set of laminated plant identification cards to assist the Missouri Department of Conservation's state foresters in recognizing running buffalo clover. Indiana's Division of Nature Preserves provides a voluntary landowner awareness program that designates Natural Areas Registry sites. One such site in Indiana contains the largest population

of running buffalo clover in the state. This non-binding agreement between the private property owner and the Department of Natural Resources encourages landowner protection of the population.

BIOLOGICAL CONSTRAINTS and NEEDS

Biological constraints of running buffalo clover include reproductive requirements (reliance on pollinators, seed scarification, and dispersal mechanisms) and dependence on disturbance to maintain a filtered sunlight habitat. Seed scarification may enhance germination of running buffalo clover (Campbell *et al.* 1988), and it appears that chemical scarification (i.e. through a digestive tract) is most effective (Hattenbach 1996). As deer do not appear to be highly successful at dispersing running buffalo clover seed (Ford *et al*. 2002), the species dependence on ungulate herbivores for seed germination and dispersal has not been resolved. If bison were the original dispersal and disturbance agent for maintenance of running buffalo clover, their disappearance from the landscape may be an irresolvable biological constraint to recovery.

Variation in seed set from year to year and population to population is also a biological trait of running buffalo clover that makes it vulnerable. Although running buffalo clover is self-compatible, it requires a pollinator to move the pollen from the anthers to the stigma (Franklin 1998). Little information exists about the effect of pollinators on seed set. It has been observed in the field that flowers sometimes appear devoid of viable seeds (Frankin 1998, M. Becus, private botanist, personal communication, 2004). Pollinators may have difficulty detecting small populations of running buffalo clover especially in marginal habitat where running buffalo clover plants are competing with other vegetation. Weather may also play a role in successful seed set as data suggest that extremely wet or dry years result in reduced seed production (Franklin 1998).

Perhaps the most critical biological constraint and need to the recovery of running buffalo clover is its dependence on disturbance. Habitat for running buffalo clover must include filtered sunlight. This requirement often means removal of competing vegetation (especially invasive plants) and selective tree removal to prevent overshading. Any recovery strategy for running buffalo clover must include a component of habitat management to ensure long-term viability of the species.

PART II. RECOVERY

RECOVERY STRATEGY

Running buffalo clover was listed under the ESA because the small numbers of known populations were threatened by habitat alteration. Other current threats to the species include habitat destruction, habitat succession, and invasive plant competition. In addition to these threats, inherent biological vulnerabilities for this species include its reliance on pollinators, seed scarification, dispersal mechanisms, and disturbance.

Since its listing in 1987, several positive outcomes have been realized due to recovery implementation: 1) more information is available regarding the species biology; and 2) the known number of populations has dramatically increased as survey efforts have expanded throughout the historic range. Although many of the threats to running buffalo clover populations still exist, two initially identified potential threats, lack of a rhizobium associate and viral pathogens do not appear to be a threat to the species.

Recovery of running buffalo clover will be achieved by implementing actions which address the species distribution, numbers, and threats. Given the known threats and constraints, this recovery effort focuses primarily on increasing the number of protected and managed populations, determining the viability of existing populations, and research into the species ecological requirements. Key to this strategy is the protection and ecological management of various-sized populations of running buffalo clover throughout its geographic range. The recovery criteria and subsequent recovery actions rely heavily on retaining and managing the habitats on which running buffalo clover needs to maintain viability. In addition, the recovery strategy relies on a greater understanding of the biotic and abiotic needs of running buffalo clover in order to apply adequate management. Numerous scientific studies have started to shed light on the ecological requirements of running buffalo clover, but more information is needed to understand the level of periodic disturbance required to maintain the species.

In order to reclassify and eventually delist running buffalo clover, adequate numbers and sizes of populations need to be monitored, protected, managed, and the ecological factors that regulate the populations need to be further defined. Additionally, until these population regulation factors are better understood, the genetic diversity of known populations of all sizes should be conserved. It follows that the recovery actions described in this Plan fall into five categories: 1) Conserve and manage running buffalo clover populations and the habitat on which they depend, 2) Define population regulation factors, 3) Conserve germplasm and genetic diversity, 4) Promote public awareness and understanding, and 5) Review and track recovery progress.

RECOVERY GOALS

The ultimate goal of this recovery program is to remove running buffalo clover from the Federal List of Threatened and Endangered Plants (50 CFR 17.12), with an intermediate goal of reclassification to Threatened.

RECOVERY OBJECTIVES

To achieve the recovery goals, a minimum number of viable populations should be protected and managed throughout a majority of the species geographic range. Populations are considered protected when there are permanent assurances that the habitat will be managed. Management objectives for running buffalo clover include 1) invasive species control, 2) reducing habitat succession, and 3) defining population regulation factors. Additional recovery objectives include 1) ensuring viability of protected populations, 2) maintaining genetic diversity and germplasm, and 3) promoting public understanding of the species.

RECOVERY CRITERIA

Running buffalo clover may be reclassified from endangered to threatened when the following criteria are met. These criteria address the numbers, distribution, and threats to the species. Numerical goals are based on most recently available scientific information and are subject to revision as new information becomes available.

1. Seventeen populations, in total, are distributed as follows: 1 A-ranked, 3 B-ranked, 3 C-ranked, and 10 D-ranked populations across at least 2 of the 3 regions in which running buffalo clover currently occurs (Appalachian, Bluegrass, and Ozark). The number of populations in each rank is based on what would be required to achieve a 95% probability of persistence within the next 20 years based on population viability analysis (see Appendix 5). Rankings refer to the Element Occurrence (EO) ranking categories (Table 1).

2. For each A-ranked and B-ranked population described in #1, population viability analysis indicates 95% probability of persistence within the next 20 years, OR for any population that does not meet the 95% persistence standard, the population meets the definition of viable. For downlisting purposes, viability is defined as follows: A) seed production is occurring; B) the population is stable or increasing, based on at least five years of censusing; and C) appropriate management techniques are in place.

3. The land on which each of the populations described in #1 occurs is owned by a government agency or private conservation organization that identifies maintenance of the species as one of the primary conservation objectives for the site, OR the population is protected by a conservation agreement that commits the private landowner to habitat management for the species. Natural Resource Management Plans on Federal lands may be suitable for meeting this criterion. This criterion will ensure that habitat-based threats for the species are addressed (see Appendix 6).

Running buffalo clover may be removed from the List of Endangered and Threatened Plants (50 CFR 17.12) when the following have been met:

1. Thirty-four populations, in total, are distributed as follows: 2 A-ranked, 6 B-ranked, 6 C-ranked, and 20 D-ranked populations across at least 2 of the 3 regions in which running buffalo clover occurs (Appalachian, Bluegrass, and Ozark). The number of populations in each rank is based on what would be required to achieve a 95% probability of persistence within the next 20 years; this number was doubled to ensure biological redundancy across the range of the species. Rankings refer to the Element Occurrence (EO) ranking categories (Table 1).

2. For each A-ranked and B-ranked population described in #1, population viability analysis indicates a 95% probability of persistence within the next 20 years, OR for any population that does not meet the 95% persistence standard, the population meets the definition of viable.[2] For delisting purposes, viability is defined as follows: 1) seed production is occurring; 2) the population is stable or increasing, based on at least 10 years of censusing; and 3) appropriate management techniques are in place.

3. Downlisting criterion #3 is met for all populations described in delisting criterion #1.

[2] C-ranked and D-ranked populations are not included for the purposes of viability in recovery criteria # 2 due to their inherently small population sizes and marginal habitat quality. Due to the cyclic nature of running buffalo clover and the high probability of small populations blinking in and out, maintaining viability for a specific C-ranked or D-ranked population at a given time may not be possible. Regardless, small populations have displayed high levels of genetic diversity that is important for survival of the species as a whole and thus are included in the recovery criteria referring to protection and management of sites.

STEPDOWN RECOVERY OUTLINE

1 Conserve and manage known running buffalo clover populations and the habitat they occupy

- 1.1 Determine appropriate habitat management techniques
- 1.2 Implement appropriate habitat management techniques
- 1.3 Protect known running buffalo clover populations and the habitat they occupy via management agreements and other land-based strategies
- 1.4 Monitor known populations rangewide
- 1.5 Survey for additional running buffalo clover populations throughout the clover's geographic range
- 1.6 Develop post-delisting monitoring and management plans

2 Define population regulation factors

- 2.1 Identify biotic factors that regulate running buffalo clover populations
  - 2.1.1 Examine the conditions necessary for flowering
  - 2.1.2 Examine the species and frequency of appropriate pollinators
  - 2.1.3 Examine the level of seed set under different management conditions
  - 2.1.4 Examine factors necessary for seed germination in the wild
  - 2.1.5 Examine the impacts of herbivory on vegetative growth, flowering, and fruit production
- 2.2 Identify abiotic factors that regulate running buffalo clover populations
  - 2.2.1 Evaluate factors in viable running buffalo clover populations such as nutrient levels, moisture, light levels, temperature, geology, and soil types to determine optimal abiotic factors
  - 2.2.2 Examine the effects of soil disturbance and shading as management options for running buffalo clover populations
  - 2.2.3 Examine additional factors that may affect seed viability including weather conditions and year-to-year variations in rainfall

3 Conserve germplasm and promote genetic diversity

- 3.1 Continue to practice seed storage which emphasizes genetic diversity
  - 3.1.1 Determine inadequacies in existing seed storage bank and collect additional seed if needed to encompass the entire range of the species

4 Outreach and enforcement

4.1 Provide public information about running buffalo clover

5 Review and track recovery progress

5.1 Communicate with the Running Buffalo Clover Recovery Team and other interested parties to evaluate progress of recovery
5.2 Revise recovery plan as appropriate

RECOVERY NARRATIVE

1 Conserve and manage known running buffalo clover populations and the habitat they occupy

1.1 Determine appropriate habitat management techniques

The number of known running buffalo clover populations is large enough to withstand some experimentation in developing effective techniques for managing the clover species. A small number of experimental sites should be established which utilize different management regimes. Such regimes may include various forms of planned disturbance, such as livestock grazing, mowing, the removal of invasive plant species, and non-disturbance methods such as signs, fences or gates. Management techniques that promote sexual reproduction (i.e., flowering) may help to increase genetic diversity of running buffalo clover. A small number of experimental management sites should be established with the goal of developing practical long-term practices that conserve or enhance running buffalo clover populations. Regular monitoring and adaptive management should be practiced at all experimental management sites, where adaptive management is described as a continuous process implementing new knowledge and corrective actions, as necessary.

1.2 Implement appropriate habitat management techniques

Management techniques that currently work well should be documented at populations that are stable or increasing in size. Running buffalo clover management techniques proven to be beneficial and effective should be implemented as soon as possible on public lands. Control of invasive plant species in known running buffalo clover populations should be a priority. Proven management techniques should also be incorporated into voluntary management agreements with willing private landowners. Management actions should include application of management techniques on large as well as small running buffalo clover populations across the range of the species since small populations may contain high levels of genetic diversity. For site-specific management recommendations landowners should contact their local FWS office or state resource agency.

1.3 Protect known running buffalo clover populations and the habitat they occupy via management agreements

Criteria in this Plan for reclassifying running buffalo clover to Federally threatened status, and subsequent delisting, specify that a minimum number of populations must be protected via written, legally binding management agreements or their equivalent. Occurrence of a running buffalo clover population on public land does not by itself assure the population's protection. Thus, it is important to achieve such agreements on both public and private land. Agreements are likely to be established on land owned by a Federal, State, or private conservation organization, or may also be established on private land with a voluntary deed restriction (e.g., conservation easement or natural area

dedication). Leases and voluntary land acquisition may be practical options at some locations.

1.4 Monitor known populations rangewide

Efforts should continue to determine or estimate the size of known running buffalo clover populations range-wide. Actual or estimated population sizes are expressed in the form of numbers of rooted crowns. Annual census data will be used to update the PVA to provide a robust tool for species status assessment. Monitoring plans for each state will be designed such that they can continue to be used post-delisting.

1.5 Survey for additional running buffalo clover populations throughout the clover's geographic range

A greater level of genetic diversity exists among populations as compared to within populations of running buffalo clover (Crawford *et al.* 1998). This means each newly found population may represent new genotypes valuable to the overall survival of running buffalo clover throughout its range. New populations continue to be found in the eastern part of the clover's range, especially in Kentucky and West Virginia. Searches in the eastern range (Indiana, Ohio, Kentucky, and West Virginia) should continue. As of 2005, only three naturally occurring populations of running buffalo clover, found in Missouri, are known to exist in the western part of the running buffalo clover's range. Running buffalo clover occurred historically in Illinois, Kansas, and Arkansas (Brooks 1983). Because of the genetic significance of finding new populations, additional searches should be conducted in the vicinity of locations not recently surveyed and where the clover historically occurred.

1.6 Develop post-delisting monitoring and management plans

Develop, adopt, and implement a plan describing habitat management and monitoring actions that will be conducted and/or continued once running buffalo clover is recovered and delisted. Post-delisting monitoring of populations will be required for not less than five years after running buffalo is removed from the protection of the Act. Habitat management plans will detail management actions that will be conducted to sustain running buffalo clover habitats. Due to State differences in running buffalo clover habitats and populations, post-delisting monitoring plans may vary for each State, but will require USFWS review and approval.

2 Define population regulation factors

2.1 Identify biotic factors that regulate running buffalo clover populations

Numerous biotic factors affecting survival of running buffalo clover populations remain unexplored. Life history factors relating to germination, vegetative vs. sexual reproduction (i.e., flowering), and the timing of life history events related to environmental events (e.g., stolon growth and rooting related to timing of

disturbance) need to be examined. The ecological significance of interactions with pollinators and herbivores also need to be documented.

2.1.1 Examine the conditions necessary for flowering including the levels of light and/or disturbance that are required.

2.1.2 Examine the species and frequency of appropriate pollinators (introduced vs. native). Are pollinators a limiting factor in small populations?

2.1.3 Examine the level of seed set in wild populations at several localities under different management conditions over multiple years.

2.1.4 Examine factors necessary for seed germination in the wild. How does this relate to populations that blink in and out?

2.1.5 Examine the impacts of herbivory on vegetative growth, flowering, and fruit production. Investigate the role of small and large animals and their contribution to dispersal. Is there a balance between ungulates as agents of herbivory and as dispersal agents?

2.2 Delineate abiotic (i.e., general environmental) limiting factors that regulate running buffalo clover populations

Many abiotic factors influencing running buffalo clover populations also remain unexplored. Key questions about nutrients, moisture, light, and temperature remain unanswered. Underlying geology, associated soil types, degree of beneficial soil disturbance, and degree of shading all need better documentation.

2.2.1 Evaluate factors in viable running buffalo clover populations such as nutrient levels, moisture, light levels, temperature, geology, and soil types to determine optimal abiotic factors.

2.2.2 Examine the effects of soil disturbance and shading as management options for running buffalo clover populations.

2.2.3 Examine additional factors that may affect seed viability including weather conditions and year-to-year variations in rainfall.

3 Conserve germ plasm and promote genetic diversity

3.1 Continue to practice seed storage which emphasizes genetic diversity

It is important that storage of running buffalo clover seeds continue in a manner that maximizes genetic diversity of stored seeds. Most of the running buffalo clover populations that currently exist are small (i.e., less than 1,000 rooted

crowns) and vulnerable to catastrophes or more subtle events. These small populations tend to disappear and sometimes reappear for no obvious reason.

Small populations contain genotypes important to overall genetic diversity of running buffalo clover. Thus, it is important to update storage efforts with seeds from currently known and newly discovered populations from each state where running buffalo clover occurs. Significant running buffalo clover seed banks are maintained at the Missouri Botanical Gardens and the USDA National Center for Genetic Resources Preservation in Ft. Collins, Colorado.

3.1.1 Determine inadequacies in existing seed storage bank and collect additional seed if needed to encompass the entire range of the species.

4 Outreach and enforcement

4.1 Provide public information about running buffalo clover

Disseminate information to the general public about running buffalo clover, how to protect it, and how to manage it. Owners of public and private land should be notified if running buffalo clover occurs on their property. Foster a sense of pride and stewardship in landowners and land managers in a manner that promotes conservation and protection of the running buffalo clover. Promote running buffalo clover conservation through project reviews, contacts with private landowners, and consultations with public agencies. Partner with other resource agencies to promote conservation of running buffalo clover.

5 Review and track recovery progress

5.1 Communicate regularly with the Running Buffalo Clover Recovery Team and other interested parties to evaluate progress of recovery

Running Buffalo Clover Recovery Team members will function as liaisons for their respective States. As such, they will provide the Recovery Team with population status, habitat management, research, and recovery updates from their respective States. Regular meetings (in person or via conference call) of the Recovery Team and other interested parties is critical to monitor recovery efforts throughout the species range and to identify additional recovery needs.

5.2 Revise Plan as appropriate

We cannot address every future development and contingency. As such, this Plan may need to be revised to better reflect current conditions, and incorporate new findings.

PART III. IMPLEMENTATION

The implementation schedule that follows lists the actions and estimated costs for the recovery program for running buffalo clover. It is a guide for meeting the recovery goals outlined in this Plan. Parties with authority, responsibility, or expressed interest to implement a specific recovery action are identified in the Implementation Schedule. The listing of a party in the Implementation Schedule does not require, nor imply a requirement, that the identified party has agreed to implement the action(s) or to secure funding for implementing the action(s). However, parties willing to participate may benefit by being able to show in their own budgets that their funding request is for a recovery action identified in an approved recovery plan and is therefore considered a necessary action for the overall coordinated effort to recover running buffalo clover. Also, section 7(a)(1) of the ESA directs all Federal agencies to utilize their authorities in furtherance of the purposes of the ESA by carrying out programs for the conservation of threatened and endangered species.

The Implementation schedule lists and ranks recovery tasks, provides task descriptions and duration, identifies responsible agencies, and provides estimated costs. This schedule will be reviewed periodically until the recovery objectives are met, and priorities and tasks will be subject to revision. Tasks are presented in order of task priority number.

KEY TO IMPLEMENTATION SCHEDULE

Column 1: Task Priority

Priority 1: An action that must be taken to prevent extinction or to prevent the species from declining irreversibly in the foreseeable future.

Priority 2: An action that must be taken to prevent a significant decline in species population/habitat quality, or some other significant negative impact short of extinction.

Priority 3: All other actions necessary to meet the recovery objectives.

No tasks have been ranked as priority one in this recovery program, which indicates there are not any actions necessary to prevent extinction of this species in the foreseeable future. As defined in this Plan, the species may have already achieved reclassification criteria by the publication of this recovery plan.

Column 2: Task Number

The number from the STEPDOWN RECOVERY OUTLINE (refer to PART II). Task number does not indicate priority.

Column 3: Task Description

A short description of the recovery task, which coincides with the STEPDOWN RECOVERY OUTLINE (refer to PART II).

Column 4: Task Duration

The number of years that it is expected to take before the task is completed. A pound sign (#) indicates that the task is currently ongoing. A plus (+) indicates that the task will be continuous throughout the recovery period. Tasks may be both ongoing and continuous.

Column 5: Participants

This lists the agencies, organizations, and participants that are expected to be involved in completing these tasks, but other partners may be included as they are identified. If a lead organization exists for a task, the lead organization is indicated by an asterisk (*). A key to the acronyms is provided here.

AR:	Arkansas Natural Heritage Commission
IL:	Illinois Department of Natural Resources
IN:	Indiana Department of Natural Resources
KS:	Kansas Biological Survey
KY:	Kentucky State Nature Preserves Commission
MO:	Missouri Department of Conservation
OH:	Ohio Department of Natural Resources
WV:	West Virginia Department of Natural Resources
NCGRP:	National Center for Genetic Resource Preservation, USDA
UNIV:	Universities and Botanic Gardens
PLO:	Private landowner
NGO:	Non-governmental organizations (e.g. The Nature Conservancy)
BGAD:	Bluegrass Army Depot, Department of Defense
USFS:	United States Forest Service
USFWS:	United States Fish and Wildlife Service

Columns 6-11: Cost Estimates for FY's 1-5

The total estimate cost to recover the species over the next 15 years, plus the estimated cost for carrying out the task during the next five fiscal years (FY). Estimated costs are listed in thousands of dollars.

Column 11: Comments

Explanatory comments and additional information.

Table 4. Implementation Schedule

TASK PRIORITY	TASK NUMBER	TASK DESCRIPTION	TASK DURATION (YRS.)	PARTICIPANTS	TOTAL COSTS	COST ESTIMATES ($000) FY1	FY2	FY3	FY4	FY5	COMMENTS
2	1.1	Determine appropriate habitat management techniques	#, 5	USFWS, USFS, BGAD, KY, OH, WV	$75,000	15	15	15	15	15	
2	1.2	Implement appropriate habitat management techniques including invasive plant control	+	USFWS, USFS, BGAD, IN, KY, MO, OH, WV	$375,000	25	25	25	25	25	May change over time as task 1.1 is determined
2	1.3	Protect known populations and the habitat they occupy via management agreements	+,3	USFWS, USFS, BGAD, IN, KY, MO, OH, WV, NGO, PLO	$75,000			15	15	15	
2	1.4	Monitor known populations in each state to document trend and update PVA	#, 10	USFWS, USFS, BGAD, IN, KY, MO, OH, WV	$260,000	26	26	26	26	26	
2	2.1.1	Examine conditions necessary for flowering	4	USFWS, USFS, BGAD, UNIV	$20,000	5	5	5	5		
2	2.1.2	Examine the types and frequency of pollinators	2	USFWS, USFS, BGAD, UNIV	$10,000	5	5				
2	2.1.3	Examine the level of seed set under various management regimes	4	USFWS, USFS, BGAD, UNIV	$20,000	5	5	5	5		
2	2.1.4	Examine factor necessary for seed germination in the wild	4	USFWS, USFS, BGAD, UNIV	$20,000	5	5	5	5		
2	2.1.5	Examine the impacts of herbivory in the wild	4	USFWS, USFS, BGAD, UNIV	$20,000	5	5	5	5		

TASK PRIORITY	TASK NUMBER	TASK DESCRIPTION	TASK DURATION (YRS.)	PARTICIPANTS	TOTAL COSTS	COST ESTIMATES ($000) FY1	FY2	FY3	FY4	FY5	COMMENTS
2	2.2.1	Evaluate factors that determine optimal abiotic conditions	4	USFWS, USFS, BGAD, UNIV	$20,000	5	5	5	5		
2	2.2.2	Examine effects of soil disturbance and shading	4	USFWS, USFS, BGAD, UNIV	$20,000	5	5	5	5		
2	2.2.3	Examine abiotic factors (e.g. weather) that may affect seed viability	4	USFWS, USFS, BGAD, UNIV	$20,000	5	5	5	5		
2	3.1.1	Additional seed collection and storage	#	USFWS, NCGRP	$15,000	1	1	1	1	1	
3	1.5	Survey for additional populations throughout range	5	USFWS, USFS, IN, KY, MO, OH, WV, KS, AR	$125,000	25	25	25	25	25	
3	1.6	Develop Post-Delisting Monitoring and Management Plan	1	USFWS, USFS, BGAD, IN, KY, MO, OH, WV	$5,000						Will occur during later years of recovery
3	4.1	Provide public information about running buffalo clover	#	ALL	$78,000	8	5	5	5	5	
3	5.1	Conduct annual recovery team conference calls and/or face-to-face meetings	+	ALL	$35,000		5		5		Face-to-face meeting every two years
3	5.2	Revise recovery plan as appropriate	+	Running Buffalo Clover Recovery Team	$5,000						

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APPENDIX 1. Population Monitoring Protocols

Population monitoring may range in the level of detail from a simple census (number of rooted crowns) to more complex demographic monitoring (following crowns over time with respect to reproduction, survival, etc.). The methodology used will depend on the questions to be answered.

Census Methodology:

1. For small populations (less than 50 rooted crowns), record the number of rooted crowns and flowering stems.

2. For large populations, including numerous subpopulations, record the number of rooted crowns and flowering stems in representative square-meter plots, estimate the area occupied by running buffalo clover, and extrapolate to determine the number of rooted crowns and flowering stems per population or subpopulation. Depending on the size of the area occupied by running buffalo clover and its density, it may be necessary to sample several square-meter plots (randomly or with an attempt to sample different densities).

All censusing should occur in May or June, preferably when running buffalo clover is flowering, and before new stolons root. As the number of rooted crowns change from spring to summer, counting at the same time each year will provide the most consistent data.

Demographic Methodology:

1. Establish permanently-marked plots (size depends on population size and density).
2. Map all rooted crowns using a grid system within the plot and record information such as number of stolons per rooted crown, number of flowering stems per rooted crown/stolon. Map any seedlings.
3. Collect these data from each plot at least once each season during May-September.
4. Search for seedlings and document fruit production.

APPENDIX 2. Populations of Running Buffalo Clover

Population	State	Region	Ranking	Habitat	Ownership	Protected?
Bell Chute Access	MO	O	X	floodplain	S	Y
Cedar Bottom Woodland	MO	O	D	open woods	P	N
Cuivre River State Park	MO	O	B	floodplain	S	Y
Graham Cave State Park	MO	O	B	floodplain	S	Y
Jerome Access	MO	O	X	floodplain	S	Y
Dearborn County Farm	IN	B	C	forested terrace	S	N
Doublelick Run	IN	B	C	floodplain	P	N
Greendale	IN	B	D	mesic upland forest	P	N
Henschen Branch	IN	B	D	wooded ravine terrace	P	N
Hidden Valley	IN	B	A	wooded ravine and lawn	P	N
Island Branch	IN	B	D	wooded ravine terrace	P	N
Ault Park	OH	B	C	mesic forest	L	N
Brown Co.	OH	B	C	mesic forest/trail	P	N
Cincinnati Nature Center	OH	B	X	mesic forest	P	N
Congress Green	OH	B	B	lawn, cemetery	S	Y
Fletcher/SR 7	OH	A	D	mesic forest	P	N
Fankhauser	OH	B	D	lawn	P	N
Gatch	OH	B	C	lawn	P	N
Halls Creek	OH	B	X	mesic forest	L	N
Mitchell Memorial-west	OH	B	A	open woods	L	N
Mitchell Memorial-east	OH	B	C	mesic forest	L	N
Morrison (Warren Co.)	OH	B	X	lawn	P	N
MWF Bowles Woods	OH	B	B	lawn	L	N
MWF Lake	OH	B	D	mesic forest	L	N
MWF Parcours Trail	OH	B	X	mesic forest	L	N
Newberry	OH	B	D	mesic forest	L	N
Pebble Creek Golf Course	OH	B	X	mesic forest	P	N
Promont	OH	B	X	lawn	P	N
Sand Run	OH	B	X	mesic forest	P	N
Shawnee Lookout (SL) Blue Jacket Trail	OH	B	X	trail	L	N
SL Bobcat/Cabin View	OH	B	D	lawn	L	N
SL Little Turtle Trail	OH	B	B	mesic forest	L	N
SL Miami Fort	OH	B	A	lawn	L	N

Population	State	Region	Ranking	Habitat	Ownership	Protected?
SL Oxbow	OH	B	C	flood plain	L	N
Sycamore Creek	OH	B	B	mesic forest	P	N
Warder-Perkins/Niehaus	OH	B	B	mesic forest	P	Y
Wayne NF - Ironton	OH	A	D	forested along trail	F	Y
Ashbys Fork	KY	B	D	floodplain	P	N
Ashland	KY	B	C	lawn	L	N
Barlow Place	KY	B	X	lawn	P	N
Beaver Branch	KY	B	B	wooded stream terrace with trails	P	N
Big Bone at Dark Hollow	KY	B	C	stream terrace	P	N
Big Bone Lick SP East	KY	B	A	lawn	S	Y
Big Bone Lick SP West	KY	B	D	lawn	S	Y
Boone Creek	KY	B	B	floodplain	P	N
BGAD 34	KY	B	A	floodplain	F	Y
BGAD 35	KY	B	D	floodplain	F	Y
BGAD 38	KY	B	X	floodplain	F	Y
BGAD 39	KY	B	X	floodplain	F	Y
BGAD 40	KY	B	B	floodplain	F	Y
BGAD 41	KY	B	D	floodplain	F	Y
BGAD 42	KY	B	X	floodplain	F	Y
BGAD 43	KY	B	X	floodplain	F	Y
BGAD 44	KY	B	X	floodplain	F	Y
BGAD 45	KY	B	D	floodplain	F	Y
BGAD 46	KY	B	A	floodplain	F	Y
BGAD 48	KY	B	X	floodplain	F	Y
BGAD 49	KY	B	X	floodplain	F	Y
BGAD 50	KY	B	C	floodplain	F	Y
BGAD 51	KY	B	B	floodplain	F	Y
BGAD 52	KY	B	B	floodplain	F	Y
BGAD 54	KY	B	X	floodplain	F	Y
BGAD 55	KY	B	X	floodplain	F	Y
BGAD 56	KY	B	B	floodplain	F	Y
BGAD 57	KY	B	D	floodplain	F	Y
BGAD 58	KY	B	X	floodplain	F	Y
BGAD 59	KY	B	B	floodplain	F	Y
BGAD 61	KY	B	D	floodplain	F	Y
BGAD 63	KY	B	C	floodplain	F	Y
BGAD 64	KY	B	B	floodplain	F	Y

Population	State	Region	Ranking	Habitat	Ownership	Protected?
BGAD 69	KY	B	C	floodplain	F	Y
BGAD 70	KY	B	X	floodplain	F	Y
BGAD 72	KY	B	X	floodplain	F	Y
Cherokee Park	KY	B	X	lawn	L	N
Clear Creek	KY	B	X	floodplain	P	N
Craig Creek Tributary	KY	B	C	floodplain	P	N
Dinsmore	KY	B	C	open woods/trail	L	N
Doe Run Lake	KY	B	D	young forest	L	N
Fowler Creek	KY	B	X	floodplain	P	N
Ft. Boonesbourough area	KY	B	X	woodland/path (recently cleared)	P	N
Gaines House	KY	B	X	lawn	P	N
Griers Creek	KY	B	D	floodplain	P	N
Griffith Woods	KY	B	X	floodplain	P	N
Gum Branch Wildlife Management Area	KY	B	A	woodland and stream terrace	S	N
Iroquois Hunt Club	KY	B	D	stream terrace/mesic woods	P	N
Kramer St	KY	B	D	driveway	P	N
Larchmont Farm	KY	B	D	lawn	P	N
Leach Farm	KY	B	X	lawn	P	N
Liberty Road Barrens	KY	B	X	young forest	P	N
Little Clover Creek	KY	B	D	floodplain	P	N
Lower Howards Creek	KY	B	C	wooded cattle trail along stream	L	Y
Lower Howards Creek South	KY	B	X	floodplain	P	N
Lulbegrud North	KY	B	C	floodplain	P	N
Montgomery Co	KY	B	D	floodplain	P	N
Mt. Zion Road	KY	B	C	floodplain/trails	P	N
Oakland Farm	KY	B	D	lawn	P	N
Paris Pike North	KY	B	C	lawn	P	N
Presbyterian Cemetery	KY	B	X	lawn	P	N
Rice Creek	KY	B	X	woodland/trail	P	N
Second Creek	KY	B	D	floodplain	P	N
Silver Creek	KY	B	D	floodplain/trails	P	N
Site 100	KY	B	D	woodland	P	N
Spears House	KY	B	D	lawn	P	N
Stonewall	KY	B	X	lawn	P	N
Sunny Hollow	KY	B	X	lawn	P	N
Sycamore Farm	KY	B	C	lawn	P	N
UK Arboretum	KY	B	X	mesic forest	S	N

Population	State	Region	Ranking	Habitat	Ownership	Protected?
Upper Howards Creek	KY	B	B	grazed wooded floodplain	P	N
Vining House	KY	B	X	lawn	P	N
Wilhoit House	KY	B	C	lawn	P	N
Willsrupard Road	KY	B	C	grazed woodland	P	N
Wolf Pen Branch	KY	B	B	woodland	P	Y
Back Fork of Elk River	WV	A	X		P	N
Bowden	WV	A	B	ORV trail	F	Y
Brush Heap Knob (Rich Mtn. East)	WV	A	D	wooded cow path	P	N
Brushy Run	WV	A	D	oak / hickory forest	F	Y
Cheat River	WV	A	H		P	N
Cotton Hill	WV	A	D	forest in floodplain	P	Y
Crouch Knob - Becky Creek	WV	A	A	old skid roads, young woodland	F,S	Y,N
Dry Fork of the Elk River	WV	A	C	old logging road	P	N
Fernow	WV	A	A	logging roads, skid trails, wildlife paths	F	Y
Franklin	WV	A	B	stream bottom	P	N
Hans McCourt	WV	A	X		P	N
Hoe Lick	WV	A	B	old logging road	F	Y
Laurel Mountain	WV	A	E	forested jeep trail	P	N
Left Fork of Clover Run	WV	A	D	old logging road	P	N
Lower John's Run	WV	A	D	old logging road or possibly railroad grade	F, P	Y,N
Marilla	WV	A	X		P	N
McGee Run-Back Fork- A,B,C	WV	A	B	old logging roads, young forest	F, P	Y,N
McGowan Mountain	WV	A	A	old skidder road, adjacent a new clear cut	F	Y
Mill Creek	WV	A	D	old road and logged clearing	P	N
Millstone Run	WV	A	B	old logging road	P	N
Mowry Run	WV	A	B	old logging road	S, P	N
Parsons	WV	A	D	ATV track in mesic woods	P	N
Pond Lick Mountain	WV	A	X	old logging road, active limestone quarry	P	N
Porterwood	WV	A	D	along ORV trail within a floodplain forest	P	N
Rafe Run (Westvaco W Tract 801)	WV	A	C		P	N
Rattlesnake Run - A	WV	A	B	mesic forest, old logging roads, deer trails	F, P	Y,N
Rich Mountain West, Lookout Tower	WV	A	C	logging roads	P	N
Rich Mountain West, Microwave	WV	A	C	old road in secondary forest	P	N
Rich Mountain West, Quarry	WV	A	D	jeep trail	P	N
Shaver's Fork Flood Plain	WV	A	C	in floodplain along footpath	P	N
Shaver's Mountain	WV	A	B	old skid trail and logging road	F	Y
Snyder Run, Rich Mountain East	WV	A	C	trail in mesic forest	P	N

Region: A = Appalachian, B = Bluegrass, O = Ozark

EO ranking for rooted crowns: A = 1000+, B = 100-999, C = 30-99, D = <30, E = Extant but not ranked, H = historical, X = Extirpated

Ownership: F = Federal, S = State, L = county, city, local government, P = private

APPENDIX 3. Associate Species of Running Buffalo Clover by State

Indiana

TREES and SHRUBS:
Acer negundo
Acer saccharum
Aesculus glabra
Asimina triloba
Fraxinus americana
Fraxinus quadrangulata
Platanus occidentalis
Quercus alba
Quercus macrocarpa
Quercus shumardii
Ulmus rubra

HERBACEOUS SPECIES:
Acalphya rhomboidea
Alliaria petiolata
Amphicarpaea bracteata
Campanula americana
Carex jamesii
Carex frankii
Cryptotaenia canadensis
Eupatorium rugosum
Laportea canadensis
Leersia virginica
Lysimachia nummularia
Pilea pumila
Poa compressa
Polemonium reptans
Podophyllum peltatum
Polygonum hydropiperoides
Polygonum punctatum
Polygonum virginianum
Taraxacum officinale
Trifolium repens
Verbesina alternifolia

Kentucky

TREES and SHRUBS:
Acer negundo
Acer saccharum
Celtis occidentalis
Cornus drummondii
Fraxinus americana
Juglans nigra
Plantanus occidentalis
Symphoricarpos orbiculata
Ulmus americana

HERBACEOUS SPECIES:
Collinsia verna
Glechoma hederacea
Elymus villosa
Iodanthus pinnatifidus
Stellaria media
Trifolium repens
Valeriana pauciflora
Verbesina alterniflora
Viola papilionacea

Ohio

TREES and SHRUBS:
Acer negundo
Acer nigrum
Acer saccharum
Aesculus flava
Aesculus glabra
Asimina triloba
Celtis occidentalis
Cercis canadensis
Fraxinus americana
Gymnocladus dioca
Juglans nigra
Lindera benzoin
Lonicera maackii
Tilia americana
Ulmus americana
Quercus macrocarpa

HERBACEOUS SPECIES:
Alliaria petiolata
Carex jamesii
Carex rosea
Cerastium spp.
Duchesnea indica
Elymus macgregorii
Elymus virginicus
Erigeron annuus
Eupatorium rugosum
Galium aparine
Geum canadense
Glechoma hederacea
Impatiens spp.
Lonicera japonica
Mertensia virginica
Muhlenbergia schreberi
Oxalis stricta
Ozmorhiza claytonii
Phlox divaricata
Pilea pumila
Poa sylvatica
Polygonatum biflorum
Polygonatum cespitosum
Stellaria media
Synandra hispidula
Taraxacum officiale
Toxicodendron radicans
Trifolium repens
Urtica dioica
Viola sororia
Viola striata

Missouri

TREES and SHRUBS:
Acer rubra
Acer saccharum
Carpinus caroliniana
Juglans nigra
Lindera benzoin
Nyssa sylvatica
Platanus occidentalis
Quercus alba
Quercus rubra
Ulmus americana

HERBACEOUS SPECIES:
Agrimonia pubescens
Alisma plantago-aquatica
Amphicarpaea bracteata
Asarum canadense
Asclepias purpurascens
Cryptotaenia canadense
Elephantopus carolinianus
Galium triflorum
Galium concinnum
Gratiola neglecta
Impatiens spp.
Mimulus alatus
Oxalis dillenii
Phlox divaricata
Pilea pumila
Poa sylvestris
Polemonium reptans
Polygonum virginianum
Rhus radicans
Samolus parviflora
Scutellaria spp.
Trifolium pretense
Trifolium repens
Viola pennsylvanica

West Virginia

TREES and SHRUBS:
Acer saccharum
Juglans nigra
Liriodendron tulipifera
Prunus serotina

HERBACEOUS SPECIES:
Amphicarpa bracteata
Aster spp.
Campanula americana
Carex pensylvanica
Circaea alpina
Cryptotaenia canadensis
Dryopteris marginalis
Erigeron pulchellus
Eupatorium rugosum
Galium asprellum
Glecoma hederacea
Hypericum mutulum
Hypericum punctatum
Junicus tennis
Leersia oryzoides
Lilium spp.
Oxalis europaea
Panicum clandestinum
Pilia pumula
Plantain virginica
Poa spp.
Polygonum pensylvanicum
Potentilla canadensis
Prunella vulgarus
Pycnantheum virginianum
Ranunculus repens
Sedum ternatum
Solidago arguta
Urtica dioica
Verbena urticifolia
Verbesina alternifolia
Viola spp.

APPENDIX 4. Management Recommendations for Running Buffalo Clover

U.S. FISH & WILDLIFE SERVICE OHIO FIELD OFFICE

MANAGEMENT RECOMMENDATIONS FOR RUNNING BUFFALO CLOVER

Background

Running buffalo clover (*Trifolium stoloniferum*) was listed as a federally endangered species in 1987. This stoloniferous perennial occurs in Missouri, Indiana, Ohio, Kentucky and West Virginia in one of two fairly distinct habitats (lawn or wooded). Consistent management of these different habitat types is critical for maintaining running buffalo clover populations. The following recommendations were compiled in Ohio to guide property owners and land managers in the management and recovery of this endangered species.

Lawn Sites
(cemeteries, parks, old home sites, etc.)

♣ Mowing – We recommend a mowing regime that reduces competition to the running buffalo clover (RBC) and allows for flowering and seed set. Mowers should be set at no lower than 3 inches.

Schedule:
Once in Mid-April
Once during the first week of May
Once during the last week of June
Anytime after July 1 is acceptable

♣ Avoid the use of herbicides around RBC.

♣ Control invasive plants manually in the vicinity of RBC. Typical invasive groundcovers growing among RBC include periwinkle, Japanese honeysuckle, and wintercreeper.

♣ We do not recommend burning as an effective management strategy for existing populations. RBC is not a prairie species and occurs in habitats that are not necessarily adapted to fire (e.g. mesic woodlands). Unlike fire-adapted species, much of the RBC plant structure is above ground. Fire will most likely kill the plants that are growing at the site.

♣ Soil disturbances/scraping may be beneficial to existing populations. Experiments involving various types and levels of soil disturbance should be conducted by experienced researchers.

Wooded Sites
(mesic woods, sites near streams, etc.)

♣ We recommend removal of individual, select trees to maintain a "dappled shade" environment. Cut stumps should be treated with a systemic herbicide to prevent resprouting.

♣ Control invasive plants through manual pulling (e.g. garlic mustard) or selective herbicide application on cut stems (e.g. Amur honeysuckle).

♣ No foliar herbicide application within 25 feet of RBC sites.

♣ No burning (see above).

♣ Minimal soil disturbance (see above).

Special thanks to the Ohio RBC Management Group: M. Becus, A. Cusick, R. Glotzhober (Ohio Historical Society), J. Klein and J. Mundy (Hamilton Co. Park District), G. Schneider and M. Moser (ODNR-Division of Natural Areas & Preserves), M. Vincent (Miami University), and J. Windus (ODNR-Division of Wildlife)

Drawing by John Myers

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APPENDIX 5. Population Viability Analysis

Population viability analysis (PVA) is a general term used to describe the suite of quantitative methods that predict the future condition of one or more populations of conservation concern. A recent review of recovery plans for threatened and endangered species found that PVA was increasingly used to manage and recover rare species in the United States. However, less than half of the recovery plans approved since 1991 incorporated some aspect of PVA (Morris *et al.* 2002). The National Research Council (1995) has urged increased use of PVA in recovery plans. After an initial assessment into the potential of using PVA, the Recovery Team concluded that additional insight into the dynamic nature of running buffalo clover populations was possible through PVA.

PVA can be broken down into four broad categories: count-based, structured, metapopulation, and spatially explicit. A concise description of these four approaches can be found in Morris *et al.* (2002). Count-based PVA relies on time series census data to estimate extinction risk. It is the simplest form of PVA and the most commonly used (Morris *et al.* 2002). We chose to use the relatively simple count-based PVA for analysis of running buffalo clover population trends. Other forms of PVA require additional data not available and are far less common in general. Morris *et al.* (1999) contend that the use of the more sophisticated models may be appealing because they incorporate more biological detail, but the benefits may be illusory if critical components of the model are unknown due to lack of data.

The statistical methods for assessing extinction risk from count-based census data are referred to as the diffusion approximation (DA) approach and were developed by Dennis *et al.* (1991). Diffusion approximation PVA has been used to assess extinction risk in numerous species, including insects, mammals and plants (Morris *et al*. 1999, Schultz and Hammond 2003). Using the DA approach, we assessed the extinction risk of each population of running buffalo clover where count-based census data existed for at least five years. We followed the guidelines found in *A Practical Handbook for Population Viability Analysis* (Morris *et al.* 1999). First, we calculated transformations of the counts and the number of years between counts and then preformed a linear regression on these transformed data. The regression result yields estimates of the average growth rate (μ) and its associated variance (σ^2) (Box 3.2 in *Morris et al.* 1999). Measures of viability were then calculated for individual populations (Box 3.3 in Morris *et al.* 1999). Four parameters were required to calculate the probability of extinction: 1) initial population size, which refers to the population size at the start of the projection period, 2) an extinction threshold, which we estimated as one plant (we considered this a conservative number because we could not estimate the size of the seedbank), and 3) μ, and σ^2. We chose to estimate extinction risk for 20 years because longer time periods were not justified by the length of our observed census counts. The average length of the time series for each element occurrence rank was 8.0, 9.2, 9.4, and 10.1 years for A, B, C, and D ranks, respectively.

Key assumptions for DA analysis were reviewed by Schultz and Hammond (2003) and are as follows: 1) counts are exhaustive or represent a constant fraction of the total population, 2) the variability between years is a result of environmental variability, 3) there are no catastrophic years in the observed data, and 4) population growth rate is not affected by density. One particular problem with using DA for plants is the invisibility of the seedbank. This problem

arises because the method assumes a constant fraction of the total population is counted during each census period. Because it is difficult to know what percentage of the population exists as seeds from year to year, projections may be invalid if the seed bank is significantly different from year to year. It should also be noted that the existence of a seed bank means that a population would still be extant even if no plants appeared aboveground for one or more years. Both critical and supportive assessments of the diffusion approximation approach are summarized by Elderd *et al.* (2003).

We also elected to estimate population viability for multiple occurrences of independent populations (Equation 5.1 in Morris *et al.* 1999 and below). The overall risk of extinction for a species drops when multiple and independent populations exist. This is seen clearly when the following formula for global extinction risk is used:

$$P_{global} = P_1 * P_2 * P_3 * P_4 * P_5 * \ldots * P_M$$

Where P_i is the probability that the *i*th population becomes extinct over time and where P_{global} is the probability that all M populations become extinct. For example, if there are three populations all with an independent probability of extinction of 0.4, then P_{global} becomes 0.064 (e.g., $P_{global} = 0.4 * 0.4 * 0.4 = 0.064$).

The results of our estimates are stratified by population rank. Overall and average rank probabilities of extinction also are presented. The average probability of extinction for each rank was used to determine the number of populations needed to have less than a 0.05 probability of extinction for each ranking. Based on the average probabilities of extinction given below, 1 A-rank , 3 B-rank, 3 C-rank, and 10 D-rank populations would be needed to achieve a 95% probability of persistence for each population rank for the next 20 years. PVA also provides criteria to maintain species viability throughout the range. Running buffalo clover recovery cannot be accomplished in one region alone, as large and small populations throughout the range contain critical levels of genetic variation.

Analysis of short-term data may yield probability of extinction risks that are either overly high or low. To offset this concern, continued monitoring of individual populations will increases the chances that the PVA incorporates year to year variation. We acknowledge that PVA is only one tool to help us understand viability of running buffalo clover populations. The determination of extinction risk should be based on the most appropriate and biologically defensible quantitative method available at the time a reclassification or delisting action is considered.

Probability of extinction after 20 years for individual populations stratified by rank with at least 5 years in the observation period.

Ecoregion	State	Population	Rank	Mu (μ)	Variance (σ^2)	Length of time series	Starting population size (nq)	Prob. of Extinction (20 Years)
Appalachians	WV	Lower Rock Camp Run	A	0.013284	0.9424	10	1127	0.071517
Appalachians	WV	Crouch Knob†	A	*-0.018193*	*1.244596*	*9*	*64998*	*0.021091*
Appalachians	WV	Fernow‡	A	-0.007087	0.5723	5	5555	0.005001
Bluegrass	OH	Mitchell Memorial Park	A	0.271306	0.1904	8	1157	0
Bluegrass	OH	Shawnee Lookout Park	A	0.127468	0.089	10	2583	0
Appalachians	WV	Becky Creek	A	0.273110	0.2113	6	1622	0
							Overall "A" Risk[1]	0
							Average "A" Risk[2]	0.01626817
Appalachians	WV	Hoe Lick	B	-0.343411	0.0947	8	197	0.893248
Appalachians	WV	Bowden	B	-0.339089	2.523	7	326	0.731014
Appalachians	WV	McGee Run/Back Fk Trib	B	-0.102819	0.0362	9	637	0
Appalachians	WV	Baker Sods	B	-0.168239	0.7644	10	111	0.504425
Appalachians	WV	Upper John's Run	B	-0.106706	0.3702	12	205	0.166849
Bluegrass	OH	Warder-Perkins/Niehaus	B	-0.069722	0.3663	11	711	0.037559
Appalachians	WV	Shaver's Mtn	B	-0.014600	0.2578	10	235	0.014353
Appalachians	WV	Upper Rock Camp Run	B	0.152604	1.9295	9	339	0.162094
Bluegrass	OH	Miami Whitewater Forest	B	0.313034	1.6147	11	134	0.09195
Bluegrass	OH	Congress Green	B	0.007301	0.1199	10	145	0.000416
							Overall "B" Risk	0
							Average "B" Risk	0.2601908
Appalachians	WV	Shaver's Fk Flood Plain	C	-0.086210	0.3604	5	34	0.332602
Bluegrass	OH	Gatch	C	-0.017971	0.7839	10	50	0.325138
Appalachians	WV	Porterwood	C	0.002561	0.8495	12	36	0.357573

Ecoregion	State	Population	Rank	Mu (μ)	Variance (σ^2)	Length of time series	Starting population size (nq)	Prob. of Extinction (20 Years)
Appalachians	WV	Dry Fk - Elk River	C	-0.005103	0.6678	9	48	0.266248
Appalachians	WV	Snyder Run	C	0.423411	6.6781	11	69	0.46707
Appalachians	WV	Brush Heap Knob	C	0.160658	1.3186	9	55	0.20869
Appalachians	WV	Laurel Mtn	C	0.331461	1.9213	10	79	0.145261
							Overall "C" Risk	0.00014577
							Average "C" Risk	0.30036886
Ozarks	MO	Cedar Bottom	D	-0.442297	0.2524	7	9	0.999883
Bluegrass	OH	Newberry Wildlife Refuge	D	-0.782229	1.272	10	1	1
Appalachians	WV	Rich Mtn West Quary	D	-0.273274	0.3433	11	8	0.961539
Appalachians	WV	Brushy Run	D	-0.178966	0.2781	7	6	0.907345
Appalachians	WV	Parsons	D	-0.220846	0.3518	8	13	0.873557
Appalachians	WV	Mill Creek	D	-0.155722	0.4982	14	7	0.827753
Appalachians	WV	Lower John's Run	D	-0.304764	7.7944	12	7	0.931021
Appalachians	WV	Rich Mtn West Microwave	D	-0.017236	0.7145	14	25	0.702432
Appalachians	WV	Left Fk - Clover Run	D	-0.055786	0.185	5	16	0.227378
Appalachians	WV	Cotton Hill	D	0.017165	0.7318	14	15	0.42979
Appalachians	WV	McGowan Mtn	D	0.073473	0.1781	9	9	0.054856
							Overall "D" Risk	0.00221143
							Average "D" Risk	0.71959582
†: Crouch Knob based on Ecoregion means for mu, variance, and length of time series.								
‡: Fernow mu and variance based on occurences observed since 1998, however projected population was the total number of plants in 2003.								
1: Overall "Rank" Risk is the product of all the populations within each rank.								
2: Average "Rank" Risk is the mean of all the populations within each rank.								

Probability of extinction after 20 years for individual populations stratified by ecoregion with at least 5 years in the observation period.

Ecoregion	State	Population	Rank	Mu (μ)	Variance (σ^2)	Length of time series	Starting population size (nq)	Prob. of extinction (20 Years)
Appalachians	WV	Becky Creek	A	0.273110	0.2113	6	1622	0
Appalachians	WV	Fernow‡	A	-0.007087	0.5723	5	5555	0.005001
Appalachians	WV	Crouch Knob†	A	-0.018193	1.244596	9	64998	0.021091
Appalachians	WV	Lower Rock Camp Run	A	0.013284	0.9424	10	1127	0.071517
Appalachians	WV	McGee Run/Back Fk Trib	B	-0.102819	0.0362	9	637	0
Appalachians	WV	Shaver's Mtn	B	-0.014600	0.2578	10	235	0.014353
Appalachians	WV	Upper Rock Camp Run	B	0.152604	1.9295	9	339	0.162094
Appalachians	WV	Upper John's Run	B	-0.106706	0.3702	12	205	0.166849
Appalachians	WV	Baker Sods	B	-0.168239	0.7644	10	111	0.504425
Appalachians	WV	Bowden	B	-0.339089	2.523	7	326	0.731014
Appalachians	WV	Hoe Lick	B	-0.343411	0.0947	8	197	0.893248
Appalachians	WV	Laurel Mtn	C	0.331461	1.9213	10	79	0.145261
Appalachians	WV	Brush Heap Knob	C	0.160658	1.3186	9	55	0.20869
Appalachians	WV	Dry Fk - Elk River	C	-0.005103	0.6678	9	48	0.266248
Appalachians	WV	Shaver's Fk Flood Plain	C	-0.086210	0.3604	5	34	0.332602
Appalachians	WV	Porterwood	C	0.002561	0.8495	12	36	0.357573
Appalachians	WV	Snyder Run	C	0.423411	6.6781	11	69	0.46707
Appalachians	WV	McGowan Mtn	D	0.073473	0.1781	9	9	0.054856
Appalachians	WV	Left Fk - Clover Run	D	-0.055786	0.185	5	16	0.227378
Appalachians	WV	Cotton Hill	D	0.017165	0.7318	14	15	0.42979
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Appalachians	WV	Mill Creek	D	-0.155722	0.4982	14	7	0.827753
Appalachians	WV	Parsons	D	-0.220846	0.3518	8	13	0.873557
Appalachians	WV	Brushy Run	D	-0.178966	0.2781	7	6	0.907345
Appalachians	WV	Lower John's Run	D	-0.304764	7.7944	12	7	0.931021
Appalachians	WV	Rich Mtn West Quary	D	-0.273274	0.3433	11	8	0.961539

Ecoregion	State	Population	Rank	Mu (μ)	Variance (σ^2)	Length of time series	Starting population size (nq)	Prob. of extinction (20 Years)
							Overall Appalachian Risk[1]	0
							Average Appalachian Risk[2]	0.3947195
Bluegrass	OH	Mitchell Memorial Park	A	0.271306	0.1904	8	1157	0
Bluegrass	OH	Shawnee Lookout Park	A	0.127468	0.089	10	2583	0
Bluegrass	OH	Congress Green	B	0.007301	0.1199	10	145	0.000416
Bluegrass	OH	Warder-Perkins/Niehaus	B	-0.069722	0.3663	11	711	0.037559
Bluegrass	OH	Miami Whitewater Forest	B	0.313034	1.6147	11	134	0.09195
Bluegrass	OH	Gatch	C	-0.017971	0.7839	10	50	0.325138
Bluegrass	OH	Newberry Wildlife Refuge	D	-0.782229	1.272	10	1	1
							Overall Bluegrass Risk	0
							Average Bluegrass Risk	0.20786614
Ozarks	MO	Cedar Bottom	D	-0.442297	0.2524	7	9	0.999883
							Overall Ozarks Risk	0.999883
							Average Ozarks Risk	0.999883

†: Crouch Knob based on Ecoregion means for mu, variance, and length of time series.

‡: Fernow mu and variance based on occurences observed since 1998, however projected population was based on the total number of plants in 2003.

1: Overall Risk is the product of all the populations within each ecoregion.

2: Average Risk is the mean of all the populations within each rank.

APPENDIX 6. Summary of Threats and Recommended Recovery Actions

Listing Factor	Threat	Downlisting Criteria	Delisting Criteria	Task
A.	Habitat succession to the point of severe shading that leads to reduced flower and fruit production	1, 2, 3	1, 2, 3	1.1, 1.2, 1.6, 2.1, 4.1, 5.1, 5.2
A.	Permanent habitat loss, such as road construction, that completely destroys the habitat and/or kills all plants and seeds	1, 2, 3	1, 2, 3	1.6, 3.1, 3.2, 4.1, 5.1, 5.2
A.	Competition from non-native invasive plant species	1, 2, 3	1, 2, 3	1.1, 1.2, 1.6, 4.1, 5.1, 5.2
D.	The majority of known populations are unprotected and/or unmanaged	1, 3	1, 3	1.3, 1.4 , 1.5, 1.6, 3.1, 3.2, 4.1, 5.1, 5.2
E.	Fluctuations in population sizes, seed production, and dispersal	1	1	1.4, 2.1, 2.2, 3.1, 3.2, 5.1, 5.2

Listing Factors:
A. The Present or Threatened Destruction, Modification, or Curtailment of Its Habitat or Range
B. Overutilization for Commercial, Recreational, Scientific, Educational Purposes (not a factor)
C. Disease or Predation (not a factor)
D. The Inadequacy of Existing Regulatory Mechanisms
E. Other Natural or Manmade Factors Affecting Its Continued Existence

Downlisting Criteria:

1. Seventeen populations, in total, are distributed as follows: 1 A-ranked, 3 B-ranked, 3 C-ranked, and 10 D-ranked populations across at least 2 of the 3 regions in which running buffalo clover currently occurs (Appalachian, Bluegrass, and Ozark). The number of populations in each rank is based on what would be required to achieve a 95% probability of persistence within the next 20 years based on population viability analysis (see Appendix 5). Rankings refer to the Element Occurrence (EO) ranking categories (Table 1).

2. For each A-ranked and B-ranked population described in #1, population viability analysis indicates a 95% probability of persistence within the next 20 years, OR for any population that does not meet the 95% persistence standard, the population meets the definition of viable. For downlisting purposes, viability is defined as follows: A) seed production is occurring; B) the population is stable or increasing, based on at least five years of censusing; and C) appropriate management techniques are in place.

3. The land on which each of the populations described in #1 occurs is owned by a government agency or private conservation organization that identifies maintenance of the species as one of the primary conservation objectives for the site, OR the population is protected by a conservation agreement that commits the private landowner to habitat management for the species. Natural Resource Management Plans on Federal lands may be suitable for meeting this criterion. This criterion will ensure that habitat-based threats for the species are addressed.

Delisting Criteria:

1. Thirty-four populations, in total, are distributed as follows: 2 A-ranked, 6 B-ranked, 6 C-ranked, and 20 D-ranked populations across at least 2 of the 3 regions in which running buffalo clover occurs (Appalachian, Bluegrass, and Ozark). The number of populations in each rank is based on what would be required to achieve a 95% probability of persistence within the next 20 years; this number was doubled to ensure biological redundancy across the range of the species. Rankings refer to the Element Occurrence (EO) ranking categories (Table 1).

2. For each A-ranked and B-ranked population described in #1, population viability analysis indicates a 95% probability of persistence within the next 20 years, OR for any population that does not meet the 95% persistence standard, the population meets the definition of viable.[3] For delisting purposes, viability is defined as follows: 1) seed production is occurring; 2) the population is stable or increasing, based on at least 10 years of censusing; and 3) appropriate management techniques are in place.

3. Downlisting criterion #3 is met for all populations described in delisting criterion #1.

[3] C-ranked and D-ranked populations are not included for the purposes of viability in recovery criteria # 2 due to their inherently small population sizes and marginal habitat quality. Due to the cyclic nature of running buffalo clover and the high probability of small populations blinking in and out, maintaining viability for a specific C-ranked or D-ranked population at a given time may not be possible. Regardless, small populations have displayed high levels of genetic diversity that is important for survival of the species as a whole and thus are included in the recovery criteria referring to protection and management of sites.

APPENDIX 7. Agency and Public Comments on the Draft Plan

On August 12, 2005, the Service released the Running Buffalo Clover Draft Revised Recovery Plan: First Revision, for a 60-day peer review and public comment period ending on October 11, 2005. Availability of the Plan was announced in the *Federal Register* (FR 70 47222) and a news release to media contacts throughout the range of the species.

In accordance with Service policy, requests for peer review of the draft Plan were sent to experts outside the Service. In particular, these experts were asked to comment on 1) threats to the species, 2) recovery strategy, 3) research needs, 4) use of population viability analysis, 5) recovery criteria, and 6) recovery tasks and implementation. Peer reviews were received from the following individuals:

Mr. Allison Cusick, The Ohio State University- Museum of Biological Diversity, Columbus, OH
Dr. Theresa Culley, University of Cincinnati, Cincinnati, OH
Dr. William Morris, Duke University, Durham, NC

During the comment period, the *Federal Register* Notice Of Availability and press release were distributed to 51 affected government agencies, organizations, and interested individuals. The recovery plan was available online at http://www.fws.gov/midwest/Endangered/ and from the Ohio Field Office upon request.

Ten comment letters were received during the official comment period. Affiliations from which the comments came from include 3 peer reviewers, 3 Federal agencies, 2 state agencies, 1 conservation organization, and 1 private citizen.

Each letter contained one or more comments, with some letters raising similar issues. Several commenters requested explanation or clarification of points made in the Plan and included suggestions for changes. Some commenters provided updated data on populations and their status. Adjustments within the text and to Appendix 2 reflect those additional data. Many commenters felt that the Plan was well-written and scientifically sound with a defensible and feasible recovery strategy. All comments received were considered and noted. Significant comments that were not incorporated or that require further clarification are addressed below.

The letters received from the independent peer reviewers, as well as other comment letters on the Draft Revised Recovery Plan, are on file at the U.S. Fish and Wildlife Service, Ohio Field Office, 6950-H Americana Parkway, Reynoldsburg, Ohio 43068.

Comment: There is conflicting research about whether running buffalo clover can self-pollinate. Should this be investigated further?

Response: Running buffalo clover is self-compatible, but requires a pollinator to transfer the pollen, thus it cannot automatically self-pollinate. This issue is clarified within the Reproduction section of the Plan.

Comment: Genetic diversity of small populations may be as important as large populations. Should we protect small populations rather than large populations since small populations are more vulnerable to extirpation? Why attach a geographic component?

Response: High genetic diversity has been shown in small populations as well as large populations of running buffalo clover. High genetic diversity also exists throughout the range of the species. Using the best data currently available, recovery criteria were developed that strive to maintain populations of all sizes throughout the range of the species. By only protecting the large or only protecting the small populations in just a limited part of the range we risk losing unique genetic diversity found in this species. With that said, the Recovery Team acknowledged that protected populations should occur in two of the three regions (Appalachian, Bluegrass, Ozark). This was decided due to the very small number of known populations outside of the Appalachian and Bluegrass regions.

Comment: Listing only plants by genus in Table 2 is not specific enough to use as an associate species for running buffalo clover. The actual species should be listed since many species in genera are widely different in habitat or occurrence.

Response: Table 2 is meant to be a quick reference for general associate species rangewide. For a complete and more specific species list by state see Appendix 3.

Comment: Catastrophic disturbance is a difficult term to interpret. If you mean habitat loss, this term is more easily understood.

Response: We agree this term may be confusing, especially since running buffalo clover is to some extent dependant on disturbance. Instead, the term permanent habitat loss will be used. This applies to actions like road construction and development. Permanent habitat loss as just described should not be confused with habitat loss through succession or invasive species. These are separate threats.

Comment: We urgently need an update on running buffalo clover survey data – should this not be at the front of the recovery plan?

Response: Much of the data used in the development of the Plan was from population censuses conducted in 2004 and 2005. Appendix 2 provides a list of all populations, location, rank, habitat types, ownership, and protection status.

Comment: In the Biological Constraints section it is mentioned that flowers appear devoid of viable seed. Given that recovery criteria 2 (A) states that “flower production is occurring”, it may be worthwhile to include additional criteria for seed production.

Response: We agree that seed production is a better measure of viability than flower production and have changed the criteria to reflect that suggestion. A recovery task aimed at understanding set seed under various conditions has also been added under 2.1.3.

Comment: The Management Recommendations for Running Buffalo Clover document is quite restrictive. These recommendations are very labor intense, restrict the use of herbicides, restrict prescribed burnings, and require mowing schedules that are impossible to handle.

Response: The Management Recommendations for Running Buffalo Clover document was prepared for use in Ohio although it may be very applicable to other states. Specific recommendations, including timeframes for mowing, may vary throughout the range of the species. We recommend that you work closely with the Service field offices to implement the best management techniques for your site (see recovery task 1.2).

Comment: Given that running buffalo clover sometimes fluctuate in size from one year to another, what criteria can be used to tell when a population is truly extirpated.

Response: Members of the Recovery Team suggests that plants should be absent from a site for 5 years before the site is considered extirpated. If the population had a long history of flowering previous to its absence from the site, this timeline may be much longer due to the seed bank. It is important to note that plants may not appear in the exact location from year to year due to their clonal nature and seed banking abilities.

Comment: The Plan is to be commended for adopting quantitative recovery criteria based on PVA. The Plan has taken the first step by applying a simple count-based PVA method to census data collected from existing running buffalo clover populations. When considering reclassification of running buffalo clover, the determination of extinction risk should be based on the most appropriate and biologically defensible quantitative method available at the time. This will not necessarily be the approach employed in Appendix 5. In particular, an advanced model could include consideration of a seed bank, density-dependant population growth, and population correlations (i.e. non-independent populations).

Response: Our current use of PVA is based on the best data available today. Continued monitoring of running buffalo clover populations and a greater understanding of the species ecology will enable us to improve our PVA in the future and incorporate other factors into the model that will further strengthen our understanding of extinction risk. Recovery task 1.4 identifies the importance of continued population censusing and revising the PVA as new information is revealed.

Comment: Shouldn't there be a minimum number of years of data before a population viability analysis can be conducted?

Response: The most predictive PVA should have several years of data included for each population. We only used populations for which we had at least 5 years of data in our PVA. Information concerning how the PVA was conducted is included in Appendix 5. As more data are collected in the future, the PVA can be modified to reflect the best available information.

Comment: One of the assumptions of PVA is that there are no catastrophic years in the observed data. Is this realistic to expect for running buffalo clover or other clonal perennials? If data for the PVA are collected during a difficult time for the plant it will reflect a different degree of viability than if the census data occurs during a stable period.

Response: Analysis of short-term data may yield probability of extinction risks that are either overly high or low. To offset this concern, continued monitoring of individual populations will increases the chances that the PVA incorporates year to year variation. We acknowledge that PVA is only one tool to help us understand viability of running buffalo clover populations. The determination of extinction risk should be based on the most appropriate and biologically defensible quantitative method available at the time a reclassification or delisting is considered.

Comment: Reclassification criteria state that a sufficient number of populations need to be protected in 2 of the 3 regions. I assume that the criteria can be met even if all populations in one region (i.e. Ozark) went extinct. Given the argument presented in the Plan that all existing genetic variants might be of value for the future persistence of the species, and that each region harbors unique variants, shouldn't germplasm from all three regions be preserved?

Response: Recovery action 3.1.1 recommends that germplasm be collected from populations in each state in which running buffalo clover occurs. In this way, germplasm would be collected from all three regions.

Comment: Substantiate the need for biological redundancy in the delisting recovery criteria.

Response: The numbers of populations of each rank used as downlisting criteria were doubled to develop the delisting criteria. We felt that twice as many populations should be protected and managed in order to delist running buffalo clover. Biological redundancy, along with representation, and resiliency are biological principals intended to provide for population conservation (Shaffer and Stein 2000).

Comment: One commenter thought that the task number was the priority number and suggested that recovery tasks investigating seed germination (2.1.4) and pollination (2.1.2) should have their task numbers swapped.

Response: Recovery actions of equal priority have varying recovery task numbers. Both tasks 2.1.2 and 2.1.4 are listed as priority 2 in the Plan. This is further clarified under the Implementation section.

Comment: There is a need for regular meetings, workshops, tighter partnerships, data exchange, and cooperative management and assessment.

Response: Recovery action 5.1 has been revised to include all interested parties to communicate regularly in evaluating the progress of running buffalo clover recovery.

Comment: The recovery plan presents the evidence for reducing running buffalo clover's status from endangered to threatened. However, complete delisting of this species is not advisable.

Response: The recovery criteria outline requirements that must be met before downlisting or delisting running buffalo clover could occur. The plan estimates delisting in 2020.

Comment: If running buffalo clover were delisted there would be little incentive for Federal agencies to consider this species a priority any longer.

Response: Delisting of running buffalo clover will only occur once the species no longer warrants a protected status. In addition, once a species is delisted, the Service is required to monitor that species for a minimum of 5 years to ensure recovery has been achieved.

Comment: The Land Management Office at the Bluegrass Army Depot (BGAD) no longer receives conservation funds for managing and monitoring running buffalo clover. The tasks shown in the Implementation Schedule are not feasible unless we receive funds and personnel from the Service to manage these populations.

Response: Actions that are identified in the BGAD's 2006-2010 Endangered Species Management Plan (ESMP) and subsequent Integrated Natural Resources Management Plan are not contingent on additional funds. The ESMP identifies actions that will protect, monitor, and manage the habitat for running buffalo clover at BGAD. It is our understanding that these actions are the responsibility of the BGAD with assistance from personnel from Kentucky State Nature Preserves Commission, Kentucky Department of Fish and Wildlife, The Nature Conservancy, and the Kentucky USFWS Field Office.