Botrychium campestre W.H. Wagner & Farrar (Iowa moonwort): A Technical Conservation Assessment

Prepared for the USDA Forest Service, Rocky Mountain Region, Species Conservation Project

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COVER PHOTO CREDIT

Botrychium campestre (Iowa moonwort). © H. Wagner 1999

Summary of Key Components for Conservation of *Botrychium campestre*

Status

Botrychium campestre (Iowa moonwort) is known from nine possible populations in Rocky Mountain Region, U.S. Forest Service (Region 2), but only five of these have been verified and observed within the last ten years. The known population in Region 2 is extremely small -- the total number of individuals documented at the five occurrences where abundance data are available is at most 28 plants. *Botrychium campestre* is ranked globally vulnerable (G3) by NatureServe. Within Region 2, it is ranked critically imperiled (S1) in Colorado, Wyoming, and Nebraska, and unrankable (S?) in South Dakota. *Botrychium campestre* has no federal status under the Endangered Species Act of 1973 (16 U.S.C. 1531-1536, 1538-1540). It is designated a sensitive species by U.S. Forest Service Region 2.

Primary Threats

Observations and quantitative data have shown that there are several tangible threats to the persistence of *Botrychium campestre*. The primary threats to *B. campestre* are habitat loss, disturbance of populations by human activities, collection, succession, and habitat fragmentation. Because the known populations in Region 2 are very small, they are also threatened by stochastic processes. Non-native species invasion may also pose a threat to *B. campestre*, but there is no evidence that this is occurring presently. Gopher disturbance has impacted occurrences of a close relative of *B. campestre* in Iowa, but there is no evidence of gopher impacts to populations in Region 2.

Primary Conservation Elements, Management Implications and Considerations

Although four populations have been documented on land managed by the U.S. Forest Service, only one of these populations (discovered in 2003 but including only two individuals) has been observed recently. Other populations are known from land owned by The Nature Conservancy, the State of Colorado, the U.S. Army Corps of Engineers, and on private land. At present, conservation efforts to protect the known populations of *Botrychium campestre* in Region 2 are most likely to be effective. Restoration of prairie and ponderosa parkland habitats may benefit *B. campestre* by buffering its habitat from edge effects and weed impacts, but because it has never been found in an area that had been plowed historically it is unlikely that restored areas will be suitable habitat. Because no members of *Botrychium* subgenus *Botrychium* have been successfully propagated, restoration of populations is not presently an option. Further species inventory is needed to better understand the full range of *B. campestre*. Research is needed to investigate the belowground life history, the role of mycorrhizae, and the role of disturbance in the autecology of *B. campestre* so that conservation efforts on its behalf can be most effective.

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INTRODUCTION

This assessment is one of many being produced to support the Species Conservation Project for the Rocky Mountain Region, U.S. Forest Service (USFS). *Botrychium campestre* (Iowa moonwort) is the focus of an assessment because it is designated a sensitive species in Region 2. Within the National Forest System, a sensitive species is a plant or animal whose population viability is identified as a concern by a Regional Forester because of significant current or predicted downward trends in abundance or significant current or predicted downward trends in habitat capability that would reduce the species' distribution (FSM 2670.5(19)). Sensitive species may require special management, so knowledge of their biology and ecology is critical.

This assessment addresses the biology of *Botrychium campestre* throughout its range in Region 2. The broad nature of the assessment leads to some constraints on the specificity of information for particular locales. This introduction defines the goal of the assessment, outlines its scope, and describes the process used in its production.

Goal of Assessment

Species conservation assessments produced as part of the Species Conservation Project are designed to provide forest managers, research biologists, and the public a thorough discussion of the biology, ecology, management, and conservation status of certain species based on available scientific knowledge. The assessment goals limit the scope of the work to critical summaries of scientific knowledge, discussion of the broad implications of that knowledge, and outlines of information needs. The assessment does not seek to develop specific management recommendations but provides the ecological background upon which management must be based. However, it does focus on the consequences of changes in the environment that result from management (that is, management implications). Furthermore, it cites management recommendations proposed elsewhere and, when management recommendations have been implemented, the assessment examines the success of the implementation.

Scope of Assessment

The *Botrychium campestre* assessment examines the biology, ecology, conservation status, and management of this species with specific reference to the geographic and ecological characteristics of the USFS Rocky Mountain Region. Although the majority of the literature on the species originates from field investigations outside the region, this document places that literature in the ecological and social contexts of the central Rockies. Similarly, this assessment is concerned with reproductive behavior, population dynamics, and other characteristics of *B. campestre* in the context of the current environment rather than under historical conditions. The evolutionary environment of the species is considered in conducting the synthesis, but it is placed in a current context.

In producing the assessment, refereed literature, non-refereed publications, research reports, and data accumulated by resource management agencies were reviewed. The assessment emphasizes refereed literature because this is the accepted standard in science. Non-refereed publications and reports were regarded with greater skepticism and used only when information was unavailable elsewhere. Unpublished data (for example, Natural Heritage Program records and herbarium specimen labels) were important in estimating the geographic distribution. These data required special attention because of the diversity of persons and methods used to collect the data.

Treatment of Uncertainty in Assessment

Science represents a rigorous, systematic approach to obtaining knowledge. Competing ideas regarding how the world works are measured against observations. However, because our descriptions of the world are always incomplete and our observations are limited, science focuses on approaches for dealing with uncertainty. A commonly accepted approach to science is based on a progression of critical experiments to develop strong inference (Platt 1964). However, it is difficult to conduct critical experiments in ecological sciences, and often observations, inference, good thinking, and models must be relied upon to guide the understanding of ecological relations.

In this assessment, the strength of evidence for particular ideas is noted, and alternative explanations are described when appropriate. While well-executed experiments represent a strong approach to developing knowledge, alternative approaches such as modeling, critical assessment of observations, and inference are accepted as sound approaches to understanding features of biology and ecology.

Publication of Assessment on the World Wide Web

To facilitate use of species assessments in the Species Conservation Project, they are being published on the Region 2 World Wide Web site. Placing the documents on the Web makes them available to agency biologists and the public more rapidly than publishing them as books or reports. More important, it facilitates revision of the assessments, which will be accomplished based on guidelines established by Region 2.

Peer Review

Assessments developed for the Species Conservation Project have been peer reviewed prior to release on the Web. This assessment was reviewed through a process administered by the Center for Plant Conservation employing at least two recognized experts on this or related taxa. Peer review was designed to improve the quality of communication and increase the rigor of the assessment.

MANAGEMENT STATUS AND NATURAL HISTORY

Management Status

Botrychium campestre is found in a small number of locations globally and within Region 2. While it has a broad geographic range, there are many small, disjunct populations separated by large areas of unsuitable habitat. *Botrychium campestre* is not listed as threatened or endangered in accordance with the Endangered Species Act of 1973 (16 U.S.C. 1531-1536, 1538-1540). It is listed on the U.S. Forest Service Sensitive Species Lists for Regions 2, 6, and 9. The Heritage Network ranks this species as globally vulnerable (G3) (NatureServe Explorer 2001). The state and province heritage ranks range from vulnerable (S3) in Minnesota, imperiled (S2) in Iowa and Michigan, critically imperiled (S1) in Alberta, New Brunswick, Ontario, Quebec, Saskatchewan, Montana, Idaho, Washington, Oregon, and Wisconsin, to historic (SH) and possibly extirpated in New York, and unrankable (S?) in South Dakota. Many records, particularly those east of the Great Lakes, have not been confirmed, and further investigation will likely result in a revision of the distribution of this species (Farrar personal communication 2002).

Within Region 2, Colorado, Wyoming, and Nebraska list *Botrychium campestre* as critically imperiled (S1), while South Dakota lists it as unrankable (S?) due to its uncertain status. *Botrychium campestre* has not been found in Kansas and has no ranking there. Although its small size, cryptic nature, and early emergence relative to related species (April to May) may account for its apparent rarity to some extent (Wagner and Wagner 1990), available data and extensive searching suggest that *B. campestre* is nonetheless very rare in Region 2. For specific information on the distribution of *B. campestre* in Region 2, see Distribution and Abundance section of this document.

Existing Regulatory Mechanisms, Management Plans, and Conservation Strategies

Botrychium campestre is not listed as threatened or endangered in accordance with the Endangered Species Act of 1973 (16 U.S.C. 1531-1536, 1538-1540), and therefore there are no federal laws concerned specifically with its conservation. However, since it is listed on the Sensitive Species Lists in USFS Regions 2, 6, and 9, Regional Foresters give consideration to this species and its habitat to maintain its long-term persistence (U.S. Forest Service 1995). For example, biological evaluations are required for sensitive species in every project undergoing National Environmental Policy Act analysis. A conservation strategy has not been written for this species at a national or regional level by the Forest Service or any other federal agency. There are no state laws in Colorado, Nebraska, South Dakota, and Wyoming that offer any protection for *B. campestre*.

Adequacy of current laws and regulations

Botrychium campestre has no legal protection unto itself that would prevent the destruction of habitat or individuals on state and private land, or on federal lands not managed by the USFS. Three of the known occurrences of this species in Region 2 are found in preserves or State Natural Areas, where management attention is given to the persistence and habitat of *B. campestre*. Four are known from lands managed by the USFS, where they also benefit from special consideration as a sensitive species. However, only one of these populations has been observed recently (Dugout Gulch, WY). Of the other three, one is probably another species (*B. lineare* at Echo Lake, CO), one has never been verified (Pawnee National Grassland, CO) and another has not been observed since 1973 (Bearlodge Campground, WY). For specific information on the known occurrences in Region 2, see the Distribution and Abundance section of this document.

Adequacy of current enforcement of laws and regulations

There are no known cases in which an occurrence of *Botrychium campestre* was extirpated due to human activities or by the failure to enforce any existing regulations in Region 2. However, this does not necessarily indicate that current regulations or their enforcement are adequate for the species' protection. There is a high probability that occurrences have already been extirpated or impacted by human activities such as grazing, tilling, and residential development.

Biology and Ecology

Classification and description

Botrychium campestre is a member of the adder's tongue family (Ophioglossaceae). The Ophioglossaceae is comprised of three genera: *Ophioglossum*, *Cheiroglossa*, and *Botrychium*. *Botrychium* (grapeferns) is the most diverse of these genera with 50 to 60 species worldwide (Wagner and Wagner 1993). The genus *Botrychium* contains three subgenera: *Osmundopteris*, *Sceptridium* and *Botrychium* (Wagner and Wagner 1993). *Botrychium* (moonworts), which contains *Botrychium campestre*, is the most diverse of the three subgenera with approximately 25 to 30 species. Members of this subgenus share many morphological traits, and subtle interspecific differences make it difficult to identify species in the field. In general, moonworts tend to grow in places that are unpromising to botanists (Wagner and Wagner 1983, Root personal communication 2003). In addition, species within this subgenus often grow together in genus communities (Wagner and Wagner 1983). Morphological and genetic analyses of genus communities have demonstrated that hybridization rarely occurs and that most hybrids have abortive spores (Wagner and Wagner 1983, Wagner et al. 1984, Wagner and Wagner 1986) thus evincing the presence of multiple species in these genus communities rather than infraspecific variants. The elucidation of interspecific differences has been confounded by the cryptic nature of *Botrychium* species (Paris et al. 1989, and Wagner 1998, Hauk and Haufler 1999). However, *B. campestre* is a highly distinctive moonwort, once the characters are recognized. The closest relative of *B. campestre* is *B. lineare* (Farrar 2001, Hauk et al. 2003). *Botrychium campestre* is the only species of moonwort known from Region 2 in prairie habitats, although it is also found in forested habitats and possibly in the alpine. It is also noteworthy for its early phenology, since the aboveground phase of its lifecycle takes place in April through June before other *Botrychium* species emerge. *Botrychium campestre* is among a few members of subgenus *Botrychium* that produce gemmae (Farrar and Johnson-Groh 1990). *Botrychium echo* and *B. gallicomontanum*, which also have gemmae, are believed to have resulted from rare hybridization events between *B. campestre* and other *Botrychium* species (**Figure 1**). Its rarity, phenology, morphology, and unique reproductive strategies make *B. campestre* a distinctive element of the flora of Region 2.

The diversity of the genus *Botrychium* in North America was not recognized until the 1980s when Drs. Herb and Florence Wagner began to work in earnest on *Botrychium*. *Botrychium campestre* was first recognized as a distinct taxon in 1982 in Iowa and Michigan, and was described as a species by Drs. H. Wagner and D. Farrar in 1986 (Wagner and Wagner 1986). The type specimen is housed at the University of Michigan Herbarium. This species was originally believed to be endemic to the loess prairies in Iowa, but surveys have since identified many populations throughout the northern United States and southern Canada (Farrar and Johnson-Groh 1986, Paris et al. 1989, Root 1993, Nekola and Schlicht 1996).

Botrychium species can be extremely difficult to identify due to their subtle diagnostic characters, frequent co-occurrence with multiple *Botrychium* species, and high morphological variability (Paris et al. 1989). Identification is facilitated by comparison with silhouette outlines of verified specimens (such as those presented in Wagner and Wagner 1986) and use of dichotomous keys (see Weber and Wittmann 2001). These do not guarantee positive identification, and it is often necessary to get verification by a *Botrychium* expert. Although it is more easily recognized than many other *Botrychium* species, *B. campestre* is among the most morphologically variable of moonwort species (Wagner and Wagner 1990b).

In Iowa and Michigan, *Botrychium campestre* has been found associated with *B. simplex*, with which it is the putative parent species of *B. gallicomontanum* (Farrar and Johnson-Groh 1991). *Botrychium campestre* was documented with *B. ascendens* and *B. virginianum* in 2003 at Dugout Gulch in Wyoming (King et al. 2003). *Botrychium michiganense* (or perhaps a yet undescribed species) was found at the Bearlodge Campground site in 2001, but *B. campestre* was not present with it.

Botrychium subgenus *Botrychium* sporophytes are simple plants recognized by their small size and distinctive leaf and spore structures. Members of this subgenus are usually less than 15 cm in height. They

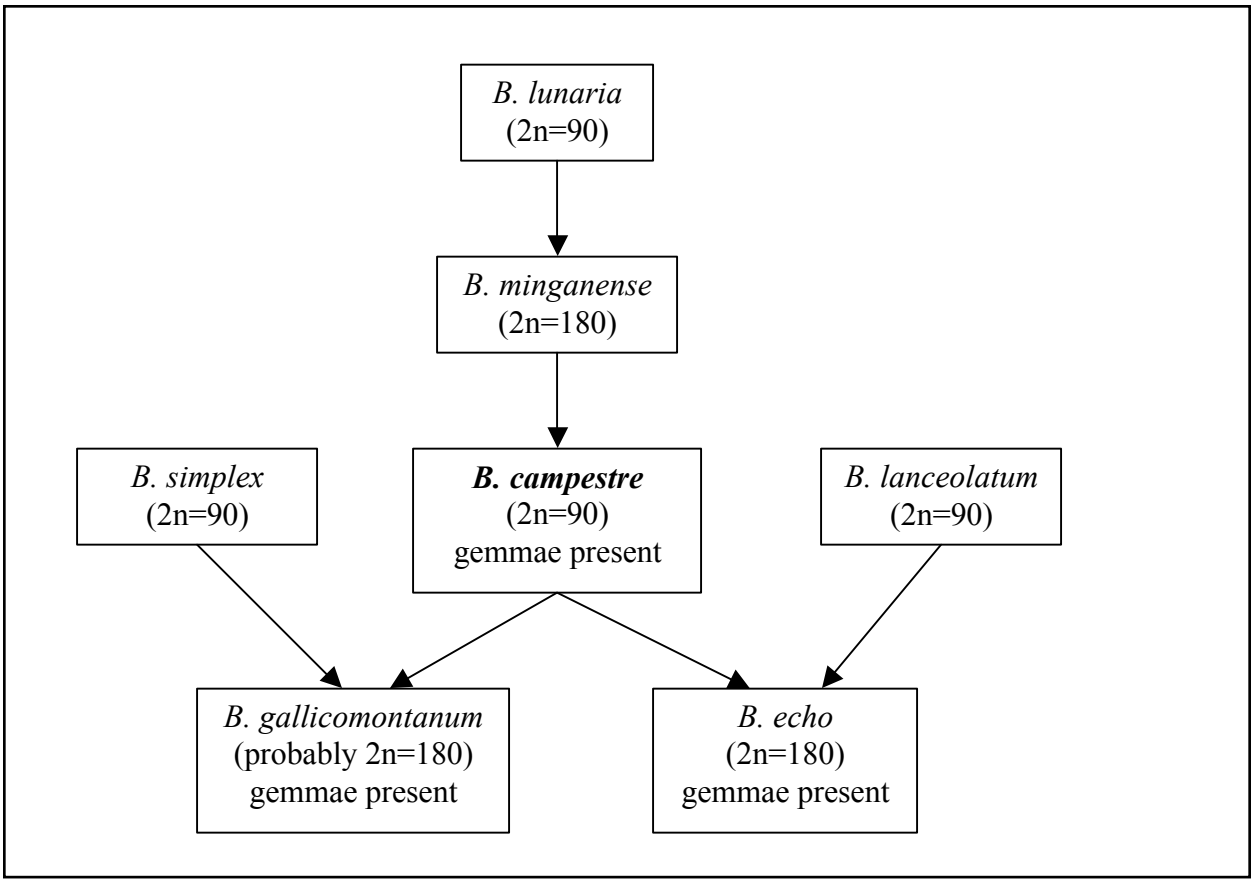

Figure 1. Ploidy levels and putative phylogenetic relationships of *Botrychium campestre*. Note that descendents of *B. campestre* have twice the number of chromosomes, suggesting an allopolyploid origin through hybridization events for these species. The descendents of *B. campestre* also possess gemmae. Gemmae production may have first evolved in *B. campestre* and was subsequently passed on to species that evolved from it.

possess a trophophore, or sterile leaf-like structure that is often heavily lobed or segmented, but rarely truly pinnate (Wagner and Wagner 1993). Members of the subgenus *Botrychium* usually produce only one leaf per year, and in some years no leaves are produced (Johnson-Groh 1998). On the same stalk sits a fertile sporophore that is often taller than the trophophore. The sporophore contains 20 to 100 grape-like sporangia, each containing possibly thousands of spores (Farrar and Johnson-Groh 1986, Wagner 1998).

In particular, *Botrychium campestre* is easily distinguished in Region 2 by its small size (5 to 10 cm), early leaf emergence, and prolific production of gemmae (minute vegetative propagules) on the lower stem (Wagner and Wagner 1986). It is the only known *Botrychium* in Region 2 to occur in prairie habitats (Weber and Wittmann 2001). It could potentially occur with the larger, non-gemmae producing *Botrychium multifidum*, however, such co-occurrence has never been documented in Region 2 and is improbable (Wagner and Wagner 1993). *Botrychium campestre* is similar to *B. lineare* and *B. minganense* in that all three species have somewhat narrow, widely-spaced pinnae on the trophophore (Spackman et al. 1997, Higman and Penskar 1999, Fertig 2000). *Botrychium campestre* tends to be smaller than *B. minganense* (Higman and Penskar 1999), and it has relatively few pinnae on the trophophore which tend also to be narrower and more dissected (Fertig 2000). The plant axis is also broader and fleshier, and the spores are smaller in *B. campestre* (Fertig 2000). It is distinguished from *B. lineare* in having wider pinnae on a fleshier axis, and a relatively fleshy sporophore (Fertig 2000).

For excellent keys and technical descriptions see Wagner and Wagner (1986, 1993). Photos and/or illustrations can be found in Farrar and Johnson-Groh (1990), Spackman et al. (1997), Higman and Penskar (1999), and Fertig (2000). See **Figure 2** and **Figure 3** for the photo and illustration included in Spackman et al. (1997).

Figure 2. *Botrychium campestre* sporophyte (from Spackman et al. 1997, photo by Dr. H. Wagner, copyright 1999).

Distribution and abundance

Botrychium campestre is known from populations across North America in 14 states and five provinces (**Figure 4**) (NatureServe Explorer 2001). Populations of *B. campestre* are widely distributed and occur in disjunct populations. It is most common in Minnesota, Iowa, and Michigan (NatureServe Explorer 2001). In Iowa there are at least 12 locations throughout the state, mostly along the Missouri River on loess deposits (Nekola and Schlicht 1996). In this portion of its range, *B. campestre* appears to be more common than elsewhere (though still quite rare), with less geographic separation of populations (Farrar and Johnson-Groh 1986). It is likely that other populations will be found as more inventory work is done. This may result in the identification of other "hot spots" for this species (Ode personal communication 2003). Prior to conversion of the loess prairies to agriculture, there was much greater habitat connectivity for *B. campestre*. Thus, it might be assumed that historically there was also greater genetic connectivity between populations of *B. campestre*.

Although *Botrychium campestre* is known from four of the five states in Region 2, populations within Region 2 are small and geographically separated from one another (**Figure 5**). In Region 2, *B. campestre* is known from only one site in Nebraska (consisting of two small occurrences), two in South Dakota, two in Wyoming, and one (possibly three) in Colorado. However, it is a difficult plant to locate and more specific surveys may lead to the discovery of more populations. **Table 1** includes summary information for the known populations in Region 2.

In Nebraska, two small occurrences of *Botrychium campestre* are known from the Niobrara Valley Preserve, which is owned by The Nature Conservancy.

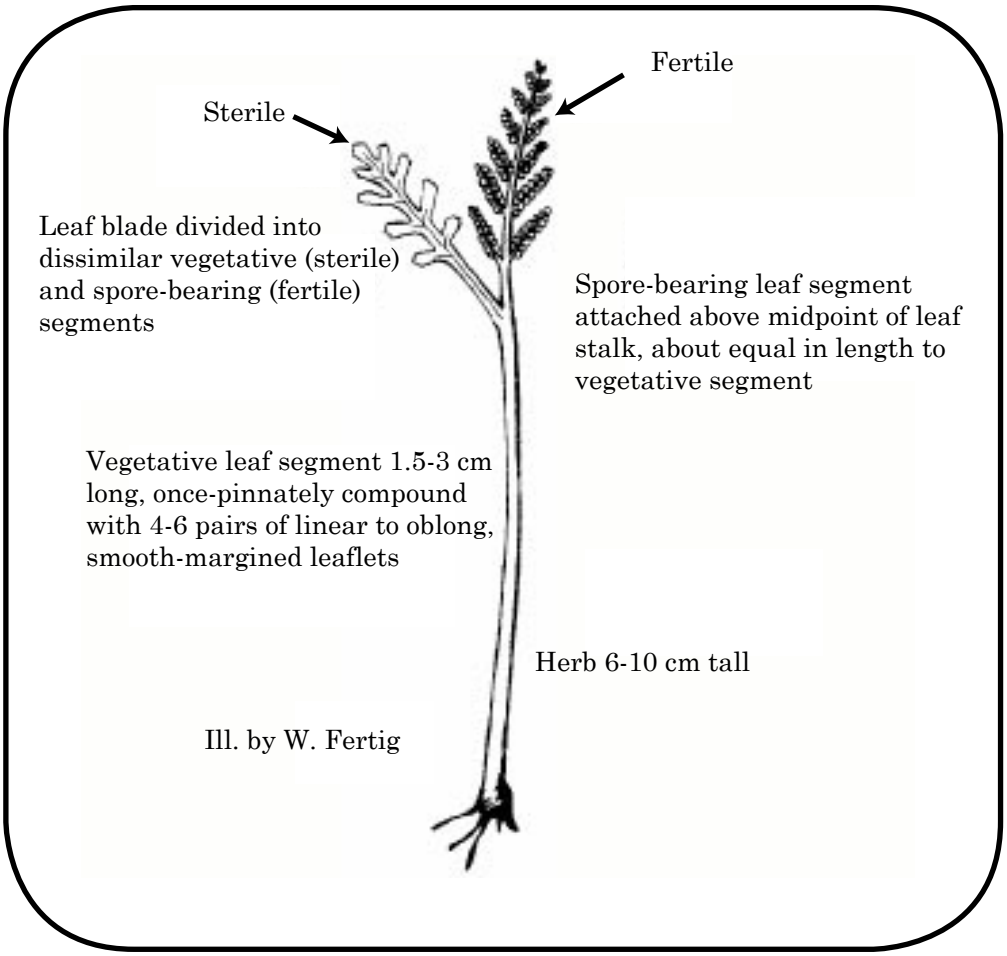

Figure 3. Illustration of *Botrychium campestre* showing diagnostic features (from Spackman et al. 1997, Illustration by Walt Fertig, copyright 1999).

One of these occurrences has not been seen in more than 10 years, despite repeated visits to the site. The other occurrence is very small, with only four plants observed in 2001 (Steinauer personal communication 2002).

In South Dakota, *Botrychium campestre* is known from two locations. One location is known from a collection made in 1997 in Brown County, in the north-central part of the state (Ode personal communication 2002). The only abundance information reported for this occurrence is "uncommon." This is the only known population in Region 2 on private land. A second occurrence was discovered in 2003 by Dr. Don Farrar in the South Shore Recreation Area in Gregory County (Ode personal communication 2003). This occurrence consists of 12 to 16 individuals and is the largest known occurrence in Region 2.

In Wyoming, *Botrychium campestre* is known from two occurrences. One occurrence is known from a collection by Dr. Robert Dorn in 1973 from the Bearlodge Mountains on the Black Hills National Forest, at the Bearlodge Campground in Crook County. No *Botrychiums* were subsequently seen at this site until 2001, when plants were collected and identified by Dr. Don Farrar as the yet undescribed taxon *B. michiganense* (Farrar personal communication 2002, Ode personal communication 2002). However, recent electrophoretic work suggests that this specimen is in fact not *B. michiganense* but probably a newly discovered taxon (Farrar 2003). No abundance information is available for this occurrence. In 2003, another population of *B. campestre* was found in the Black Hills of Wyoming in Crook County. This population is very small (two individuals), but further searching of the area is needed. This and other discoveries of *Botrychium* occurrences resulting from this survey work suggest that the Black Hills may be another important area for *Botrychium* species (Burkhart personal communication 2003, Ode personal communication 2003).

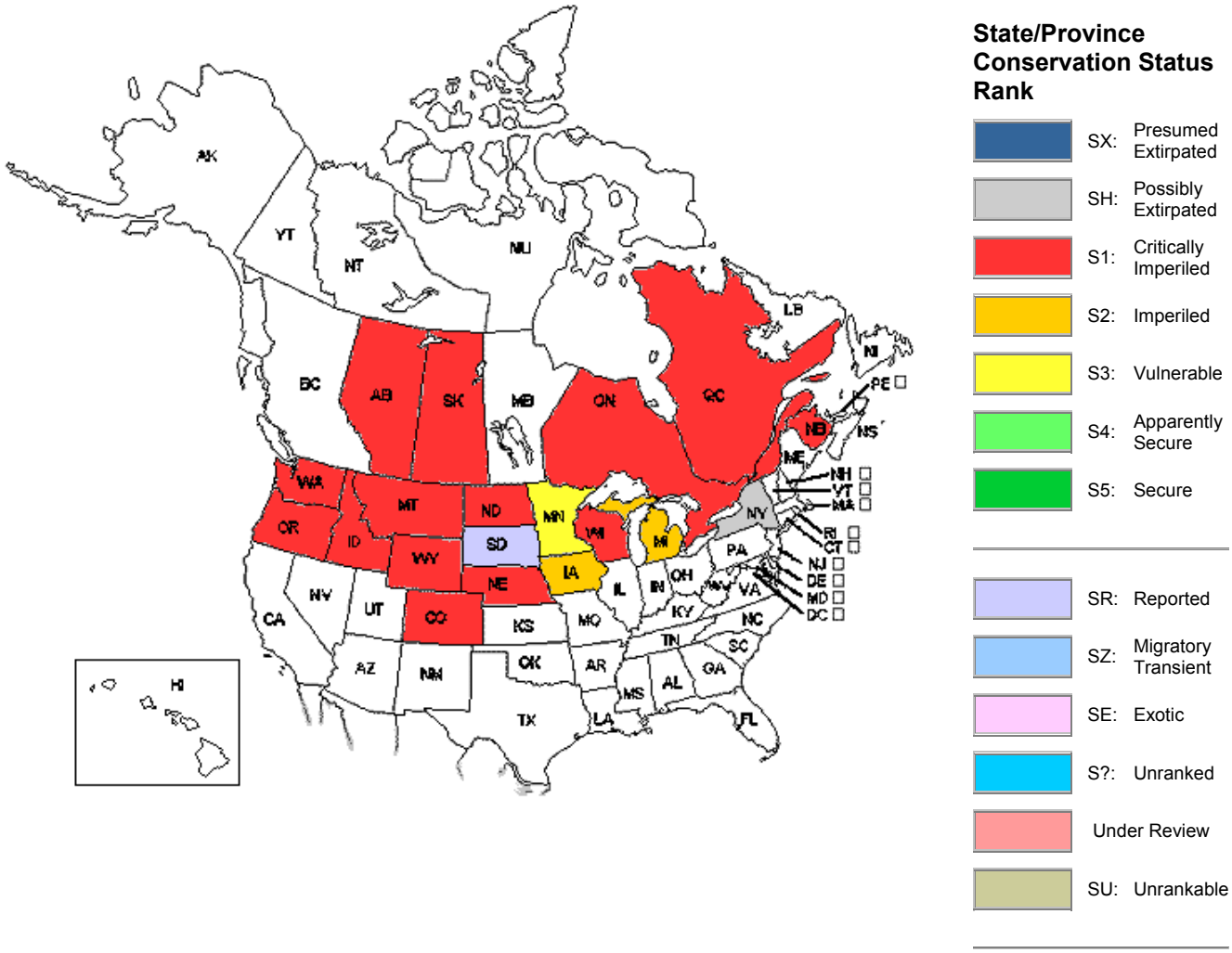

Figure 4. Global distribution map for *Botrychium campestre*, showing state/province conservation status ranks based on the number of occurrences and other factors within each jurisdiction. (NatureServe Explorer 2002). In South Dakota, *B. campestre* is now ranked S? (Ode personal communication 2003).

In Colorado, *Botrychium campestre* is known from a small occurrence in the Bonny Prairie Natural Area (Colorado Natural Areas Program 1998, Root 2000, Root personal communication 2002). In 1993, Peter Root observed six sporophytes at this location, but three or less have been seen in every other year (Root personal communication 2002). A specimen collected from Echo Lake in Clear Creek County was also identified as *B. campestre* by Dr. Herb Wagner. However, in the opinions of Dr. Farrar and Peter Root, this specimen is probably *B. lineare* or a hybrid of unknown origin (Root personal communication 2002, 2003). The habitat and elevation for this occurrence are highly atypical of *B. campestre*. Continued searches for *B. campestre* in this and other high elevation areas of Colorado are ongoing by Peter Root (Root personal communication 2002). A third site for Colorado may be the Pawnee National Grassland, where it was observed on a field trip, possibly with the Colorado Native Plant Society, in the 1980s (Brune personal communication 2002, Root personal communication Brune 2002). Attempts by Peter Root, Rick Brune, and Janet Wingate (on April 30, 2002, and again in May 2003), and the authors (on April 28, 2002) to verify this sighting yielded no plants. The severe drought conditions of 2002 may have precluded sporophyte emergence, hence further inventory work is still needed to attempt to verify this report.

Botrychium campestre has not been found in Kansas, despite searches in loess prairie remnants in the eastern portion of the state (Freeman personal communication 2002). Further searching is warranted in this area as well.

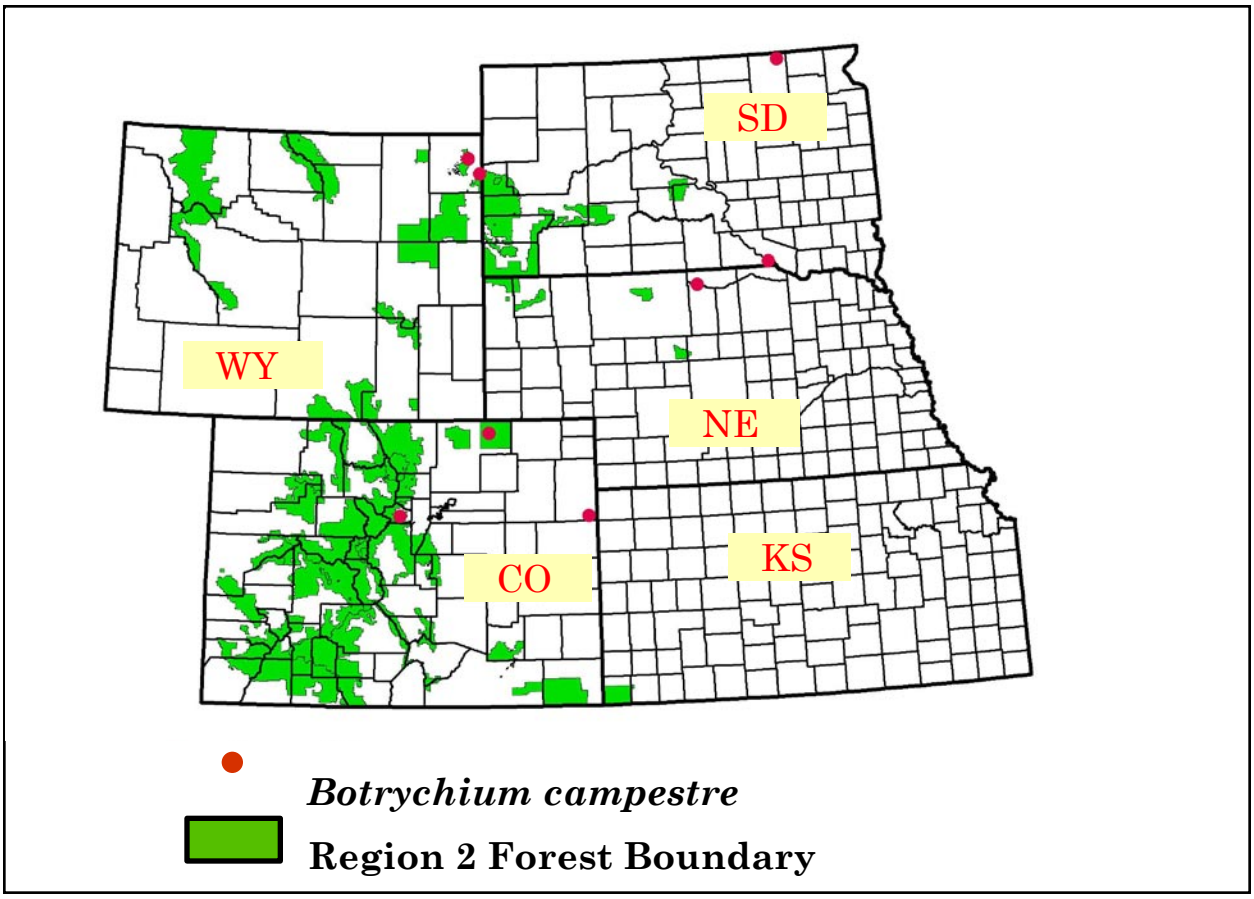

Figure 5. Distribution of *Botrychium campestre* reports in the states of Region 2.

Other areas in Region 2 possess apparently suitable habitat for *Botrychium campestre* (Root personal communication 2002), including Region 2 U.S. Forest Service lands. Dr. Farrar and Peter Root have searched tallgrass prairies of the piedmont west of Boulder, Colorado with negative results, and Peter Root and Dave Steinman have also searched the prairie open space areas east of Boulder, Colorado with negative results (Root personal communication 2002). Other areas warranting further searching in Region 2 include South Park, Great Sand Dunes National Park (Root personal communication 2002), and other tallgrass and midgrass prairies. Appropriate *Botrychium* habitat in the mountains of Region 2 should also be searched using the genus community methods of Wagner and Wagner (1983) and Wagner (1998). Given the possible collection of *B. campestre* from Echo Lake, Colorado, further work is needed to determine if viable populations of this species also occur in mountain habitats. However, prairie habitats (including tallgrass, midgrass, and shortgrass prairies), and parklands dominated by *Pinus ponderosa* (ponderosa pine), such as in the Black Hills of South Dakota and Wyoming are the highest priority for further inventory work on *B. campestre*.

In general, more accurate distribution information is needed for *Botrychium* species. Wagner and Wagner (1990) list reasons for this deficiency of information. Finding *Botrychium* species is made difficult by their small size (sometimes less than 2 cm) and the difficulty in finding them among similarly colored vegetation. Their identification is complicated by the presence of few distinguishing morphological characters, subtle species-specific morphological markers in those few characters, and high levels of site-dependent variation in characters. In addition, many species occur together in genus communities (Wagner and Wagner 1983), making it difficult to separate species based on habitat. As mentioned above, *B. campestre* is restricted primarily to prairie habitats, but it occurs in other habitats in Region 2, including ponderosa pine parkland in the Black Hills, and possibly in alpine sites in the unlikely event that the Echo Lake population in Colorado turns out to be *B. campestre*. Farrar suspects that many purported populations of *B. campestre* east of the Great Lakes need verification. One location was revisited in Newfoundland and found to be *B. ascendens* (Farrar personal communication 2002).

Table 1. Summary information on the known occurrences of *Botrychium campestre* in Region 2.

State	Location	Last Observed	Land Ownership	Population Size	Notes
Colorado	Yuma County, Bonny Prairie State Natural Area	2001	State of Colorado	3-6	Only definite population in Colorado
Colorado	Clear Creek County, Echo Lake	1994	USFS	1?	Probably *B. lineare*
Colorado	Weld County, Pawnee National Grassland	approximately 1980	USFS	unknown	Location and ID uncertain; no specimen
Nebraska	Brown County, Niobrara Valley Preserve	1990s	TNC	unknown	
Nebraska	Brown County, Niobrara Valley Preserve	2001	TNC	4	
South Dakota	Gregory County, South Shore Recreation Area, Francis Case Reservoir	6/11/2003	U.S. Army Corps of Engineers	12-16	Newly discovered-Largest known population in Region 2
South Dakota	Brown County, 1 mi. east of Sand Lake National Wildlife Refuge	5/29/1997	Private	"uncommon"	
Wyoming	Crook County, Bearlodge Mountains-Bearlodge Campground	1973	USFS	"very rare"	Not seen again in searches in 1989, 1993, and 1999; another *Botrychium* spp. found in 2001
Wyoming	Crook County, Bearlodge Mountains-Dugout Gulch	6/19/2003	USFS	2	Newly discovered

Herbarium specimens are challenging to create for *Botrychiums*, often misidentified, and may be erroneously mounted with multiple species on a single herbarium page (Wagner and Wagner 1990, Root personal communication 2002). Factors such as early, brief, and seasonally-dependent emergence times, difficulty in seeing this small plant among grasses, superficial similarity with newly-emerging forbs, and rarity also make accurate distribution data difficult to obtain.

Botrychium campestre has a wide range, yet it is globally rare. It may be among the rarest ferns in North America (Farrar and Johnson-Groh 1986). Monitoring data from 5-Ridge Prairie, Iowa suggest that populations are in decline (Johnson-Groh 1999). Monitoring efforts at Bonny Prairie State Natural Area and the Niobrara Valley Preserve have never observed large populations at these sites, and the small population sizes observed at these sites suggest that these occurrences are highly vulnerable to extirpation. The failure to observe *B.*

campestre at the Bearlodge Campground, Wyoming since 1973 also suggests that this population may no longer exist. *Botrychium campestre* is most common in Minnesota, Iowa, and Michigan, though still rare in those areas, and extremely rare throughout the rest of its range. Many records, particularly those east of the Great Lakes in Canada, have not been confirmed, and further investigation will likely result in a downward revision of the distribution of this species (Farrar personal communication 2002).

Population trend

While data are insufficient to assess the rangewide population trend for *Botrychium campestre*, changes in its habitat caused by humans strongly suggests that it has declined significantly. Appropriate habitat for *B. campestre* has been fragmented and destroyed as the loess prairies of the Midwest were plowed and cultivated during the last 100 to 150 years. This probably resulted in the extirpation of many populations, since most of the remaining populations occur on prairie remnants. Currently there remain only tiny islands of suitable habitat surrounded by vast areas devoid of suitable habitat. *Botrychium campestre* is found almost exclusively on native prairies and surveys on plowed fields have revealed no occurrences (Wagner and Wagner 1993, Colorado Natural Heritage Program 2002). Native prairie habitats have decreased precipitously since European settlement. Loess prairies in particular, with their deep fertile soils, have been eagerly sought for cultivation, and most of these areas are now used to grow corn and wheat (Colorado Natural Areas Program 1998). Within the states of Region 2, large tracts of loess prairie have been plowed and converted to agriculture. Due to this species' apparent affinity for this habitat, it is likely that *B. campestre* populations have decreased with the decrease in this habitat.

Population trends resulting from impacts to other habitats in Region 2, particularly forested habitats, is unclear. There is insufficient information to make inferences regarding the effects of various forest management practices and human use patterns in these habitats on the population trend of *Botrychium campestre*.

Few researchers have followed trends in local populations (but see Johnson-Groh 1998 and 1999). However, populations show high variation in the number of emergent stalks among years, like most members of the subgenus (Johnson-Groh 1999, Root personal communication 2002). Some plants and entire populations may not produce stalks every year (Johnson-Groh 1999, Root personal communication 2002). Drought may be the most significant factor determining stalk emergence (Johnson-Groh 1999). Monitored populations of *Botrychium campestre* in Minnesota exhibited overall declines between 1989 and 1999 (Johnson-Groh 1999). Whether these observations represent decline of the entire species or merely a local response to climate variation or other regional temporal environmental patterns is unclear, but these observations are not encouraging.

Habitat

Little is known about the exact habitat associations and environmental tolerances of *Botrychium campestre*. However, it has been found primarily in native, unplowed prairies (Wagner and Wagner 1993, Colorado Natural Heritage Program 2002) and in sites with some disturbance, such as grazing (Farrar and Johnson-Groh 1986, Colorado Natural Areas Program 1998, Ode personal communication 2002). It is commonly associated with loess prairie throughout much of its range. It is also known to occur in the dunes around Lake Michigan and in sandy soils, along railroad tracks, and in areas of calcareous soils underlain by limestone (Wagner and Wagner 1986, Wagner and Wagner 1993). The Bonny Prairie occurrence in Colorado (underlain by Pierre Shale), the Pawnee National Grassland (underlain by the Laramie Formation), and the Niobrara Valley occurrences in Nebraska are found in areas underlain by calcareous sedimentary rocks.

Botrychium campestre is usually found in tall to midgrass prairie systems where dead leaf litter from grasses (thatch) is present (Root 1993, Colorado Natural Areas Program 1998). *Botrychium campestre* populations are susceptible to drought (Johnson-Groh 1999), and a light cover of thatch is thought to shade soils and keep soils moist by reducing evaporative water loss (Root 1993, Colorado Natural Areas Program 1998). However, a heavy cover of thatch may impede emergence (Root 1993, Colorado Natural Areas Program 1998). Available sources do not offer quantitative estimates of thatch cover that is appropriate for *B. campestre*.

Unsearched potential habitat exists in Region 2 (Wagner and Wagner 1986, Root 1993), and future surveys may reveal more populations (see the Tools and Practices section of this document). Such surveys could clarify *Botrychium campestre*'s habitat needs and associations.

The two occurrences in Wyoming are documented from areas dominated by ponderosa pine forests. At Dugout Gulch, the plants were found in an open, grassy swale on an old roadbed. There are no specific habitat data available for the Bearlodge Campground location.

Historically, fire played a major role in the ecology of prairie ecosystems, including tallgrass and midgrass prairies (Launchbaugh 1972, Launchbaugh and Owensby 1978). Thus it might be assumed that fire is necessary to maintain suitable habitat for *B. campestre*, particularly in areas such as the Niobrara Valley Preserve and in Gregory County, South Dakota where the lack of fire has allowed the encroachment of *Juniperus virginiana* (red cedar). However, at the Gregory County occurrence the plants were observed at the drip line of *J. virginiana* on the north side of the tree, suggesting that it may benefit from the presence of this species. Because this phenomenon has not been reported previously for *B. campestre*, further investigation is needed to study the nature of this relationship. Fire may also serve an important role in nutrient cycling and in the reduction of thatch loads (Colorado Natural Areas Program 1998).

Little information exists pertaining to specific microhabitat associations. As mentioned above, *Botrychium campestre* is susceptible to drought and probably benefits from a moderate amount of thatch. Much of the life cycle of *Botrychium* species occurs underground. Scientists understand very little about these subterranean factors, yet they are crucial since *Botrychiums* rely on mycorrhizal interactions in each of their life stages (Campbell 1922, Bower 1926, Scagel et al. 1966, Foster and Gifford 1989, Schmid and Oberwinkler 1994). Johnson-Groh (1999) hypothesizes that this is the most important factor in the presence and persistence of *Botrychium* populations. However almost nothing is known about which species of mycorrhizae interact with *Botrychiums*, what factors affect the mycorrhizae, and what factors affect the interaction between the mycorrhizae and *Botrychium* (see Community Ecology section of this document for discussion on mycorrhizal interactions). Determining the impact of these factors will provide valuable insight into the management of *B. campestre*.

The periodicity of disturbance is probably a very strong determining factor for the suitability of a given site for *Botrychium* species. Observations of other taxa have shown that *Botrychium* species are often found in sites that are disturbed and then left alone for a considerable period of time. Buell (2001) found that *B. echo* is common on man-made ski slopes in Summit County, Colorado. Interestingly, ski slopes that had been created more than 30 years ago harbored 97 percent of the *B. echo* individuals. The mechanisms responsible for this pattern are unknown, and more research is needed to determine the types and periodicity of disturbance that create and maintain suitable habitat for *Botrychium* species.

Buell (2001) notes that *Botrychium* subgenus *Botrychium* species in Summit County, Colorado (including *B. minganense*, *B. lanceolatum*, *B. lunaria*, *B. echo*, and *B. hesperium*) have a decidedly patchy within-site distribution pattern. This could be the result of patchy distributions of mycorrhizae, the nature of *Botrychium* dispersal and establishment, or the result of random dispersal. Wagner (1998) hypothesizes that some spores may disperse when mammals eat the fertile sporophytes, and this mechanism of dispersal could account for a patchy distribution. More information pertaining to underground factors, which affect establishment, in conjunction with information on dispersal mechanisms could help elucidate the causes of these observed distribution patterns.

Habitat for *Botrychium campestre* in Region 2 might be tightly constrained by water availability. In other parts of its range, *B. campestre* is found in areas that receive in excess of 28 inches of precipitation per year. However, populations of *B. campestre* in Colorado and Wyoming are in areas that typically receive 10 to 20 inches of precipitation per year (Western Regional Climate Center 2003).

Botrychium campestre is found in loamy (loess, alluvium, and on "black soil" prairies) and in sandy soils (Farrar and Johnson-Groh 1986). Peter Root (personal communication 2002) speculates that the arid climate (low humidity and annual rainfall) in much of Region 2 prevents the occurrence of *B. campestre* in sandy, upland substrates, while loess soils are less prone to desiccation. Searches of sandhills in Colorado have failed to yield any occurrences (Root personal communication 2002). The observation suggesting that *B. campestre* may be present in the Pawnee National Grassland of Colorado was reported from the vicinity of a dry lake (playa), where soil moisture is probably higher. The soils are sandy at the Bearlodge Campground occurrence and at both South Dakota occurrences, but these sites are probably less drought-stressed than areas further south with similar edaphic characteristics.

Reproductive biology and autecology

In the Competitive/Stress-Tolerant/Ruderal (CSR) model of Grime (2001) characteristics of *Botrychiums* most closely approximate those of stress-tolerant ruderals. Like many epiphytes, lichens, and bryophytes, they are characterized by small stature, slow relative growth rates, and small propagules. A distinguishing characteristic of plants in this category is that stressful conditions are experienced during growth. *Botrychium* species have high reproductive outputs (Wagner 1998), which likens them to other "r" selected species (using the classification scheme of MacArthur and Wilson 1967), although their longevity and slow growth do not. Unlike other species of *Botrychium* for which the growing season is more protracted, *B. campestre* has a narrow window of opportunity for growth and reproduction due to ephemeral soil moisture availability. This also characterizes ruderals in the CSR model (Grime 2001).

Moderate to light disturbance may be a critical part of the autecology of *Botrychium* species including *Botrychium campestre* (Lellinger 1985, Wagner and Wagner 1993). For *B. campestre*, fire probably plays an important role in limiting the development of an overstory, since it is primarily responsible for the maintenance of the grasslands of the plains (Wright and Bailey 1980). Other localized disturbances, including human disturbance, also apparently create and maintain habitat for *B. campestre*, such as the old roadbed on which it was found at Dugout Gulch, Wyoming.

Botrychium campestre is a perennial plant. The root and stem are underground, and the leaves may not emerge every season (Johnson-Groh 1999). However, when leaves emerge, *Botrychiums* produce a multitude of spores. *Botrychiums* have between 20 and 100 sporangia per sporophore, and each sporophyte may contain thousands of spores, possibly the highest number of spores per case of all vascular plants (Wagner 1998).

Like all Pteridophytes, but unlike angiosperms and gymnosperms, *Botrychium* spores develop into gametophytes that live independently of the sporophyte. Alternation of generations occurs in all plants, but in the ferns the gametophyte lives independently of the sporophyte, and the two often have different ecological requirements. The gametophyte produces male and female sex cells in the antheridia and archegonia respectively. Male sex cells must move through a fluid environment to fertilize a female egg cell. The subterranean nature of *Botrychium* gametophytes probably restricts many *Botrychiums* to self-fertilization (McCauley et al. 1985, Soltis and Soltis 1986). See **Figure 6** for a diagrammatic representation of the life cycle of *B. campestre*, and **Figure 7** for a life cycle graph (after Caswell 2001).

In addition to spore production, *Botrychium campestre* is one of several *Botrychiums* that produce gemmae (Farrar and Johnson-Groh 1990). Gemmae are minute "vegetative propagules abscised at maturity from the parent plant" (Farrar and Johnson-Groh 1990). This mode of reproduction has not been previously reported in any fern genus (Farrar and Johnson-Groh 1986). A single *B. campestre* stem may contain between 20 and 100 of these 0.5 to 1 mm asexual propagules (Farrar and Johnson-Groh 1990). Older gemmae may also produce additional gemmae, and 500 to 600 gemmae can sometimes be found surrounding a single plant (Mason and Farrar 1989, Farrar and Johnson-Groh 1990). Thus, the tendency for *B. campestre* to grow in clumps is probably the result of reproduction via gemmae. Gametophytes may have a higher susceptibility to desiccation than sporophytes, and gemmae (which produce sporophytes) may be a more reliable form of reproduction in the dry environments inhabited by *B. campestre* (Farrar and Johnson-Groh 1990).

Botrychium campestre sporophytes typically emerge very early in the spring, usually in mid-April to mid-May (Wagner and Wagner 1986, Wagner 1998). This probably allows them to capitalize on ephemeral early spring moisture and to avoid competition with grasses and other more competitive species. By mid- to late-June the aboveground portions of the plant have withered and senesced. The early emergence and senescence of the sporophyte distinguishes *B. campestre* from other North American *Botrychium* species and is a helpful diagnostic characteristic for identification in the field.

Botrychium spores are small and light and are likely carried by winds. Researchers have hypothesized that the dispersal distance for some *Botrychium* spores range from a few centimeters (Hoefferle 1999, Casson et al. 1998) to up to three meters (Peck et al. 1990). However, these studies have focused on either taller or forest-dwelling *Botrychiums*, and few studies have adequately examined the dispersal distances of other similar species that inhabit windy prairie ecosystems. While most spores land close to the parent plant, they occasionally travel considerable distances (Briggs and Walters 1997). In addition to wind dispersal, it has been speculated that animals may disperse *Botrychium* spores (Wagner and Wagner 1993, F. Wagner personal

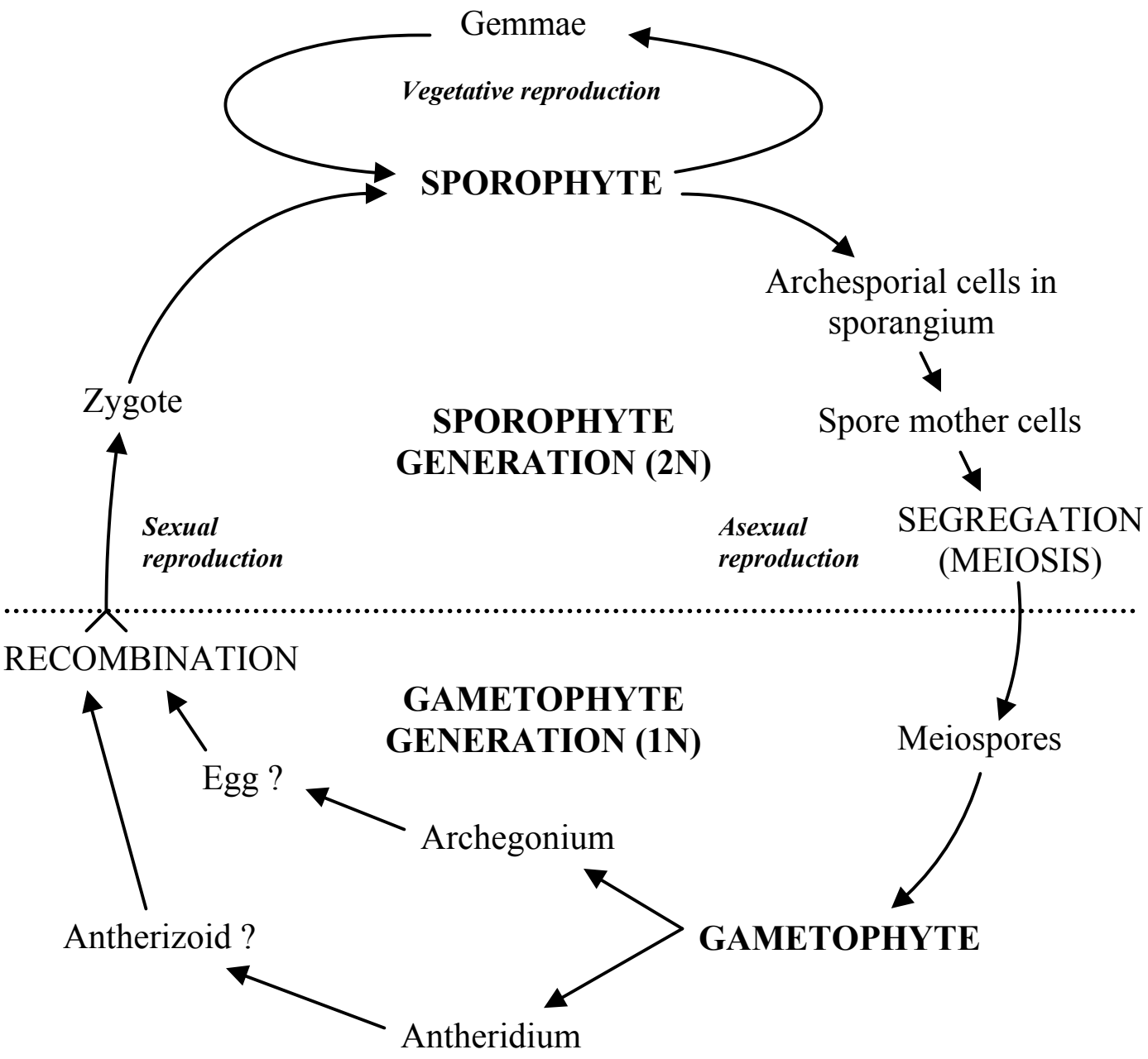

Figure 6. Life cycle diagram for *Botrychium campestre* (after Lellinger 1985), illustrating the alternation of generations. Only the sporophyte is typically found, the gametophyte being subterranean.

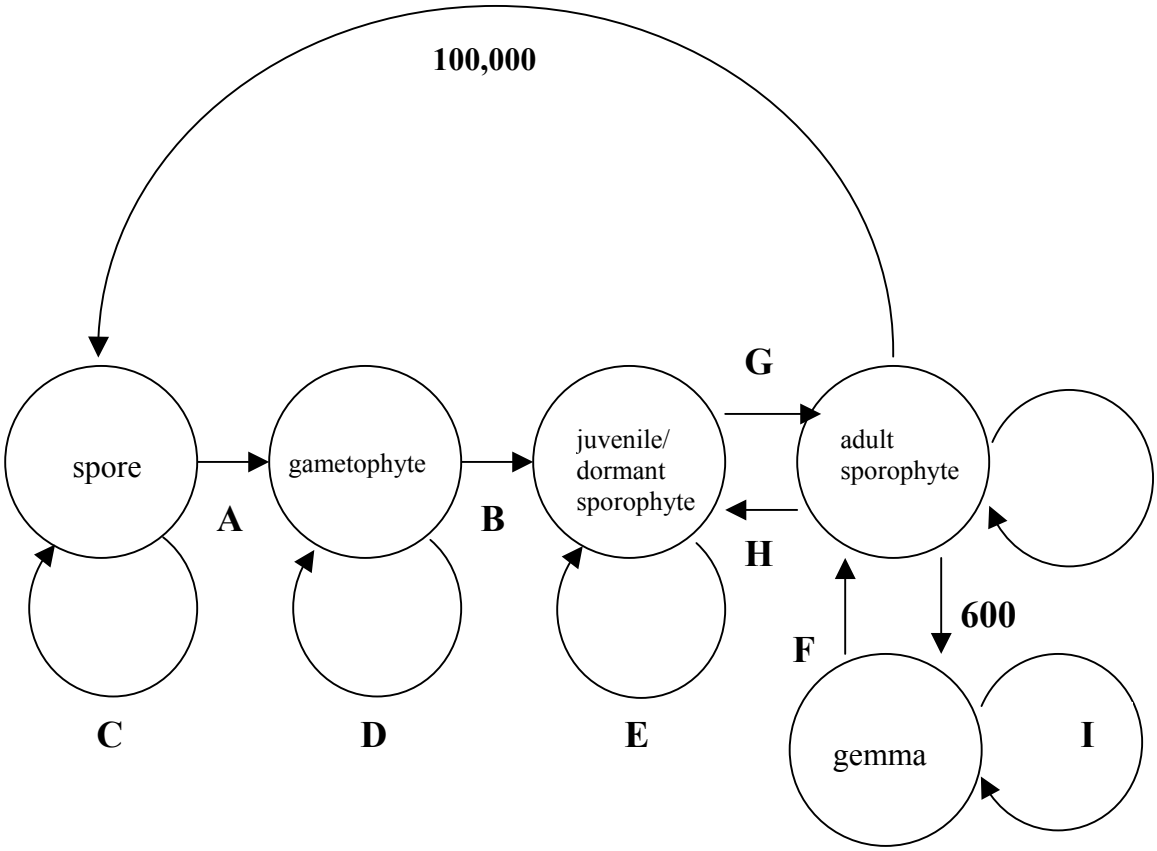

Figure 7. Hypothetical life cycle graph (after Caswell 2001) for *Botrychium campestre*. Transition probabilities are not known and are difficult to quantify since important stages of the lifecycle occur underground (A-I). Please see Johnson-Groh et al. (2002) for the best information currently available regarding these parameters. The number of years needed for a juvenile sporophyte to reach adulthood and emerge from the ground is not known. Spore production is estimated from Wagner (1998), and gemma production is from studies by Mason and Farrar (1989) and Farrar and Johnson-Groh (1990). No transition probabilities are known for *B. campestre*.

communication 2002). The spores have thick walls that may help to retain their viability as they pass through an animal's digestive tract (Johnson-Groh 1998, F. Wagner personal communication 2002). It has been thought that deer and small mammals may disperse the spores of forest species such as *B. dissectum* along trails and roads. J.D. Montgomery recovered the spores of *B. virginianum* from the droppings of a vole, and they appeared to be intact (Root personal communication 2003). Buell (2001) observed that distribution patterns of *Botrychium* in Summit County, Colorado often followed swales, heavy equipment tracks, and erosion rills, suggesting that water may play a role in the dispersal of spores down slopes.

The average lifespan is unknown for *Botrychium campestre*. Johnson-Groh (1998) found that most individuals emerged for four continuous years. However, with gemmae and possible short distance spore-dispersal, offspring may grow near the parent, and it is difficult to discriminate individuals between seasons (Johnson-Groh 1999).

Botrychiums rely upon mycorrhizae in both the sporophytic (Bower 1926, Foster and Gifford 1989) and gametophytic (Campbell 1922, Bower 1926, Scagel et al. 1966, Foster and Gifford 1989, Schmid and Oberwinkler 1994) stages. *Botrychium* spores need three to four weeks of darkness before they can germinate, with longer periods of darkness increasing the probability of germination (Whittier 1973). Germination can occur without mycorrhizal infection, however the gametophyte will not mature without an arbuscular mycorrhizal symbiont (Campbell 1911, Whittier 1972, Whittier 1973). The subterranean, achlorophyllous gametophyte of the related *B. lunaria* may live underground for up to five years (Winther personal communication 2002) using carbohydrates

and minerals gained from the mycorrhizal interaction (Schmid and Oberwinkler 1994).

It is unknown how or if the mycorrhizal interaction changes when the gametophyte develops into a sporophyte. However, *Botrychium* sporophytes have reduced, non-proliferous roots that lack hairs (Wagner and Wagner 1993) and they are dependent upon mycorrhizae (Bower 1926, Foster and Gifford 1989). Winther (personal communication 2002) found that congeners had both endomycorrhizal and ectomycorrhizal associations.

Mycorrhizae

Arbuscular (also referred to in the literature as vesicular-arbuscular) mycorrhizae are the known fungal symbiont with *Botrychium* species (Berch and Kendrick 1982, Schmid and Oberwinkler 1994). Johnson-Groh (1999) hypothesizes that the most important factor in the establishment and persistence of *Botrychium* populations is the presence of mycorrhizae. However, little is known about the specific nature of this interaction. Farrar (1998) notes that mycorrhizal fungi are low in species diversity, ubiquitous in disturbed and undisturbed sites, and generalist in whom they infect (Smith and Read 1997). However, recent studies have measured surprisingly high species diversity of arbuscular mycorrhizal (AM) fungi in a single hectare (Bever et al. 2001). A single plant root has been observed to host up to 49 species of AM fungi (Vandenkoornhuyse et al. 2002). These observations, coupled with the ubiquity and low host specificity of AM fungi, suggest that mycorrhizae may not be a limiting factor in the distribution of *B. campestre*.

Mycorrhizae can have large impacts on the composition of a plant community by shifting the intensity of competitive interactions (Read 1998, Van Der Heijden et al. 1998). Marler et al. (1999) found that the exotic *Centaurea maculosa* (spotted knapweed) had more intense competitive effects on *Festuca idahoensis* (Idaho fescue) when grown together in the presence of mycorrhizal fungi. Given their tight association with mycorrhizae, similar work with *Botrychiums* is needed to understand the effects of mycorrhizae-mediated interspecific competition.

Hybridization

Botrychium campestre is one of the few diploid (2n= 90) members of the genus (Wagner and Wagner 1986). Hybrids are rare in *Botrychium*, and when they are found they are typically sterile (Wagner 1998). However, *B. campestre* is the purported ancestor of a complex of other species that may have arisen from hybridization events. The descendants of *B. campestre* are tetraploids that produce some gemmae but not in the same profusion as *B. campestre*. *Botrychium gallicomontanum* was described in 1991 and is also found in prairie habitats (Farrar and Johnson-Groh 1991). This species was described from Frenchman's Bluff in Minnesota, where it occurs with both *B. campestre* and *B. simplex*, its probable ancestors (Farrar and Johnson-Groh 1986, Farrar and Johnson-Groh 1991, Wagner and Wagner 1993). *Botrychium campestre* is also probably an ancestor of *B. minganense*, which is morphologically intermediate between *B. campestre* and *B. lunaria* (Farrar and Johnson-Groh 1986). *Botrychium echo* is a probable descendant of *B. campestre* and *B. lanceolatum* (Colorado Native Plant Society 1997, Root personal communication Root 2002). *Botrychium campestre* is also the nearest relative to a recently described moonwort, *B. lineare* (Wagner and Wagner 1994). See **Figure 1** for a diagrammatic representation of the ploidy and phylogenetic relationships of *B. campestre*.

Demography

Members of the genus *Botrychium* appear to have naturally low rates of outcrossing (Farrar 1998). Detailed anatomical studies of the gametophytes of members of subgenus *Botrychium* have not been performed, but the anatomy of the gametophyte of *B. virginianum* (subgenus *Sceptridium*) appears to be designed for self-fertilization, since the antheridia are positioned just above the archegonia. Water moving through the soil is likely to bring the male sex cells to the archegonia on the same plant (Bower 1926). Soltis and Soltis (1986) used electrophoretic techniques to confirm that there are extremely high levels of inbreeding in this species. Allelic variability within each moonwort species consistently shows very low intraspecific variation when compared with other ferns and seed plants (Farrar 1998). McCauley et al. (1985) found the congener *Botrychium dissectum* (subgenus *Sceptridium*) to have an outcrossing rate of less than 5 percent. However, the rare presence of interspecific hybrids in natural settings indicates the ability for cross-fertilization hybridization to occur (Wagner et al. 1984). Much, if not most, of *Botrychium campestre*'s reproductive output is through the vegetative, asexual production of gemmae (Farrar and Johnson-Groh 1990, Johnson-Groh et al. 2002). Thus, *B. campestre* and other *Botrychium* species may not be particularly sensitive to the effects of inbreeding depression, since the opportunities for sexual recombination are rare.

Due to anatomical and environmental constraints that limit its success as an outcrosser, *Botrychium campestre* appears predisposed to low outcrossing rates. Although populations may have been less disjunct prior to the conversion of the loess prairies to agriculture, gene flow (via spore or, less frequently, gamete movement) between populations has probably always been limited. Soil movement by mammals may have improved the genetic connectivity of populations if propagules were also transported.

Farrar (1998, personal communication 2002) hypothesizes that low genetic diversity could lead to high genetic stability, which might benefit *Botrychium* species by assuring that they remain attractive hosts to mycorrhizal fungi. As obligate mycorrhizal hosts that obtain their mineral nutrition and some carbohydrates from their fungal symbionts, the *Botrychium* species depend on the establishment and maintenance of this relationship. Genetic diversity would be more useful to *Botrychium* species when present in their fungal symbionts, since they are the intermediaries between the roots and the rhizosphere and must adapt to environmental change.

Demographic studies of *Botrychium campestre* are lacking, and basic parameters circumscribing life history characteristics are unknown. However, available information strongly suggests that the number of aboveground sporophytes observed in a given year is a poor indicator of population size and viability. Sporophytes can remain dormant for one or more years (Johnson-Groh 1999), and most of the population at a given site resides underground (Johnson-Groh 1999, Johnson-Groh et al. 2002). Studies of the belowground portions of *B. campestre* populations in Iowa yielded 21 gametophytes, 198 belowground juvenile sporophytes, and 5907 gemmae per m^2, where aboveground sporophyte density averaged only 6.7 plants per m^2 (Johnson-Groh 2002). Thus, the ratio of belowground structures to emergent sporophytes is 914:1 for *B. campestre*. This is only exceeded by *B. hesperium* among the species of *Botrychium* studied by Johnson-Groh et al. (2002). For these reasons long term studies are needed for determining the sporophyte population size at a given site (Johnson-Groh and Farrar 2003).

Botrychium campestre produces gemmae prolifically, and gemmae can produce other gemmae (Mason and Farrar 1989). This proliferation can result in as many as 600 gemmae in the vicinity of one plant (Mason and Farrar 1989).

The study of establishment of individuals is problematic due to important events in the life cycle of *Botrychium* that occur underground. Spores of *Botrychium virginianum* germinated on agar showed a 90 percent germination rate (Peck et al. 1990). Therefore, most spores are probably deposited in sites inappropriate for growth. The requirement of darkness for spore germination (Whittier 1973) is not surprising, given the need to establish a mycorrhizal symbiosis within a few cell divisions (Campbell 1911). However, this need probably greatly reduces the number of successful spores. The mechanism by which spores get underground is not known, but they somehow get from the soil surface to a depth of one to two inches. Water and frost action (freezing and thawing) are probably involved (Root personal communication 2003). The importance of spore banks is unknown for *Botrychium campestre*, but recent studies suggest that they play a vital role in the survival strategies of some ferns (Dyer and Lindsay 1992). The longevity of the spores of *B. campestre* is unknown, but spores of other fern genera (not *Botrychium*) have been germinated from 50-year old herbarium specimens (Dyer and Lindsay 1992).

Botrychium gametophytes are reported to persist underground for up to five years (Winther personal communication 2002), and they grow very slowly from an embryo into an adult gametophyte that produces gametes (Wagner 1998). Sporophytes also may live heterotrophically underground for several years before producing aboveground structures (Kelly 1994). Upon emergence aboveground, the sporophytes begin spore production on their fertile lamina (sporophore). The aboveground longevity of *B. campestre* is approximately four years, which is long compared to the closely related *B. mormo* (Johnson-Groh 1998), but short compared to more distantly related species, such as *B. australe* (11.2 years) (Kelly 1994) and *B. dissectum* (at least a few decades) (Montgomery 1990, Kelly 1994).

No population habitat viability analysis has been done for *Botrychium campestre* to date. The only *Botrychium* species for which this analysis has been conducted is *B. mormo* (Berlin et al. 1998), which differs in many significant ways from *B. campestre* and from most other moonworts as well. Nonetheless, some of the conclusions drawn from the model are relevant to most members of the genus. Three factors were cited that have the most control in the model, although these are also the factors about which the least is known. These factors are viable spore set per sporophyte, the nature and extent of a spore bank, and spore germination rate. Given its heavy reliance on reproduction via gemmae, these factors are probably secondary in *B. campestre*.

Botrychium species are often found in areas with light to moderate disturbance (Lellinger 1985, Wagner and Wagner 1993). Because most *Botrychium* species are early to mid-seral species, they may be expected to drop out as succession proceeds to conditions unsuitable to them. While the prairie habitats of *B. campestre* are not seral per se, they are maintained by natural disturbance processes, that prevent the invasion of woody species, most importantly fire. The typically small populations of *Botrychium* species leave them vulnerable to local extirpation from stochastic processes (Johnson-Groh et al. 1998). Thus, *B. campestre* and other species of *Botrychium* may depend on a shifting mosaic of suitable habitats for their long-term persistence (Chadde and Kudray 2001), as does *Pedicularis furbishiae* (Furbish's lousewort) (Pickett and Thompson 1978, Menges and Gawler 1986). Spores would necessarily be the means by which *B. campestre* migrate to new locations. Given the more persistent nature of the habitats for *B. campestre* and *B. gallicomontanum*, it might be argued that the metapopulation dynamics of these species are less important for their persistence than for most other members of the genus *Botrychium*.

Populations of many *Botrychium* species tend to be small and localized (Colorado Natural Heritage Program 2002). This is probably due to the patchy nature of their habitat, which is a direct result of the nature of the natural disturbance that creates it. Nonetheless, apparently suitable habitat is often not occupied by *Botrychium*. This may be due to limitations in successful migration to the site, or other unknown ecological parameters. The observations of Buell (2001) are interesting in this regard: *Botrychium* species were found only on ski slopes that had been cleared for more than 30 years. A lack of appropriate mycorrhizal symbionts may be one factor limiting population growth in *Botrychium* populations.

Community ecology

Botrychium campestre is found with other prairie species. Coffin and Pfanmuller (1988) cite *Schizachyrium scoparium* (little bluestem), *Muhlenbergia cuspidata* (plains muhly), *Astragalus crassicarpus* (groundplum milkvetch), and *Amorpha canescens* (leadplant) as associated species in Minnesota. Numerous associated species are noted by Nekola and Schlicht (1996) for populations in Iowa, with *S. scoparium* and *Carex richardsonii* (Richardson's sedge) as the most consistent associates. They also noted that all *B. campestre* individuals were found within 10 cm of clumps of *S. scoparium*. In Colorado, *B. campestre* is also associated with *S. scoparium* at the Bonny Prairie State Natural Area (Root 1993, Colorado Natural Areas Program 1998, Colorado Natural Heritage Program 2002). At all of the known verified occurrences in Region 2, *B. campestre* is closely associated with grasses, although the exact species have not been noted in most available sources. For a list of associated species that have been documented with *B. campestre* in the states of Region 2, see **Table 2**.

In Nebraska's Niobrara Valley Preserve, which is owned by The Nature Conservancy, management efforts have focused on the control of *Juniperus virginiana* and *Quercus macrocarpa* (bur oak), which are encroaching on the habitat of *B. campestre* due to the lack of a natural fire regime (Steinauer personal communication 2002). The invasion of prairie by *J. virginiana* has also been noted at the Gregory County occurrence in South Dakota. However, at this site *B. campestre* is found in close association with *J. virginiana*, and its presence at the drip line of *J. virginiana* has the appearance of a commensal relationship (that is, an interaction between species populations in which one species benefits from another, but the other, in this case *J. virginiana*, is not affected).

At both locations in Wyoming, *Botrychium campestre* was documented in sites within areas forested by *Pinus ponderosa*. At Dugout Gulch, *B. campestre* was found to be closely associated with *Poa* spp. (bluegrass), an introduced species, *Medicago lupulina* (black medick), an introduced species, and several other species with "trace" as the cover class (King et al. 2003). It was also found with *B. ascendens* and *B. virginianum*. The adjacent slopes are dominated by *Quercus macrocarpa*, *Corylus cornuta* (beaked hazelnut), and *Betula papyrifera* (paper birch), with the surrounding uplands forested by *P. ponderosa*, but *B. campestre* is located in an open area on an old roadbed. Associated species noted for the occurrence at the Bearlodge Campground are *P. ponderosa* and *Populus tremuloides* (quaking aspen), but no cover data are available.

Since the Pleistocene, pronghorn, elk, deer, prairie dogs, rabbits, other small mammals, and insects have been present on the prairies of the Great Plains (Launchbaugh and Owensby 1978). Bison were the dominant grazers for approximately 5,000 years (McDonald 1981). Historically, bison and elk moved in large herds in Region 2 and elsewhere, grazing areas intensively for a short time, but not revisiting sites for long periods. Current cattle grazing practices involve much more protracted and intensive use. Species turnover in heavily-grazed midgrass prairies may result

Table 2. Associated species that have been documented with *Botrychium campestre* at five locations in the states of Region 2. DG = Dugout Gulch, WY; BC = Bearlodge Campground, WY; BP = Bonny Prairie, CO; NV = Niobrara Valley Preserve, NE; GC = Gregory County, SD. Designation: R = rare, E = exotic. Nomenclature follows that of Kartesz 1999.

Species	Dominant or common associate	Designation	DG	BC	BP	NV	GC
Achillea millefolium			X				
Antennaria spp.			X				
Aristida purpurea					X		
Botrychium ascendens		R	X				
Botrychium virginianum		R	X				
Bouteloua curtipendula	X				X		
Bouteloua gracilis					X		
Bromus tectorum		E			X		
Campanula rotundifolia			X				
Carex inops			X				
Cirsium arvense		E			X		
Corylus cornuta			X				
Crataegus chrysocarpa			X				
Dalea enneandra					X		
Erigeron spp.			X				
Erysimum asperum					X		
Fragaria virginiana			X				
Juniperus virginiana	X					X	X
Maianthemum canadense			X				
Maianthemum stellatum			X				
Medicago lupulina	X	E	X				
Melilotus alba		E			X		
Monarda fistulosa			X				
Pinus ponderosa			X	X			
Poa spp.	X		X				
Polygala senega			X				
Populus tremuloides				X			
Quercus macrocarpa			X			X	
Ratibida columnifera					X		
Rosa spp.			X				
Schizachyrium scoparium	X				X		
Sphaeralcea coccinea					X		
Stipa comata					X		
Symphoricarpos spp.			X				
Taraxacum spp.			X				
Trifolium repens		E	X				
Verbena stricta					X		
Verbascum thapsus		E			X		
Veronica spp.			X				

in their conversion to shortgrass prairies (Launchbaugh 1972). Cattle grazing, as indicated by the presence of cow pies, was documented at the Brown County, South Dakota occurrence and at the newly discovered occurrence in Crook County, Wyoming.

It is likely that if it is a stress-tolerant ruderal (Grime 2001), *Botrychium campestre* is not in competition with the dominant species in its habitat, such as *Schizachyrium scoparium*. The early spring phenology of the species allows it to utilize ephemeral, early season water resources that other perennial prairie species do not depend on. Light resources are not limited, since *B. campestre* grows in open sites in May and June before the canopy of grass is well developed.

The coexistence of many species of *Botrychium* in genus communities is interesting from a community ecology standpoint. "Genus community" is a term coined by Wagner and Wagner (1983) to describe the peculiar tendency for two to several *Botrychium* species to be found in close association with one another (see the Definitions section of this document). If the members of genus communities occupy the same niche, then they coexist in violation of Gause's competitive exclusion principle (Krebs 1972). Because water, nutrient, and some carbohydrate uptake are mediated by mycorrhizae, it is possible that even if genus community members are dependent on the same resources, coexisting plants are not engaged in direct intraspecific competition. Competition may be for access to the mycorrhizae, if it is occurring at all. No research has been done on *Botrychium* species with respect to these issues, and these issues are perhaps less relevant for *B. campestre* since it is not typically found in close association with congeners. Wagner and Wagner (1983) offer an interesting discussion of this issue from a population biology standpoint. There are no reports of parasitism or disease in the literature for any *Botrychium* species.

There is some speculation that spores of at least some *Botrychium* are dispersed by mammals, based primarily on observations of herbivory on the sporophores of *B. mormo* (Casson et al. 1998, F. Wagner personal communication 2002). Although dispersal by animals has not been demonstrated for *B. campestre*, bison or other animals could have functioned as spore dispersal vectors in the past. Small mammals may still function as spore disperal vectors. The large, thick walled spores of *Botrychium* species may be an adaptation to dispersal by herbivores (Wagner 1998).

See **Figure 8** and **Figure 9** for envirograms outlining the resources and malentities for *Botrychium campestre* and summarizing the relationships among different biotic and abiotic factors that weigh heavily in the autecology of this species.

CONSERVATION

Threats

The primary threat to *Botrychium campestre* in Region 2 is loss of habitat. Appropriate loess prairie habitat for this species has greatly diminished due to conversion to agriculture, and existing habitat remnants are also at risk due to succession, fragmentation, and non-native species invasions. Loss of habitat due to development and conversion to agriculture may be extirpating occurrences that are currently unknown. If this species is dependent on a natural disturbance regime that results in a shifting mosaic of seral communities (Pickett and Thompson 1978), then these remaining fragments may be important for its long-term survival.

Due to its extreme rarity in Region 2 and the very small number of individuals in the known occurrences (at most 16 sporophytes where abundance data are available), any land use activity within an occurrence of *Botrychium campestre* may potentially threaten it. Although in other parts of its range this species is found in moderately disturbed areas and is dependent on some level of natural disturbance, these same disturbance regimes could serve to extirpate a very small population, particularly in a small habitat unit. The small populations documented in Region 2 are at risk from stochastic events beyond the control of managers.

In the deciduous hardwood forest habitats of *Botrychium mormo*, invasion of non-native earthworms has resulted in dramatic decreases in mycorrhizal fungi (Nielsen and Hole 1963, Cothrel et al. 1997, Berlin et al. 1998, Gundale 2002). As an obligate mycorrizal symbiont, *B. mormo* is significantly threatened by this decrease. Most earthworm activity takes place in the O soil horizon (Langmaid 1964), while mycorrhizal activity is greatest at the interface of the O and A soil horizons (Smith and Read 1997). The activity of earthworms has resulted in the elimination of the duff layer and a shift in species composition in *B. mormo* habitat (Berlin et al. 1998, Gundale 2002). Although earthworms present a possible threat to *B. campestre*, no research has shown that species of *Botrychium* other than *B. mormo* are being affected by them.

			Centrum
n 2		1	Resources

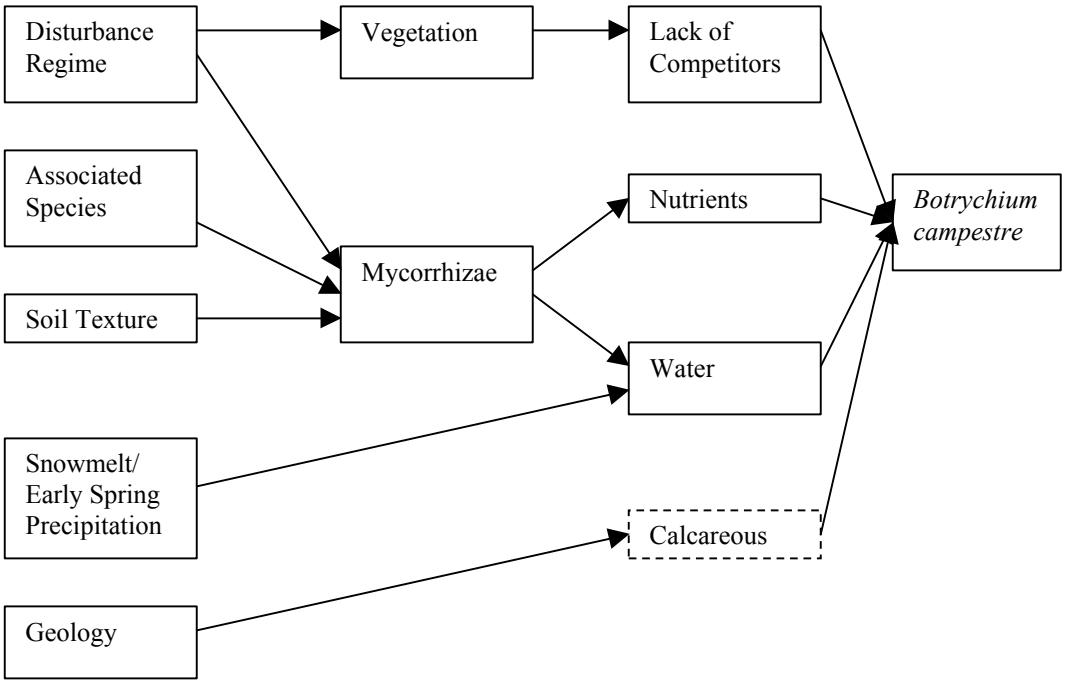

Figure 8. Envirogram outlining the resources of *Botrychium campestre*. Cell with dotted borders is speculative.

Global climate change is likely to have wide-ranging effects in the near future. Projections based on current atmospheric CO_2 trends suggest that average temperatures will increase while precipitation will decrease in Colorado (Manabe and Wetherald 1986). This will have significant effects on nutrient cycling, vapor pressure gradients, and a suite of other environmental variables. Temperature increase could cause vegetation zones to climb 350 feet in elevation for every degree F of warming (U.S. Environmental Protection Agency 1997). Lower soil moistures in the growing season induced by decreased precipitation could have serious impacts for *Botrychium campestre*.

Atmospheric nitrogen deposition, both organic and inorganic, is increasing worldwide. Relatively low levels of nitrogen enrichment are advantageous to some species but deleterious to others, making it difficult to predict species- and community-level responses.

Influence of management activities or natural disturbances on habitat quality

In *Botrychium campestre* habitat, grazing and fire are the most relevant disturbance issues for managers to consider. Johnson-Groh (1999) observed that burning in either spring or fall may not be damaging to *B. campestre* in monitored populations in Iowa and Minnesota. However, fire combined with drought and erosion, both of which are exacerbated by fire, can be harmful. She also notes that it is difficult to draw inferences from these data due to small sample sizes and imprecise control of burns.

A variety of practices has been used in attempts to improve habitat quality for *Botrychium campestre* (Colorado Natural Areas Program 1998, Steinauer personal communication 2002). Fall or late winter burning appears to have the most beneficial effects, whereas spring burning results in plant injury or mortality and exaggerates the effects of summer drought (Colorado Natural Areas Program 1998). However, burning at any time of the year is likely to impact some stage in the lifecycle of *B. campestre*. While spring burning will impact sporophytes, summer and autumn burning would destroy the current year's spore crop (Root personal communication 2003). In Nebraska's Niobrara Valley Preserve, management efforts have focused on the removal of *Juniperus virginiana* that has encroached on occurrences and habitat for *B. campestre*

			Centrum
n	2	1	Malentities

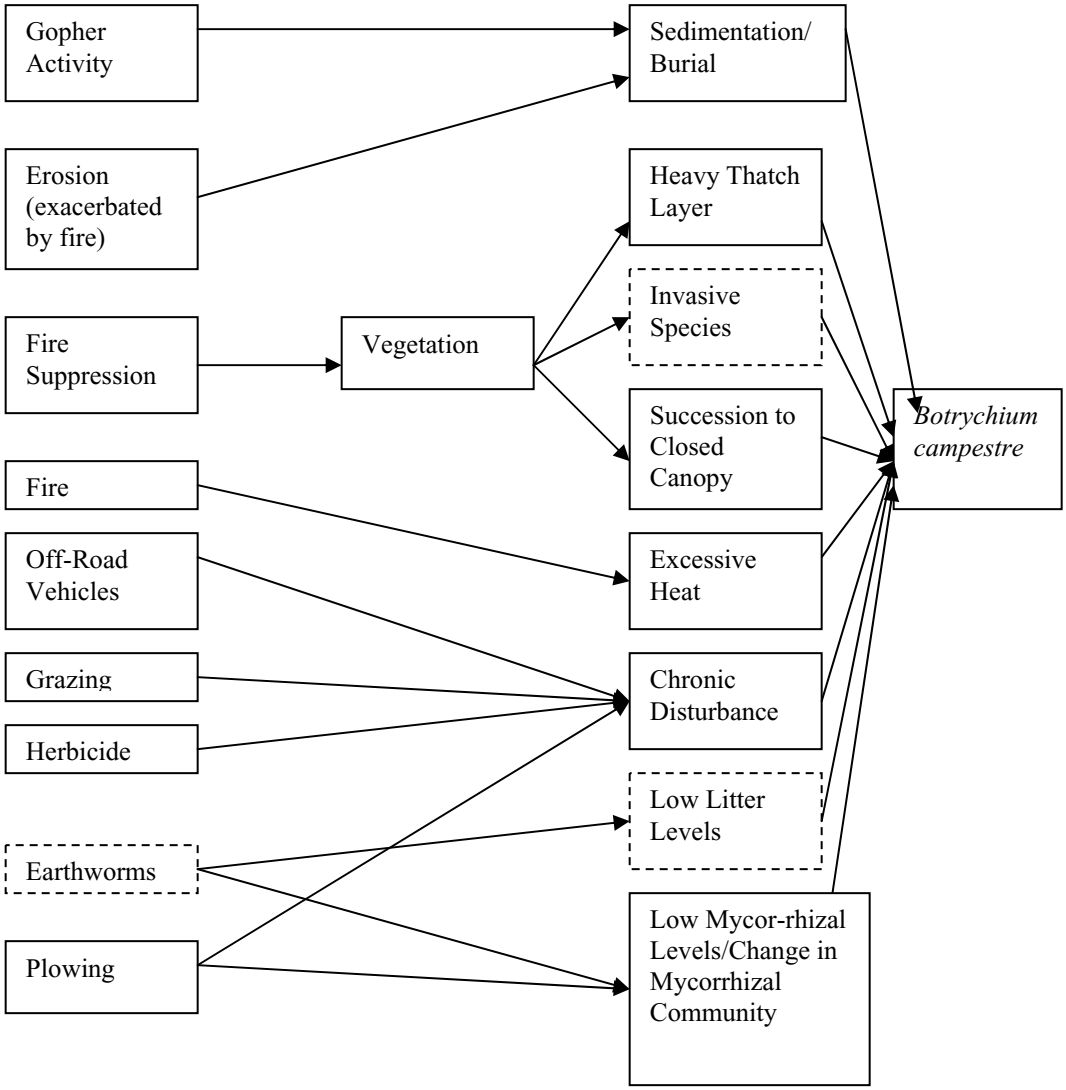

Figure 9. Envirogram outlining the malentities to *Botrychium campestre*. Cells with dotted borders are speculative.

with the lack of a natural fire regime (Steinauer personal communication 2002). Although the *B. campestre* plants are located in a bison pasture, the effects of bison on the species are unknown because the bison cannot access them due to the surrounding heavy woody vegetation.

Excess litter accumulation may negatively affect *Botrychium campestre* at the Bonny Prairie Natural Area in Colorado (Root 1993, Colorado Natural Areas Program 1998). In the absence of fire and the pre-settlement grazing regime, a thick layer of thatch tends to build up; this may impede the growth of *B. campestre*. However, removal of all thatch is likely to reduce soil moisture levels and may also imperil this occurrence of the species. Because a maximum of six individuals has been observed at this site since its discovery in 1990, it is impossible to conduct experiments to determine appropriate management practices without risking extirpation. Raking has been offered as a way to manage thatch loads in the vicinity of the known plants, with an experimental burn planned for a portion of the tallgrass prairie portion of the Natural Area to observe the effects of burning on habitat quality.

Gopher excavation has resulted in the temporary loss of the closely related, gemmae-bearing *Botrychium*

gallicomontanum in permanent plots at Frenchman's Bluff, Minnesota. Part of a plot was buried by soil excavated by gophers, but after 11 years of monitoring at this site the *B. gallicomontanum* population had largely rebounded (Johnson-Groh 1999).

Influence of management activities or natural disturbances on individuals

Because *Botrychium campestre* is inconspicuous and many populations may remain undocumented, clearances prior to management actions within potential habitat would help alleviate threats to this species from human impacts.

Burning may result in damage to the aboveground portions of the sporophyte plant, particularly if it occurs at phenologically sensitive times. For *Botrychium campestre*, this is from April to July when the aboveground portion of the life cycle is taking place. Burning could result in mortality, but due to the strong dependence of the species on mycorrhizae, removal of leaf tissue via burning or other means is probably inconsequential to the plant's survival (Wagner and Wagner 1993, Johnson-Groh and Farrar 1996b, Johnson-Groh 1999), unless the fire is hot enough to sterilize the soil.

Grazing offers both potential benefits and detriments with regard to *Botrychium campestre*. Disturbance of the surface by cattle may injure some individuals, but at carefully monitored intensities, cattle grazing may have positive effects on litter reduction (Colorado Natural Areas Program 1998). The use of cattle grazing as a management tool for the enhancement of habitat is risky for a plant as rare as *B. campestre*, since it is likely to cause some level of erosion, trampling, alteration of plant community composition, damage to the soil structure (particularly when wet), and introduction of invasive plants.

Interaction of the species with exotic species

There are no data suggesting a direct impact of weeds on *Botrychium* species (Johnson-Groh and Farrar 2003), but their mutual affinity for disturbance may cause *Botrychium* species and their habitats to be vulnerable to negative impacts from weeds. Although high quality habitat for *B. campestre* tends to be areas that are minimally disturbed by human activities, the potential impact from weeds on *B. campestre* remains high for two reasons. *Botrychium campestre* occurs at fairly low elevations in Region 2 where noxious weeds are more prevalent than at higher elevations, where most other *Botrychium* species in the region are found. Also, undisturbed prairie ecosystems may still be highly susceptible to weed invasion. Marler et al. (1999) observed indirect enhancement of the competitive ability of *Centaurea maculosa* with a native bunchgrass in the presence of arbuscular mycorrhizal fungi. *Centaurea maculosa* is extensively mycorrhizal. Thus mycorrhizae (possibly the species on which *Botrychium* species depend, since arbuscular mycorrhizae are often generalists) augment the ability of *C. maculosa* and perhaps other noxious weeds to invade native grasslands. While previous research has suggested that undisturbed areas with high biodiversity of native species are less susceptible to weed invasion, recent studies have observed the opposite (Stohlgren et al. 2003). Some exotic plant species have demonstrated the ability to invade native prairie. *Bromus japonicus* (Japanese brome) now occurs in large monotypic stands in the badlands of South Dakota, and this and other annual brome species may pose a threat to areas such as Bonny Prairie (Carpenter personal communication 2003).

Three weed species (*Medicago lupulina*, *Trifolium repens* and *Taraxacum* spp.) were documented with *Botrychium campestre* at the newly discovered site in Crook County, Wyoming (King et al. 2003). *Trifolium repens* (white clover) and *Taraxacum* spp. (dandelion) were seen in the area but not in close association with *B. campestre*. However, *M. lupulina* was observed directly associated with *B. campestre* at 5 percent cover. The threat to this occurrence from these species is unknown, but their behavior in this vicinity needs to be monitored. At the Bonny Prairie State Natural Area, several exotic plant species are well established including *Melilotus alba* (white sweetclover), *Cirsium arvense* (Canada thistle), *Verbascum thapsus* (common mullein), and *Bromus tectorum* (cheatgrass).

Burning could increase the prevalence of *Bromus tectorum* in *Botrychium campestre* habitat (Colorado Natural Areas Program 1998). Thus, managers using burning as a tool for prairie management must take care to minimize opportunities for cheatgrass to be introduced in newly burned areas. *Bromus tectorum* grows vigorously early in the spring, when the sporophyte of *B. campestre* is also actively growing. This phenological overlap could result in direct or indirect competition between them. Management of *B. tectorum* in populations of *B. campestre* would be difficult and risky, since treatments meant to eradicate cheatgrass would also likely have impacts on *B. campestre*.

Threats from over-utilization

Although evidence suggests that leaf removal does not have a significant long-term effect on *Botrychium campestre*, collection of the species in Region 2 is not advisable due to extremely low population sizes. Johnson-Groh and Farrar (2003) state that no collections should be made in populations of less than 20 plants. Instead, they recommend that photos be taken. Root has never collected material from the Bonny Prairie site because of its small population size (Root personal communication 2002). Where populations are already of questionable viability, collection of material from them is a risky endeavor, even if the plants will probably survive. For example, in a population of two sporophytes, there is almost no margin of error, and accidentally removing the apical bud could easily result in the extirpation of the species at this site. This is a difficult issue for some *Botrychium* species (including *B. campestre*), since collection and verification by an expert is the only way to be absolutely sure of proper identification. Vouchers are valuable and assist greatly with taxonomic research on the species. Weber and Wittmann (2001) recommend not collecting plants with the roots, because there are no diagnostic characteristics associated with the roots and collecting them kills the plant. To minimize the risk of infection and of removing the apical bud, Johnson-Groh and Farrar (2003) recommend cutting the leaf with a knife near ground level rather than pinching or pulling with the fingers. They also recommend that no more than 10 percent of a population be collected.

Inappropriate recreational use of the habitat of *Botrychium campestre* is a tangible threat. Off-road vehicle use in an occurrence of this species could quickly extirpate it by directly killing sporophytes and disturbing the soil, which could kill the gametophytes. Similarly, intensive grazing would probably result in extirpation of occurrences.

There are no known commercial uses for *Botrychium campestre*. According to Gerard in his 1633 herbal (p. 407), "moonewort" (referring to *B. lunaria*) "is singular to heale greene and fresh wounds: it staieth the bloudy flix. It hath beene used among the alchymistes and witches to doe wonders withall." Currently *Botrychium* species are not widely sold in the herb trade, but they are mentioned as ingredients in tinctures and poultices for the treatment of external or internal injuries. There is potential for over-utilization of *Botrychium* species if their popularity increases in the herb trade. Because they cannot be cultivated, any commercial use would require the harvest of wild populations.

Conservation Status of the Species in Region 2

Is distribution or abundance declining in all or part of its range in Region 2?

No rigorous quantitative research has been conducted on population trends for this species in Region 2. Quantitative data from 12 years of monitoring in Minnesota and from 5-Ridge Prairie, Iowa suggest that populations are in decline (Johnson-Groh 1999). Steinauer (personal communication 2002) reported that one of the two known populations at the Niobrara Valley Preserve has not been seen in more than 10 years. The other occurrence at the Niobrara Valley Preserve is very small (four plants observed in 2001). Despite searches, the population at the Bearlodge Campground in Wyoming has not been seen in 30 years. After 10 years of observations at the Bonny Prairie Natural Area, Colorado no more than six sporophytes have been recorded at this location. There are no known populations that are currently increasing in Region 2 or elsewhere, and the largest population ever observed in Region 2 is 16 individuals, in Gregory County, South Dakota.

Do habitats vary in their capacity to support this species?

Succession may lead to unsuitable conditions for *Botrychium campestre* at a site in the absence of a disturbance regime. Variables such as burning regime, amount of litter, soil moisture variation, and soil texture may affect habitat suitability. Suitability of a site to the appropriate mycorrhizae is equally important for *Botrychium* species as they are obligate mycorrhizal symbionts. Soil moisture and texture, as well as the associated species in the plant community, are perhaps the most relevant factors with respect to mycorrhizae.

Vulnerability due to life history and ecology

Botrychium campestre is vulnerable to habitat change (erosion, weeds, and succession) due to its dependence on a disturbance regime to maintain the suitability of its habitat. Several adaptations leave *B. campestre* well-suited to its prairie habitat. The early season growth allows it to capitalize on spring water resources, while avoiding some danger of fires that tend to occur later in the summer or fall. The use of gemmae

for asexual reproduction allows *B. campestre* to persist without dependence on the gametophyte portion of its life cycle, which requires relatively moist soils to permit fertilization. Also, the ability to remain dormant for one or more years enables plants to survive drought conditions (Johnson-Groh 1999). While reproduction by cloning is good in static environments, sexual reproduction and long-distance dispersal are better suited to facile environments where recombination of alleles and higher genetic diversity leave some individuals better suited to new conditions.

Evidence of populations in Region 2 at risk

The rarity and extremely small population sizes of *Botrychium campestre* in Region 2 suggest that it is highly vulnerable to extirpation locally or even regionally, and that all the Region 2 populations are at risk. No more than a total of 28 individuals are known from the five occurrences in Region 2 where abundance data are available. Of the nine documented occurrences of *B. campestre* in Region 2, population size is not known for four occurrences, and four occurrences are highly questionable, due to uncertain identification and failure to observe sporophytes at the site in over 10 years (**Table 1**). Thus the current status of these occurrences is uncertain. Stochastic processes and normal environmental variation could easily result in extirpation of any or all of the small and localized populations known from Region 2. Available habitat in Region 2 is probably still in decline due to fragmentation, exotic species invasion, and edge effects that decrease the quality of small patches of natural vegetation. However, the rate of decline has certainly abated since the large scale conversion of loess and other midgrass prairies to agriculture during the last century. Because the ecology of this species is poorly understood, current management may be placing demands on the species despite good intentions. Monitored populations of *B. campestre* in Minnesota and Iowa have exhibited overall declining numbers (Johnson-Groh 1999).

Current data suggest a high degree of imperilment for this species in Region 2, but these data are sparse. The likelihood of finding more populations in Region 2 is high with further targeted surveys and involvement of experts on *Botrychium* spp. However, it is unlikely that large, robust populations will be found, and further searching is unlikely to identify large numbers of new populations. This underscores the need to conduct additional survey work for this species and to continue to rigorously monitor the known populations.

Management of the Species in Region 2

Implications and potential conservation elements

The conversion of much of the Great Plains to agriculture has resulted in the destruction and fragmentation of habitat for *Botrychium campestre*, but it is not known to what extent the species was impacted rangewide and on USFS land. We can only infer that many occurrences were extirpated when contiguous habitat was disrupted. Obviously the remaining few occurrences became more insular and more widely separated from other occurrences. Population genetics and island biogeographic theory suggest that this results in reduced gene flow, reduced heterozygosity, and a higher probability of local extirpation. Although habitat connectivity for *B. campestre* has been impacted, it is not certain whether this translates to genetic connectivity and decreased gene flow, or whether this even matters for *B. campestre* which reproduces primarily by asexual gemmae (Farrar 1998).

When we assume that there are more populations to be discovered, it is implicit that some populations are less isolated than we think. Similarly, finding other occurrences may broaden our definition of "appropriate habitat," which would lead to a realization that habitats are in fact more connected or perhaps less imperiled.

Maintaining the ecosystem processes needed by *Botrychium campestre* is undoubtedly a conservation measure. In the absence of natural phenomena to maintain certain ecosystem processes (e.g., bison and prairie fires), activities that mimic these processes could benefit *B. campestre*.

Desired environmental conditions for *Botrychium campestre* include greater habitat connectivity and natural ecosystem processes in place at all scales. However, this is difficult to achieve because once an area is plowed it is not known when, if ever, the site will once again be suitable for *B. campestre*. It is uncertain whether habitat for *B. campestre* can realistically be restored due to the importance of mycorrhizae and our lack of understanding of the underground ecology of this species. Therefore, increasing the connectivity of habitat around highly insular populations by restoring the prairie is not presently a viable option, and may be insufficient for the conservation of *B. campestre*. However, conservation efforts that work towards improving habitat quality and connectivity

will offer ancillary benefits such as reducing weed invasions at the perimeters of habitat units. Perhaps with greater knowledge of the ecology and propagation of *B. campestre* this will become feasible, but now the priorities for this species lie with conserving the known extant occurrences. Managing areas of appropriate habitat in the vicinity of known occurrences in ways that favor the persistence of *B. campestre* is a worthwhile endeavor, since it may provide areas for colonization and may conserve occurrences that remain undiscovered at present.

Tools and practices

Species inventory

Botrychium campestre, like other species of subgenus *Botrychium*, is small, inconspicuous, and thus difficult to find. Although the probability that other occurrences remain to be found in Region 2 is high, it is nonetheless extremely rare in the West. Its early season growth and the year-to-year fluctuation in aboveground sporophytes (with years of no individuals aboveground in a population possible) also make inventory work difficult with this species. This species was only recently described (Wagner and Wagner 1986) and is difficult to identify (Wagner and Wagner 1990). For these reasons inventory work remains a high priority for this species in Region 2.

Often, due to limitations in time and funding, attempts to search for rare plants involve looking for multiple species in large areas. While this approach has been effective in finding many rare plant occurrences, it may not be effective for *Botrychium campestre* given the difficulty in finding and identifying the species, and annual fluctuations in aboveground sporophyte numbers. Because searching for *B. campestre* requires one's full attention, attempts to search for this species are more likely to be successful if it is the only species being sought (Root personal communication 2002). Having experts (contractors, agency botanists, or others trained and experienced with searching for *Botrychiums*) conduct searches in appropriate habitat may be the most effective approach to expanding our knowledge of the distribution of this species in Region 2.

Johnson-Groh and Farrar (2003) offer excellent suggestions for conducting surveys for *Botrychium* species. The protocols defined in this document will serve as standard protocols for all survey work on subgenus *Botrychium*. Suggestions for surveys include search methods, marking plants, collection protocols, documentation, and other concerns. Limitations of these methods are also discussed and are important considerations.

Habitat attributes can be used as a filter to refine areas to search for *Botrychium* species. Root (2003) notes that because *Botrychium campestre* is an ephemeral species, it has a relatively short window in which it can be sought in the field. At the Pawnee National Grassland, searches are most likely to be effective before the grass greens up. *Botrychium campestre* has not been seen after May 20 in Colorado, and late April is the ideal time for searching in normal years. In higher latitudes it may be sought in May and June. *Botrychium campestre* is typically found in grassy areas.

Habitat inventory

Identifying suitable habitat in which to focus searches for *Botrychium campestre* could be done by intersecting soil and vegetation maps to identify loess areas with native vegetation in Region 2. High priority search areas should be sites where *Schizachyrium scoparium* is the dominant species, since it commonly occurs with *B. campestre*. The scale of most vegetation maps (e.g., GAP, Basin-Wide Maps) is too coarse to be used to detect small remnants of unplowed loess, so fine-scale habitat inventory will necessitate much field work and inquiries with managers and land owners regarding the history of sites. Aerial photography and satellite imagery may also assist with the identification of areas worth searching.

Areas with vegetation similar to that of the occurrences known from the Black Hills of Wyoming and South Dakota should also be targeted for inventory work. Areas dominated by ponderosa pine are found in all states of Region 2 except Kansas, and are particularly widespread in Colorado and Wyoming. It is unknown whether suitable habitat is present near ponderosa pines outside of the Black Hills in Region 2, since this is the only location in the region where *Botrychium campestre* is found in this type of habitat. Given promising recent discoveries in the Black Hills, a full inventory of this area by experts is needed using the protocols defined by Johnson-Groh and Farrar (2003).

Monitoring

Annual monitoring of every occurrence of *Botrychium campestre* in Region 2 could help in understanding its ecology and population trends. This is made relatively simple by the small population sizes known within the Region at present. Counting the number

of emergent sporophytes at each occurrence every year, as recommended by Root (1993), would provide valuable data on the status of the species. Population trend monitoring protocols pertaining to members of subgenus *Botrychium* are defined in Johnson-Groh and Farrar (2003). Protocols for monitoring *B. lineare* have been drafted for the Pike-San Isabel National Forest (Carpenter 1996), and these may have some relevancy for *B. campestre* as well. Tracking individuals by marking or mapping them within each sampling unit could help to better understand life span, dormancy, recruitment success, and population trends. Monitoring the Region 2 populations is a high conservation priority, along with species inventory work.

Adding a photoplot component to this work, following recommendations offered in Elzinga et al. (1998), might facilitate the tracking of individuals and add valuable qualitative information. However, the small size of *B. campestre* could make it difficult to use this technique for tracking individuals. A new handbook on photo point monitoring (Hall 2002) offers excellent guidance on establishing photo point monitoring plots. This technique can be done quickly in the field, and although it does not provide detailed cover or abundance data, it can help to elucidate patterns observed in quantitative data.

The number of emergent sporophytes is highly variable and is an incomplete indicator of total population numbers in *Botrychium campestre*. Mason and Farrar (1989) and Johnson-Groh (1998) describe methods for extracting gemmae, gametophytes, and non-emergent sporophytes from soil samples. Using these methods, Johnson-Groh (1998) recovered as many as 7000 gametophytes and 250 sporophytes per m^2 of the closely related *B. mormo*, although an unknown number of gametophytes may have been *B. virginianum*. In an earlier study, Bierhorst (1958) found 20 to 50 gametophytes per ft^2 of the distantly related *B. dissectum*, with relatively few mature gametophytes with attached juvenile sporophytes. These studies suggest that the observation of a single emergent sporophyte may indicate the presence of a viable population (Casson et al. 1998), or an early stage of colonization.

A detailed and scientifically rigorous monitoring effort employing the destructive sampling methods of Johnson-Groh (1998) is not advisable in the very small populations of Region 2. Thus, at present the priorities for Region 2 lie in basic survey work and in a complete census of the few known populations annually. Marking and mapping individuals would also provide valuable information if done with great care, although is much more labor intensive. Johnson-Groh (1999) notes that it can be difficult to be assured that an individual marked in a previous year is the same individual again in subsequent years. This problem is exacerbated in *Botrychium campestre*, because it reproduces with gemmae, which remain close to the parent plant (Johnson-Groh 2001). Thus many sporophytes may emerge in close proximity to each other.

Presence/absence monitoring is not suitable for *Botrychium campestre* due to the small population sizes in Region 2. Gathering population size data can be done rapidly and may require only a small amount of additional time and effort (Elzinga et al.1998).

Beneficial management actions

Populations of *Botrychium* are inherently variable (Johnson-Groh 1999). Many populations are small, increasing the likelihood of local extirpation. The long-term viability of the species may depend on the availability of a "shifting mosaic of suitable habitats" in appropriate successional stages that *B. campestre* can colonize (Chadde and Kudray 2001). If this is the case, then the metapopulation dynamics of this species become crucial to its management and conservation, and underscore the need to conserve nearby areas of suitable habitat that are not currently inhabited by *B. campestre*. At present, metapopulation level research in Region 2 could merely involve searching areas of suitable habitat not known to be occupied. Future studies could include investigation of migration, extinction, and colonization rates (Elzinga et al.1998). These would be extremely difficult to assess (time consuming but technically feasible) for any *Botrychium* species.

The ecology of *Botrychium campestre* remains poorly understood, and the species has not been recognized long enough to develop effective conservation strategies. However, some generalities regarding conservation can be made based on current knowledge. Because *B. campestre* shows a general preference for open sites, it may benefit from reduced canopy cover. However, many such activities have not been tested and using the small populations in Region 2 to try management tools would put them at great risk. The largest known occurrence in Region 2 is found in the drip line of *Juniperus virginiana*, which suggests that woody species invasion may not pose a great threat to *B. campestre*. However, this remains highly speculative at present, and unless other populations are found with *J. virginiana*, it is probably wise to continue to remove encroaching woody species from native prairie areas.

Removal of woody species when the ground is frozen would minimize the risk to *B. campestre*. Maintaining the health of the mycorrhizae is certainly crucial to the species as well. Low-intensity burning is probably an effective management tool for *B. campestre* (Johnson-Groh 1999), but it has not been applied in Region 2. Though burning appears to have positive effects on *B. campestre* populations in Iowa, fire combined with erosion and desiccation, both natural results of fire, may be deleterious (Johnson-Groh and Farrar 1996a, Johnson-Groh 1999). The Bonny Prairie Management Plan (Colorado Natural Areas Program 1998) offers preliminary management recommendations and protocols pertinent to *B. campestre*, but these have not yet been implemented. These include the use of raking and fire for habitat maintenance.

Restoration

It is extremely difficult to grow any *Botrychium* species in the greenhouse or lab (Whittier 1972). No spores or gemmae are currently in storage for *B. campestre* at the National Center for Genetic Resource Preservation (Miller personal communication 2002). Collection of spores and gemmae for long-term storage may be useful for future restoration work. Herbarium specimens and wild populations (with careful restraint) may also offer sources of propagules for restoration purposes.

Information Needs

Distribution

Given the high probability that more populations await discovery in Region 2, further survey work is among the greatest research needs for *Botrychium campestre*. Revisiting known occurrences of other *Botrychium* species at high elevations is needed to determine if significant populations also inhabit these sites, although the current opinions of experts suggest that the probability of finding populations in these habitats is low. Careful surveys in appropriate habitat in April and May are warranted throughout the prairies of Region 2. Priority areas include the Black Hills of Wyoming and South Dakota, Pawnee National Grassland on the Arapahoe Roosevelt National Forest in Colorado, loess prairies in eastern Kansas, and any other loess remnants in Region 2. Given the proximity of Bonny Prairie to Kansas, any loess remnants in western Kansas must be searched. Revisiting known populations, particularly those that have not been revisited recently (e.g., South Dakota) or have been sought but not found (e.g., a population at the Niobrara Valley Preserve in Nebraska) is also a high priority. Coordination with other USFS Regions for survey work and for reporting and sharing results will also help to facilitate more effective searching.

Life cycle, habitat, and population trend

Very little is known about the population ecology of *Botrychium campestre*. In particular, the belowground portion of the life cycle remains poorly understood, although much of their lifespan occurs underground.

Survey work throughout the range of *Botrychium campestre* has found populations on loess soils, and searching unplowed loess remnants has led to success in finding a new occurrence at Bonny Prairie by Peter Root and David Kuntze (Root 1993). However, an understanding of the affinity of this species for other habitat types, particularly mountain areas and areas with a woody overstory, is lacking.

Annual revisits are needed for all the populations known in Region 2 to monitor their status. Marking and tracking individuals at these sites would provide population trend data, which is currently unknown. Determining population trends for populations in Region 2 will require several years given, the inherent difficulties in monitoring *Botrychium* species.

Response to change

Broad scale changes in habitat, such as conversion of habitat for agriculture, clearly would result in extirpation of populations. The specific responses to disturbance and succession are less clear and warrant further investigation. The amount of disturbance that *Botrychium campestre* can tolerate is not known but has considerable importance from a management perspective. Monitoring plots in Minnesota address fire management issues but not with regard to reproduction, dispersal of spores and gemmae, and establishment. The effects of exotic species on the viability of *B. campestre* populations have not been investigated.

Metapopulation dynamics

The metapopulation dynamics of *Botrychium campestre* and other *Botrychium* species are not understood. Migration, extinction, and colonization rates are unknown for all *Botrychium* species and will be difficult to determine, given the difficulties in finding and observing this species.

Demography

There have been very few demographic studies of *Botrychium* species. Currently our knowledge of demographic processes is not advanced for any member of this genus. *Botrychium mormo* is the best-studied member of the genus (Berlin et al. 1998), but many assumptions were made in estimating crucial life history parameters even for this species. Thus, any analyses made using current data for *B. campestre* would be largely conjectural, and would necessarily be based on populations in Iowa and Minnesota. No demographic data are available for populations in Region 2.

Population trend monitoring methods

Methods have been suggested to monitor population trends (Berlin et al. 1998, Johnson-Groh 1999). However, because there may be some unknown populations (NatureServe Explorer 2001), observations at known sites may or may not reflect real population trends (Johnson-Groh 1999). Thus the available methods are less likely to be effective for understanding Region-wide trends. Nonetheless, monitoring sites using the established protocols should be considered and will yield valuable data.

Restoration methods

There are many barriers to habitat restoration for Botychium campestre and other *Botrychium* species. *Botrychium* species are extremely difficult to propagate (Whittier 1972), and propagating them for reintroduction to the wild is probably not feasible. The belowground ecology of these species is crucial to understanding their autecology, yet it is also very poorly understood. As obligate mycorrhizal symbionts, they cannot survive without suitable fungal partners, but very little is known about the specifics of this relationship. The mycobionts of *B. campestre* have not been identified.

Restoration or maintenance of dominant prairie grasses will certainly be a crucial part of any restoration effort on behalf of *Botrychium campestre*. Restoration of prairie in the vicinity of known *B. campestre* occurrences is likely to benefit these populations as possible colonization sites, buffers, and in reducing influx of exotic species.

Research priorities for Region 2

The most obvious research priority in Region 2 is the need for a better understanding of the range and distribution of *Botrychium campestre*. It is likely that some, if not many, populations remain to be discovered for this and other moonwort species (Farrar and Johnson-Groh 1986, Wagner and Wagner 1986), and many of these may fall within Region 2. Mountain habitats should not be ruled out in future inventory work. Numerous other research needs are cited by Farrar and Johnson-Groh (1986), Berlin et al. (1998), and Johnson-Groh (1999). Some of these apply to *Botrychium* species in general, while others pertain specifically to *B. campestre*. With respect to *Botrychium* species in general, further research is needed on life history and demography, focusing on underground life history stages. The specific role of gemmae in the life history of *B. campestre* also needs further investigation. A clearer understanding of the relationship between *B. campestre* and its mycorrhizal symbionts will also have considerable practical value. An assessment of the effects of disturbance quality and periodicity, fire, and grazing on mycorrhizae will assist managers in ascribing appropriate management protocols. Research is also needed to assess the effect of different mycorrhizal species and infection levels on spore output. Studies on the role of fossorial mammals and other potential dispersal vectors in the dispersal of spores and gemmae will assist with the management of *B. campestre*. Investigating the relationship of *B. campestre* with woody species, particularly those that are invading prairie habitats, will help to better determine the management needs for this species. Estimating cover and/or abundance of associated species could permit the investigation of interspecific relationships through ordination or other statistical techniques. Understanding environmental constraints on *B. campestre* could facilitate the conservation of this species. Gathering data on edaphic variation (primarily moisture and texture) from permanent plots could permit the analysis of species-environment relationships. Such data gathered carefully at the known populations in Region 2, when compared with census data could provide some basic insight into the causes of the fluctuation in aboveground sporophyte abundance in Region 2, and could help with hypothesis generation for further studies of the ecology of this species. Finally, research on the autecology of *B. campestre*, particularly with regard to its responses to burning, grazing, succession, and invasive species, is needed.

Additional research and data resources

An assessment of a suite of *Botrychium* species (including *Botrychium campestre*) for Washington and Oregon is currently in progress by Farrar, Ahlenslager, and Johnson-Groh. Monitoring protocols for

Botrychium species are being drafted by Johnson-Groh and Farrar (Johnson-Groh and Farrar 2003) but are in draft form at present.

Definitions

Achlorophyllous: A plant lacking chlorophyll and thus dependent on obtaining carbon from a host or symbiont.

Antheridium: The male sex organ of the gametophyte, where male sex cells are produced by mitosis (Allaby 1998).

Archegonium: The female sex organ of the gametophyte, where female sex cells are produced by mitosis (Allaby 1998).

Commensal relationship: An interaction between species populations in which one species benefits from another, but the other is not affected (Allaby 1998).

Congener: A member of the same genus. *Botrychium hesperium* is a congener of *B. campestre*.

Competive/Stress-tolerant/Ruderal (CSR) Model: A model developed by J.P. Grime in 1977 in which plants are characterized as Competitive, Stress-tolerant, or Ruderal, based on their allocation of resources. Competitive species allocate resources primarily to growth; stress-tolerant species allocate resources primarily to maintenance; and ruderal species allocate resources primarily to reproduction. A suite of other adaptive patterns also characterize species under this model (Barbour et al. 1987). Some species, including *Botrychium campestre*, show characteristics of more than one strategy.

Ectomycorrhiza: A type of mycorrhiza where the fungal hyphae do not penetrate the cells of the root, but instead form a sheath around the root (Allaby 1998).

Endomycorrhiza: A type of mycorrhiza where the fungal hyphae penetrate the cells of the root. Arbuscular mycorrhizae are a type of endomycorrhizae (Allaby 1998).

Gametophyte: The haploid stage in the life cycle of a plant. This stage lives independently of the sporophyte in ferns. In *Botrychium* species, the gametophyte is subterranean and is parasitic on mycorrhyzal fungi (Foster and Gifford 1989).

Gemma: A minute vegetative propagule abscised at maturity from the parent plant (Farrar and Johnson-Groh 1990).

Genus community: Several *Botrychium* species are commonly found growing together in close proximity. This is unusual in the plant world, since members of the same plant genus often do not occur together, probably because of competitive interactions that would occur between them. The Wagners coined the term "genus community" to describe these peculiar assemblages of *Botrychium* species (Wagner and Wagner 1983).

Lamina: The leaf blade of a fern, including the sporophore and trophophore (Lellinger 1985).

Loess: Soil deposited by wind. In the Midwest, vast quantities of fine-grained material were blown from rivers draining Pleistocene glaciers (Farrar 1985).

Mycobiont: The fungal partner in a mycorrhizal symbiosis.

Ranks: NatureServe and the Heritage Programs Ranking system (Internet site: http://www.natureserve.org/explorer/granks.htm). G3 indicates *Botrychium campestre* is "vulnerable globally either because it is very rare and local throughout its range, found only in a restricted range (even if abundant at some locations), or because of other factors making it vulnerable to extinction or elimination". S1 Critically imperiled globally because of extreme rarity (five or fewer occurrences or very few remaining individuals) or because of some factor making it especially vulnerable to extinction.

Ruderal: Plants with an adaptive suite of characteristics, including high reproductive rate, that makes them effective colonists and well-suited to disturbed habitats (Barbour et al. 1987).

Sporophore: The fertile, spore-bearing portion of the leaf of *Botrychium* species (Foster and Gifford 1989).

Sporophyte: The diploid portion of the lifecycle of plants. Haploid spores are produced by meiosis that give rise to gametophytes (Allaby 1998).

Trophophore: The vegetative portion of the leaf of *Botrychium* species (Foster and Gifford 1989).

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