Botrychium multifidum (Gmel.) Rupr. (leathery grapefern): A Technical Conservation Assessment

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COVER PHOTO CREDIT

Botrychium multifidum (leathery grapefern). Photograph by Katherine Zacharkevics, North Zone Botanist Black Hills National Forest.

Summary of Key Components for Conservation of *Botrychium multifidum*

Status

Botrychium multifidum (leathery grapefern) is known from 20 locations in USDA Forest Service (USFS) Region 2; five of these have not been seen within the last 20 years. Reports of abundance for occurrences in Region 2 suggest that there may be cause for concern regarding the viability of this population. However, a complete census is difficult to obtain for this species, and more individuals may be present at some locations than reports indicate. Some occurrences may support significant numbers, but the population sizes of these sites were not estimated. *Botrychium multifidum* is ranked globally demonstrably secure (G5) by NatureServe. Within Region 2, it is ranked critically imperiled (S1) in Colorado and South Dakota, and imperiled (S2) in Wyoming. There is one questionable report of this species from Nebraska. *Botrychium multifidum* is not listed under the federal Endangered Species Act, but the USDA FS Region 2 Regional Forester (USDA Forest Service 2003) designate it a sensitive species.

Primary Threats

Observations and quantitative data have shown that there are several potential threats to the persistence of *Botrychium multifidum*. The primary threats are road and trail construction and maintenance, recreation, exotic species invasion, grazing, effects of small population size, timber harvest, global climate change, and pollution. However, these threats are highly speculative because very little is known about this species in Region 2.

Primary Conservation Elements, Management Implications and Considerations

Of the 20 reports of *Botrychium multifidum* within Region 2, 18 occur on land managed by the USFS, one is on privately owned land, and one is known from Rocky Mountain National Park. Five of the 20 occurrences have not been seen in more than 20 years, and four of these have not been seen in more than 50 years. The occurrence reported on private land in Nebraska in 1895 is highly questionable. Efforts are needed to determine if *B. multifidum* remains extant at these locations. Numerous additional occurrences of this species are known from Yellowstone and Grand Teton national parks, which fall just outside the Region 2 administrative boundary. Because most occurrences of this species in Region 2 are on National Forest System lands, the responsibility of maintaining viable populations within the administrative boundary of Region 2 falls largely on the USFS.

Conservation elements essential to maintaining viable populations of *Botrychium multifidum* in Region 2 include a stable, appropriate hydrological regime, and early- to mid-seral stage or other suitable open habitats. Demographic studies of other species of *Botrychium* suggest that managing occurrences to prevent adult mortality is important for maintaining population viability in Region 2.

Given the rate at which new data are becoming available and given the incompleteness of our current knowledge of *Botrychium multifidum* in Region 2, it is difficult to formulate conservation strategies for this species at present. More complete knowledge of the distribution of *B. multifidum* in Region 2 will facilitate the development of conservation strategies and permit the identification of areas most suitable for conservation management. Further survey efforts, along with monitoring studies designed to determine the impacts of grazing, succession, fire, and exotic species on population viability, are high priorities for research on *B. multifidum* in Region 2. Finally, so that conservation efforts on the behalf of this species can be most effective, research is needed to investigate the belowground life history, ecology, and reproductive biology, as well as the roles of mycorrhizae and disturbance in the autecology of *B. multifidum*. Restoration of populations of members of *Botrychium* subgenus *Sceptridium* is probably precluded by difficulty in propagation.

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INTRODUCTION

This assessment is one of many being produced to support the Species Conservation Project for the Rocky Mountain Region (Region 2), USDA Forest Service (USFS). *Botrychium multifidum* is the focus of an assessment because it is designated a sensitive species (USDA Forest Service 2003) and has a high degree of rarity and imperilment in Region 2. *Botrychium* species have been the focus of increasing interest by the USFS and other federal and state agencies due to their apparent rarity, difficulty of detection, and highly variable populations (Johnson-Groh and Farrar 2003). This assessment addresses the biology of *B. multifidum* throughout its range in Region 2. This introduction defines the goal of the assessment, outlines its scope, and describes the process used in its production.

Goal of Assessment

Species assessments produced as part of the Species Conservation Project are designed to provide forest managers, research biologists, and the public with a thorough discussion of the biology, ecology, conservation status, and management 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 broad implications of that knowledge, and outlines of information needs. The assessment does not seek to develop specific management recommendations. Rather, it provides the ecological background upon which management must be based and focuses on the consequences of changes in the environment that result from management (i.e., management implications). Furthermore, it cites management recommendations proposed elsewhere and examines the success of those recommendations that have been implemented.

Scope of Assessment

This assessment examines the biology, ecology, conservation status, and management of *Botrychium multifidum* with specific reference to the geographic and ecological characteristics of Region 2. While some of the literature on this species originates from field investigations outside Region 2, this document places that literature in the ecological and social context of the central Rocky Mountains. Similarly, this assessment is concerned with the reproductive behavior, population dynamics, and other characteristics of *B. multifidum* 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 and other investigators were reviewed. Because there have been no studies of most facets of *Botrychium multifidum* biology, literature on its congeners was used to make inferences in many cases. The refereed and non-refereed literature on the genus *Botrychium* and its included species is more extensive and includes other endemic or rare species. All known publications on *B. multifidum* are referenced in this assessment, and many of the experts on this species were consulted during its synthesis. All available specimens of *B. multifidum* in Region 2 were viewed to verify occurrences and incorporate specimen label data. Specimens were searched for at COLO (University of Colorado Herbarium), CS (CSU Herbarium), RM (Rocky Mountain Herbarium), KHD (Kalmbach Herbarium, Denver Botanic Gardens), SJNM (San Juan College Herbarium), CC (Carter Herbarium), GREE (University of Northern Colorado Herbarium), NMCR (New Mexico State University Range Science Herbarium), and UNM (University of New Mexico Herbarium). The assessment emphasizes refereed literature because this is the accepted standard in science. Non-refereed publications or reports were used in the assessment because there is very little refereed literature that specifically treats *B. multifidum* in Region 2, but these were regarded with greater skepticism. Unpublished data (e.g., Natural Heritage Program records, reports to state and federal agencies, specimen labels) were important in establishing the geographic distribution of this species. These data required special attention because of the diversity of persons and methods used in their collection.

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 experiments that produce clean results in the ecological sciences. Often, observations, inference, good thinking, and models must be relied on to guide our understanding of ecological relations. Confronting uncertainty, then, is not prescriptive. In this assessment, the strength of evidence for particular ideas is noted, and alternative explanations are described when appropriate.

Treatment of This Document as a Web Publication

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

Peer Review of This Document

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

Management Status and Natural History

Management Status

Botrychium multifidum is included on the Region 2 sensitive species list (USDA Forest Service 2003). The USFS designates a species as sensitive when it meets one or more of the following criteria:

1) the species is declining in numbers or occurrences, and evidence indicates that it could be proposed for federal listing as threatened or endangered under the Endangered Species Act if action is not taken to reverse or stop the downward trend

2) the species' habitat is declining, and continued loss could result in population declines that lead to federal listing as threatened or endangered under the Endangered Species Act if action is not taken to reverse or stop the decline

3) the species' population or habitat is stable but limited (USDA Forest Service 2003).

Because it is designated sensitive in Region 2, the Regional Forester must give consideration to this species so as to maintain its habitat and populations (see Forest Service Manual 2670). Potential effects on sensitive species must be addressed in all environmental assessments that include suitable habitat. The USFS can modify allotment management plans and projects or contracts to give consideration to *Botrychium multifidum* on a discretionary basis. Biological assessments and evaluations are conducted for *B. multifidum* when applications for permits for various land uses are considered, and impacts can be mitigated. The collection of sensitive species is prohibited without a permit (see Forest Service Manual 2670).

Botrychium multifidum is not considered sensitive by the Bureau of Land Management (BLM). *Botrychium multifidum* is not listed as threatened or endangered in accordance with the Endangered Species Act (16 USC 1531-1036, 1538-1540). It is not listed as endangered or vulnerable by the International Union for Conservation of Nature and Natural Resources (1978). NatureServe considers *B. multifidum* to be globally secure (G5) (NatureServe 2005).

While *Botrychium multifidum* is a widely distributed, circumboreal species, there are concerns for its viability in some portions of its range. It is a target for The Nature Conservancy in their conservation plan for the Southern Rocky Mountain Ecoregion (Neely et al. 2001). It is listed on several state endangered or threatened species lists, and is considered extirpated in three states. For detailed information regarding its range wide status please see the Distribution and Abundance section of this assessment.

In Region 2, *Botrychium multifidum* is ranked by the state natural heritage programs as imperiled (S2) in Wyoming and critically imperiled (S1) in Colorado and South Dakota. For explanations of NatureServe's ranking system, see the Definitions section of this document. A reported occurrence from Nebraska is highly questionable and is excluded from the state's species list (Rolfsmeier et al. 2001, NatureServe 2005). It is not known from Kansas, but the uncertainty of the location in Nebraska leaves the possibility that it was actually collected in Kansas.

Botrychium multifidum is known from 20 locations within Region 2, five of which have not been revisited in more than 20 years and four of which have not been revisited in more than 50 years. Eighteen of the known occurrences can be found on lands managed by the San Juan, Roosevelt, Medicine Bow, Bighorn, and Black Hills national forests in Colorado, South Dakota, and Wyoming. Four occurrences of *B. multifidum* are known from designated wilderness areas on the national forests of Region 2. Three occurrences are found within the Black Elk Wilderness in South Dakota (Black Hills National Forest), and another is known from the South San Juan Wilderness (San Juan National Forest) in Colorado. The use of mechanized or motorized equipment, including mountain bikes, is prohibited in wilderness areas. However, a broad range of other activities, including hiking, horseback riding, camping, hunting, fishing and grazing, is permitted. Although wilderness area designations do not explicitly protect *B. multifidum*, occurrences in wilderness areas are likely to be more protected than occurrences on lands where more uses are allowed. There is no grazing currently occurring within the Black Elk Wilderness on the Black Hills National Forest (Burkhart personal communication 2005). One occurrence is located on the Norbeck Wildlife Preserve, which was established in 1920 to protect game animals and birds and provide breeding habitat for them (US Code, Title 16, Chapter 6, Section 675). Hunting, trapping, and killing game animals and birds is not permitted within the preserve, but timber harvest and mining are still permitted. There are no use restrictions within the Preserve that offer protection to *B. multifidum* beyond those that apply to all National Forest System lands in Region 2.

Existing Regulatory Mechanisms, Management Plans, and Conservation Strategies

Adequacy of current laws and regulations

No state or federal laws explicitly disallow the destruction of habitat or individuals of *Botrychium multifidum* in Region 2. The species has no legal protection that would prevent the destruction of habitat or individuals on non-federal land in Region 2, or on federal land not managed by the USFS. Most of the known occurrences in Region 2 are found on National Forest System land, where the species is likely to benefit from its sensitive status. *Botrychium multifidum* is searched for and documented during pre-project surveys and is addressed in Biological Evaluations.

Adequacy of current enforcement of laws and regulations

There are no known cases in which an occurrence of *Botrychium multifidum* was extirpated due to human activities or by the failure to enforce any existing regulations in Region 2. It is not known if federal, state, or other laws could have prevented the extirpation of *B. multifidum* in Indiana, Maryland, and North Carolina. Thus it cannot be determined if current regulations or their enforcement are adequate for its protection.

Biology and Ecology

Classification and description

Botrychium multifidum is a member of the adder's tongue family (Ophioglossaceae), which is eusporangiate and shares a suite of characters that are less derived (more primitive) than those of most other pteridophytes (Gifford and Foster 1989). In North America, the Ophioglossaceae is composed of three genera as circumscribed by Wagner and Wagner (1993): *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 multifidum* belongs to subgenus *Sceptridium* (the evergreen grapeferns), which includes 13 species, six of which are known from North America (Clausen 1938, Wagner 1960a, Wagner and Wagner 1993). The classification of taxa in this subgenus is controversial due to many subtle and overlapping characters (Wagner 1960a). In a recent investigation of the molecular phylogenetics of the Ophioglossaceae, subgenus *Sceptridium* was found to be monophyletic (Hauk et al. 2003). However, there is little dispute of the taxonomic validity of *B. multifidum* (Wagner 1960a). Please see **Table 1** for the classification hierarchy of *B. multifidum*.

There are inconsistencies among published sources regarding the authorship of *Botrychium multifidum*. According to Wagner and Wagner (1993), the taxon was first described in 1768 by Samuel Gottlieb Gmelin (ca. 1745-1774) as *Osmunda multifida* (Gmelin

Table 1. Classification of *Botrychium multifidum* after USDA Natural Resources Conservation Service (2002), with sources (not necessarily the original source) of particular portions cited below.

Kingdom	Plantae (Plants)
Subkingdom	Tracheobionta (Vascular Plants)A
Division	Pteridophyta (Ferns)
Class	Filicopsida
Order	Ophioglossales
Family	Ophioglossaceae (Adder's Tongue Family)
Genus	*Botrychium (*Grapeferns)
Subgenus	*Sceptridium* (Evergreen Grapeferns)[1]
Section	*Multifidae*[2]
Species	*Botrychium multifidum* (Gmel.) Rupr.[1]

[1]Wagner and Wagner 1993

[2]Clausen 1938

1768). However, the International Plant Names Index (The Plant Names Project 1999) ascribes authorship of this species to Johann Friedrich Gmelin (1748-1804). Credit for the name *B. multifidum* is ascribed to three different authors. Wagner and Wagner (1993) credit this name to Franz Josef Ivanovich Ruprecht (Ruprecht 1859); this is the most commonly recognized authorship of *B. multifidum*. However, Kartesz (1999) and USDA Natural Resources Conservation Service (2002) cite "Trev." as the authority for *B. multifidum*. According to Mabberley (1997), this refers to Ludolf Christian Treviranus (1799-1864). To further muddy the waters, Great Plains Flora Association (1986) and Hartman and Nelson (2001) cite "Trevis." as the authority, referring to Vittore Benedetto Antonio Trevisan de Saint-Leon (1818-1897) according to Mabberley (1997). Dorn and Dorn (1972) attribute authorship to "Trevisan," suggesting that the latter name above is intended. The designation of authorship to Ruprecht given by Wagner and Wagner (1993) should be considered the standard citation, as it was selected by the world's experts on the Ophioglossaceae and approved by the Flora of North America Editorial Committee. However, the authors appended to the name *B. multifidum* may vary in other treatments due to lack of clarity and perhaps still-to-be-resolved correct application of authorship.

Botrychium multifidum was first collected near Region 2 by John M. Coulter in 1872 at "Lower Fire Hole Basin, Yellowstone National Park" (New York Botanical Garden 2003). It was collected again in Yellowstone in 1897 by Per Axel Rydberg (*#3500*) and E.A. Bessey. This specimen became the type specimen for *B. coulteri*, described by Lucien M. Underwood in 1898 and named after its original collector (Underwood 1898). *Botrychium coulteri* was later included as a subspecies under *B. multifidum* by R.T. Clausen in his monograph of the Ophioglossaceae (Clausen 1938). Most contemporary treatments no longer recognize subspecies within *B. multifidum*. *Botrychium multifidum* ssp. *coulteri* is included in Beidleman et al. (2000) and Weber and Wittmann (2000), but Weber and Wittmann (2001a and 2001b) recognize Colorado plants only at the species level.

There are a great many synonyms for *Botrychium multifidum* in the literature (**Table 2**). Because there is considerable regional variation in the stature, dentation, and leaf shape of *B. multifidum*, many taxa have been described over the years to describe these variants. For example, in California *B. multifidum* is often very large, with broad leaf blades (20 to 35 cm across). This led Underwood (1905) to describe these plants as a new species, *B. californicum*. The type specimen of *B. californicum* (R.M. Austin, *s.n.*, NY) nearly fills a herbarium sheet (an image of this specimen can be viewed at New York Botanical Garden 2003). Clausen (1938) promulgated numerous subspecies under *B. multifidum*. Clausen (1938) noted that *B. californicum* intergrades with both *B. coulteri* and *B. silaifolium*, and he included all of these taxa as infraspecies under *B. multifidum*. In describing *B. multifidum* forma *dentatum*, Tryon (1939) wrote of the distinctness of plants in open and shaded places, asserting that this provided strong evidence of their genetic distinctness. However, Wagner (1962) determined that *B. multifidum* forma dentatum is actually a dwarfed form of *B. ternatum*. While some regional variants are quite morphologically distinctive (including *B. multifidum* ssp. *coulteri*), the degree of intergradation of the diagnostic characters that define the infraspecies of *B. multifidum* is great enough that none of them warrant taxonomic recognition. Wagner (1962) cited much observational evidence that the dwarfed forms of *B. multifidum* do not warrant treatment

Table 2. A partial list of synonyms for *Botrychium multifidum*. In bold are the most commonly seen names applied to this taxon.

Source	Taxon
1	*Botrychium californicum* Underwood
1	*Botrychium coulteri* Underwood
1	*Botrychium matricariae* (Schrank) Spreng.
1,3	***Botrychium multifidum* (Gmel.) Trev.**
2	***Botrychium multifidum* (J.F. Gmelin) Rupr.**
2	*Botrychium multifidum* forma *dentatum* R.M. Tryon
1,2,3	*Botrychium multifidum* ssp. *californicum* (Underw.) R.T. Clausen
1,2,3	*Botrychium multifidum* ssp. *coulteri* (Underw.) R.T. Clausen
2	*Botrychium multifidum* ssp. *robustum* (Rupr.) R.T. Clausen
1,2,3	*Botrychium multifidum* ssp. *silaifolium* (C. Presl) R.T. Clausen
2	*Botrychium multifidum* ssp. *typicum* R.T. Clausen
2	*Botrychium multifidum* var. *australe* Farw.
1,2,3	*Botrychium multifidum* var. *californicum* (Underw.) M. Broun
1,2,3	*Botrychium multifidum* var. *coulteri* (Underw.) M. Broun
2	*Botrychium multifidum* var. *dichotomum* Farw.
2	*Botrychium multifidum* var. *habereri* (Gilbert) Farw.
1,2,3	*Botrychium multifidum* var. *intermedium* (D.C. Eaton) Farw.
2	*Botrychium multifidum* var. *intermedium* Farw.
2	*Botrychium multifidum* var. *oneidense* Farw.A
2	*Botrychium multifidum* var. *robustum* C. Chr.
1,2,3	*Botrychium multifidum* var. *silaifolium* (C. Presl) M. Broun
2	*Botrychium multifidum* var. *simplicius* Farw.
2	*Botrychium multifidum* var. *typicum* Wherry
1	*Botrychium silaifolium* C. Presl
1	*Botrychium silaifolium* var. *coulteri* (Underwood) Jepson
2	*Sceptridium multifidum* (Gmel) M. Nishida
2	*Sceptridium multifidum* (J.F. Gmelin) M. Nishida

[1]Kartesz (1999)

[2]International Plant Names Index

[3]USDA Natural Resources Conservation Service 2003

as infrataxa since the differences observed appear to be the result of environmental variability. Wagner (1960b) commented that Clausen relied too heavily on characteristics that are readily modified by the environment in his circumscription of many subspecies and varieties. Stevenson (1975) notes that the leaves of a single, copiously branched, large plant in nature can fit the descriptions of two or more subspecies, suggesting that the recognition of several subspecies of *B. multifidum* is unnecessary. Wagner and Wagner (1993), Kartesz (1999), The Plant Names Project (1999), and USDA Natural Resources Conservation Service (2002) recognize all of the infraspecific taxa as synonyms of *B. multifidum*, and this treatment is used in most sources for Region 2.

Lyon (1905) placed the evergreen grapeferns in their own genus (*Sceptridium*), and used this genus for the taxa described by Underwood (1898, 1905) and by others (these are not all included in **Table 2**). *Sceptridium* as a genus is in current use by some (e.g., Watano and Sahashi 1992, Barker and Hauk 2003, Hauk et al. 2003). Clausen (1938) was the first to use *Sceptridium* as a subgenus for the evergreen grapeferns, and this is the most widely accepted usage.

Description

Only two extant genera of vascular cryptogams, *Isoëtes* and *Botrychium*, are capable of secondary growth, or wood production, although secondary growth was common in many extinct vascular cryptogams (Esau 1965, Gifford and Foster 1989). The stems of *B. multifidum* are often quite woody.

Wagner and Wagner (1993) recognize six species of *Botrychium* in subgenus *Sceptridium* in North America. Species of *Sceptridium* produce one leaf per year, which is divided into a sterile trophophore and a fertile sporophore (Clausen 1938). Unlike members of subgenus *Botrychium*, members of subgenus *Sceptridium*, including *B. multifidum*, are often observed without a sporophore (Wagner 1946, Wagner 1962).

Relative to most other members of the genus, *Botrychium multifidum* is a large plant and the largest member of subgenus *Sceptridium* (Rook 2002). The roots are few but thick (2 to 4 mm in diameter) and coarse, and lack root hairs. Stipes are 1.5 to 6 cm long, and the sporophore (if present) is 8 to 40 cm long (Lellinger 1985). The trophophore is ternate, green or possibly reddish or copper-tinged, broadly triangular, deltate or deltate-lanceolate, three to four pinnate, 6 to 30 cm long, and 3.5 to 20 cm wide (Clausen 1938, Wagner 1960a, Lellinger 1985, Rook 2002). Pinnae and pinnules are quite variable, with entire, acute, obtuse, or rarely round apices, and entire to crenate margins (Lellinger 1985). As its common name (leathery grapefern) implies, the leaves of *B. multifidum* tend to be leathery. The previous year's leaf remains green through the winter until a new leaf appears in the spring (Wagner and Wagner 1993). The previous year's leaf often appears yellowed and shriveled by spring but may persist through much of the following summer (Wagner 1960a, Dorn and Dorn 1972). Plants described as *B. multifidum* ssp. *coulteri* are rather distinctive, typically very stout, fleshy, and compact (Clausen 1938). They are 15 to 23 cm high, with small and crowded ultimate leaf divisions (**Figure 1**, **Figure 2**, and **Figure 3**). However, tiny shade forms of *B. multifidum* that are difficult to detect (**Figure 4**) are also found in Region 2. The sporophytes of *B. multifidum* are diploid (2n = 90) (Wagner and Wagner 1993). Please see the Reproductive Biology and Autecology section of this assessment for information on the life history and reproduction of *Botrychium* species.

The gametophytes of *Botrychium* species remain poorly understood. They are achlorophyllous and wholly dependent on mycorrhizal fungi for obtaining water, mineral nutrients, and carbohydrates (Campbell 1922, Bower 1926, Scagel et al. 1966, Gifford and Foster 1989, Schmid and Oberwinkler 1994). The gametophytes of other *Botrychium* species have been cultured and studied (Campbell 1911, Whittier 1973, Whittier 1981, Melan and Whittier 1989, Thomas and Whittier 1993), but unlike other members of the genus, such as *B. campestre* (Johnson-Groh et al. 2002), the gametophytes of *B. multifidum* have proven extremely difficult to observe in nature (Stevenson 1975, Gifford and Brandon 1978). In lieu of observing material from the wild, Gifford and Brandon (1978) grew them in axenic culture for study. To grow them to a suitable

Figure 1. *Botrychium multifidum* at Bear Park, Medicine Bow National Forest, Jackson County, Colorado. Photograph courtesy of John Proctor.

Figure 2. A sporulating individual at Big Creek Park, Carbon County, Wyoming. Photograph provided by John Proctor.

Figure 3. A reproductive individual at East Fork Campground, Bighorn National Forest, Carbon County, Wyoming. *Polytrichum* (with microphylls that look like tiny juniper leaves) can be seen below and right of the trophophore. Photograph provided by Greg Karow.

Figure 4. Shade form of *Botrychium multifidum* at Palmer Gulch (BOMU 3), Black Hills National Forest, Pennington County, South Dakota. Photograph by Katherine Zacharkevics, provided by Reed Crook.

size for analysis required nine months in a dark growth chamber. Gametophytes were first observed in nature by Daigobo (1979) and were found to be very similar to those grown in culture (Daigobo 1979, Gifford and Foster 1989). Like all other *Botrychium* species studied, the gametophyte of *B. multifidum* has a dorsal ridge on which the gamete-producing structures are borne. The gametophyte is up to 5 mm long and is usually obovoid or club-shaped. The rhizoids (root-like structures) form on the lower surface. Gifford and Brandon (1978) observed antheridia (the male gamete-producing structures) in the cultured gametophytes but no archegonia (female gamete-producing structures). Daigobo (1979) observed antheridia at the top of the dorsal (antheridial) ridge and archegonia on either side of the dorsal ridge. The gametophytes of *B. multifidum* are monoecious (with both male and female gamete producing structures) and protandrous (produces male structures before producing female structures). Please see the Demography section of this document for the implications of the lack of information regarding gametophytes and for the implications of a possible lack of archegonia.

It is not uncommon to find more than one species in subgenus *Sceptridium* at a single site (Wagner 1960b), but no other members of this subgenus occur in Region 2. *Botrychium multifidum* could occur with the smaller, gemma-producing *B. campestre*, but such a co-occurrence has never been documented in Region 2 and is improbable (Wagner and Wagner 1993). In the northeastern United States and southeastern Canada, *B. multifidum* is often found with *B. ternatum* and *B. dissectum* (also members of subgenus *Sceptridium*) in the northeastern United States and southeastern Canada (Wagner 1961, Wagner and Rawlings 1962, Wagner and Wagner 1983, Wagner and Wagner 1993). *Botrychium multifidum* is rather similar to *B. robustum*, but this species is known from Japan, eastern China, the former Soviet republics, and Unalaska Island, Alaska (Wagner and Wagner 1993). *Botrychium multifidum* can also be difficult to distinguish from *B. rugulosum* (Johnson-Groh personal communication 2003). There are reports from Yellowstone National Park of co-occurrence of *B. multifidum* with *B. simplex* and another *Botrychium* species, probably *B. lanceolatum* (Whipple personal communication 2003). *Botrychium multifidum* is smaller, has a coarse leathery texture, and has a persistent evergreen trophophore, which distinguish it from the otherwise-similar and sympatric *B. virginianum*.

Sources for keys, photographs, illustrations, and descriptions

There are numerous sources of keys, photographs, illustrations, and descriptions that are very valuable in identifying *Botrychium multifidum*. The most useful keys for identifying *B. multifidum* in the field in Region 2 include Weber and Wittmann (2001a and 2001b, for Colorado), Dorn (2001, for Wyoming), and Dorn and Dorn (1977, for the Black Hills of South Dakota). Lellinger (1985) includes a good description, key, and photograph. A photograph of a sporophyte and a small illustration are available in Pojar and MacKinnon (1994). Many photographs are available on the Internet (e.g., Wisconsin State Herbarium [2003]). See **Figure 1**, **Figure 2**, **Figure 3**, **Figure 4** and the cover of this assessment for photographs of *B. multifidum* as it appears in Region 2. See also Rook (2002) for photographs, habitat information, and links to other sources. Daigobo (1979) includes photographs, illustrations, and a description of the gametophyte of *B. multifidum*. Gifford and Brandon (1978) and Gifford and Foster (1989) include photographs of the gametophyte of *B. multifidum* grown in axenic culture.

Wagner and Wagner (1993) include a description and illustration of *Botrychium multifidum*. Hitchcock et al. (1969) include the best illustration of *B. multifidum*, which is also included in Lackschewitz (1991). Another excellent illustration is found in Dorn and Dorn (1972). Cody (1988) also includes a good illustration. Cobb (1956) includes a good illustration, but it depicts a large individual more typical of those found in the eastern United States. A description and illustration (under *B. silaifolium*) are included in Britton and Brown (1913). Because it is in the public domain, this illustration is available online from USDA Natural Resources Conservation (2002) and is in this assessment (**Figure 5**).

Because of its wide distribution, many floras and field guides offer descriptions of *Botrychium multifidum* (e.g., Harrington 1954, Kearney and Peebles 1960, Gleason and Cronquist 1963, Tutin et al. 1964, Munz and Keck 1968, Huxley 1972, Great Plains Flora Association 1986, Lackschewitz 1991, Wagner and Wagner 1993, Kershaw et al. 1998, and Beidleman et al. 2000). Full technical descriptions for *B. multifidum* ssp. *coulteri* are available in Underwood (1898) and Clausen (1938) and are quite useful. Coulter and Nelson (1909) also include a briefer description of subspecies *coulteri*. Other technical descriptions for the full species and other infrataxa can be found in Clausen (1938), which is also useful for looking up older literature on *B. multifidum*.

The type specimen of *Botrychium multifidum* ssp. *coulteri* (collected by P. A. Rydberg, *#3500*, with E. A. Bessey on August 7, 1897) is housed at the New York Botanical Garden Herbarium. Images of this specimen and three others (including the first collection of this subspecies by Coulter in 1872) can be viewed on its website (New York Botanical Garden 2003).

There have been many studies of members of subgenus *Sceptridium*, including chemotaxonomic (Tanaka et al. 1986), phylogenetic (Stevenson 1980, Hauk et al. 2003), and anatomical (Chau 1986, Watkins and Dute 1998) perspectives. Stevenson (1976) includes very technical and detailed anatomical descriptions of the shoot apex and vascular system of *Botrychium multifidum*. It has been studied very closely as a representative species of the Ophioglossaceae in detailed morphological and phylogenetic studies. Another study has investigated novel calcium crystals found in the vascular tissue of *B. multifidum*, and includes scanning electron micrograph photos of the crystals (Morrow and Dute 2002).

Distribution and abundance

Members of subgenus *Sceptridium* are distributed worldwide in temperate and north temperate habitats (Wagner and Wagner 1983). In North America, the area of highest diversity for this subgenus is east of the Mississippi River from the southern Gulf Coast to the northern coasts of the Great Lakes (Wagner and Wagner 1983, Barker and Hauk 2003). *Botrychium multifidum* is the only member of subgenus *Sceptridium* found in Region 2.

In some parts of its range, *Botrychium multifidum* is common relative to other members of the genus *Botrychium* (Johnson-Groh personal communication 2003), thus its G5 rank (globally demonstrably secure). However, it is locally rare in many parts of its range, including Region 2. Where it is found in Region 2, it is never abundant, and all of the known occurrences for which counts are available are small. Although it is known from numerous occurrences in Wyoming (almost all of which are in Yellowstone and Grand Teton national parks), it is uncommon and usually present in low numbers when it is found (Dorn and Dorn 1972, Whipple personal communication 2003).

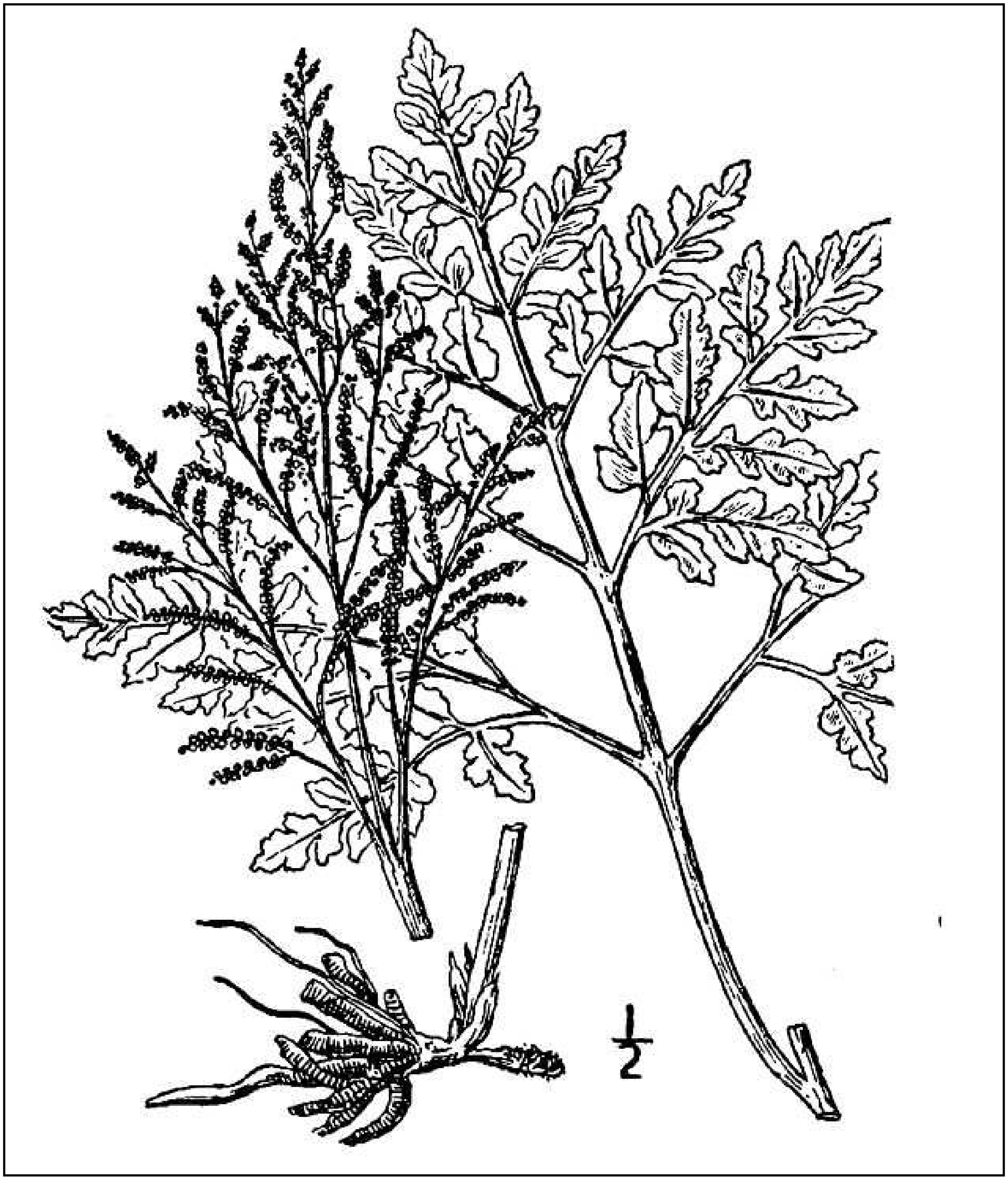

Figure 5. Illustration of *Botrychium multifidum*.

Moonwort (subgenus *Botrychium*) population sizes vary considerably, from fewer than ten to thousands of sporophytes (Johnson-Groh and Farrar 2003), and this appears to be true for *B. multifidum* (subgenus *Sceptridium*) as well. Because of difficulties in accounting for all reproductive plants in a population, and because much of the population at any given time resides under ground as gametophytes or possibly as dormant sporophytes, any count of plants at a given location must be thought of as an underestimate of the true population size (Johnson-Groh and Farrar 2003).

Botrychium multifidum is a circumboreal species (Clausen 1938, Wagner 1960a, Ollgaard 1971). It has been documented in Europe from France to Romania and Czechoslovakia, reaching its northern limit in the subarctic of Sweden and Finland (Tutin et al. 1964, Ollgaard 1971, Somsak 1978). It is also known from central Russia, southern Siberia, Yunnan, and the Himalayas. In the western hemisphere it is known from Greenland and across the Canadian arctic and subarctic to Alaska, south to North Carolina, Colorado, Arizona, and California. It is reported from 12 provinces in Canada and 36 states. However, it has apparently been extirpated in three states (Maryland, North Carolina, and Indiana; Kartesz 1999). *Botrychium multifidum* has an erratic distribution in western North America, even where it is more common (Zika personal communication 2003). **Figure 6** shows the global range of several subspecies of *B. multifidum*. See **Table 3** for a list of states and provinces where *B. multifidum* is found, including subnational conservation status ranks and state designations.

Within the administrative boundary of Region 2, *Botrychium multifidum* is known from 20 occurrences, 18 of which occur within national forests (**Figure 7**). Numerous additional occurrences are known from Yellowstone and Grand Teton national parks just outside Region 2. Information on these occurrences is included in this assessment in order to contribute to a better understanding of *B. multifidum* within the region. Please see **Table 4** for summary information for documented locations in and adjacent to Region 2. Details on the distribution of *B. multifidum* in Region 2 are discussed below.

Colorado

In Colorado, *Botrychium multifidum* is known from eight locations in Larimer, Routt, Jackson, San Miguel, and Conejos counties. However, plants have not been seen at three of these locations in more than 50 years; they are known only from herbarium specimens. The occurrence on San Juan National Forest in San Miguel County included three individuals in 1999, and because of the small number of individuals present, a specimen was not collected. This occurrence is somewhat questionable because it is in atypical habitat; it may actually be *B. lanceolatum*. One location in Colorado

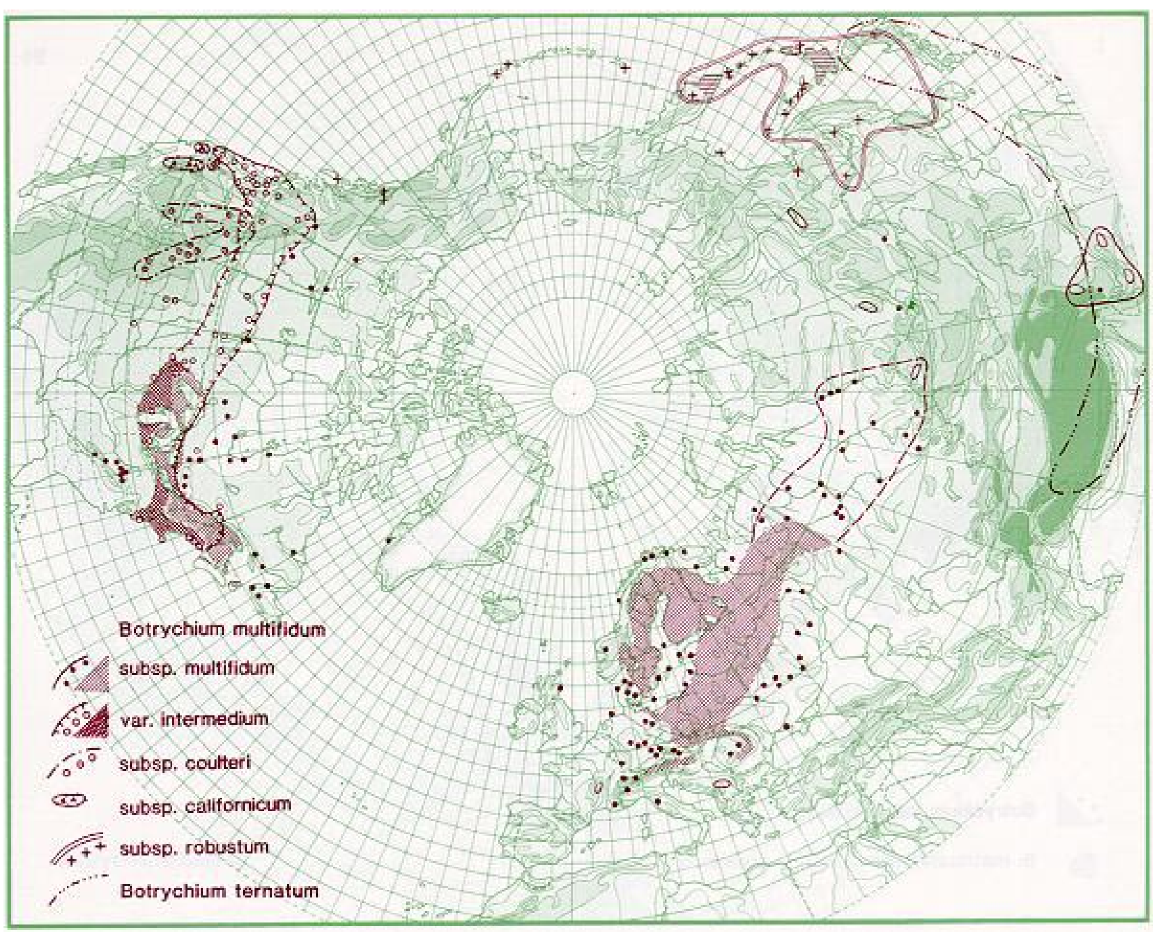

Figure 6. Global distribution map of *Botrychium multifidum* showing the distribution of several subspecies and varieties (Anderberg 2003).

Table 3. Known distribution and subnational conservation status ranks of *Botrychium multifidum* in the western hemisphere (from Ollgaard 1971, Kartesz 1999, USDA Natural Resources Conservation Service 2002, South Dakota Natural Heritage Program 2003, and NatureServe 2005). USDA Forest Service Region 2 states are in bold. Please see the Definitions section of this assessment for explanation of the conservation status ranks.

Nation	State/Province/District	S rank	State Designation	Status
Canada	Alberta	S2		
Canada	British Columbia	S4		
Canada	Labrador (Newfoundland)	S1		
Canada	Manitoba	S3		
Canada	New Brunswick	S4		
Canada	Newfoundland Island (Newfoundland)	S2S3		
Canada	Northwest Territories	SR		
Canada	Nova Scotia	S4		
Canada	Ontario	S5		
Canada	Prince Edward Island	S3		
Canada	Quebec	SR		
Canada	Saskatchewan	S3		
Canada	Yukon Territory	SR		
Greenland	Julianehaab	None		
USA	Alaska	SR		
USA	Arizona	S2		
USA	California	SR		
USA	**Colorado**	**S1**		
USA	Connecticut	SR		
USA	Delaware	SR		
USA	Idaho	SR		
USA	Illinois	S1	Endangered	
USA	Indiana	S?		Extirpated
USA	Iowa	S2	Threatened	
USA	Maine	SR		
USA	Maryland	SH	Endangered	Extirpated
USA	Massachusetts	SR		
USA	Michigan	S?		
USA	Minnesota	SR		
USA	Montana	SR		
USA	Nevada	SR		
USA	New Hampshire	SR		
USA	New Jersey	S1	Endangered	
USA	New Mexico	SR		
USA	New York	SR	Exploitably Vulnerable	
USA	North Carolina	SH		Extirpated
USA	North Dakota	S1		
USA	Ohio	S1	Threatened	
USA	Oregon	S1		
USA	Pennsylvania	S?		

Table 3 (concluded).

Nation	State/Province/District	S rank	State Designation	Status
	Rhode Island	S?		
	South Dakota	**S1**		
	Utah	S1		
	Vermont	S3		
	Virginia	S1		
	Washington	SR		
	West Virginia	S?		
	Wisconsin	SR		
	Wyoming	**S2**		

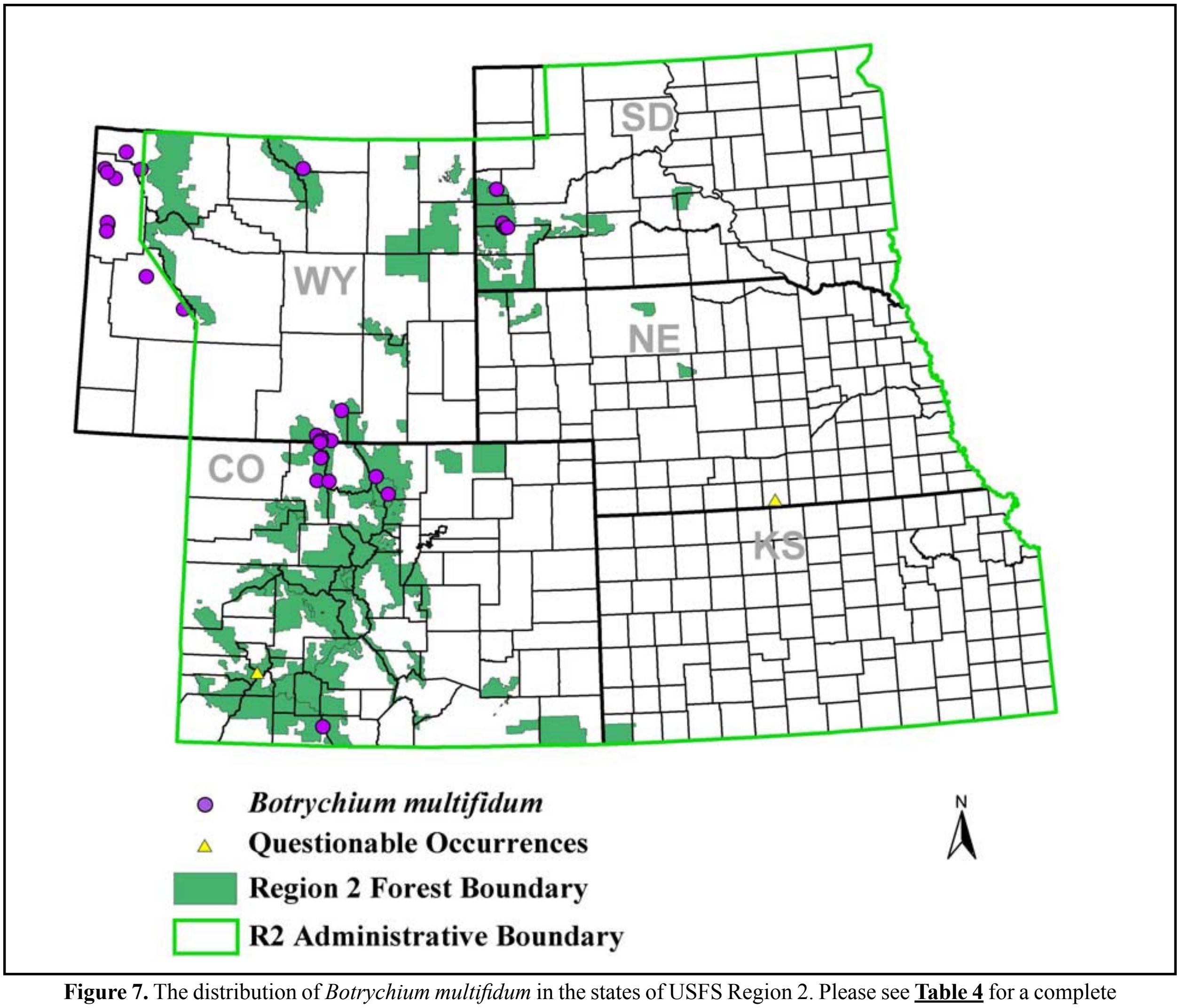

Figure 7. The distribution of *Botrychium multifidum* in the states of USFS Region 2. Please see **Table 4** for a complete summary of all occurrences in the states of USFS Region 2.

was discovered in 2003 at Bear Park on Medicine Bow National Forest (Proctor personal communication 2004); it was the first known occurrence of this species from Jackson County. A second Jackson County occurrence was observed in Ryan Park on Routt National Forest in 2005 (Proctor personal communication 2005). An occurrence in Rocky Mountain National Park at Cabin Lake was discovered in 1989 and has been periodically observed since then by Peter Root and others (Root personal communication 2003). The only other recent observation of this species in Colorado occurred in 2001 in South San Juan Wilderness in Conejos County.

Table 4. Summary information for the known reports of *Botrychium multifidum* in USDA Forest Service Region 2. Source ID is Colorado Natural Heritage Program, South Dakota Natural Heritage Program, and Wyoming Natural Diversity Database element occurrence number unless otherwise noted. Lands within the Region 2 are in bold. Shaded records represent occurrences in Wyoming that fall outside the administrative boundary of Region 2.

Source ID	County/ State	Location	Owner	Last obs	Abundance	Elevation (ft.)	Habitat and Notes
Steve O'Kane, Arnold Clifford, and Dave Jamieson (*#5871* at SJNM)	Conejos, CO	Between Fish Lake and Blue Lake	**USDA Forest Service (USFS; San Juan National Forest, South San Juan Wilderness)**	8/8/2001	Unknown	11,512	Rolling tundra with tree islands of *Picea* with many ponds. Bare spots in grassy matrix on S-facing slope. With *Botrychium simplex*.
Proctor personal communication 2004	Jackson, CO	Bear Park	**USFS (Medicine Bow National Forest)**	8/18/2003	~25	8,885	Margin of an open water pond near peaty area and beaver dams.
Proctor personal communication 2005	Jackson, CO	Ryan Park	**USFS (Routt National Forest)**	10/18/2005	1	8,600	Plant is located on a drier microsite on a raised hump in the meadow beside the creek in full sun. No tree cover; dominated by *Carex* spp. and *Deschampsia caespitosa* in a meadow. More are probably present but cannot be found easily due to overstory vegetation. Plant is vegetative.
CO EO#1	Larimer, CO	Cabin Lake	National Park Service (Rocky Mountain)	9/25/2003	~6	8,710	Revegetating lakebed and *Pinus contorta* forest; more than 1000 individuals have been documented here in the past, although only 6 in evidence in 2003. Area appears to be gradually drying, as indicated by a test well and by vegetation change since 1989. *Cirsium arvense* occupies approximately 30 percent of the lakebed. Construction of a stable facility and extensive horse trails was completed in 2003 approximately 100 m west of this occurrence.
CO EO#4	Larimer, CO	Trap Lake	**USFS (Arapaho Roosevelt National Forest)**	1936	Unknown	9,060	South border of Trap Lakes of Cameron Pass.
CO EO#3	Routt, CO	Steamboat Springs	**USFS (Routt National Forest)**	1891	Unknown	~6,750	Not reported.
CO EO#2	Routt, CO	Elk River	**USFS (Routt National Forest)**	1951	Unknown	8,000	[near] beaver dams off Diamond Park Road.
CO EO#5	San Miguel, CO	Savage Basin	**USFS (San Juan National Forest)**	7/29/1999	3	11,000 to 11,200	Steep eroded bank above road on road cut of 4 wheel drive road; atypical habitat and questionable location; may be a misidentification of *Botrychium lanceolatum*.
Rolfsmeier et al. 2001	Franklin, NE	Near Franklin	Private (?)	1895	Unknown	~2000	Copse and meadows on Mr. Ewing's farm near Franklin. A highly questionable location that is not included in the text of this assessment.

Table 4 (cont.).

Source ID	County/ State	Location	Owner	Last obs	Abundance	Elevation (ft.)	Habitat and Notes
BOMU 1, BOMU 2, BOMU 6; SD EO# 1	Custer, SD	Lost Cabin Creek	**USFS (Black Hills National Forest, Black Elk Wilderness)**	9/8/2005	471	6,200 to 6,280	BOMU 1: In a wet meadow along the edges of spruce stands. The ground around the plants is moist with a thick mossy layer. Plants appeared unhealthy and dry. Most of the plants had a few brown spots on their leaves, and many had shriveled leaves. Other plants in the area appeared pale and shriveled also. 65 plants were seen at this location in 2003, when plants were not noted to appear unhealthy. In 2004, 35 plants were seen. In 2005, 383 individuals were seen, 13 of which were sporulating. Most are tiny (less than 1/2 inch) and in shade; most large sporulating individuals are in a sunny meadow. Density was as high as 25 plants per square foot in places. BOMU 2: nine plants (in 2004) in spruce/aspen community on granite parent material, in spruce dominated area. Plants are in a shallow grassy depression under spruce limbs. Not mossy. Mesic, but not as wet as some sites. Grassy with abundant needle litter. Plants in two subpopulations of one square meter each, near a trail. BOMU 6: Rich, mossy, spruce dominated bottom, with several species of moss, lichen, and abundant Athyrium filix-femina and Equisetum sylvaticum, abundant downed spruce. Bottom is wide and undulating with shallow depressions and old stream channels common. Plants occur near edges of depressions. SD EO# 1: In drainage bottom beneath spruce and aspen in canopy opening.
SD EO# 3, BOMU 7	Custer, SD	Upper Iron Creek	**USFS (Black Hills National Forest, Black Elk Wilderness)**	10/1/2004	12	5,520 to 5,600	Spruce dominated bottom with gently flowing stream, pine and birch scattered. Occurs in low somewhat grassy areas with thick underlying moss (usually *Climacium*). Soil is gravelly and rocky- of decomposed granite under thick moss layer. Plants occur near the creek (within 7 meters), sometimes in old stream channels.
SD EO# 4; BOMU 4, BOMU 5	Custer, SD	Iron Creek	**USFS (Black Hills National Forest, Black Elk Wilderness)**	9/4/2004	30	5,075 to 5,200	BOMU 4: Mostly open floodplain, dominated by grasses (*Agrostis stolonifera* and *Calamagrostis canadensis*), sedges, and forbs. Soil is sandy with abundant pebbles and small cobbles. *Botrychium multifidum* is found mostly with *Climacium* sp. *Cirsium arvense* is present in the occurrence. BOMU 5: Gravelly bar (dominated by graminoids) of decomposed granite with small depressions, with moss in lower areas. Site is fairly open. Adjacent forests are spruce-dominated.

Table 4 (cont.).

Source ID	County/ State	Location	Owner	Last obs	Abundance	Elevation (ft.)	Habitat and Notes
Corey personal communication 2003, Crook personal communication 2003	Lawrence, SD	Butcher Gulch	**USFS (Black Hills National Forest, Northern Hills Ranger District)**	July 1994	Unknown	5,000	With spruce and hazelnut near the creek, on limestone substrate. Soil is moist to saturated. Sought by Farrar and Crook and not found in 2003, or by Burkhart in 2005. Area burned in 2002. This area has been noted to be botanically diverse.
SD EO# 2, BOMU 3	Pennington, SD	Palmer Gulch	**USFS (Black Hills National Forest, Norbeck Wildlife Preserve)**	5/21/2004	8	6,300	SD EO#2: In rich woods along stream. BOMU 3: In a lush mossy drainage bottom with a gently flowing winding creek. Overstory is dominated by *Picea glauca* with *Betula papyrifera*, *Corylus cornuta*, and *Cornus sericea* in the shrub layer. All plants are found within four meters of the creek (mostly within one meter of the creek). All plants were growing in moss (probably *Climacium*). One individual sporulating in partial sun along an old stream channel; seven other nonreproductive plants in heavier shade. *Cirsium arvense* is present at this occurrence.
WY EO#1	Carbon, WY	Head of Encampment River	**USFS (Medicine Bow National Forest, Hayden Ranger District)**	10/10/1973	Unknown	10,000	Wet meadow.
WY EO#18	Carbon, WY	Headwaters of Soldier Creek	**USFS (Medicine Bow National Forest, Hayden Ranger District)**	9/30/2004	101 (37 sporulating)	9,280	Drier portions of a meadow on raised mounds that appear to be remnants of very old beaver dams, also on terrace above creek. 37 of 101 plants have sporangia. 10 individuals are grazed.
Proctor personal communication 2004	Carbon, WY	Brush Creek	**USFS (Medicine Bow National Forest, Hayden Ranger District)**	9/27/2004	6 (2 sporulating, 4 vegetative	9,340	Drier microsite in sedge-hairgrass meadow. Slope: 2 percent. Open site. All plants in a 4 ft. diameter area. Livestock and wildlife grazing, especially elk, is occurring.
Proctor personal communication 2005	Carbon, WY	Big Creek Park	Private	8/5/2005	~20	8,500	Floodplain/tall willow carr below a large turnout. Found near and upslope of stream banks in bottomlands in partial shade, downslope of carbonate slopes. Often observed with *Spiranthes romanzoffiana*. Some indication of the presence of peat (*Carex simulata* is present). Most plants are concentrated in groups but a few individuals are scattered. Livestock grazing is occurring; heavy livestock use has been observed in the past. This pasture was apparently rested this year. Within 100 meters of Medicine Bow NF boundary.
Proctor personal communication 2005	Carbon, WY	Beaver Dam Park	**USFS (Medicine Bow National Forest, Hayden Ranger District)**	10/18/2005	27	8,550	Drier microsite within meadow, dominated by low growing forbs in full sun. Slope flat. Soil moisture is seasonally moist. Size: One acre. Previously grazed by wildlife and livestock; grazing ended in early October 2005 due to construction of an exclosure to rest the area and allow for stream channel improvement (plants found afterward). About 30% of the individuals had at least part of leaf grazed off. One individual sporulating.

Table 4 (cont.).

Source ID	County/ State	Location	Owner	Last obs	Abundance	Elevation (ft.)	Habitat and Notes
Proctor personal communication 2005	Carbon, WY	East Fork Meadow	**USFS (Medicine Bow National Forest, Hayden Ranger District)**	10/18/2005	37	8,600	In a drier microsite within a *Carex* meadow. Dry area is dominated by low-growing forbs. In full sun and moist soil, occupying one acre. Land use includes livestock and wildlife grazing, gopher pockets, other rodents, fishing, hunting, and hiking. Approximately 5 percent of plants grazed but retaining part of their trophophore. Drier areas of the meadow receive heavier grazing than the surrounding wet *Carex* meadow. More suitable habitat is in the area that was not surveyed.
WY EO#12	Park, WY	Lewis Lake	NPS (Yellowstone)	early July 1978	several individuals	7,780	West shore in thermal area near large hot spring.
WY EO#13	Park, WY	North of Biscuit Basin	NPS (Yellowstone)	8/13/1992	1 (not relocated in subsequent years)	7,265	Wetland area.
WY EO#14	Park, WY	Midway Geyser Basin	NPS (Yellowstone)	8/25/1992	Unknown	7,250	South end of the thermal area near a trailhead.
WY EO#15	Park, WY	Lower Geyser Basin	NPS (Yellowstone)	6/24/1994	Unknown; “best site known in YNP”	7,390	Not reported.
WY EO#16	Park, WY	Obsidian Cliff Wetland Complex	NPS (Yellowstone)	Aug-03	at least 25	7,390	In wetland complex.
WY EO#17	Park, WY	Obsidian Creek	NPS (Yellowstone)	8/27/2003	at least 2	7,310	On the edge of the 1988 burn with a few *Picea engelmannii* that survived the fire.
WY EO#11	Park, WY	Heart Lake Geyser Basin	NPS (Yellowstone)	8/10/1979 (6/27/1995)	“a few”	7,460	Just off the trail in a dry hot spring area; in an area adjacent to ground that is too hot to support vascular plants; sparse cover, 10% slope, east aspect.
WY EO#19	Park, WY	1 mile southeast of Upper Falls	NPS (Yellowstone)	6/7/2001	Unknown	7,700	Wet meadow. Plants in vegetative condition.
WY EO#20	Sheridan, WY	East Fork Campground	**USFS (Bighorn National Forest, Tongue Ranger District)**	10/6/2004	60 vegetative, 40 sporulating	7,620	In an alluvial depression on the edge of a willow/graminoid meadow; plants located on an ever so slight rise above a wetter meadow. Associated vegetation is short in stature. In a cattle exclosure that was established in 1976; moose, deer, elk, and occasional domestic cow or calf forage within the exclosure. A prescribed burn was taking place during the discovery of this occurrence.
Walter Fertig (#7464 at RM)	Sublette, WY	Meeks Lake/ Iron Creek Meadows	Private (?)	6/11/1991	Unknown	9,100 to 9,300	Shady lodgepole pine bog on east shore of Meeks Lake.

Table 4 (concluded).

Source ID	County/ State	Location	Owner	Last obs	Abundance	Elevation (ft.)	Habitat and Notes
WY EO#2	Teton, WY	Jenny Lake	NPS (Grand Teton)	8/3/1934	Unknown	6,700 to 7,000	Stony Margin and bay near outlet of Jenny Lake.
WY EO#3	Teton, WY	Leigh Lake	NPS (Grand Teton)	7/24/1901	Unknown	6,900	Wet ground.
WY EO#4	Teton, WY	Lonestar Geyser	NPS (Yellowstone)	1976	Few	7,620	Wet meadow.
WY EO#5	Teton, WY	Between Shoshone and Lewis Lakes	NPS (Yellowstone)	8/24/1958	Unknown	7,800	Shade of lodgepole, border of stream.
WY EO#7	Teton, WY	Snake District Ranger Station	NPS (Yellowstone)	8/23/1991	10 to 15 in four small sub-populations	6,900	Edge of creek.
WY EO#8	Teton, WY	Bechler Area-Calf Creek and Falls River Confluence	NPS (Yellowstone)	7/28/1991	Unknown	6,450	Willow swamp.
WY EO#9	Teton, WY	Bechler Area-Boundary Creek East Fork	NPS (Yellowstone)	8/17/1991	Very small population	7,300	Edge of lodgepole island in thermal swamp in a pristine area in backcountry.
WY EO#10	Teton, WY	Southeast of Moose-Wilson Road	NPS (Grand Teton)	8/29/2002	30	6,440	Sunny opening on level, damp substrate among *Alnus incana*, *Rhamnus alnifolia*.
J. Whipple personal communication 2003	Teton, WY	Bechler Area-Dunanda Falls	NPS (Yellowstone)	unknown	Unknown	6,900	Not reported.
Erwin F. Evert (#9123 at RM)	Teton, WY	Black Sand Geyser Basin	NPS (Yellowstone)	8/4/1985	Unknown	7,500	Wet, peaty soil.
J. M. Coulter (s.n. at NY)	Teton, WY	Lower Fire Hole Basin	NPS (Yellowstone)	1872	Unknown	?	Not reported.
James D. Montgomery (22087 in private collection of Peter Root)	Teton, WY	Potts Hot Spring	NPS (Yellowstone)	6/12/1966	Unknown	7,750	In open grassy area at Potts Hot Spring Basin, near West Thumb.

Nebraska

In Nebraska, *Botrychium multifidum* is known from a single dubious collection by E.M. Hussong in 1895 (*#4689* at the University of Nebraska State Museum [NEB]; Rolfsmeier et al. 2001). The locality given is "Copse and meadows on Mr. Ewing's farm near Franklin." No county or state was documented, but "Franklin Co., NE" was added later in a different handwriting. The handwriting is recognized by the curator of NEB, Margaret Bolick, as that of "someone who worked during the 1930's and messed up a lot of localities by assuming specimens with incomplete locality data were from Nebraska" (Bolick personal communication 2003). Thus, this sheet has been annotated as a dubious locality. Rolfsmeier et al. (2001) omitted *B. multifidum* from the Ferns and Fern Allies of Nebraska, with the following justification: "A specimen (Hussong s.n.) at NEB, supposedly from Franklin County, was so identified by Petrik-Ott (1975, 1979). The specimen is from a large collection of very doubtful provenance, and the record was excluded by GPFA (1977, 1986) and is rejected by us." Given the close proximity of Franklin, Nebraska to the state line, it is possible that this specimen was collected in Kansas instead.

South Dakota

In South Dakota, *Botrychium multifidum* is known from five sites on the Black Hills National Forest. Three of these are in the Black Elk Wilderness near Harney Peak (Crook personal communication 2003, South Dakota Natural Heritage Program 2003). These sites (SD EO# 1, #3, #4) are all within a few hundred feet of each other and are best thought of as a single occurrence. More than 500 individuals have been documented at these aggregated sites between 2003 and 2005, making them collectively the largest population currently known from Region 2. Norbeck Wildlife Preserve in Pennington County contains a fourth occurrence. Despite three attempts to relocate *B. multifidum* at Butcher Gulch in the northern Black Hills, the fifth South Dakota occurrence has not been observed since 1994 (Corey personal communication 2003, Crook personal communication 2003, Burkhart personal communication 2005).

Wyoming

Botrychium multifidum is known from three distinct areas in Wyoming. Most of the occurrences of *B. multifidum* in Wyoming fall outside the administrative boundary of Region 2, and these are discussed below. Seven occurrences in Wyoming fall within Region 2. Six of these are in Carbon County in southern Wyoming; five of the six are on the Medicine Bow National Forest (WY EO #1, WY EO #18, Proctor personal communication 2004, 2005), and the other is on private land at Big Creek Park, within 100 m of the Medicine Bow National Forest boundary (Proctor personal communication 2005). *Botrychium multifidum* was discovered on the Bighorn National Forest in 2004 at the East Fork Campground (WY EO #20) in Sheridan County.

Twenty one of the 28 known occurrences of *Botrychium multifidum* in Wyoming are found in the Greater Yellowstone Ecosystem of Wyoming in the Teton and Wind River Ranges in Park, Teton, and Sublette counties (Rocky Mountain Herbarium 1998, Dorn 2001, Fertig 2002, Wyoming Natural Diversity Database 2003). These occurrences are protected in national parks and wilderness areas in the Greater Yellowstone Ecosystem (Fertig 2002). Many locations in Yellowstone National Park are known from historic records, but Whipple (personal communication 2003) has found six more occurrences since 1994 (**Table 4**). Despite her recent observations, Whipple considers *B. multifidum* to be rare overall, but it is found consistently in certain habitat types. As observed elsewhere in Region 2, *B. multifidum* occurrences in Yellowstone National Park are almost always small.

Region-wide distribution

Botrychium multifidum has been targeted in recent and ongoing search efforts within the states of Region 2, on National Forest System land and elsewhere. In 2002, an extensive survey of sensitive and rare plants in the Bearlodge District of the Black Hills National Forest in Wyoming did not yield any new records for *B. multifidum* (Heidel personal communication 2003). Searches by Crook, Farrar, Zacharkevics, and Burkhart in the Black Hills of South Dakota have yielded discoveries of new occurrences since 2003 (Crook personal communication 2003, Burkhart personal communication 2005). Peter Root has conducted numerous searches in potential habitat in Colorado over many years. John Proctor, Andy Kratz, and Wendy Haas have searched many locations on the Medicine Bow National Forest, leading to the discovery of six new occurrences in Colorado and Wyoming (see **Table 4** and the Habitat section of this document for details).

In general, more accurate distribution information is needed for *Botrychium* species. Wagner and Wagner (1990) list reasons for this deficiency of information.

The small size of many *Botrychium* species, particularly members of subgenus *Botrychium*, makes them very difficult to find in similarly colored vegetation. While *B. multifidum* is typically larger than members of subgenus *Botrychium*, very small plants (smaller than a dime) have been reported in shaded habitats (Crook personal communication 2003, Root personal communication 2003). *Botrychium* 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. Herbarium specimens are often misidentified and may be prepared with multiple species on a single sheet (Wagner and Wagner 1990, Root personal communication 2002). The difficulty in seeing *B. multifidum* among grasses, its superficial similarity to newly emerging forbs, and its rarity also make accurate distribution data difficult to obtain for this species.

Moonwort populations (subgenus *Botrychium*) are described as "spatially and temporally scattered, clustered and isolated" (Johnson-Groh and Farrar 2003, p. 8). This is also largely true of grapeferns (subgenus *Sceptridium*) although the life history characteristics of *B. multifidum* suggest that its populations are likely to be more persistent than those of moonworts. As seen in other species of *Botrychium*, occurrences of *B. multifidum* are widespread but highly disjunct throughout much of its range (Wagner 1972). However, in wetter areas where *B. multifidum* is fairly common, such as northeastern North America (e.g., Ontario, Nova Scotia) and the Pacific Northwest (e.g., British Columbia), occurrences are more continuous. Occurrences in Region 2 are typically isolated, but less so in the high precipitation areas of northwestern Wyoming and northeast of Steamboat Springs, Colorado. Because precipitation in most of Region 2 is insufficient to provide the elevated soil moisture that the species requires (particularly in the sandy soils that it prefers), its distribution is limited to areas with a high water table.

Wagner (1998) hypothesizes that some spores disperse when mammals eat the fertile sporophytes, and this mechanism could account for patchy distributions such as those seen for *Botrychium multifidum*. More information pertaining to dispersal mechanisms and underground factors that affect establishment could help to elucidate the causes of these observed distribution patterns.

Population trend

There are no rigorous quantitative data on population trends for *Botrychium multifidum* in Region 2. However, there is evidence suggesting declines within Region 2 as well as range-wide. *Botrychium multifidum* is believed to have been extirpated from Indiana (Crovello et al. 1983), Maryland (Brown and Brown 1984), and North Carolina (North Carolina Natural Heritage Program 1999). In Ohio there are records of *B. multifidum* from three counties where it has not been seen again (Schneider 1993). In Region 2, five of the 20 known occurrences have not been seen within the last 20 years, and many locations of *B. multifidum* are known only from very old herbarium specimens. Because of the uncertain locations of these occurrences, it is difficult to implement protective management practices, and it will be difficult to determine whether some of these occurrences remain extant. Fertig (2002) noted that Colorado and South Dakota occurrences appear to be in decline while Wyoming occurrences are considered stable overall, but there are no data included to support this statement. The recent discovery of many occurrences in Region 2 is probably a product of increased interest in and searches for this species rather than a population increase. Further searches are likely to result in the discovery of more occurrences. The known occurrences in Region 2 are small, and theoretical concepts of minimum viable population size in plant populations suggest that these occurrences are highly susceptible to extirpation by demographic and environmental stochasticity. However, it appears likely that this species has always existed in small, disjunct populations in Region 2 and elsewhere.

At Cabin Lake, Colorado, more than 1000 plants were counted in the center of a dried-out lakebed in 1989 (Root personal communication 2003). Approximately 100 small plants were recorded the same year under a canopy of *Pinus contorta* (lodgepole pine) at the margin of the lake bed (Colorado Natural Heritage Program 2003). By 2003, only six large individuals persisted in the center of the lake bed and the shaded individuals had largely disappeared. Because prolonged dormancy (remaining dormant over one or more growing seasons) is not typically observed in *Botrychium multifidum* (Johnson-Groh personal communication 2003), it is likely that these plants have died. Please see the Cryptic Phases section under Reproductive biology and autecology of this assessment for further discussion of dormancy in *Botrychium* species.

At Butcher Gulch, South Dakota, *Botrychium multifidum* has not been seen since 1994 (Corey personal communication 2003, Crook personal communication 2003). This area burned in 2002, and *B. multifidum* was not found during surveys in 2003 and 2005 (Crook personal communication 2003, Burkhart personal communication 2005).

At a location along Lost Cabin Creek in the Black Hills (BOMU 1), repeated visits have yielded different numbers of individuals. In 2003, 65 plants were found; in 2004, 35 plants were found; and in 2005, 383 plants were found. This apparent fluctuation may be due to real variation in population size, but it is more likely to have resulted from a combination of search effort, timing, and annual climate variation that leads to differences in vegetative cover and detectability of *Botrychium multifidum*. The varied observations at this occurrence highlight the difficulties in making an accurate assessment of population trend, even for a single occurrence.

The severe drought that affected much of the western United States from 2001 through 2003 may have reduced populations of *Botrychium multifidum* in Region 2. It is not known how *B. multifidum* responds to and recovers from drought. Drought may also have favored the spread of Canada thistle (*Cirsium arvense*) at Cabin Lake in Rocky Mountain National Park (Connor personal communication 2003). Although *B. multifidum* may be sensitive to factors that affect its hydrologic regime, it is probably quite resilient and can survive ephemeral aberrant conditions such as drought (Zika personal communication 2003).

As a long-lived perennial that does not often exhibit dormancy, *Botrychium multifidum* populations might be expected to be fairly constant. The degree to which it depends on favorable years (perhaps with respect to precipitation) for successful reproduction is not known.

Populations of many *Botrychium* species, including *B. multifidum*, tend to be small and localized (Colorado Natural Heritage Program 2003). This is probably due to the patchy nature of their habitat. Habitat that appears to be suitable for *Botrychium* is often not occupied. This may be due to stochastic factors such as local extinctions or the failure of gametophytes to establish, incomplete understanding of habitat attributes, or other ecological parameters.

Habitat

Habitat descriptions

Botrychium multifidum has been documented from a variety of habitats throughout northern North America. Most published descriptions are for areas outside of Region 2. Lellinger (1985) cites *B. multifidum* habitats as "terrestrial in old pastures, meadows, woodland margins, riverbanks, and bottomlands in subacid soil." Rook (2002) describes the habitat affinities for *B. multifidum* as "mainly in fields; open grassy places; acid, often sandy soils." Gleason and Cronquist (1963) describe *B. multifidum* habitat as "moist and sandy places, rarely in woods" while variety *intermedium* is found in "open fields and woods in acid soil." Scoggan (1978) describes its habitats in Canada as "dry to moist, grassy or sandy fields or open thickets and woods." In Ohio, *B. multifidum* is known from "Meadows and open woods, cemeteries and lawns, even when closely mowed" (Schneider 1993). Habitat types listed for Wisconsin include boreal forest, bracken grassland, savanna, sedge meadow, and southern upland forest (Umbanhowar 2003). In Greenland, *B. multifidum* is found in very warm sites, typically with south facing aspects in somewhat sandy soils (Ollgaard 1971). In Montana, some habitats include thickets, marshes near lakes, lakeshores, on a rotting log, and on rocky soil at the edge of spruce forest (from specimens at RM). In Idaho, *B. multifidum* was collected in moist coniferous forest dominated by *Pinus contorta*. It is also known from low elevation prairie remnants in western Oregon, and the mountains of Oregon and Washington where it is occasional on trail sides and in riparian zones and in moist montane meadows (Zika personal communication 2003). In Yellowstone National Park, *B. multifidum* is probably most abundant on "geyser formations", as reported by Coulter and Nelson (1909) and Rydberg (1922).

In Region 2, *Botrychium multifidum* has been documented in a variety of habitats including wet meadows, forest edges, lake shores, stony lake margins, in willow clumps at the edge of a pond, next to trails, and near beaver dams (**Figure 8**, **Figure 9**, **Figure 10**, **Figure 11**, **Figure 12**). These sites may be open (a drained lake bed) or shaded (*Pinus contorta* bog). Forest edges and sites with an open canopy are slightly more frequently reported in Region 2 than other types of sites (see **Table 4**) and are noted more commonly in the literature. Dorn and Dorn (1972) report *B. multifidum*

Figure 8. The lakebed of Cabin Lake, Rocky Mountain National Park, Larimer County, Colorado. Photograph taken by the author on September 7, 2003. Note the dominance of Deschampsia caespitosa in the lakebed. The white pipe at center photo is a test well, where steady drying of the site has been documented.

Figure 9. Habitat for *Botrychium multifidum* at Bear Park, Jackson County, Colorado. Plants are visible at the base of the willow (*Salix planifolia*). Photograph courtesy of John Proctor.

Figure 10. Habitat for *Botrychium multifidum* at Bear Park, Jackson County, Colorado, showing the proximity to water. Plants are visible at the lower right. Photograph courtesy of John Proctor.

Figure 11. *Botrychium multifidum* habitat at Big Creek Park, Carbon County, Wyoming. Photograph provided by John Proctor.

Figure 12. *Botrychium multifidum* habitat at Iron Creek (BOMU 4), Black Hills National Forest, Custer County, South Dakota. Photograph provided by Reed Crook.

from "moist, usually open areas at low to high elevations." Dorn and Dorn (1977) describe habitats in the Black Hills as "moist woods." From Colorado, Weber and Wittmann (2001a and b) report *B. multifidum* from "mountain meadows."

Local habitat descriptions

In Rocky Mountain National Park, Colorado, *Botrychium multifidum* is known from the grassy bed of a drained reservoir where large, robust plants have been observed, and from the edges of the *Pinus contorta* forest around the lake bed (Root 1995, Root personal communication 2003). *Botrychium multifidum* has been documented from drained lakes in Wisconsin as well.

Cabin Lake was a small reservoir that was probably built in the early 1900s (Connor personal communication 2003). The small dam was breached in 1969, and the lake drained, leaving a silty basin. *Botrychium multifidum* was discovered here in 1989, and observations since then suggest that successional changes are occurring at this site and that it has dried considerably. In 1989 the lakebed was characterized as a sedge meadow, but it is now dominated by grasses such as *Deschampsia caespitosa* (**Figure 8**). In 1989, *Pedicularis groenlandica* and bog orchids were found at this site, but they are no longer present. A groundwater test well was installed in the lakebed in 2001 when there was still water at the surface. However, in September 2003 the water table had fallen to 2.5 ft. (Root personal communication 2003).

In Bear Park, Colorado *Botrychium multifidum* was found in the transition zone between a peatland on the margin of a pond and the upland (**Figure 9** and **Figure 10**). This location may be flooded in the spring. At this location, about 25 plants are found on a peninsula between two beaver dams (about 20 ft. from the beaver dams, and about 20 ft. from the water's edge).

Botrychium multifidum has also been reported from a steep, eroded bank above a 4-wheel drive road in Savage Basin on San Juan National Forest (Colorado Natural Heritage Program 2003). This is highly atypical habitat, leaving some doubt regarding this report. The habitat for this report is more typical of members of subgenus *Botrychium* such as *B. 'colorado,'* a large form of *B. minganense*, or possibly a large *B. lanceolatum*.

In Yellowstone National Park, *Botrychium multifidum* appears to be strongly associated with geothermally-influenced meadows (Whipple personal communication 2003). These meadows contain unusual plant assemblages. They tend to be slightly disturbed but not recently disturbed, where competitive species (e.g., *Carex* spp. [sedges]) are not common. Geothermal activity may play a role in maintaining a natural disturbance regime favorable to *B. multifidum*.

In 2003 Whipple found *Botrychium multifidum* at two sites in Yellowstone. One site, a wetland complex at Obsidian Cliffs, is a geologically complex area with low-temperature geothermal features and a geothermally influenced creek. At this location, and in many others at Yellowstone, *B. multifidum* was found in areas where *Carex* species did not form a monoculture. The area is seasonally wet but dries out slightly during the growing season. Soil organic matter was very high at this location, which had the appearance of a peatland (Whipple personal communication 2003).

A second site, found in 2003 along Obsidian Creek, burned in 1988. This site and the Northern Hills site in South Dakota are the only known sites in or near Region 2 with a recent fire history. The Obsidian Creek occurrence was found among *Picea engelmannii* (Engelmann's spruce) that had survived the fire, near a geothermally influenced creek.

At Harney Peak in the Black Hills of South Dakota, *Botrychium multifidum* occurs in shaded sites in *Picea glauca* (white spruce)-dominated forests. These plants are typically less than one inch in diameter and have not been observed to sporulate. Soils at this site are coarse, sandy, and derived from granite (Crook personal communication 2003).

An occurrence of *Botrychium multifidum* was discovered in South Dakota in 2003 north of the Harney Peak area. At this site eight plants were found, one of which was sporulating. The sporulating plant was growing in an old streambed where it was less shaded than the other seven plants, which were scattered within 100 meters of the sporulating plant. The occurrence was located in a narrow draw dominated by *Picea glauca*, and the stream channel was incised approximately 1 to 2 ft.

The site located in Butcher Gulch in the Northern Hills Ranger District of the Black Hills National Forest in South Dakota is unusual in that it is underlain by limestone, which tends to create less acidic soils. Very little information is available on the habitat in Butcher Gulch, and *Botrychium multifidum* has not been seen since 1994. Butcher Gulch burned in 2002, and two attempts to find *B. multifidum* at this location failed (Corey personal communication 2003, Crook personal communication 2003).

Some common themes emerge in *Botrychium multifidum* habitat descriptions from Region 2 and elsewhere, which are treated individually below.

Elevation, latitude, slope, and aspect

The elevation of known occurrences of *Botrychium multifidum* within Region 2 ranges from 6,300 to 11,500 ft. Occurrences in Colorado are found at slightly higher elevations (~6,750 to 11,500 ft.) than those in Wyoming (6,450 to 10,000 ft.) and South Dakota (5,000 to 6,550 ft.). This is probably latitudinally-influenced; a downward shift in habitat elevation occurs with increasing latitude.

Botrychium multifidum usually occurs in flat sites in Region 2. This is probably due to its moisture requirements that are not typically met on better-drained slopes. However, in three sites it occurs on south facing exposures in alpine and arctic habitats. Two of these are alpine occurrences in Colorado (in San Miguel and Conejos counties). It has also been found on south-facing slopes in Greenland (Ollgaard 1971). The warming effects of greater insolation on south-facing slopes are well studied (Barbour et al. 1987). Thus, the presence of suitable habitat on these south-facing slopes may allow *B. multifidum* to persist at these higher elevations and latitudes that might normally lie beyond its physiological tolerance limit. This phenomenon has often been observed in the alpine and arctic (e.g., Bliss 1987). *Botrychium multifidum* is reported from a 10 percent slope on an east aspect at Heart Lake Geyser Basin in Yellowstone National Park (Whipple personal communication 2003).

Many reports of *Botrychium multifidum* are from drier microsites within wet meadows, where soils are seasonally wet but somewhat better drained and thus dry out in the summer. This pattern is particularly evident among occurrences in Carbon County, Wyoming where most occurrences were found on "humps" within wet meadows dominated by *Carex* species. Common associates with *B. multifidum* on these slightly drier microsites include *Antennaria* species, *Prunella vulgaris*, *Potentilla* species, *Fragaria virginiana*, and moss (Proctor personal communication 2005).

Soil

Unlike the majority of *Botrychium* species, which are calciphlic (Root personal communication 2003), *B. multifidum* is typically found in subacidic or acidic soils that may be high in organic matter. This has been well documented in Region 2 and elsewhere. However, *B. multifidum* is found in calcareous soils at Butcher Gulch in South Dakota and at Big Creek Park in Wyoming. *Botrychium multifidum* often occurs on sandy soils as reviewed in the literature (e.g., Gleason and Cronquist 1963) and documented from many herbarium specimens. However, there are also reports of *B. multifidum* from highly organic soils (in Yellowstone National Park), granitic soils (at Harney Peak, South Dakota), and in silty soils (Cabin Lake, Rocky Mountain National Park). It has been found in peatlands in southeastern Alaska (Zika personal communication 2003).

Moisture

Habitat for *Botrychium multifidum* in Region 2 is constrained by soil moisture availability. The species is often found in wetlands (**Table 5**) and is a wetland indicator species (U.S. Fish and Wildlife Service 1988). *Botrychium multifidum* is more common in mesic eastern North America. In Region 2, mesic sites with appropriate habitat characteristics are limited largely to montane or subalpine areas. Throughout Region 2, *B. multifidum* is only found in very mesic sites, usually riparian areas, wet meadows, and near lake margins, where water is near the surface throughout the growing season. However, it is typically found in microsites that are slightly less wet than the surrounding vegetation, where soils may become seasonally dry. In the Yellowstone area *B. multifidum* is found near hot springs and geysers.

Disturbance as a habitat attribute

Most members of subgenus *Sceptridium* are found in moderately disturbed habitats including second-growth woods, old fields, next to trails, and on grazed grassy slopes (Clausen 1938, Barker and Hauk 2003). Although information on the disturbance regime in occurrences in Region 2 is sparse, some observations (within and outside of Region 2) suggest that disturbance is involved in the creation and maintenance of suitable habitat for *Botrychium multifidum*. However, Farrar (personal communication 2003) has most commonly found *B. multifidum* in relatively undisturbed sites in mesic to wet meadows. In Massachusetts, it has been found "opposite a cellar hole" and "just beyond a ski jump" (Bemis 2003). In Vermont, it has been found in "thickets at roadside, old tailings dump area" near an asbestos mine, and on serpentine tailings in the mine pit (*Root #86-25*). It was also documented on serpentine soils in Quebec (Sharpe 1997). Reports in Ohio from "cemeteries and lawns, even when closely mowed" also suggest that it tolerates disturbance or at least soil compaction (Schneider 1993). Some sites in Region 2 are near creeks, in drained lake basins, and in the vicinity of beaver dams where water action may cause periodic disturbance, as well as adjacent to trails in South Dakota. In Colorado it has been documented from a steep, eroding road cut, but there is some doubt regarding the validity of this occurrence. In Yellowstone National Park, *B. multifidum* has a strong affinity for areas that are disturbed by geothermal activity. Please see the Reproductive Biology and Autecology section of this document for a more detailed treatment of the ecological role of disturbance for *B. multifidum*.

Beaver activity has been reported at three occurrences of *Botrychium multifidum* in Region 2, and

Table 5. Wetland Indicator Status for *Botrychium multifidum* (U.S. Fish and Wildlife Service 1988).

Code	Region	Georaphic Areas in Region	Wetland Indicator Status
4	North Plains	MT (Eastern), ND, SD, WY (Eastern)	FAC
5	Central Plains	CO (Eastern), NE, KS	FAC
8	Intermountain	CO (Western), NV, UT	FACU
9	Northwest	ID, OR, MT (Western),WA,WY (Western)	FAC
Wetland Indicator Status Explanations:			
FAC	Facultative	Equally likely to occur in wetlands or non-wetlands (estimated probability 34-66 percent).	
FACU	Facultative Upland	Usually occurs in non-wetlands (estimated probability 67-99 percent), but occasionally found on wetlands (estimated probability 1-33 percent).	

at many sites outside the region as well. The activity of beavers has considerable impacts on the structure and function of their riparian habitats, and they have been referred to as "ecosystem engineers." Beaver activity may create or augment habitats for *B. multifidum*. The population dynamics of beaver can affect species richness by altering the age distribution of meadows across the landscape (Wright et al. 2003), which may in turn affect the distribution of *B. multifidum*.

Fire

Pinus contorta is the tree species most commonly associated with occurrences of *Botrychium multifidum*. This is a fire-adapted species, and fire is a natural and relatively frequent part of forests dominated by this tree. Stand ages of subalpine lodgepole forests south of Yellowstone National Park suggest a 200 to 400 year fire return interval, but at lower elevations the interval may be as short as 50 to 150 years (Peet 2000). The role of fire in the ecology *B. multifidum* is unclear, but there is no evidence that it has an affinity for recently burned areas. In Yellowstone National Park, an occurrence of *B. multifidum* was discovered in 2003 in an area that had burned in 1988, but the intensity of the fire was low and portions of the area did not burn completely (Whipple personal communication 2003). In South Dakota, Butcher Gulch burned in 2002. Surveys by Farrar and Crook have not found *B. multifidum* at this site since the fire, but it had not been seen for eight years prior to the fire either (Crook personal communication 2003). Although *B. multifidum* is often associated with *P. contorta*, it is also often in mesic, open areas adjacent to forest where burning may be less frequent or intense. Please see the Community Ecology section of this document for more details on the vegetation associated with *B. multifidum*.

Meadows

Botrychium multifidum is often found in meadows and openings in forested areas in Region 2 and elsewhere. Meadows are treeless areas dominated by various species of grasses, sedges, and forbs that are scattered throughout forests of the Rocky Mountains (Peet 2000). There is little agreement as to the ecological processes that are responsible for the creation and maintenance of meadows. Some wet meadows in Rocky Mountain National Park are maintained by a combination of saturated, fine-textured soils, high snow accumulation, and cold air drainage (Peet 2000). Wet meadows are extremely variable in their species composition, depending on water chemistry and water availability. Subtle hydrologic gradients result in significant changes in species dominance in wet meadows (Wilson 1969).

Numerous occurrences of *Botrychium multifidum* are known from geothermally influenced meadows in Yellowstone National Park. These meadows are interesting from an ecological standpoint and usually support unique plant assemblages. They are probably maintained by a mild disturbance regime imposed by geothermal activity (Whipple personal communication 2003). *Botrychium multifidum* shows a strong affinity for these habitats. Please see the Community Ecology section of this document for more details on the plants associated with *B. multifidum*.

Mycorrhizae as a habitat attribute

Most of the life cycle of *Botrychium* species occurs under ground, including several important life history stages, yet scientists understand very little about this part of the life cycle. *Botrychium* species rely on mycorrhizal interactions in each of their life stages (Campbell 1922, Bower 1926, Scagel et al. 1966, Gifford and Foster 1989, Schmid and Oberwinkler 1994). Johnson-Groh (1999) hypothesizes that mycorrhizae are the most important factor in the persistence of *Botrychium* occurrences. However, almost nothing is known about which species of mycorrhizal fungi interact with *Botrychium* species, what factors affect the mycorrhizal fungi, and what factors affect the interaction between the mycorrhizal fungi and *Botrychium* (please see the Reproductive biology and autecology section of this document for discussion on mycorrhizal interactions).

Reproductive biology and autecology

In the Competitive/Stress-Tolerant/Ruderal (CSR) model of Grime (2001), characteristics of *Botrychium* species 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. These characteristics are typical of stress-tolerant species in the CSR model (Grime 2001).

Moderate disturbance may be a critical part of the autecology of *Botrychium* species, including *B. multifidum* (Clausen 1938, Lellinger 1985, Wagner and Wagner 1993, Barker and Hauk 2003, Johnson-Groh and Farrar 2003). The disturbance regime required by *B. multifidum* has not been studied and is not well understood. There is much less information on the role of disturbance in the autecology of *B. multifidum* than for members of subgenus *Botrychium* (Farrar personal communication 2003). Although there have been no formal studies, it appears that *B. multifidum* is similar to other *Botrychium* species in that it is often found in older disturbed areas (areas disturbed more than 10 years ago) (Johnson-Groh personal communication 2003). However, Farrar (personal communication 2003) notes that he typically finds it in relatively undisturbed sites.

Disturbance may play a role in the reproductive biology of *Botrychium multifidum*. Published research from outside Region 2 and observations from within Region 2 strongly suggest that open sites are needed for successful reproduction. Wagner (1946, 1962) noted that plants with sporophores are usually found in open sites with greater insolation. This has also been reported in Region 2 at Cabin Lake (Root personal communication 2003) and in the Black Hills (Crook personal communication 2003). These observations suggest that the shaded plants are "waiting" for a disturbance to occur that would create an opening in the forest canopy and allow them to reproduce (Crook personal communication 2003, Farrar personal communication 2003). Given that their lifespan approaches the length of the average fire return interval in some forest habitat types of Region 2 (Stevenson [1975] reported that *B. multifidum* can live 100 years), it is plausible to speculate that *B. multifidum* would be capable of taking advantage of rare disturbance events. If disturbance does not occur or fails to create openings, the shaded plants may persist in these sites without ever garnering the resources needed to reproduce. The number of plants in the forest at Cabin Lake has declined precipitously since 1989, but this may also be due to recent hydrologic alteration of the site or competition for water with *Pinus contorta* (Root personal communication 2003). Plants with sporophores occur in shaded sites in Oregon, Washington, and Vermont (Zika personal communication 2003), and at Cabin Lake in Colorado (Root personal communication 2003), suggesting that this may not be an accurate portrayal of the population ecology of *B. multifidum*. It is possible that the small shaded plants were reproductive when the site was more open, and that they are now suppressed by closed canopy conditions that arose later.

Crook (personal communication 2003) offers interesting thoughts regarding the role of fire in the reproductive biology of *Botrychium multifidum*. There are many similarities between the life histories of *Botrychium* species and the club mosses (*Lycopodium* species). Both have subterranean, mycoparasitic gametophytes that live for years under ground before the sporophytes emerge. *Lycopodium* species have been observed to establish after fire, possibly due to the presence of mineral soil at the surface into which the spores can fall and establish under ground. Because the gametophytes require years to mature, sporophytes are not observed until long after the fire and require about 80 years before population levels return to pre-fire levels.

The wet habitats where *Botrychium multifidum* is found are often dominated by highly competitive plants. However, observations suggest that *B. multifidum* is not particularly competitive. Whipple (personal communication 2003) notes that it is often found with typical competitive wetland species, but usually in disturbed sites where these species are not dominant. As noted in the CSR model, highly competitive species are not successful in stressed or disturbed habitats because they allocate much of their available resources to growth. This is maladaptive in stressed or disturbed habitats (Grime 2001). Root (personal communication 2003) speculates that small plants under a *Pinus contorta* canopy are suffering from competition for water as groundwater levels at Cabin Lake drop and the trees mature.

Reproduction

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 (**Figure 13**). 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 *Botrychium* species to self-fertilization (McCauley et al. 1985, Soltis and Soltis 1986). Cross-fertilization may occur (Wagner et al. 1985); however, the antheridia and archegonia are near each other, and inbreeding is prevalent (McCauley et al. 1985, Soltis and Soltis 1986, Farrar and Wendel 1996).

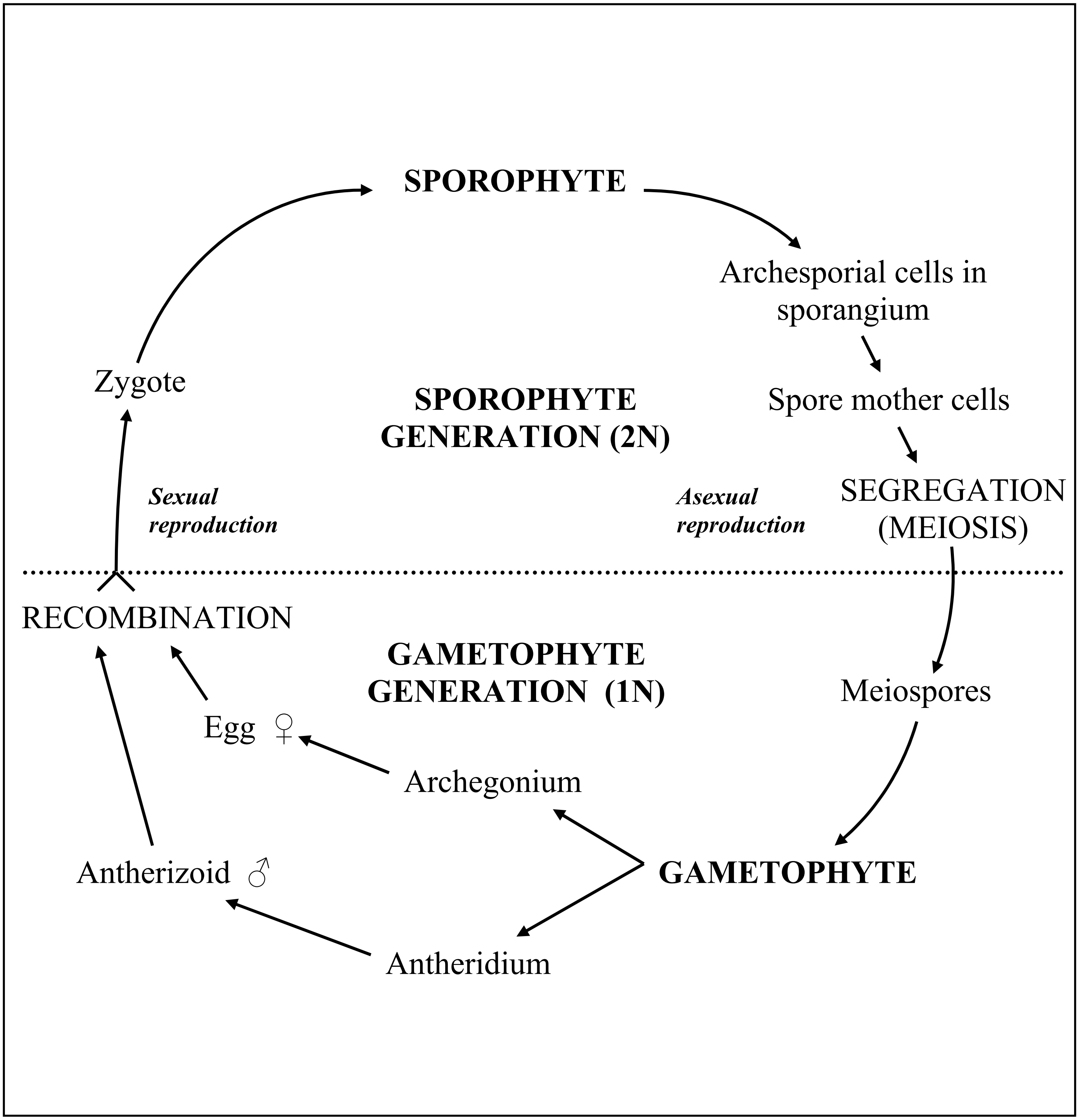

Figure 13. Life cycle diagram for *Botrychium multifidum* (Lellinger 1985), illustrating the alternation of generations. Only the sporophyte is typically found, the gametophyte being subterranean and extremely difficult to find.

Phenology

When compared with other species of *Botrychium*, *B. multifidum* has a protracted period of activity through the growing season. It begins leaf production earlier in the year than all other members of subgenus *Sceptridium* (Wagner 1960a, Wagner 1962), and its leaves mature earlier in the growing season than those of *B. dissectum* and *B. ternatum* (Wagner 1961). The production of this leaf is "astonishingly gradual," taking three or four months (Wagner 1960a, p. 306). The leathery trophophore is persistent through the winter, hence the common name "evergreen grapefern" for subgenus *Sceptridium* (Root 1995, Barker and Hauk 2003). The previous year's leaf is often retained well into the growing season, after which it withers and falls from the stem (Wagner 1960a). The retention of the previous year's leaf for part of the growing season may be advantageous in northern latitudes by increasing the amount of carbohydrate acquisition through photosynthesis (Root personal communication 2003).

Spores are released in July through September (Clausen 1938, Wagner 1960a, Root personal communication 2003), and as late as October (Gleason and Cronquist 1963, Root 1995). Root (personal communication 2003) suggested that it be called the "Hunting Season Grapefern" because it is best seen around the beginning of hunting season in September when it is actively sporulating. As the sporangia mature they turn from green to yellow to brown (Wagner 1960a). *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).

Fertility

Members of subgenus *Sceptridium* sometimes do not produce a sporophore (Wagner 1946, Wagner 1962). *Botrychium multifidum* has often been observed without sporophores in shaded locations in Region 2. For a discussion of this phenomenon please see the introduction to this section and the Phenotypic Plasticity section below.

Each *Botrychium* sporophyte may bear thousands of spores in the 20 to 100 (or more) sporangia per sporophore, possibly the highest number of spores per case of all vascular plants (Wagner 1998). There has been no rigorous assessment of the viability of the spores of *B. multifidum*. Spores of *B. virginianum* germinated on agar showed a 90 percent germination rate (Peck et al. 1990).

Dispersal

Dispersal capability is unknown for *Botrychium multifidum*, but inferences can be drawn from studies of *Botrychium* and other fern genera. Researchers have hypothesized that the dispersal distance for *Botrychium* spores ranges from a few centimeters (Casson et al. 1998, Hoefferle 1999) up to 3 m (Peck et al. 1990). Dyer (1994) found that spore banks were largest in soil samples underneath ferns, but they were considerably smaller only 2 m away from sources. While most spores land close to the parent plant, they occasionally are capable of long-distance dispersal because they are light enough to travel considerable distances (Briggs and Walters 1997). In addition to wind dispersal, animals may disperse *Botrychium* spores (Wagner and Wagner 1993, Wagner personal communication 2002). Spores have thick walls that may help to retain their viability as they pass through an animal's digestive tract (Johnson-Groh 1998, Wagner personal communication 2002). Deer and small mammals may disperse the spores of forest species such as *B. dissectum* along trails and roads. After feeding the spores of *B. virginianum* to a vole, J.D. Montgomery recovered apparently intact spores from the droppings (Root personal communication 2003). Buell (2001) noted that distribution patterns of *Botrychium* in Summit County, Colorado often followed swales, heavy equipment tracks, and erosion rills, suggesting that water plays a role in dispersing spores down slope. Elk that eat the sporophores of *B. multifidum* could act as dispersal agents that would move spores directly to other potentially suitable meadow habitats. Gifford and Brandon (1978) suggested that soil movement by soil insects, worms, or larger animals may break up and disperse gametophytes that proliferated vegetatively in culture.

Cryptic phases

Like other species of *Botrychium*, *B. multifidum* can remain dormant for at least one year (Wagner 1960a) and perhaps more. While long periods of dormancy are well documented for many species in subgenus *Botrychium* (Muller 1993, Kelly 1994, Lesica and Ahlenslager 1996, Johnson-Groh 1998, Johnson-Groh 1999), preliminary monitoring data suggest that species in subgenera *Osmundopteris* and *Sceptridium* do not go dormant for as long or as frequently (Johnson-Groh personal communication 2003). This may be because the members of *Osmundopteris* and *Sceptridium* depend more on photosynthesis for their carbohydrate acquisition than members of subgenus *Botrychium*, many of which rely heavily on mycorrhizae for their carbohydrate supply (Johnson-

Groh personal communication 2003). *Botrychium multifidum* is probably more similar to *B. virginianum* (subgenus *Osmundopteris*) than to members of subgenus *Botrychium* (the moonworts). Johnson-Groh (personal communication 2003) has monitored a population of *B. virginianum* since 1997 and has observed dormancy very infrequently. Two years of preliminary monitoring of *B. multifidum* also suggest that dormancy is infrequent (Johnson-Groh personal communication 2003). Montgomery (1990) commonly observed dormant periods of two years in *B. dissectum* (subgenus *Sceptridium*).

The importance of the underground lifecycle phases of *Botrychium* species are highlighted by the recent work of Johnson-Groh et al. (2002) who reports very high ratios of underground (gametophytes and juvenile sporophytes) to aboveground structures.

The importance of spore banks for *Botrychium multifidum* is unknown, but recent studies suggest that they play a vital role in the survival strategies of some ferns (Dyer and Lindsay 1992). Because of the limited ability to grow *Botrychium* spores in culture, it has been difficult to observe and quantify spore banks (Johnson-Groh and Farrar 2003), but studies of other ferns have found diverse spore banks that persist for many years (Milberg 1991, Dyer 1994).

The longevity of the spores of *Botrychium multifidum* is unknown. Measurements of non-chlorophyll-bearing spores of other fern families indicate an average viability length of 1045 days (Lloyd and Klekowski 1970). However, the spores of some fern genera may remain viable for longer periods of time (Miller 1968, Lloyd and Klekowski 1970, Windham et al. 1986). Spores of other fern genera (not *Botrychium*) have been germinated from 50-year old herbarium specimens (Dyer and Lindsay 1992). However, these spores were of leptosporangiate ferns, which may behave very differently than those of *Botrychium* (Spellenberg personal communication 2004).

Phenotypic plasticity

As discussed in the Classification and Description section of this document, *Botrychium multifidum* is morphologically variable (Wagner 1962). In Michigan and New England, variations in color, texture, size, and leaf shape have been observed (Wagner 1946, Wagner 1960a, Wagner 1962). Leaf thickness does not differ greatly between members of subgenus *Sceptridium*, but this is altered by habitat (Wagner 1960a). The descriptions of infrataxa in Clausen (1938) give a good indication of the degree of variability in *B. multifidum*. The variability in leaf form from a single county in Virginia also illustrates the wide range of variation in this species (Wagner 1946). The leaves of plants at some sites in South Dakota appear less dissected than others (Burkhart personal communication 2005). This variability often leads to misidentification of *B. multifidum* as *B. dissectum* where their ranges overlap. It also led to the proliferation of descriptions of infrataxa persisting through the early 20th century.

Observations suggest that *Botrychium multifidum* has the greatest reproductive output in open sunny sites (Wagner 1946, Wagner 1962, Crook personal communication 2003, Root personal communication 2003). Reports conflict on the effect of insolation on plant morphology and size. Wagner (1962) noted that *B. multifidum* is small and compact (with blades approaching 35 mm in length) in open sites along a horse trail in Michigan, but these plants are fully fertile. In contrast, plants in deep shade had much larger leaves (with blades approaching 140 mm in length) but were often sterile. In Region 2, similar observations have been made regarding the fertility of shade versus sun forms. A specimen housed at RM (*Fertig #7464*) illustrates the infertility of shade forms; this specimen was collected from a "shady lodgepole pine bog" and has a very small sporophore with very few sporangia. However, at Cabin Lake and Harney Peak, most plants are small and infertile under the forest canopy but large and fertile in open areas (Crook personal communication 2003) although large plants have also been seen in shaded sites at the forest edge (Root personal communication 2003). It is likely that ecological factors such as water availability elicit this phenotypic response. There does not appear to be a relationship between size of the trophophore and age; small shade forms may have large rootstocks when excavated, suggesting that they are many years old (Burkhart personal communication 2005). The sporophore of a plant found in Greenland had shriveled or aborted, suggesting that *B. multifidum* is probably very near its physiological limit to survive and persist at this latitude (Ollgaard 1971).

Botrychium multifidum plants in shaded sites in Region 2 are quite small. Very small, non-reproductive plants grow under a *Pinus contorta* canopy at Cabin Lake, Colorado (Colorado Natural Heritage Program 2003, Root personal communication 2003) and under white spruce in the Harney Peak area, South Dakota (Crook personal communication 2003, Farrar personal communication 2003). Despite their size, these small, non-reproductive plants may be very old (Crook personal communication 2003). In other, generally more mesic regions, the reverse tends to be the case, with smaller plants in openings and larger, lax forms in the shade (Wagner 1946, Wagner 1962). Some large plants have been seen in shaded locations in Region 2 also (Root personal communication 2002). There may be some correlation between plant size and fertility, with larger plants more likely to produce a sporophore (Farrar personal communication 2003). However, one sporulating plant found in South Dakota in the Hell Canyon Ranger District was only 1.5 or 2 inches tall (Crook personal communication 2003).

Mycorrhizae

Botrychium species rely upon mycorrhizae in both the sporophytic (Bower 1926, Gifford and Foster 1989) and gametophytic stages (Campbell 1922, Bower 1926, Scagel et al. 1966, Gifford and Foster 1989, Schmid and Oberwinkler 1994). 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 *B. lunaria* (subgenus *Botrychium*) may live under ground for up to five years (Winther personal communication 2002) using carbohydrates and minerals gained from its mycorrhizae (Schmid and Oberwinkler 1994). Daigobo (1979) observed infection of *B. multifidum* gametophytes with an endophytic (arbuscular mycorrhizal) fungus in all developmental stages.

It is unknown how or if the mycorrhizal relationship changes as the gametophyte develops into a sporophyte, but it certainly shifts from a parasitic relationship to a more mutualistic relationship since the sporophyte is chlorophyllous and no longer wholly dependent on a mycobiont for carbohydrates. *Botrychium* sporophytes have reduced roots that lack hairs (Wagner and Wagner 1993), and they depend upon mycorrhizae (Bower 1926, Foster and Gifford 1989). Observations of root samples of *B. multifidum* and *B. virginianum* showed 100 percent infection, with all roots having the same degree of infection (Kempema et al. 2003).

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. Smith and Read (1997) and Farrar (1998) note that mycorrhizal fungi are low in species diversity, ubiquitous in disturbed and undisturbed sites, and non-selective in the species they infect. In contrast, 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 as many as 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 *Botrychium multifidum*.

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 spotted knapweed (*Centaurea maculosa*) had more intense competitive effects on Idaho fescue (*Festuca idahoensis*) when grown together in the presence of mycorrhizal fungi. Because of their tight association with mycorrhizae, similar work with *Botrychium* species is needed to understand the potential for mycorrhizae-mediated interspecific competition.

Hybridization

Hybrids between *Botrychium* species are rare (Wagner and Wagner 1993, Wagner 1998). However, at least ten records of sterile hybrid combinations have been documented (Wagner et al. 1984, Wagner et al. 1985, Wagner 1993). Sterile hybrids between *B. hesperium* and *B. echo* have been observed in sites where these species occur together (Wagner and Wagner 1983). The nothospecies *B.* x *watertonense* (n = 90) is the result of a cross between *B. paradoxum* and *B. hesperium* (Wagner et al. 1984). It was discovered in Waterton Lakes National Park where it co-occurs with its putative parents. Though spores are apparently abortive in *B.* x *watertonense*, this species may be capable of some reproduction through the apogamous production of spores (Wagner et al. 1984, Ahlenslager and Lesica 1996). Allopolyploidy may also have resulted in new species of *Botrychium* historically (Wagner 1993).

The anatomy of the gametophyte (Bower 1926) and the difficulty gametes encounter when traveling through the soil (McCauley et al. 1985, Soltis and Soltis 1986) serve to limit outcrossing and the opportunities for hybridization. Morphological and genetic analyses of genus communities have demonstrated that hybridization rarely occurs and most hybrids have abortive spores (Wagner and Wagner 1983, Wagner et al. 1984, Wagner and Wagner 1986).

There has been some speculation that *Botrychium* oneidense is a hybrid with *B. multifidum* and *B. dissectum* as parent species (Clausen 1943, Wagner 1960b, Fosberg 1961). However, recent molecular phylogenetic research indicates that *B. oneidense* is not genetically distinct enough from *B. dissectum* to warrant full species recognition (Barker and Hauk 2003), and there is no molecular evidence to suggest a hybrid origin involving *B. multifidum*.

Demography

There have been no demographic studies of *Botrychium multifidum* in Region 2 or elsewhere. In a long-term demographic study of *B. dissectum* (subgenus *Sceptridium*), Montgomery (1990) showed that this species is long-lived, with a very high survival rate for adults from year to year. This suggests that recruitment is less important for population persistence. A review of the relative contribution of adult survivorship and fecundity to fitness suggests that adult survivorship relates more directly to fitness than does fecundity for long-lived species (Crone 2001). Thus, factors that increase adult mortality of *B. multifidum* are most likely to threaten the viability of populations.

Members of the genus *Botrychium* appear to have naturally low rates of outcrossing (Farrar 1998). The anatomy of the gametophyte of *B. virginianum* (subgenus *Osmundopteris*) 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. Alleles within each moonwort species consistently show very low intraspecific variation when compared with other ferns and seed plants (Farrar 1998). Watano and Sahashi (1992) observed that heterozygous genotypes were very rare in four *Sceptridium* species due to high rates of inbreeding. McCauley et al. (1985) found *B. dissectum* (subgenus *Sceptridium*) to have an outcrossing rate of less than 5 percent. However, the occasional presence of interspecific hybrids in natural settings indicates the ability of the genus to hybridize (Wagner et al. 1984). In observations of axenically reared gametophytes of *B. multifidum*, Gifford and Brandon (1978) noted that these plants lacked archegonia, structures that produce female gametophytes. However, reduced, leaf-like structures that appeared to be sporophytes were also present in the cultures. Thus it appears that *B. multifidum* is capable of producing haploid sporophytes through apogamy (Gifford and Brandon 1978). Apogamy is the "formation of a sporophyte, without the act of fertilization, from vegetative cells of the gametophyte" (Gifford and Foster 1989, p. 16). While this may be possible in wild occurrences as well, apogamously-produced offspring have not been observed in nature and are unlikely to be fully fertile since they would be incapable of meiosis to produce spores. Given the breeding system of *B. multifidum*, it is possible that this species may not be particularly sensitive to the effects of inbreeding depression, since the opportunities for sexual recombination are rare. The spatial distribution of *B. multifidum* occurrences suggests that gene flow (via spore or, less frequently, gamete movement) between populations is probably very low.

Farrar (1998, personal communication 2003) suggests 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 establishment and maintenance of this relationship is of paramount importance to *Botrychium* species. As such, genetic diversity would be more useful to *Botrychium* when present in their fungal symbionts, since they are the intermediaries between the roots and the rhizosphere and are more likely to be affected by environmental change.

As a long-lived perennial, *Botrychium multifidum* is distinct from members of the subgenus *Botrychium*. Individual sporophytes have been observed with more than 100 leaf scars, and since they produce one leaf per year, *B. multifidum* may live for at least 100 years (Stevenson 1975). No other species of *Botrychium* are known to be as long-lived. The aboveground longevity of *B. campestre* is approximately four years, much longer than the closely related *B. mormo* (Johnson-Groh 1998). *Botrychium australe* may live as long as 11.2 years (Kelly 1994), but *B. dissectum* (subgenus *Sceptridium*) individuals can live at least a few decades (Montgomery 1990, Kelly 1994).

Botrychium gametophytes are reported to persist under ground for up to five years (Winther personal communication 2002), growing very slowly from an embryo into a fertile adult gametophyte (Wagner 1998). Sporophytes also may live heterotrophically under ground for several years before producing aboveground structures (Kelly 1994). Upon emergence above ground, the sporophytes begin spore production on their fertile lamina (sporophore). Please see **Figure**

13 for a diagrammatic representation of the life cycle of *B. multifidum*, and **Figure 14** for a life cycle graph (after Caswell 2001).

No population habitat viability analysis (PHVA) has been done for *Botrychium multifidum* to date. The only *Botrychium* species for which a PHVA has been conducted is *B. mormo* (Berlin et al. 1998), which differs in many significant ways from *B. multifidum* 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, but 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.

Botrychium occurrences appear to have a metapopulation structure that may be an important component of their viability. Johnson-Groh and Farrar (2003) offer an overview of the metapopulation structure of subgenus *Botrychium* that applies to *B. multifidum* as well. Using the metapopulation classes defined by Hanski and Simberloff (1997), they note that *Botrychium* occurrences do not conform to a single metapopulation class. Rather, complex spatial interactions in multiple metapopulation classes probably best characterize the structure of *Botrychium* occurrences. *Botrychium multifidum* 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* (Pickett and Thompson 1978, Menges and Gawler 1986). If this is the case, spores are the only means by which *B. multifidum* could migrate to new locations. Because most *Botrychium* species are early to mid-seral species, they may be expected to drop out as succession proceeds. Succession is of particular concern for meadow species including *B. multifidum* (Schneider 1993, Johnson-Groh and Farrar 2003).

As discussed in the Reproductive Biology and Autecology section of this document, evidence suggests that shaded occurrences in Region 2 may represent population sinks where successful spore production occurs very infrequently.

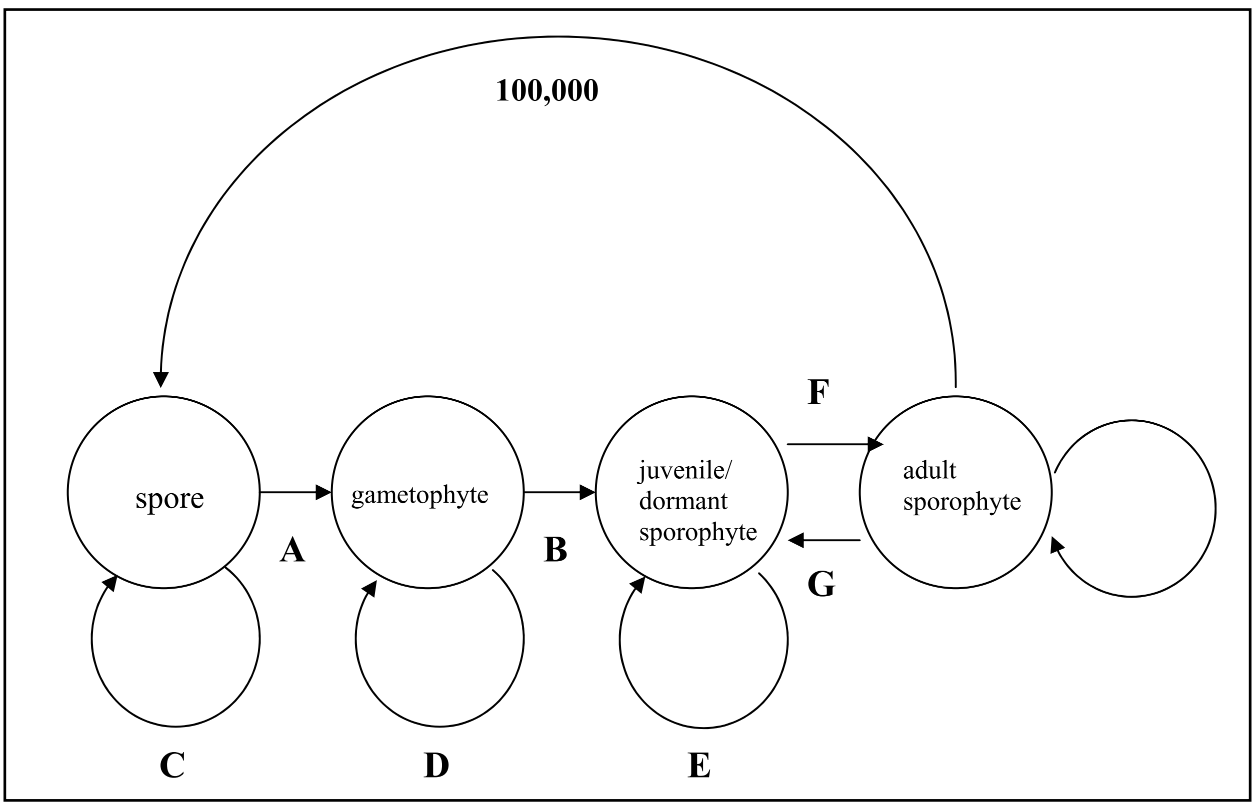

Figure 14. Hypothetical life cycle graph (after Caswell 2001) for *Botrychium multifidum*. Transition probabilities are not known and are difficult to quantify since important stages of the lifecycle occur under ground (A-G). Please see Johnson-Groh et al. (2002) for the best information currently available 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). No transition probabilities are known for B. multifidum.

Demographic studies of *Botrychium multifidum* are lacking, and basic parameters circumscribing life history characteristics are unknown. Sporophytes can remain dormant for one or possibly more years (Johnson-Groh personal communication 2003), and most of the population at a given site probably resides under ground in the gametophyte stage (Johnson-Groh et al. 2002). In a recent investigation of the belowground distribution and abundance of *Botrychium* gametophytes, the ratio of gametophytes to sporophytes of *B. virginianum* ranged from 25:1 to 122:1 (Johnson-Groh et al. 2002). For these reasons, long term studies are needed to estimate the true population size at a given site (Johnson-Groh and Farrar 2003). In an earlier study, Bierhorst (1958) found 20 to 50 gametophytes of *B. dissectum* per square foot, 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.

The study of how individual *Botrychium* plants become established is problematic due the fact that important events in their life cycle occur under ground. 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). Spores that fail to develop the symbiosis do not develop into gameotphytes. The mechanism by which spores get from the soil surface to a depth of 1 to 2 inches is not known. Water, frost action (freezing and thawing), and possibly fire are probably involved (Crook personal communication 2003, Root personal communication 2003). From predictions based on observations of survivorship, Johnson-Groh et al. (2002) estimate that 95 percent of *Botrychium* spores are unsuccessful. A density of 100 spores per square meter was predicted for *B. virginianum*, which may be similar to *B. multifidum* spore densities.

The typically small occurrences of *Botrychium* species leave them vulnerable to local extirpation from demographic and environmental stochasticity (Johnson-Groh et al. 1998). However, extinction probability models show that belowground stages buffer local *Botrychium* occurrences against extinction (Johnson-Groh et al. 1998).

Community ecology

Data from Region 2 and elsewhere suggest that *Botrychium multifidum* is strongly associated with a suite of species and genera throughout much of western North America. A specimen label from Oregon (*S. Markow #12181* at RM) describes the habitat for *Botrychium multifidum* as "Meadow edge with *Pinus contorta*, *Picea engelmannii*, *Deschampsia caespitosa*, *Viola adunca*." This description could also apply to a typical location in Colorado or Wyoming. Locations in eastern North America and even in Europe also share many species or genera. See **Table 6** for a complete list of species documented with *B. multifidum* in Region 2.

Table 6. Known associated species with *Botrychium multifidum* in USDA Forest Service Region 2; compiled from all known records. E= exotic species, R= rare species, C= common associate.

Species	Location	Status
Abies lasiocarpa	WY	
Achillea millefolium	CO, SD	
Aconitum columbianum	WY	
Actaea rubra	SD	
Agoseris glauca	SD	
Agrostis gigantea	CO	E
Agrostis stolonifera	SD	E
Agrostis spp.	WY	
Alnus incana	WY	
Anaphalis margaritacea	SD	E
Antennaria corymbosa	WY	
Antennaria rosea	WY	
Antennaria spp.	SD, WY	
Aralia nudicaulis	SD	

Table 6 (cont.).

Species	Location	Status
Athyrium filix-femina	SD	
Betula occidentalis	SD	
Betula papyrifera	SD	
Botrychium c.f. *lanceolatum*	WY	
Botrychium simplex	CO, WY	R
Bromus ciliatus	SD	
Bromus inermis	SD	E
Calamagrostis canadensis	SD, WY	
Calamagrostis spp.	SD	
Carex aquatilis	CO, WY	
Carex aurea	SD	
Carex intumescens	SD	
Carex microptera	WY	
Carex nebrascensis	WY	
Carex simulata	WY	
Carex utriculata	CO, SD, WY	
Carex spp.	SD, WY	
Cerastium arvense	WY	
Circaea spp.	SD	
Cirsium arvense	CO, SD	E
Cirsium scopulorum	WY	
Cladonia spp.	SD	
Climacium dendroides	SD	
Cornus canadensis	SD	
Cornus sericea	SD	
Corylus cornuta	SD	
Danthonia spp.	WY	
Dasiphora fruticosa	WY	
Delphinium spp.	CO	
Deschampsia caespitosa	CO, WY	
Epilobium angustifolium	SD	
Epilobium spp.	SD	
Equisetum arvense	WY	
Equisetum palustris	SD	
Equisetum spp.	SD	
Equisetum sylvaticum	SD	
Erigeron spp.	SD	
Eupatoriadelphus maculatus	SD	
Eupatorium maculatum	SD	
Fragaria vesca	CO, SD, WY	
Fragaria virginiana ssp. *glauca*	CO, SD, WY	
Galium boreale	SD	
Galium triflorum	SD	

Table 6 (cont.).

Species	Location	Status
Geranium richardsonii	SD, WY	
Geranium spp.	SD	
Geum macrophyllum	WY	
Geum spp.	SD	
Halenia deflexa	SD	
Heracleum maximum	SD	
Heracleum sphondylium	WY	
Juncus ensifolius	CO	
Juncus tracyi	CO	
Lactuca spp.	SD	
Lathyrus ochroleucus	SD	
Linnaea borealis	SD	
Lycopus spp.	SD	
Maianthemum canadense	SD	
Maianthemum stellatum	SD	
Marchantia spp.	SD	
Mentha spp.	WY	
Mimulus moschatus	CO	
Monarda fistulosa	SD	
Orthilia secunda	SD	
Osmorhiza spp.	SD	
Ostrya virginiana	SD	
Panicum acuminatum	WY	
Pedicularis groenlandica	CO	
Peltigera spp.	SD	
Phleum alpine	WY	
Phleum pratense	SD, WY	E
Physocarpus spp.	SD	
Picea engelmannii	CO, WY	
Picea glauca	SD	C
Pinus contorta	CO, WY	C
Pinus ponderosa	SD	
Poa palustris	WY	
Poa pratensis	WY	E
Poa spp.	SD	
Polytrichum spp.	CO, WY	
Populus tremuloides	SD	
Prunella vulgaris	WY	
Pteridium aquilinum	WY	
Pyrola spp.	SD	
Rhamnus alnifolia	WY	
Ribes spp.	SD	
Rosa spp.	SD	

Table 6 (concluded).

Species	Location	Status
Rubus idaeus	SD	
Rubus pubescens	SD	
Rudbeckia hirta	SD	
Rumex salicifolius	SD	
Rumex spp.	SD	
Salix geyeriana	WY	
Salix lemmonii	WY	
Salix monticola	WY	
Salix planifolia	CO, WY	
Salix spp.	SD	
Sanicula marilandica	WY	
Scirpus acutus	WY	
Sibbaldia procumbens	WY	
Sisyrinchium montanum	SD	
Solidago canadensis	WY	
Solidago spp.	SD	
Spiranthes romanzoffiana	CO, SD, WY	C
Symphoricarpos spp.	SD	
Symphyotrichum spp.	SD	
Taraxacum officinale	CO, WY	E
Taraxacum spp.	SD	
Thalictrum spp.	SD	
Thlaspi arvense	CO	E
Trautvetteria carolinense	WY	
Trifolium pratense	SD	
Trifolium spp.	SD	
Veratrum californicum	CO	
Vicia americana	CO	
Viola adunca	CO	
Viola renifolia	SD	
Viola spp.	SD, WY	

Coniferous trees are common associates of *Botrychium multifidum*, particularly in Region 2. Numerous specimens from Oregon, Idaho, Montana, Wyoming, and Colorado note the presence of *Pinus contorta* with *B. multifidum*. *Botrychium multifidum* has been documented from open sites and wet meadows where *P. contorta* is the dominant species of the surrounding matrix community, with a few trees also present in the meadow (e.g., Bear Park, Colorado). In the Black Hills of South Dakota, *P. contorta* is uncommon, and *Picea glauca* is the dominant coniferous forest species in wet areas, while *Pinus ponderosa* (ponderosa pine) is dominant in drier sites (Kartesz 1999, Crook personal communication 2003). *Picea glauca* grows in all *B. multifidum* occurrences found in South Dakota (Crook personal communication 2003). Two hardwood species (*Ostrya virginiana* and *Corylus cornuta*) are also important forest species in the Black Hills, and each has been documented with one occurrence of *B. multifidum*. In Wyoming and Colorado, *Picea engelmannii* is often reported with *B. multifidum*.

Strawberry species (*Fragaria vesca* and *F. virginiana*) are common associates of many *Botrychium* species including *B. multifidum* (Root personal communication 2003). Like *Botrychium* species, virtually all members of the family Rosaceae have strong arbuscular mycorrhizal relationships (St. John 1996), and it is possible that the co-occurrence of

Botrychium species with strawberries is related to the local mycoflora. Strawberries have been documented with *B. multifidum* in Idaho, Oregon, and in sites in eastern North America. Fragaria vesca is also associated with *B. multifidum* in Czechoslovakia (Somsak 1978).

Carex species are also very frequently documented with *Botrychium multifidum* range-wide and with most occurrences in Region 2. Among the more commonly documented sedges in Region 2 are *C. aquatilis* and *C. utriculata*, both of which occur in *B. multifidum* occurrences in Colorado and Wyoming. *Carex aquatilis* has also been documented with *B. multifidum* in Oregon.

Several species of cool season grasses commonly occur with *Botrychium multifidum*, as documented on numerous herbarium specimens. *Deschampsia caespitosa* is a common associate with *B. multifidum* outside of Region 2 (including Oregon) and is a dominant species in the lakebed at Cabin Lake. Other herbaceous plant species that have been documented repeatedly with *B. multifidum* include *Viola adunca* (OR, CO) and *Pedicularis groenlandica* (ID, CO). Please see the Threats section of this document for a discussion of the exotic species documented with *B. multifidum*.

Mosses are a common component of *Botrychium multifidum* habitat, and due to the close association of some moss genera with *B. multifidum*, they have been used to refine search efforts and locate occurrences. *Botrychium multifidum* is commonly associated with *Polytrichum* species in Virginia, and the presence of this moss at a site in Virginia led to the discovery of an occurrence of *B. multifidum* by Wagner (1946). *Polytrichum* occurs with *B. multifidum* at East Fork Campground in Wyoming (WY EO# 20; **Figure 3**). In the Black Hills, *Climacium* species have been found with almost all the known occurrences of *B. multifidum*, to such an extent that finding it is a signal to start looking for *B. multifidum* (Crook personal communication 2003). Farrar has noted that Rhytidiadelphus species are another common associate of *B. multifidum*, as this moss has similar moisture requirements (Crook personal communication 2003).

While *Botrychium multifidum* is most often found in mossy microhabitats, it may also be found in sites without moss. In the Black Hills, it was observed under dense spruce cover in needle litter with no other green plants (Burkhart personal communication 2005).

Elk grazing occurs at the Cabin Lake site in Colorado (Bernier personal communication 2003) and probably at most other Region 2 occurrences as well. Elk may even selectively graze *Botrychium multifidum* (Root personal communication 2003). The sensitivity of *B. multifidum* to grazing is not known, but some observations suggest that it is at least somewhat tolerant of grazing. It occurs in pastures throughout North America and in mowed lawns in Ohio. Montgomery (1990) found that repeated removal of the leaf of *B. dissectum* (subgenus *Sceptridium*) for three years did not kill plants, and he commented (p. 178), "It is certainly remarkable that these plants persist." Because elk may act as a dispersal agent for *B. multifidum*, grazing in the fall during sporulation may serve an important role in the population biology of this species.

Given its small stature, *Botrychium multifidum* is likely to be outcompeted for light resources. *Pinus contorta* may outcompete *B. multifidum* for water under forested canopies, which may account for the small size of plants near these trees at Cabin Lake (Root personal communication 2003).

Although they do so less commonly than members of subgenus *Botrychium*, species in subgenus *Sceptridium* may grow together in genus communities (Wagner and Wagner 1983). "Genus community" is a term coined by Wagner and Wagner (1983) to describe the peculiar tendency for several *Botrychium* species to be found in close physical proximity. Genus communities of members of *Sceptridium* have not been documented in Region 2, as there are no other members of subgenus *Sceptridium* currently known from the region. In eastern North America, *B. multifidum* has been documented with *B. dissectum*, *B. oneidense*, and *B. ternatum* (Wagner and Wagner 1983). It has been found with *B. simplex* at two sites in Yellowstone National Park (Whipple personal communication 2003) and in Greenland (Ollgaard 1971). *Botrychium multifidum* was also found with another *Botrychium* species in Yellowstone in 2003, probably *B. lanceolatum* (Whipple personal communication 2003).

The coexistence of multiple species of *Botrychium* in genus communities is interesting from a community ecology standpoint. 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 depend on the same resources, coexisting plants are not engaged in direct intraspecific competition. Competition, if it is occurring at all, may be for access to the mycorrhizae. No research has been done on *Botrychium* species with respect to these issues, and these issues are perhaps less relevant for *B. multifidum* since it is less often found in genus communities than other *Botrychium* species. Wagner and Wagner (1983) offer an interesting discussion of this issue from a population biology standpoint.

There are no reports of parasitism or disease reported 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 reports of herbivory on the sporophores of *B. mormo* (Casson et al. 1998, Wagner personal communication 2002). Although dispersal by browsing animals has not been demonstrated for *B. multifidum*, it is possible that elk act as dispersal agents by eating the ripe sporophores. This is potentially advantageous to *B. multifidum* since elk frequent wet meadows and would tend to deposit the spores in favorable germination sites. Small mammals may also function as spore dispersal vectors. The large, thick-walled spores of *Botrychium* species may be an adaptation to dispersal by herbivores (Wagner 1998).

Conservation

Threats

Observations suggest several threats to the persistence of *Botrychium multifidum* in Region 2. In decreasing priority, the primary threats to this species include road and trail construction and maintenance, recreation, exotic species invasion, grazing, effects of small population size, timber harvest, global climate change, and pollution. Fertig (2002) cited logging activity, grazing, habitat conversion, and recreational activities as threats to occurrences in the South Dakota Black Hills and northern Colorado. These threats and the hierarchy ascribed to them are somewhat speculative given the lack of information for Region 2 occurrences. More complete information on the biology and ecology of this species may elucidate other threats. An accurate assessment of threats to this species is an important component of future inventory and monitoring work. The following sections describe the potential of each threat to affect habitats and populations of *B. multifidum*.

Due to its rarity in Region 2 and the small number of plants in known occurrences, any land use activity or unusual stochastic natural even affecting an occurrence of *Botrychium multifidum* may threaten it. Although in other parts of its range this species is found in moderately disturbed areas and depends on some level of natural disturbance, a slight increase in frequency or magnitude of disturbance could extirpate a small population.

Influence of management activities or natural disturbances on habitat quality and individuals

Road and trail construction and maintenance

The potential secondary effects of road construction and maintenance, including hydrologic alteration and weeds, should be considered for their potential impacts on this species. Road construction, widening, maintenance, or re-routing can impact habitat and individuals in occurrences near roads, such as Big Creek Park (Proctor personal communication 2005) as well as several occurrences in Yellowstone National Park.

New road construction threatens occurrences of *Botrychium multifidum* where they pass through occupied habitat. The proliferation of roads and disturbance from construction are likely to encourage the spread of weeds throughout *B. multifidum* habitat, which will increase the threat of exotic species. Roads also threaten occurrences through indirect effects such as erosion, sediment deposition, and hydrologic alteration. The barrier effect of roads is known to have broad demographic and genetic consequences, reviewed thoroughly in Forman and Alexander (1998).

Recreation

The history of the Cabin Lake site in Colorado and the decline of *Botrychium multifidum* at this location suggest several threats to habitat quality relating primarily to hydrologic alteration. After a legal dispute, the Glacier Creek Stables were moved from Sprague Lake to a site west of Cabin Lake and less than 100 m upslope. This facility became fully operational in 2003. Horse trails were also constructed in the area, including one on the east side of the stable, close to the lake. There is speculation that construction of the stable and trail contributed to the drying out of the lake bed by intercepting slope runoff, and that this has resulted in a decline in *B. multifidum* (Root personal communication

2003). However, it is difficult to separate the effects of gradual drying since the dam breached in 1969, drought, and the construction of the trail and stables upslope of the occurrence. In any case, the population trend at this site suggests that *B. multifidum* does not respond well to hydrologic alteration that dries out habitat.

Occurrences of *Botrychium multifidum* in popular hiking areas are threatened by trampling and illegal camping. All known occurrences on the Black Hills National Forest are close to trails. The occurrence at East Fork Campground on the Bighorn National Forest (WY EO# 20) is vulnerable to trailing, trampling, illegal campsites, and other impacts from concentrated human use of the area. A trail passes through the meadow occupied by *B. multifidum* at Ryan Park, which is used by hikers, fishermen, and hunters and may receive seasonally heavy use. Some recreational use also occurs at the Beaver Dam Park occurrence (Proctor personal communication 2005). There is no evidence that occurrences in Region 2 have experienced significant impacts due to trail use.

Off-highway vehicle use is a common and growing threat to rare plants and to biodiversity in general. Off-highway vehicle use of public lands has burgeoned in recent years, and illegal off-road activity in closed areas has also increased (Bureau of Land Management 2001). The proximity of known occurrences of *Botrychium multifidum* in Region 2 to large metropolitan areas and to popular tourist destinations leaves this species especially vulnerable to off-highway vehicle impacts since some areas may be heavily used. However, there is no existing evidence that any occurrence has been impacted by off-road vehicle use in Region 2.

Grazing

Grazing offers both potential benefits and detriments with regard to *Botrychium multifidum*, but the impacts of grazing on population viability remain poorly understood. *Botrychium multifidum* is found in pastures outside of Region 2, suggesting that it tolerates some level of grazing. Similarly, elk use the meadow habitats of *B. multifidum* and may even selectively graze the species. Disturbance of the surface by cattle may injure some individuals (above and below ground). Grazing can eliminate a season's contribution to the spore bank (**Figure 15**; Johnson-Groh and Farrar 2003). Nine occurrences of *B. multifidum* in Region 2 are known to occur within active grazing allotments (**Table 7**). Grazing and trampling are potential threats to the occurrence of *B. multifidum* at Bear Park, Colorado (Proctor personal communication 2003). Grazing is also

Figure 15. A grazed individual at headwaters of Soldier Creek, Medicine Bow National Forest, Carbon County, Wyoming. Photograph provided by John Proctor.

Table 7. Status of grazing allotments on National Forest System lands of Region 2 where *Botrychium multifidum* has been reported

Occurrence	Location	Allotment Name	State	National Forest	Allotment Status
Steve O'Kane, Arnold Clifford, and Dave Jamieson (# 5871 at SJNM)	Between Fish Lake and Blue Lake	Fish Lake	CO	San Juan	Unknown.
Proctor personal communication 2004	Bear Park	Sawmill Creek	CO	Medicine Bow	Active, 300 cow-calf pairs, grazed July 26- September 30.
CO EO#4	Trap Lake	Corral Park	CO	Arapahoe Roosevelt	Not active. Last active in 1984 or 1985; was grazed from July–September, 240 cow-calf pairs. Sheep grazing may have taken place up until the 1920's or 1930's. Area is now heavily used by moose and elk.
CO EO#2	Elk River	Farwell Mountain	CO	Routt	Active sheep allotment. Has been combined with the Big Red Park Allotment. Used July 4-September 25. 1200 ewe-lamb pairs on 20,126 acres.
CO EO#5	Savage Basin	Bridal Veil	CO	San Juan	Unknown.
BOMU 1, BOMU 2, BOMU 6; SD EO #1	Lost Cabin Creek	Spokane and non-allotment	SD	Black Hills	Not grazed.
BOMU 7; SD EO #3	Upper Iron Creek	Non-allotment	SD	Black Hills	Not grazed.
BOMU 4 and 5/ SD EO #4	Iron Creek	Spokane	SD	Black Hills	Not grazed
Corey personal communication 2003, Crook personal communication 2003	Butcher Gulch	Bear Butte	SD	Black Hills	Active, but no evidence of cattle use in 2005; some possibility of impacts from trailing.
BOMU 3/ SD EO#2	Palmer Gulch	Palmer Gulch	SD	Black Hills	Not grazed.
WY EO#1	Head of Encampment River	Encampment	WY	Medicine Bow	Active, three permittees, 381 cow-calf pairs total. Grazed July through October (specific dates depend on the permit).
WY EO#18, Beaver Dam Park, East Fork Meadow (Proctor personal communication 2005)	Headwaters of Soldier Creek	Wood Mountain	WY	Medicine Bow	Active, moderately stocked, two permittees, 300 cow-calf pairs. Grazed July 15 through September 30. Some individuals are within an exclosure constructed in 2005 to improve functional condition of the riparian area.
Proctor personal communication 2004	Brush Creek	North Brush Creek	WY	Medicine Bow	Active, 264 cow-calf pairs. Grazed July 1-October 10.
WY EO#20	East Fork Campground	Big Goose C&H	WY	Bighorn	Active; plants in an exclosure but cattle were grazing inside.

occurring at Big Creek Park on private land (Proctor personal communication 2005). At Beaver Dam Park, *B. multifidum* persists in an area that received concentrated historic livestock use, suggesting that it is somewhat tolerant of grazing and soil compaction (Proctor personal communication 2005). However, Burkhart (personal communication 2005) noted that occurrences in the Black Hills tend to be found in ungrazed areas. At Ryan Park, microsites occupied by *B. multifidum* appear be grazed more heavily than adjacent wet *Carex* meadows (Proctor personal communication 2005). The tolerance of *B. multifidum* to horse grazing is unknown, but this is a potential threat to the plants at Cabin Lake if horses (stabled within 100 meters of the occurrence) graze there.

Livestock tend to favor grasses, permitting the competitive release of broad-leaved plants, which may benefit *Botrychium multifidum* in some situations. However, using cattle grazing as a management tool for the enhancement of habitat is risky for a plant as locally rare as *B. multifidum*, 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 may introduce invasive plants. Although *B. multifidum* appears to have survived considerable degradation of a riparian area resulting from overgrazing, the effects this had on its population cannot be known without baseline data.

Effects of small population size

Demographic stochasticity is the variation over time in vital rates such as recruitment and survival, and it is generally only a concern for very small populations. Because there are limited data for population sizes in Region 2, the degree to which many occurrences are threatened by demographic stochasticity is unknown. However, reported numbers of individuals at all occurrences of *Botrychium multifidum* in Region 2 fall below the generally accepted minimum effective population size of 50 reproductive individuals (after Soulé 1980). This number is considered to be what is necessary to buffer against the probability that a fluctuation in vital rates will take a population to the extinction threshold.

Environmental stochasticity generally refers to variation over time in the physical and biological environment. For a single population, this includes natural events occurring at random intervals that kill a large proportion of the individuals present. Such events may occur very rarely, yet still significantly impact population persistence (Menges 1991). Multiple occurrences located in close proximity can have a mitigating effect against the impacts of environmental stochasticity. However, studies of other *Botrychium* species suggest that there is little genetic connectivity among the known occurrences of *B. multifidum* in Region 2. Thus, these occurrences are more vulnerable to extirpation by catastrophic local disturbances and events such as fire, landslides, and disease.

Timber harvest

The impacts from timber harvest include soil compaction, disruption of the surface soil horizons, changes in light levels, and loss of soil nutrients and moisture (Johnson-Groh and Farrar 2003). Thus it might be expected that occurrences of *Botrychium multifidum* within a timber harvest area would be negatively impacted. However, most occurrences in Region 2 are protected from direct impacts of timber harvest because they occur adjacent to streams or within wetlands, and therefore are within buffer zones established to protect these resources. For example, the occurrence in Bear Park, Colorado (Proctor personal communication 2004) is in the Saw Mill Creek timber allotment, but it is not directly threatened by timber harvest activities since it is adjacent to a creek. Secondary impacts of timber harvest such as road building or other activities may directly impact occurrences of *B. multifidum*, or indirectly impact them by altering the hydrology or increasing sedimentation and erosion in *Botrychium* habitat. Burial by sediment has killed individuals of other *Botrychium* species (Johnson-Groh and Farrar 2003).

Global climate change

Global climate change is likely to have wide-ranging effects in the near future for all habitats, although the direction of projected trends are yet to be determined, and predictions vary based on environmental parameters used in predictive models. For example, Manabe and Wetherald (1986) demonstrate projections based on current atmospheric CO_2 trends that suggest average temperatures will increase while precipitation will decrease in the western United States. Decreased precipitation could have dire consequences for occurrences of *Botrychium multifidum* in Region 2 if it results in drying of the required moist habitats. However, Giorgi et al. (1998) showed that temperature and precipitation in the western United States increased under simulated doubling of atmospheric CO_2 levels. Either scenario could significantly affect the distribution of montane grasslands in Region 2. Temperature increase, predicted by both models, could cause vegetation zones to climb 350 ft. in elevation for every degree F of warming (U.S. Environmental Protection Agency 1997). Global climate change can be expected to severely impact occurrences at the southern edge of this species' natural range, pushing it north in latitude and up in elevation. With extensive habitat disturbance in the path of potential migration, many organisms will not be able to move. Thus, preserves may provide short-term protection, but these may be ineffectual if organisms are trapped without suitable corridors for migration. Although *B. multifidum* has the potential for long-distance dispersal of its spores and thus may be able to colonize new sites, the pace of climate change and habitat shift may be too rapid for the species to establish new colonies before old ones are extirpated.

Pollution

Atmospheric nitrogen deposition has become an important agent of vegetation change in densely populated regions (Köchy and Wilson 2001). Nitrogen loading and vegetation change have been observed to be greatest near large metropolitan areas (Schwartz and Brigham 2003). Nitrogen enrichment experiments show that nitrogen is limited in pristine habitats (Gross et al. 2000). Measurable impacts from nitrogen pollution might therefore be expected in many parts of Region 2, especially in Colorado. Elevation of nitrogen levels is likely to cause a few generalist species to increase in abundance while many others decline (Schwartz and Brigham 2003).

Interaction of the species with exotic species

Noxious weeds have not been documented as directly causing a decline in any *Botrychium* species (Johnson-Groh and Farrar 2003), but their mutual affinity for disturbance may leave *Botrychium* species and their habitat vulnerable to negative impacts from weeds. Impacts include competition for water and light and possibly allelopathic effects. Chemical, biological, and mechanical measures to control weeds may also negatively affect non-target plant species. At Cabin Lake, Colorado, exotic species observed by the author include Canada thistle (*Cirsium arvense*), field pennycress (*Thlaspi arvense*), timothy (*Phleum pratense*), and dandelion (*Taraxacum officinale*). None of these species are currently managed in Rocky Mountain National Park (Bernier personal communication 2003). *Cirsium arvense* is considered a noxious weed (Colorado Department of Agriculture 2001) and is particularly invasive. It has spread over approximately 30 percent of the bed of Cabin Lake and poses a significant threat to this occurrence of *B. multifidum*. *Cirsium arvense* is also present in two occurrences in South Dakota (BOMU 3 and BOMU 4). Somsak (1978) noted the presence of *C. arvense* and white clover (*Trifolium repens*) with *B. multifidum* in Czechoslovakia. Kentucky bluegrass (*Poa pratensis*) is also a species of concern at Cabin Lake, although it has not been documented there, because it is often invasive in these habitats (Connor personal communication 2003) and could easily be introduced by the horses that use the area.

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). Because *B. mormo* is an obligate mycorrhizal symbiont, this poses a significant threat. Most earthworm activity takes place in the O horizon (Langmaid 1964) while mycorrhizal activity is greatest at the boundary of the O and A horizons (Smith and Read 1997). The activity of earthworms eliminates the duff layer and shifts plant species composition in *B. mormo* habitat (Berlin et al. 1998, Gundale 2002). Although earthworms present a possible threat to *B. multifidum*, no research has shown that species of *Botrychium* other than *B. mormo* are currently being affected by them. Earthworms are a diverse group of more than 3,500 species worldwide, and the expansion of global commerce and recreational fishing may increase the potential for exotic earthworm invasions and related adverse effects on soil processes and plant species (Hendrix and Bohlen 2002).

Threats from over-utilization

There are no immediate threats to *Botrychium multifidum* from over-utilization. Vouchers are valuable and assist greatly with taxonomic research on *Botrychium* species. Weber and Wittmann (2001a) recommend collecting plants without 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.

Where occurrences are already of questionable viability (fewer than 50 plants), collection of material from them, even if the plants survive, is a risky endeavor. For example, in a population of three sporophytes, there is almost no margin of error, and accidentally removing an apical bud could easily result in the extirpation of the species at this site. In occurrences of fewer than 20 plants, Johnson-Groh and Farrar (2003) state that no collections should be made and instead recommend that photographs be taken and deposited at an herbarium. This is a difficult issue for some *Botrychium* species, since collection and verification by an expert is the only way to be certain of correct identification.

There are no known commercial uses for *Botrychium multifidum*. 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 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 readily cultivated, any commercial use would require the harvest of wild populations.

Conservation Status of Botrychium multifidum in Region 2

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

No quantitative research has been conducted on population trends for *Botrychium multifidum* in Region 2, and there is little information available on which to base estimates of population trend. Some occurrences in Region 2 appear to be declining. Dramatic declines have been seen at Cabin Lake, Colorado, where more than 100 plants were reported in 1989, but only six in 2003. The occurrence at Butcher Gulch, South Dakota has not been seen since 1994 despite repeated search efforts, and the area burned in 2002. Several occurrences in Region 2 are known only from specimens collected more than 50 years ago; their status is highly uncertain. Fertig (2002) noted that "populations appear to be in decline in South Dakota and may be in decline in Colorado. Wyoming populations are considered stable…", but he did not include data to support this statement. As discussed in the Threats section of this assessment, all occurrences of *B. multifidum* in Region 2 are small enough to be vulnerable to the effects of demographic and environmental stochasticity. Please see the Population Trend section of this assessment for more information on occurrence status in Region 2.

Do habitats vary in their capacity to support this species?

Ecological succession naturally leads to declining habitat suitability for *Botrychium multifidum* in the absence of a disturbance regime. Variables such as fire frequency, amount of litter, light availability, soil moisture variation, and soil texture may affect habitat suitability. The ability of a site to support mycorrhizae is equally important for *Botrychium* species. 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

The wetland and riparian habitats required by *Botrychium multifidum* are the most threatened habitats in the semi-arid western United States. These sites are often heavily used by humans and are often subject to hydrologic alterations that could render them unsuitable for *B. multifidum*. Abusive land use practices, such as overgrazing, have led to erosion and stream entrenchment in many areas, with the result that many potential sites for *B. multifidum* in Region 2 have dried out. The slightly drier microsites occupied by *B. multifidum* at some locations appear to be more heavily grazed by livestock, who prefer these areas to keep their hooves dry.

The apparent tendency of *Botrychium* species, including *B. multifidum*, to reproduce by selfing may leave them genetically inelastic and vulnerable to ecosystem change. While reproduction by cloning may be beneficial 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.

Managing habitats to maintain the viability of any plant species that appears to benefit from disturbance is challenging. The lack of a natural disturbance regime can be as detrimental as the presence of an unnatural one. For many *Botrychium* species, probably including *B. multifidum*, disturbance creates suitable habitats, but it also sets in motion processes of succession that may ultimately result in unsuitable habitat. The frequency and intensity of the disturbance that most benefits *B. multifidum* is not known, but the availability of early- to mid-seral habitats appears to be critical to the persistence of this species.

Because much of their life history occurs under ground, and because they are generally small cryptic plants, *Botrychium multifidum* plants are easily overlooked and are thus poorly understood and easily missed. Occurrences that have not been noticed are at particular risk of extirpation.

Evidence of populations in Region 2 at risk

The rarity and small populations of *Botrychium multifidum* in Region 2 suggest that it is highly vulnerable to extirpation locally or even regionally, and that all occurrences in Region 2 are at risk. Of the 20 documented occurrences of *B. multifidum* in Region 2, five have not been seen again in more than 20 years (**Table 4**). The current status of these occurrences is uncertain. Stochastic processes and normal environmental variation could easily result in extirpation of the small and localized occurrences known in Region 2. The quality and availability of habitat in Region 2 may have declined due to fragmentation, exotic species invasion, hydrologic alteration, and edge effects that decrease the number and quality of patches of wet meadow habitat. Because the ecology of this species is poorly understood, current management may be negatively impacting this species despite good intentions.

Current data suggest a high degree of imperilment for *Botrychium multifidum* in Region 2, but these data are sparse and probably incomplete. It is very likely that more occurrences will be found in Region 2 with further targeted surveys and the involvement *Botrychium* experts, but the known patterns of distribution and abundance of *B. multifidum* in Region 2 suggest that large, robust populations are unlikely to be found. This underscores the need to conduct additional survey work for this species and to rigorously monitor the known occurrences.

Management of Botrychium multifidum in Region 2

Implications and potential conservation elements

The viability of all occurrences of *Botrychium multifidum* in Region 2 may be affected by factors contributing to habitat loss, alteration of hydrologic regime, succession, and fire regime.

Succession and woody plant encroachment threaten many *Botrychium* species; few are found in heavily shaded sites. Succession is a particular threat to *Botrychium* species in meadow habitats (Johnson-Groh and Farrar 2003). The degree to which *B. multifidum* is threatened by succession is less clear since it is reported from shaded forest sites. However, there is evidence to suggest that the reproductive output of shaded plants is lower than that of plants growing in full sun. Dispersal may also be more effective in open sites where the wind can be a dispersal agent. In Ohio, overshading by woody species as a result of succession is considered a threat (Schneider 1993). Succession is a plausible threat in some Region 2 occurrences but is unlikely in others, such as those near permanent water sources such as lake shores, streams, and some wet meadows. Succession may occur in Yellowstone occurrences as geothermal activity shifts, but open conditions are likely to persist in most locations.

The activity of beavers alters the hydrology and physiography of mesic bottomland habitats and may be beneficial or detrimental to *Botrychium multifidum* depending on the situation. Beaver dams have been reported near two occurrences of *B. multifidum* in Jackson (Bear Park) and Routt (Elk River) counties, Colorado (Proctor personal communication 2004). It is possible that the persistence of *B. multifidum* at these sites depends on beaver dams raising the water table. Beaver dam construction may open the canopy in forests near wet meadows, and it may also drown plants.

The work of Montgomery (1990) on the demography of *Botrychium dissectum* suggests that factors contributing to increased adult mortality are more likely to reduce fitness than factors that reduce fecundity. This suggests that the removal of sporophores by herbivores, or even ecological succession that increases shading and reduced reproductive output, is less deleterious than factors such as ground disturbance or hydrologic alteration that kill adult *B. multifidum* plants.

Although fire is not known to be directly detrimental to *Botrychium*, secondary effects (i.e., erosion, sedimentation, drying of the soil due to greater insolation) may have a greater impact than the fire itself (Johnson-Groh and Farrar 2003). Fire may have impacted the Butcher Gulch occurrence in South Dakota (Crook personal communication 2003). Fire impacts individuals directly by burning their aerial portions, but *Botrychium* species including *B. multifidum* appear to suffer no ill consequences of this (Johnson-Groh and Farrar 2003). Unusually hot fires could result in mortality if the apical bud is killed, but because the species is strongly dependent on mycorrhizae, removal of leaf tissue via burning or other means is probably inconsequential to the plant's survival (Montgomery 1990, Wagner and Wagner 1993, Farrar and Johnson-Groh and Farrar 1996, Johnson-Groh 1999). Fires that occur in the summer or fall (when forest fires are most common) would destroy reproductive output for that year and might kill spores lying near the surface (Root personal communication 2003).

The most current data available suggest that *Botrychium multifidum* is imperiled in most of Region 2 because the few known occurrences all have small population numbers and are therefore vulnerable to extirpation. The loss of any occurrence is significant and may result in the loss of genetic diversity of the species. Further research is needed before meaningful suggestions can be offered regarding restoration policy. Please see the Tools and Practices and Threats sections of this assessment for information on mitigating threats resulting from management.

Desired environmental conditions for *Botrychium multifidum* include sufficiently large areas where the natural ecosystem processes on which *B. multifidum* depends can occur, permitting it to persist unimpeded by human activities and their secondary effects. This includes sufficient ecological connectivity between known occurrences and suitable unoccupied habitat to allow *B. multifidum* metapopulation dynamics to operate. Critical habitat variables for *B. multifidum* include maintaining a stable, relatively high water table and early- to mid- seral or other open conditions. Given the current lack of detailed information for this species in Region 2, it is unknown how far this ideal is from being achieved. It is possible that most or all of the ecosystem processes on which *B. multifidum* depends are functioning properly at most occurrences. Further research on the ecology and distribution of *B. multifidum* will help to develop effective approaches to management and conservation. Until a more complete picture of the distribution and ecology of this species is obtained, priorities lie with conserving the known occurrences.

Since the majority of *Botrychium multifidum* occurrences in Region 2 are located on National Forest System lands, designation of special interest areas and research natural areas could help to ensure the protection of this species. What we know of *B. multifidum* in Region 2 suggests that the occurrences of highest conservation priority are the robust populations found in the Black Hills National Forest in South Dakota. While they are within protected areas on the Black Hills National Forest, actions may be needed to ensure that current management practices, which appear to be compatible with the needs of *B. multifidum*, continue. Conservation action may be needed to ensure the viability of other occurrences found in areas currently managed for multiple use, including Bear Park on the Medicine Bow National Forest (Proctor personal communication 2004), where the largest occurrence in Colorado is located, the East Fork Campground on the Bighorn National Forest (WY EO#20), and at the headwaters of Soldier Creek in southwestern Carbon County, Wyoming on the Medicine Bow National Forest (WY EO#18). Further survey work may reveal additional occurrences to be added to the conservation priorities for *B. multifidum* in Region 2.

Tools and practices

Species and habitat inventory

Grapeferns are difficult to find, and success in finding them requires considerable effort (Wagner 1946, Wagner and Wagner 1976). Because of its protracted period of vernation and its evergreen leaf, *Botrychium multifidum* can be found throughout much of the year. However, the plant is much easier to see in the fall when it is most likely to be sporulating and surrounding vegetation is no longer green (Root personal communication 2003). Late in sporulation, the sporophore turns yellow, which can make these plants easier to find, but this does not occur until late in the growing season when most botanical field work is finished (Root 1995). To some extent, the lack of botanizing that occurs during the fall may account for the lack of information and apparent rarity of this species (Root personal communication 2003).

Shade forms of *Botrychium multifidum* are very small and especially difficult to find (**Figure 4**). The small leaves look like patches of liverwort and are easily overlooked (Root 1995). However, small plants growing on the open floor of a *Pinus contorta* forest may be easier to find than larger plants growing under dense meadow vegetation (Farrar personal communication 2003).

Johnson-Groh and Farrar (2003) offer excellent suggestions for conducting *Botrychium* surveys. Their protocols provide a standard methodology for all survey work for subgenus *Botrychium* and subgenus *Sceptridium*. The methodology assumes that one week is available to search approximately 25 high priority sites per year, for about one hour each. Four or five people walk transects at each site for better coverage during the search. When plants are discovered, they are marked with pin flags and the surrounding area searched intensively (but carefully) on hands and knees. Limitations of these methods include low confidence in locating occurrences due to population variability (more problematic for species with frequent dormancy), difficulty in seeing the plants, predominance of below-ground lifecycle stages, and the occurrence of many species in genus communities, which complicates identification. Popovich (personal communication 2003) implemented this protocol and noted that the area searched appeared to have been heavily impacted by the searchers. A crew of four or five qualified personnel can also be expensive. Johnson-Groh and Farrar (2003) recommend using the "timed meander search procedure" described by Goff et al. (1982).

Limitations in time and funding result in attempts to locate rare plants by searching for multiple species in large areas. While this approach can be effective in finding occurrences of large flowering plants, it may not be effective for *Botrychium multifidum* given the difficulty in finding and identifying the species. Because searching for *B. multifidum* requires the searcher's undivided attention, attempts to search for this species are more likely to be successful if the search is limited to other *Botrychium* species. Using experts (i.e., contractors, agency botanists, or others trained and experienced in searching for *Botrychium*) to conduct searches in appropriate habitat may be the most effective approach to expanding our knowledge of the distribution of this species in Region 2.

Given the known habitat affinities of *Botrychium multifidum*, there are areas in Region 2 where further species inventory work is likely to reveal previously unknown occurrences (detailed below). While these are good places to target searches, *B. multifidum* is found over a wide range of elevation and is somewhat erratically distributed, suggesting that it could be found in any mountain wetland in Region 2.

In Colorado, *Botrychium multifidum* is "to be expected on the east base of the Park Range in North Park" (Weber and Wittmann 2001a, p. 26). The Routt National Forest has much wetland habitat (Fertig 2002, Proctor personal communication 2003). Ongoing surveys by Proctor, Haas, Heidel, and others on the Medicine Bow National Forest have led to discoveries of new occurrences every year since 2003. The alluvial fan area in Horseshoe Park (Rocky Mountain National Park), including the bed of the now-drained Fan Lake, also contains potentially suitable habitat. However, an assessment of this area in 2003 suggests that it has dried out and may no longer be suitable for *B. multifidum* (Root personal communication 2003). Further searching in the areas around Cabin Lake, Sprague Lake, and Bierstadt Lake is also needed (Root 1995). Further inventory work is also needed to reassess historic occurrences throughout Colorado.

In Wyoming, occurrences are most likely to be found in the Medicine Bow and Shoshone national forests (Fertig 2002). Further exploration in the Greater Yellowstone Ecosystem will certainly yield more discoveries. Although suitable habitat is present, there have been no surveys for *Botrychium multifidum* in the Shoshone National Forest immediately east of Yellowstone National Park (Heidel personal communication 2003, Whipple personal communication 2003).

The Black Hills National Forest is in need of further species inventory and is likely to yield additional occurrences (Fertig 2002, Crook personal communication 2003, Burkhart personal communication 2005). Some areas where further inventories are needed include the Harney Peak area near Custer and the Northern Hills region. There is also more unsearched habitat similar to known occurrences in South Dakota north of Harney Peak and the Black Elk Wilderness, from Custer to Hill City and to the east. *Picea glauca* is abundant in drainages in the Hell Canyon, Mystic, and Northern Hills Ranger Districts, particularly where these three districts join (Crook personal communication 2003). It is likely that *Botrychium multifidum* occurs sporadically along most creeks in the vicinity of the known occurrences on the Black Hills National Forest (Burkhart personal communication 2005). Farrar is of the opinion that *B. multifidum* will probably be found at Cheyenne Crossing on US 85 in South Dakota, near the Wyoming border (Crook personal communication 2003).

Aerial photographs and topographic, soil, and geology maps are effective tools for defining survey areas. These tools are most effective for species for which we have basic knowledge of the habitat requirements such as *Botrychium multifidum*, and from which distribution patterns and potential search areas can be deduced. The use of deductive and inductive techniques to model the distribution of *B. multifidum* will aid in refining areas targeted in survey efforts in Region 2. Species distribution modeling is an effective means of determining the extent of suitable habitat on USFS lands. Classification and Regression Tree (CART) analysis has been used to model the distribution of other sensitive plant species in Wyoming (e.g., Fertig and Thurston 2003). Combining CART analysis with envelope models such as DOMAIN, BIOCLIM, or

MaxEnt can help to refine a potential distribution map further by adding inference on the probability of *B. multifidum* occurrence (Thuiller et al. 2003, Beauvais et al. 2004). CART techniques for predicting species distributions are reviewed extensively by Scott et al. (2002). A problem with the models described above is that they do not account for ecologically relevant events that occurred in the past. Historic land use practices may have extirpated occurrences of *B. multifidum* in Region 2, but without a geospatially explicit dataset of these practices this factor cannot be included in the model. Plans are underway to develop a potential habitat map for *B. multifidum* to facilitate search efforts in the Black Hills (Crook personal communication 2003).

In general, the best areas to search for any *Botrychium* species are places that have been disturbed in the past one to three decades (Wagner and Wagner 1976, Buell 2001, Johnson-Groh personal communication 2003, Root personal communication 2003, Whipple personal communication 2003). Wagner and Wagner (1976) included old pastures, pasture-woods, second-growth fields, edges of paths, old apple orchards, fencerows, floodplains subject to immersion, young pine woods, deserted homesteads, and natural deer pastures among a list of good areas to search. Wagner and Wagner (1976) noted that areas within 10 to 20 ft. of the forest edge are particularly good sites to search, and specimen label data from Region 2 and elsewhere support this statement. Drained lakes and wet sites such as beaver dam areas where there has been some disturbance are also promising habitats for *B. multifidum*. However, undisturbed or little disturbed wetlands with peaty soils are also good areas to search for *B. multifidum*.

Using commonly associated species to identify and refine search areas is a very effective approach to species inventory work for *Botrychium multifidum*. Most Region 2 occurrences have been found within *Pinus contorta* or *Picea glauca* forests. The presence of haircap moss (*Polytrichum* spp.) was used successfully to identify search areas in Virginia (Wagner 1946, Wagner and Wagner 1976). The presence of *Polytrichum* species at a newly discovered occurrence in Jackson County, Colorado suggests that this may also be a useful filter in Region 2. Other moss species (*Climacium* spp., *Rhydidiadelphus* spp.) have been used to identify suitable habitat for *B. multifidum* on the Black Hills National Forest (Crook personal communication 2003). Please see the Community Ecology and Habitat sections of this document for other environmental correlates and commonly associated species.

Soil properties can be used to refine search areas for *Botrychium multifidum*. It is almost always found in sandy, slightly acidic soils in Region 2 and elsewhere, but it has also been found in silty and granitic soils in Region 2. Soils that support moonwort communities in four states outside Region 2 averaged 916 ppm of calcium and 140 ppm of magnesium, suggesting that soil nutrient levels may be a useful filter in the selection of potential search areas (Hansen and Johnson-Groh 2003).

Population monitoring

Although the <u>*Botrychium* Inventory and Monitoring Technical Guide</u> (Johnson-Groh and Farrar 2003) has been designed for members of subgenus *Botrychium*, the monitoring protocols it sets forth are also appropriate for use with *B. multifidum* (Johnson-Groh personal communication 2003). Many different methods have been used to monitor other *Botrychium* species (e.g., Montgomery 1990, Mueller 1992, Kelly 1994, Johnson-Groh and Lee 2002) that could be suitable for use in monitoring *B. multifidum*, depending on the question to be addressed. Protocols for monitoring *B. lineare* have been drafted for the Pike-San Isabel National Forest (Carpenter 1996); these may have some relevancy for *B. multifidum*. However, using the protocols described by Johnson-Groh and Farrar (2003) consistently will facilitate comparison of monitoring data from sites throughout the United States.

It is difficult to accurately monitor population trends for species such as *Botrychium multifidum* where the proportion of dormant plants may vary from year to year (Lesica and Steele 1994). Recent advances in mark-recapture methods may be useful for sampling *B. multifidum* occurrences. These methods have been applied to plants successfully, and are useful for sampling populations that may exhibit prolonged dormancy or may be difficult to find in concealing vegetation (Alexander et al. 1997). Prolonged dormancy of individual plants also requires consideration in sampling design.

Population trend monitoring protocols pertaining to members of subgenus *Botrychium* are defined in Johnson-Groh and Farrar (2003). Trend monitoring is most appropriate because demographic monitoring is expensive and generally does not yield information on range-wide trends. However, tracking individuals within sampling units can help to better understand life span, dormancy, recruitment success, and population trends. Conducting an annual census of above-ground plants at as many occurrences as possible will provide data from which regional population trends can be estimated. Individual *B. multifidum* plants have been marked using pins with flagging at Cabin Lake (Bernier personal communication 2003).

Establishing photopoints and photoplots following the protocols described in Elzinga et al. (1998) and Hall (2002) will help to track habitat suitability and ecological succession. These techniques can be done quickly in the field, and although they do not provide cover or abundance data, they can help to explain patterns observed in quantitative data.

Monitoring the reproductive output over several years of *Botrychium multifidum* is needed to compare the fertility of plants in shaded versus open sites (Crook personal communication 2003). Experimental manipulation could reveal whether canopy removal can spur reproduction in *B. multifidum* plants growing in shaded sites. Such studies could help to determine appropriate management protocols for *B. multifidum*.

At present the priorities for Region 2 lie in inventory work and in annual censusing of the few known occurrences. Marking and mapping individuals would also provide valuable information if done with great care, but this is much more labor intensive. Johnson-Groh (1999) notes that it can be difficult to be assured that an individual that had been marked in one year is the same individual in subsequent years.

Presence/absence monitoring is not suitable for *Botrychium multifidum* due to the small occurrence 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).

Root (1995) established population monitoring plots for *Botrychium multifidum* at Cabin Lake in Colorado. Ten 1-m^2 quadrats were established and sampled in 1994. Three quadrats are in the open zone outside the band of young pines, four are under the young pines, and three are in the meadow that occupies the center of the lake bed. The quadrats were not placed randomly but to include the clusters of plants at this location. The study was initially designed to monitor this occurrence for at least five years, but only the data from 1995 are available and a census from 2003. Plans are underway to monitor *B. multifidum* within a cattle exclosure on the Wood Mountain grazing allotment on the Medicine Bow National Forest. This will provide an opportunity to study the response of *B. multifidum* to the removal of grazing (Proctor personal communication 2005). Monitoring is also planned for *B. multifidum* occurrences on the Black Hills National Forest (Burkhart personal communication 2005).

Beneficial management actions

Further inventory and monitoring efforts would be highly beneficial to *Botrychium multifidum*. Locating high quality occurrences with robust populations will help managers to prioritize conservation efforts. Developing a better understanding of the species' centers of distribution will assist with the development of regional management protocols that favor the persistence of *B. multifidum*. Because *B. multifidum* is inconspicuous and many occurrences may remain undocumented, searches prior to ground-disturbing management actions within potential habitat would help to alleviate human-related threats to this species.

Some observations suggest that disturbance that creates forest openings may be necessary for successful reproduction of *Botrychium multifidum*. However, these observations are anecdotal and have not been verified by using a systematic approach. The observation of non-sporulating juveniles under lodgepole canopies in the western United States raises the question of whether managing occurrences by cutting trees or trimming lower limbs might promote reproduction in these plants (Farrar personal communication 2003). Maintaining habitat in an open condition is the most prudent management decision until more is known about how plants respond to a closing canopy (Johnson-Groh and Farrar 2003). Removing woody species when the ground is frozen would minimize the risk to *B. multifidum* sporophytes and gametophytes.

The utility of fire as a habitat management tool for *Botrychium multifidum* is not known. Evidence suggests that fire is not directly detrimental to *Botrychium* (Johnson-Groh and Farrar 2003). Although burning appears to have positive effects on *B. campestre* occurrences in Iowa, fire combined with erosion and desiccation, both natural results of severe fire, may be deleterious (Johnson-Groh and Farrar 1996, Johnson-Groh 1999). The role of fire in the autecology of *Lycopodium* suggests that it might also play a role in the autecology of *B. multifidum* (Crook personal communication 2003). Fire may play a role in preventing succession to closed canopy and in creating openings upon which *B. multifidum* apparently depends for successful reproduction. There have been no recommendations of the appropriate season for prescribed fire to minimize impacts to *B. multifidum*. It is likely that fire outside the growing season (after sporulation in the fall, or before spring growth) is least deleterious.

Beneficial management actions with respect to grazing are unclear, but there is evidence to suggest that *Botrychium multifidum* is at least somewhat tolerant of grazing. With respect to grazing, Johnson-Groh and Farrar (2003) wrote: "Managers must not arbitrarily increase or decrease grazing because of the moonworts. Understanding the history of land management, including frequency of grazing, number of grazing animals, and timing of grazing will allow managers to determine appropriate levels of grazing to maintain populations. Removing grazing or increasing grazing cannot be expected to maintain populations." Please see the Community Ecology and Threats sections of this document for more information on grazing.

Any management strategies designed to prevent weed infestation of *Botrychium multifidum* habitat are likely to confer benefits. Eradication of noxious weeds from *B. multifidum* occurrences is a beneficial action, considering the potential negative impacts of weeds. Aggressive management of *Cirsium arvense* in all locations where it is found in or near occurrences of *B. multifidum* could avoid expensive future eradication efforts. Where possible, hand pulling should be the preferred method of managing weed populations within occurrences of *B. multifidum*. However, this is not suitable for managing *C. arvense*. Use of herbicides within *B. multifidum* occurrences should be limited to hand application directly to the target weed using a wick.

Mitigating threats to occurrences of *Botrychium multifidum* from ground-disturbing uses such as off-road vehicles is likely to confer benefits to the species. Off-road vehicle impacts are potentially highly deleterious to a long-lived species such as *B. multifidum*. Controlling motorized access to habitat and providing appropriate signage at access points may decrease impacts to *B. multifidum*.

Occurrences of *Botrychium* are naturally variable in size (Johnson-Groh 1999). All Region 2 occurrences are small, increasing the likelihood of local extirpation. *Botrychium multifidum* may depend on a metapopulation structure that relies on the availability of a "shifting mosaic of suitable habitats" in appropriate successional stages that *B. multifidum* can colonize (as described by Pickett and Thompson 1978). If this is the case, understanding the metapopulation dynamics of *B. multifidum* becomes crucial to its management and conservation, and underscores the need to conserve nearby areas of suitable habitat that are not currently inhabited by *B. multifidum*.

Restoration

Botrychium multifidum can be propagated from spores with some difficulty in humus-rich, acidic, loamy soil (Whittier 1972, Gifford and Brandon 1978, Lellinger 1985, Rook 2003). No spores of *B. multifidum* are currently in storage at the National Center for Genetic Resource Preservation (Miller personal communication 2003). Collection of spores for long-term storage may be useful for restoration work. Herbarium specimens and wild populations (with careful restraint) may also provide spores for restoration. The required length of winter dormancy of *B. virginianum* is known (Riedel and Johnson-Groh 1999) and may assist with the cultivation of *B. multifidum* if restoration becomes necessary. *Botrychium multifidum* is hardy to USDA Zone 3 (average minimum annual temperature –40F), but it is not generally cultivated (Rook 2002). *Botrychium* species are in general very difficult to transplant, but attempts to transplant *B. virginianum* have been relatively successful (Wagner and Wagner 1976).

Information Needs

Distribution

Further inventory work is the greatest research need for *Botrychium multifidum* in Region 2. As concern and awareness of *Botrychium* species has increased, more effort has been made to find these species. Consequently, there are many recent discoveries of *B. multifidum* and other *Botrychium* species in Region 2. It is likely that further searches by trained personnel will lead to more discoveries. Given the rate at which new data is becoming available and the incompleteness of our current knowledge of *B. multifidum* in Region 2, it is unwise to formulate conservation strategies at present. More complete knowledge of the distribution of *B. multifidum* in Region 2 will facilitate the identification of areas most suitable for conservation management.

Life cycle, habitat, and population trend

Additional life history information is needed for *Botrychium multifidum* (Fertig 2002). In particular, the belowground portion of the life cycle remains poorly understood, although sexual reproduction and much of its lifespan occurs under ground. Several habitat attributes are commonly observed in occurrences of *B. multifidum* in Region 2 and elsewhere. These attributes can be useful in refining and prioritizing search areas. However, the relative importance of habitat attributes for *B. multifidum* is unknown.

Response to change

The specific responses of *Botrychium multifidum* to disturbance and succession warrant further investigation. The amount of disturbance that *B. multifidum* can tolerate is not known but is important to inform management. The species' response to water table fluctuation, periodic inundation, and drought are particularly relevant throughout Region 2. Reproductive rates and the ability to colonize new sites are important in better understanding the species' response to environmental change, but these remain poorly understood. Investigation is warranted into the effects of herbivores and exotic species on the viability of *B. multifidum* populations, given the known impacts to *B. multifidum* and its habitat from these factors. Monitoring of *B. dissectum* suggests that it can withstand grazing (Montgomery 1990), but protracted periods of leaf removal would probably be detrimental (Zika personal communication 2003).

Metapopulation dynamics

The metapopulation dynamics of *Botrychium multifidum* 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 tracking this species. Johnson-Groh and Farrar (2003) note four factors that complicate the characterization of the metapopulation structure of *Botrychium* species:

- ❖ difficulty in finding plants, resulting in low confidence that all plants are accounted for and poor understanding of their true distribution on the landscape
- ❖ the predominance of underground life history stages, precluding the determination of true population size and population dynamics
- ❖ the nature of an underground population, making it impossible to determine if a new population arose from spores or dormant gametophytes
- ❖ the need for very long term studies to determine population dynamics and the vulnerability of occurrences to extirpation.

Demography

There have been very few demographic studies of *Botrychium* species, but one of the most thorough was a study of *B. dissectum*, which, like *B. multifidum*, is in the subgenus *Sceptridium* (Montgomery 1990). There have been no investigations of the demography of *B. multifidum*. Therefore, any study addressing demographic variables in *B. multifidum* would advance our understanding of this species, and would probably also offer practical benefits to managers and stewards. No quantitative demographic data are available for *B. multifidum* in Region 2, and any analyses using current data for *B. multifidum* would be largely conjectural. *Botrychium mormo* is the best-studied member of the genus (see Berlin et al. 1998), but many assumptions were made in estimating crucial life history parameters even for this species.

Population trend monitoring methods

Standard population monitoring trend protocols have been developed by Johnson-Groh and Farrar (2003) and have been used successfully in studies of other *Botrychium* species. Other sampling designs have also been used in the study of *Botrychium* species (e.g., Berlin et al. 1998, Johnson-Groh 1999). The problem with any methodology is that it is very difficult to determine the true population size of any *Botrychium* species due to the proportion of the population that exists under ground as gametophytes and juvenile sporophytes, and due to the high annual variation in the aboveground sporophyte population. The second problem is probably less of a concern for *B. multifidum* than for other *Botrychium* species, but monitoring its life history to determine the degree to which dormancy occurs has not been conducted. Due to the inherent difficulties in monitoring *Botrychium* occurrences, determination of population trend will require decades of study (Johnson-Groh and Farrar 2003).

Restoration methods

There are many barriers to habitat restoration for *Botrychium multifidum* and other *Botrychium* species. *Botrychium* species are extremely difficult to propagate (Whittier 1972, Gifford and Brandon 1978), 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 hosts, they cannot survive without suitable fungal partners, but very little is known about the specifics of this relationship. The mycobionts of *B.*

multifidum have not been identified. It is not known if *B. multifidum* individuals tolerate transplanting.

Restoration and maintenance of native vegetation is a crucial part of any restoration effort on behalf of *Botrychium multifidum*. Restoration of native vegetation in the vicinity of known *B. multifidum* occurrences is likely to benefit these occurrences by reducing the influx of exotic species.

Research priorities for Region 2

A replicated monitoring study that compares reproductive and mortality rates of *Botrychium multifidum* in burned vs. unburned, grazed vs. ungrazed, weedy vs. natural, and shaded vs. unshaded would answer many questions about this species. Such a study is a high priority for determining beneficial management practices for *B. multifidum*.

The abundance and distribution of *Botrychium multifidum* in Region 2 is poorly known. Recent discoveries of new occurrences in Region 2, as well as range extensions outside the region (e.g., Etcheberry 1998), suggest that many more occurrences await discovery. Descriptive data for the habitat and community ecology of occurrences should be gathered whenever *B. multifidum* plants (newly discovered or revisited) are encountered. Having an accurate habitat description will help to prevent the inadvertent destruction of occurrences, as managers can identify and avoid potential habitat when planning projects such as road building and herbicide application.

Numerous research needs are cited by Farrar and Johnson-Groh (1986), Berlin et al. (1998), and Johnson-Groh (1999), and because of similarities in the life history and ecological needs of *Botrychium* species, many of these apply to *B. multifidum* as well. These include research on the life history and demography, focusing on underground life history stages.

More research is needed to determine the role of non-reproductive plants in the population biology of *Botrychium multifidum*. A comparison of the reproductive output of *B. multifidum* in shaded and open habitats is needed to determine the relative importance of plants in these settings with respect to population viability. Comparison of other ecological factors (e.g., hydrologic regime) in shaded and open habitats would add to our understanding of the ecological needs of *B. multifidum*. This could also address the magnitude of the threat of succession to *B. multifidum*. Determining if opening the canopy in shaded, non-reproductive populations results in sporulation would determine if this practice is a suitable habitat management policy for *B. multifidum*.

The ecological requirements of *Botrychium multifidum* are poorly understood (Crook personal communication 2003). Research on the autecology of *B. multifidum* is needed, particularly regarding its response to burning, grazing, succession, and invasive species. Studies to determine the intensity and frequency of different types of disturbance in order to create and maintain suitable habitat for *B. multifidum* would constitute an ideal series of graduate research projects.

A clearer understanding of the relationship between *Botrychium multifidum* and its mycorrhizal symbionts will also have considerable practical value. An assessment of the effects of disturbance type, intensity, and frequency (especially fire and grazing) on the mycorrhizal flora will assist managers in prescribing appropriate management. Research is also needed to assess the effect on spore output of different mycorrhizal species and infection levels.

Metapopulation studies are very difficult to conduct for *Botrychium* species, but it is likely that the metapopulation dynamics are important (Johnson-Groh and Farrar 2003). Investigation of migration, extinction, and colonization rates could yield valuable data for the conservation of *B. multifidum*. Studies on the role of elk and other potential dispersal vectors in the transport of spores will also assist with the management of *B. multifidum*. Gene flow can be studied among and within populations by analyzing the frequency of molecular-genetic markers. However, geneflow is very limited in *Botrychium* species, which reduces the utility of these methods for inferring rates of migration, extinction, and colonization.

Studies that produce estimates of cover and/or abundance of associated species could permit the investigation of interspecific relationships through ordination or other quantitative analysis techniques. Understanding environmental constraints on *Botrychium multifidum* could facilitate the conservation of this species. Gathering data on edaphic characteristics (i.e., moisture, texture, and soil chemistry, particularly pH, if possible) from permanent plots established for ecological studies would permit the analysis of species-environment relationships. These data would facilitate hypothesis generation for further studies of the ecology of this species.

Additional research and data resources

An assessment of a suite of *Botrychium* species in 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) and are available in draft form at present. Johnson-Groh has conducted two years of monitoring of *B. multifidum*, but the results of this work have not been published (Johnson-Groh personal communication 2003).

As this assessment was being prepared for publication, additional data became available for WY EO# 20 in Sheridan County, Wyoming. This occurrence was revisited in October 2005 and additional surveys were conducted. For further information on this occurrence, please contact the Wyoming Natural Diversity Database or Greg Karow with the Bighorn National Forest.

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).

Congener – a member of the same genus. *Botrychium simplex* is a congener of *B. multifidum*.

Conservation Status Rank – the Global (G) Conservation Status (Rank) of a species or ecological community is based on the *range-wide* status of that species or community. The rank is regularly reviewed and updated by experts, and takes into account such factors as number and quality/condition of occurrences, population size, range of distribution, population trends, protection status, and fragility. A subnational (S) rank is determined based on the same criteria applied within a subnation (state or province). The definitions of these ranks, which are not to be interpreted as legal designations, are as follows:

- **GX** **Presumed Extinct:** Not located despite intensive searches and virtually no likelihood of rediscovery.
- **GH** **Possibly Extinct:** Missing; known only from historical occurrences but still some hope of rediscovery.
- **G1** **Critically Imperiled:** At high risk of extinction due to extreme rarity (often five or fewer occurrences), very steep declines, or other factors.
- **G2** **Imperiled:** At high risk of extinction due to very restricted range, very few populations (often 20 or fewer), steep declines, or other factors.
- **G3** **Vulnerable:** At moderate risk of extinction due to a restricted range, relatively few populations (often 80 or fewer), recent and widespread declines, or other factors.
- **G4** Apparently Secure: Uncommon but not rare; some cause for long-term concern due to declines or other factors.
- **G5** **Secure:** Common; widespread and abundant.

Competitive/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 multifidum*, 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).

Eusporangiate – a primitive condition in which the cells that give rise to the sporangia are superficial (i.e., lie at the surface) (Gifford and Foster 1989)

Gametophyte – the haploid stage in the life cycle of a plant. This stage lives independently of the sporophyte in ferns. In *Botrychium* the gametophyte is subterranean and is parasitic on mycorrhizal fungi (Gifford and Foster 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 *Botrychiums* (Wagner and Wagner 1983).

Lamina – the leaf blade of a fern. In *Botrychium*, the lamina is divided into a fertile segment (the sporophore) and a sterile segment (the trophophore) (Lellinger 1985).

Monoecious – gametophytes with both male and female gamete producing structures.

Mycobiont – the fungal partner in a mycorrhizal symbiosis.

Protandrous – a condition in which the male reproductive structures mature before the female reproductive structures, encouraging outcrossing.

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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