Botrychium simplex E. Hitchcock
(little grapefern)
A Technical Conservation Assessment

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

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David G. Anderson
Colorado Natural Heritage Program
Colorado State University
Fort Collins, CO

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AUTHOR'S BIOGRAPHY

David G. Anderson is a botanist with the Colorado Natural Heritage Program (CNHP). Mr. Anderson's work at CNHP includes inventory and mapping of rare plants throughout Colorado, mapping weeds, maintaining and updating CNHP's database, and writing reports on the rare plants of Colorado. He has worked with CNHP since 1999. Much of Mr. Anderson's prior experience comes from five years of fieldwork studying the flora and ecosystem processes of the Alaskan and Canadian Arctic. Mr. Anderson also served in the Peace Corps as a science teacher in the Solomon Islands from 1996 to 1998. Mr. Anderson received his B.A. in Environmental, Populational, and Organismic Biology from the University of Colorado, Boulder (1991) and his M.S. in Botany from the University of Washington, Seattle (1996).

COVER PHOTO CREDIT

Botrychium simplex (little grapefern). (Left) photo of the first illustration of *B. simplex*, published with the description of the species (Hitchcock 1823). Photo by the author. (Right) photo by Katherine Zacharkevics, Black Hills National Forest Botanist, North Hills District of a plant collected at Dugout Gulch, Wyoming in 2005.

One reluctant leaf,
A moonwort sprouts in the fen
Risking its secrets

—Jean Anderson

SUMMARY OF KEY COMPONENTS FOR CONSERVATION OF *BOTRYCHIUM SIMPLEX*

Status

Botrychium simplex E. Hitchcock (little grapefern) is known from 50 locations in Region 2, 17 of which have not been seen within the last 20 years. The population size in Region 2 is unknown, but the estimated total population from locations where plant counts have been made is 500 to 600 plants. Other occurrences are known to support significant populations of this species, but these have not been counted. *Botrychium simplex* is not designated as a sensitive species in USDA Forest Service (USFS) Region 2, but it is considered a sensitive species in USFS Region 1 and in the Washington portion of USFS Region 6, and it is considered important for biodiversity analysis in USFS Region 4. NatureServe ranks *B. simplex* as globally secure (G5). Within Region 2, it is ranked imperiled (S2) in Colorado and Wyoming, and unrankable (SU) in South Dakota. It has no rank in Nebraska, but it probably warrants a rank of critically imperiled (S1) based on the one known occurrence in that state. *Botrychium simplex* is not listed as threatened or endangered under the Federal Endangered Species Act.

Primary Threats

Observations and quantitative data suggest several threats to the persistence of *Botrychium simplex*. The primary threats are ski area development and maintenance, road construction and maintenance, timber harvest, recreation, fire, grazing, effects of small population size, woody plant encroachment, exotic species invasion, succession, global climate change, and pollution.

Primary Conservation Elements, Management Implications and Considerations

The responsibility of maintaining viable populations of *Botrychium simplex* within Region 2 falls largely on the USFS because most occurrences and suitable habitat are on National Forest System land. Forty-one of the 50 known occurrences of this species in Region 2 are on National Forest System land, and 24 of these have been observed since 1999. Eight occurrences are known from national parks in the states of Region 2: two occurrences are in Rocky Mountain National Park within Region 2 and six are in Yellowstone National Park outside Region 2. Two additional occurrences are under unknown management, and two are on public lands managed by the Bureau of Land Management. The City of Denver, State of Colorado, and The Nature Conservancy also each have one occurrence. Seventeen occurrences have not been seen in more than 20 years, and it is important to determine if *B. simplex* is extant at these locations. Additional inventories are needed to better understand the full range and distribution of this species.

Restoring populations of *Botrychium simplex* is probably precluded by the difficulty in propagating this species. Research is needed to investigate the underground life history, ecology, reproductive biology, the role of mycorrhizae, and the role of disturbance in the autecology of *B. simplex* so that conservation efforts on its behalf can be most effective.

The major conservation element essential to ensuring viable populations of *Botrychium simplex* in Region 2 is promoting the processes that create and maintain the early- to mid-seral or other suitable habitats required by *B. simplex*. Unfortunately, these processes are poorly understood. Because new data are just now becoming available and our current knowledge of *B. simplex* in Region 2 is incomplete, it is difficult to formulate conservation strategies at present. More complete knowledge of the species' distribution will permit the identification of areas most suitable for conservation management in Region 2. New surveys are needed to better understand how the subspecies of *B. simplex* differ in habitat affinities and autecology in Region 2. Demographic studies designed to determine the impacts of grazing, succession, fire, and exotic species on population viability are also high priorities for research on *B. simplex* in Region 2.

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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 simplex* is the focus of an assessment because of its rarity, degree of imperilment, and concern for its viability in Region 2. It is not currently listed as sensitive by Region 2 or by the Bureau of Land Management (BLM) in Colorado or Wyoming. *Botrychium* species have been the focus of increasing interest by the USFS and other federal and state agencies due to their rarity, difficulty in detection, and highly variable populations (Johnson-Groh and Farrar 2003).

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

Goal of Assessment

Species conservation assessments produced as part of the Species Conservation Project are designed to provide forest managers, research biologists, and the public 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 backgrounds 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 simplex* with specific reference to the geographic and ecological characteristics of Region 2. Although some of the literature on the species may originate from field investigations outside the region, this document places that literature in the ecological and social context of the central Rocky Mountains. Similarly, this assessment is concerned with reproductive behavior, population dynamics, and other characteristics of *B. simplex* 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 placed in a current context.

In producing the assessment, I reviewed refereed literature, non-refereed publications, research reports, and data accumulated by resource management agencies and other investigators. Because basic research has not been conducted on many facets of the biology of *Botrychium simplex*, 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. simplex* are referenced in this assessment, and many of the experts on this species were consulted during its synthesis. All available specimens of *B. simplex* in Region 2 were viewed to verify occurrences and to 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 peer-reviewed literature because this is the accepted standard in science. Non-refereed publications or reports were regarded with greater skepticism, but they were used in the assessment because there is very little refereed literature that specifically treats *B. simplex* in Region 2. Unpublished data (e.g., Natural Heritage Program records, reports to state and federal agencies, specimen labels) were important in estimating the geographic distribution of this species. These data required special attention because of the diversity of persons and methods used in collection.

Treatment of Uncertainty in Assessment

Science is a rigorous, systematic approach to obtaining knowledge. Competing ideas regarding how the world works are measured against observations. 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). 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 use of species assessments in the Species Conservation Project, assessments are being published on the Region 2 World Wide Web site. Placing the documents on the Web makes them available to agency biologists and the public more rapidly than publishing them as reports. More important, Web publication will facilitate revision of the assessments, 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 before 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.

MANAGEMENT STATUS AND NATURAL HISTORY

Management Status

Botrychium simplex is not currently listed as a sensitive species in Region 2 (USDA Forest Service 2003). Its merits as a sensitive species were evaluated in 2001 (Ode 2001) and 2002 (Burkhart 2002), but it was determined that insufficient information is available to determine whether *B. simplex* meets the requirements for sensitive status (Warren 2003). *Botrychium simplex* is considered a sensitive species in Region 1 and in the Washington portion of Region 6, and it is considered important for biodiversity analysis in Region 4 (Zika et al. 1995).

Botrychium simplex is not included on the BLM State Sensitive Species lists in Colorado (Bureau of Land Management Colorado 2000) or Wyoming (Bureau of Land Management Wyoming 2002), nor is it listed as threatened, endangered, or candidate under the federal Endangered Species Act (16 USC 1531-1536, 1538-1540). This species is not listed as endangered or vulnerable by the International Union for Conservation of Nature and Natural Resources (1978). NatureServe considers *B. simplex* to be globally secure (G5).

Botrychium simplex is a widely distributed, circumboreal species, but there are concerns for its viability in many portions of its range. It is classified as endangered in Sweden (Nilsson 1981), and it is considered endangered in Illinois, Indiana, Maryland, and Ohio (USDA Natural Resources Conservation Service 2002). In North America, it has been documented from 35 states and 10 provinces as well as from Greenland. *Botrychium simplex* has been extirpated in three states. For detailed information regarding its range-wide status, see the Distribution and abundance section.

Botrychium simplex is known from 50 locations in Region 2; 17 of these have not been revisited in more than 20 years, and five have not been revisited for at least 50 years. In the states of Region 2, *B. simplex* is ranked imperiled (S2) in Colorado and in Wyoming, where it has not been tracked by the Wyoming Natural Diversity Database since 1986. This species has been reported from Nebraska, and while it has not yet been assigned a state rank, it will probably receive a rank of S1. *Botrychium simplex* is considered unrankable (SU) in South Dakota and it is not known from Kansas. For explanations of NatureServe's ranking system, see the **Definitions** section.

Existing Regulatory Mechanisms, Management Plans, and Conservation Strategies

Adequacy of current laws and regulations

Botrychium simplex has no legal protection that would prevent the destruction of habitat or individuals on state and private land in Region 2, or on federal land not managed by the USFS. Because it is not listed as a sensitive species in Region 2, the USFS does not explicitly consider it for special management. As a *Botrychium* species, however, it is documented during Biological Evaluations where it may receive some special consideration.

Adequacy of current enforcement of laws and regulations

There are no known cases in which an occurrence of *Botrychium simplex* was extirpated due to human activities or due to the failure to enforce existing regulations in Region 2. It is not known if federal, state, or other laws could have prevented the extirpation of *B. simplex* in Connecticut, Maryland, Virginia, and possibly Ohio. Thus, it cannot be determined if current regulations or their enforcement are adequate for the species' protection.

Biology and Ecology

Classification and description

Botrychium simplex is a member of the adder's tongue family (Ophioglossaceae). Members of the Ophioglossaceae are eusporangiate and share a suite of characters that are less derived than those of most other Pteridophytes (Gifford and Foster 1989). In North America, the Ophioglossaceae as circumscribed by Wagner and Wagner (1993) is composed of three genera: *Ophioglossum*, *Cheiroglossa*, and *Botrychium*. *Botrychium* (grapeferns) is the most diverse of these genera with 50 to 60 species worldwide (Wagner and Wagner 1993).

The genus *Botrychium* contains three subgenera: *Osmundopteris*, *Sceptridium*, and *Botrychium* (Wagner and Wagner 1993). Subgenus *Botrychium* (the moonworts) is the most diverse of the three, with approximately 25 to 30 species. Members of this subgenus share many morphological traits, and subtle morphological differences make it difficult to identify species in the field. Based on nuclear and chloroplast DNA, recent phylogenetic research on the Ophioglossaceae has shown that the members of subgenus *Botrychium* comprise a monophyletic group (Hauk et al. 2003). The diversity of subgenus *Botrychium* in North America was not recognized until the 1980s when Drs. Herb and Florence Wagner began work in earnest on *Botrychium*. **Table 1** is a summary of the classification of *B. simplex*.

Taxonomic status

The taxonomic status of *Botrychium simplex* has been in turmoil for more than 100 years, and there remains much uncertainty regarding the appropriate circumscription of this taxon. Reverend Edward Hitchcock first described *B. simplex* from plants found in Massachusetts (Hitchcock 1823). *Botrychium simplex* was the second species after *B. lunaria* to be described in subgenus *Botrychium*. Hitchcock's description includes a hand-painted plate of *B. simplex* (**Figure 1**) and the following introduction: "This species grows, not very abundantly, in Conway, Massachusetts. It was first noticed, two years since, and with some doubt, referred to *B. lunaria* of Swartz and Wildenow. But upon a suggestion of Dr. Torrey that it might be a new species, I have several times re-examined it during the two past summers, and feel so confident that it is specifically distinct from any described *Botrychium*, that I take the liberty to propose for it the name '*B. simplex*.'"

At least seven varieties of *Botrychium simplex* have been described (**Table 2**). The description of varietal taxa began with A.A. Eaton's (1899) account

Table 1. Classification of *Botrychium simplex* 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)
Division	Pteridophyta (Ferns)
Class	Filicopsida
Order	Ophioglossales
Family	Ophioglossaceae (Adder's Tongue Family)
Genus	*Botrychium* (Grapeferns)
Subgenus	*Botrychium* (Moonworts)[1]
Section	*Lunariae*[2], *Simplex*[3]
Species	*Botrychium simplex* E. Hitchcock[1]

[1]Wagner and Wagner 1993
[2]Clausen 1938
[3]Hauk 1995

Figure 1. The first illustration of *Botrychium simplex* (from Hitchcock 1823).

of *B. tenebrosum*. Clute (1901) demoted this taxon to varietal status under *B. matricariifolium*. Clausen (1938) recognized the similarity of this taxon to *B. simplex* and included *tenebrosum* as a variety of *B. simplex*. Other varieties that have been described for *B. simplex* include var. *cordatum* (Wherry 1937), var. *compositum* and var. *typicum* (Clausen 1938), and var. *laxifolium* (Clausen 1938, Fernald 1949). The plants from Colorado and Wyoming observed by Clausen 1938 were placed in var. *typicum* (including unnamed specimens from Gilpin and El Paso counties, where the former is probably the 1919 collection of Hazel Schmoll) and var. *compositum* (*I.M. Johnson #2415* on Pikes Peak, Colorado and *C.C. Parry #306* from Yellowstone Lake, Wyoming). Plants not falling into one of the other varieties are referred to as var. *simplex*.

The varieties of *Botrychium simplex* are often morphologically distinct. During the second half of the 20th century, Drs. Herb and Florence Wagner began to question the taxonomic validity of these varieties. Wagner and Wagner (1983) noted that "low, dark,

Table 2. A partial list of synonyms for *Botrychium simplex*.

Source	Taxon
2	*Botrychium simplex* E. Hitchcock
2	*Botrychium simplex* var. *cordatum* (Fr.) Wherry
2,3	*Botrychium simplex* var. *laxifolium* (R.T. Clausen) Fernald
2	*Botrychium simplex* var. *laxifolium* R.T. Clausen
2,3	*Botrychium simplex* var. *tenebrosum* (A.A. Eaton) R.T. Clausen
2,3	*Botrychium simplex* var. *typicum* R.T. Clausen
1,3	*Botrychium simplex* var. *compositum* (Lasch) Milde
1,3	*Botrychium tenebrosum* A.A. Eaton
1	*Botrychium simplex* ssp. *typicum* R.T. Clausen
2	*Botrychium virginicum* var. *simplex* (Hitchc.) A.Gray
2	*Botrychium lunaria* var. *simplex* (Hitchc.) Watt
2	*Botrychium kannenbergii* forma *compositum* Lasch

1 = Kartesz (1999)

2 = The Plant Names Project (1999)

3 = USDA-Natural Resources Conservation Service (2002)

acidic forest floors yield var. *laxifolium*, shaded bog edges var. *tenebrosum*, dry upland fields var. *typicum*, and moist low meadows var. *compositum*. All of these are connected with the others in intermediate habitats and there is little consistency." Thus they suggested that *B. simplex* is an extremely plastic species that exhibits a phenotypic response to different habitats, as Paris et al. (1989) also suggested. Wagner and Wagner's deconstruction of varietal concepts in *B. simplex* is culminated in their treatment in the Flora of North America, where they write "The many environmental forms and juvenile stages of *B. simplex* have resulted in the naming of numerous, mostly taxonomically worthless, infraspecific taxa" (Wagner and Wagner 1993, p. 101). Instead, they offer an alternate concept of *B. simplex* in which "eastern" and "western" forms are described. The Wagner's' western plants conform roughly to var. *compositum* while their eastern plants fall into var. *simplex*. However, var. *compositum* may not properly apply to populations of the Rocky Mountain Cordillera where a varietal name was not applied (Cronquist et al. 1972). The following paragraphs provide detailed descriptions of these taxa. Lellinger (1985, p. 112) noted that "eastern, western, and Colorado forms exist in this species, [and] additional study is needed." The "Colorado" form of *B. simplex* noted by Lellinger is probably *B. minganense*, which can be mistaken for *B. simplex* (Root personal communication 2003).

Ongoing genetic research by Dr. Don Farrar and Dr. Warren Hauk suggests another concept of *Botrychium simplex*. Significant intraspecific variation in plastid DNA sequences occurs in *B. simplex*, indicating the need for more study to evaluate the possibility that *B. simplex* (as currently circumscribed) includes multiple taxa (Hauk 1995, Farrar 1998). Current taxonomy recognizes four varieties of *B. simplex*: var. *simplex*, var. *tenebrosum*, var. *compositum*, and var. *fontanum* (**Table 3**; Farrar personal communication 2003, Farrar 2005). Var. *compositum* includes plants from Minnesota west to Oregon and Washington (Farrar 2001, Farrar personal communication 2003). Most plants found in Colorado appear to be var. *compositum*. Var. *simplex* is found in the Black Hills of South Dakota and has been found once in Colorado (Farrar personal communication 2003, Farrar 2005). At least five Wyoming specimens are labeled var. *tenebrosum*; this taxon is recognized by Hartman and Nelson (2001) for Wyoming plants. Thus it appears that three of the currently recognized varieties (var. *compositum*, var. *simplex*, and var. *tenebrosum*) may occur in Region 2. However, most Colorado and Wyoming specimens have not been evaluated by Farrar to assess their taxonomic status (Farrar personal communication 2003).

Farrar (personal communication 2003, 2005) described the fourth variety, var. *fontanum*, for genetically distinct plants from southern California, southern Nevada, northeastern Oregon, and southeastern Washington. Var. *fontanum* is typically found in calcareous fens and seeps (Farrar 2005). The presence of this variety in Colorado or elsewhere in Region 2 has not been confirmed, but calcareous fens and seeps in Region 2 need to be searched for this taxon (Farrar personal communication 2003).

Table 3. Summary information for the varieties of *Botrychium simplex* recognized by Farrar (2005).

Variety	Diagnostics	Range	Habitat and notes
simplex	Plants with mostly undivided basal pinnae and trophophore stalk equal to or exceeding length of the common stalk. Larger plants have pinnae that become progressively more dissected, with lower pinnae more elongated.	Northeastern US, west through the Great Lakes Region, with disjunct reports in Nebraska and the Black Hills, apparently also in Colorado (*Douglass #62-25*)	meadow, woodland
tenebrosum	Slender plants with undivided basal pinnae and very short trophophore and sporophore stalks but relatively long common stalks.	Northeastern US and Great Lakes Region west to Iowa and Minnesota; reported in Wyoming (Hartman and Nelson 2001)	forest, swamp margin, dune
compositum	Plants with secondarily divided basal pinnae and trophophore stalk equal to or exceeding length of the common stalk.	Mountains of the western US (including Colorado) and Canada	meadow, roadside
fontanum	Robust plants with basal pinnae divided or not, thick and fleshy with broad, bluish green pinnae and terminal pinnae with broadly rounded apices.	California, Nevada, NE Oregon, SE Washington	Calcareous fens and hardwater seeps. Possibly in CO; further surveys are needed.

Isozyme analysis demonstrates a close relationship of most western plants to var. *compositum* (Farrar 2001). Although Farrar and Wendel (1996) and Farrar (1998) note that the genetic distance between three varieties of *Botrychium simplex* approaches that of full species, current genetic evidence suggests that the range of variability in *B. simplex* falls within that of a single species (**Table 4**). As noted by Wagner and Wagner (1983), plants in different ecological situations (e.g., seasonally dry meadows, saturated fens) have marked morphological differences, but they also have marked genetic differences (Farrar 2001). Given the current uncertainties regarding the proper circumscription of *B. simplex*, it is likely that future investigation will result in further changes and refinements that may include the description of other varietal taxa or even new species.

Botrychium simplex is closely related to two rare and narrowly endemic moonworts, *B. mormo* and *B. pumicola* (Hauk 1995, Hauk et al. 2003). *Botrychium mormo* was described in 1981 after three decades of study (Wagner and Wagner 1981). It is a tiny moonwort

Table 4. Genetic variability in eastern diploid species of *Botrychium* subgenus *Botrychium* from Farrar (1998). These results support the genetic and taxonomic uniqueness of the varieties *simplex*, *tenebrosum*, and *compositum*. Most moonworts show little heterozygosity (number of alleles per locus), and do not have a high percentage of polymorphic loci. However, *B. simplex sensu lato* shows very high percentage of polymorphic loci relative to other eastern moonworts. When the varieties are analyzed separately they fall into a more normal range, suggesting that they may even warrant treatment as full species.

Species	Mean sample size per locus	Mean number of alleles per locus	Percentage of loci polymorphic
B. lunaria	41.1	1.1	11.1
B. campestre	96.9	1.2	16.7
B. pallidum	20.6	1.1	5.6
B. simplex sensu lato	**96.2**	**1.7**	**61.1**
var. *simplex*	**27**	**1.1**	**5.6**
var. *tenebrosum*	**27.8**	**1.1**	**11.1**
var. *compositum*	**15.8**	**1.1**	**5.6**
B. mormo	48.8	1.1	5.6
B. lanceolatum ssp. lanceolatum	24.6	1	0
B. lanceolatum ssp. angustifolium	29.2	1.1	5.6

that is found in rich woods of Michigan, Wisconsin, and Minnesota (Wagner and Wagner 1981). *Botrychium simplex* is the most similar species to *B. mormo*, and the two can be difficult to distinguish (Chadde and Kudray 2001a). These species do not occur together in Region 2. *Botrychium mormo* is genetically distinct from all varieties of *B. simplex* (Farrar and Wendel 1996).

Botrychium pumicola is known only from volcanic substrates and frost pockets in Oregon (Hopkins et al. 2001). The morphological distinctness of *B. pumicola* from *B. simplex* has been long recognized. *Botrychium pumicola* was described in 1900 (Coville 1900) and has now been shown to be clearly genetically distinct from *B. simplex* (Farrar 2000). Using isozyme data (Hauk and Haufler 1999) and *rbcL* data (Hauk et al. 2003), *B. pumicola* appears to be the most closely related to *B. simplex* of the species analyzed. *Botrychium pumicola* was also the closest relative in a combined analysis of *rbcL*, *trnL-F* and morphological data, with *B. montanum* the next closest relative (Hauk et al. 2003). *Botrychium mormo* was not included in this phylogenetic study.

Botrychium pumicola is one of a handful of moonwort species that have been observed to produce gemmae (Camacho 1996, Camacho and Liston 2001), which are minute vegetative propagules abscised at maturity from the parent plant (Farrar and Johnson-Groh 1990). Camacho (1996) found 0 to 10 gemmae per plant in *B. pumicola*.

Botrychium campestre also produces gemmae and was the first moonwort species in which they were documented (Farrar and Johnson-Groh 1986, Farrar and Johnson-Groh 1990, Johnson-Groh et al. 2002). Subsequent research has found them on other diploid species, including *B. pumicola*. *Botrychium gallicomontanum*, a rare allotetraploid species for which *B. campestre* and *B. simplex* are the putative parent species, is also known to reproduce with gemmae (Farrar and Johnson-Groh 1991). The production of gemmae as vegetative propagules by these three species, all of which are found in relatively xeric sites, suggests that it is an adaptation for reproduction in dry sites (Camacho 1996). Farrar and Johnson-Groh (1986) examined *B. simplex* for gemmae, but none were found. Gemmae may confer a lesser advantage to *B. simplex*, which is typically found in habitats that are at least seasonally wet.

Botrychium pumicola is unusual in that its spores are dispersed in loose groups of four spores also called tetrads (Wagner 1998). Despite the close relationship between *B. simplex* and *B. pumicola*, this phenomenon has not been documented in *B. simplex*.

Botrychium simplex is a putative parent species for several polyploid nothospecies in subgenus *Botrychium* (**Table 5**; Wagner 1993, Farrar and Wendel 1996).

Description

Botrychium subgenus *Botrychium* sporophytes are simple plants recognized by their small size and distinctive leaf and spore structures. Members of this subgenus are usually less than 15 cm in height. They possess a trophophore, or sterile leaf-like structure that is often heavily lobed or segmented, but rarely truly pinnate (Wagner and Wagner 1993). Members of the subgenus *Botrychium* usually only produce one leaf each year and in some years produce no leaves (Johnson-Groh 1998). On the same stalk sits a fertile sporophore that is often taller than the trophophore. The sporophore contains 20 to 100 grape-like sporangia, each containing possibly thousands of spores (Farrar and Johnson-Groh 1986, Wagner 1998).

Botrychium species can be difficult to identify due to their subtle diagnostic characters, frequent occurrence with other *Botrychium* species, and morphological variability (Paris et al. 1989). Because they are such simple plants, there are few morphological characters that can be used to distinguish species; identification is often based on very subtle characters (Hauk and Haufler 1999). Identification is facilitated by the use of dichotomous keys (see Weber and Wittmann 2001a and Weber and Wittmann 2001b); however, these do not guarantee a positive identification, and it is often necessary to get verification by a *Botrychium* expert.

Table 5. Hypothetical parents for polyploid species of *Botrychium* subgenus *Botrychium* (after Hauk 1995, from Wagner 1993 and Farrar and Wendel 1996).

Polyploid species	Hypothetical parents
B. hesperium	*B. lanceolatum* x *B. simplex*
B. pseudopinnatum	*B. pinnatum* x *B. simplex*
B. gallicomontanum	*B. campestre* x *B. simplex*

Botrychium simplex is challenging to identify with confidence since it is highly variable, small, and cryptic. Farrar (2001) notes that "*B. simplex* is by far the most variable of diploid moonworts," and Wagner and Wagner (1983) wrote that "Only a few botrychiums have such astonishing variability as *B. simplex*." They also noted that "no species approaches *B. simplex* in the extent of its variability. Var. *compositum* stands in vivid contrast to var. *tenebrosum*."

Within Region 2, both the eastern and western forms described by Wagner and Wagner (1993) are present, and it appears that all three of the varieties recognized by Wagner and Wagner (1993) occur here. Wagner and Wagner (1993) provide a summary of the diagnostic characters of *Botrychium simplex*; these are summarized in **Table 6**. A comparison of the varieties recognized by Farrar (2005) is included in **Table 3**.

Botrychium simplex is a small perennial fern, seldom exceeding 8 cm tall (Lorain 1990). It has small roots (0.5 to 1 mm in diameter) and a highly variable trophophore, which is 1 to 7 cm long, oblong to long-elliptic, 0.3 to 2 cm wide, truncate to round at the base, round at the apex, pinnate or sometimes nearly simple, entire, round pinna apices, and entire or crenulate margins (Lellinger 1985, Farrar 2005). Useful field marks for *B. simplex* include its diminutive size, succulent stem, single compound leaf that is often clasping the sporophore, its unbranched fertile frond, and the tendency for the trophophore and sporophore to connect at ground level (**Figure 2**; Farrar 2005). It is highly variable and only distinguished absolutely from other grapeferns by its larger spores, which are unusually large for a diploid species, ranging in diameter from 0.035 to 0.050 mm (Rook 2002, Farrar 2005). Occasional plants are found that have sporangia dotting the margins of the trophophore (**Figure 3**). These are called supernumerary sporangia and are seen infrequently in all moonwort species (Farrar 2005). *Botrychium simplex* is diploid with 45 chromosomes (2n = 90) (Wagner 1993, Wagner and Wagner 1993).

Detailed descriptions for both "eastern" and "western" *Botrychium simplex* appear in Wagner and Wagner 1993. Several field characteristics are useful for distinguishing these two types of *B. simplex*. Western *B. simplex* has a sporophore that is longer relative to the trophophore, lacks a common stalk, and has fan-shaped pinnae. See **Table 6** for a comparison of diagnostic characteristics between these types, and **Figure 3**, **Figure 4**, and **Figure 5** for specimens representing these types.

Botrychium simplex var. *compositum* is roughly equivalent to the "western" *B. simplex* of Wagner and Wagner 1993 (**Figure 3**). It is usually has a three-parted leaf as shown in Gray (1908), Hitchcock and Cronquist (1969), and Cronquist et al. (1972). The shape of the trophophore is distinctive in having three main branches (Lorain 1990). The sporophore diverges at or just above ground level (Welsh et al. 1993, Farrar 2005) or sometimes below ground level (as noted on some specimens collected by Peter Root).

Botrychium simplex var. *tenebrosum* occurs in eastern North America (Wagner and Wagner 1993) and elsewhere, including Wyoming (**Figure 4**; Hartman and Nelson 2001). It was thought by Wagner and Wagner (1993) and others to be a persistent juvenile, but current genetic evidence suggests otherwise. Var. *tenebrosum* is distinguished from var. *simplex* (**Figure 5**) by its smaller size, slender stature, and its simple and rudimentary trophophore attached near the top of an exaggerated common stalk. Wagner and Wagner (1993) describe a western equivalent to *B. simplex* var. *tenebrosum*, with a lower attachment of the trophophore, which is longer and more herbaceous in texture. The spores of var. *tenebrosum* are larger than those of var. *simplex* (Eaton 1899). Clausen (1938) includes photographs of specimens of *B. simplex* var. *tenebrosum*. Var. *tenebrosum* has been mistaken for *B. simplex* var. *simplex* and *B. matricariifolium*.

Botrychium simplex is frequently confused with other species of *Botrychium* in Region 2 and elsewhere. Within Region 2, *B. simplex* specimens have been misidentified as *B. lunaria* and *B. hesperium*. In several cases, one plant on an herbarium sheet containing several plants has been annotated as *B. lunaria* or in one case *B. paradoxum*. Many medium or large specimens of *B. simplex* have flabellate pinnae that strongly resemble *B. lunaria* (Wagner and Wagner 1981). In Oregon, well-formed plants can be mistaken for *B. pumicola*, and immature plants are easily confused with *B. minganense* and *B. lunaria* (Zika et al. 1995). A key point in separating *B. simplex* from *simplex*-like minganense (*B. "colorado"*) is that in *B. simplex*, the sporophore separates from the stipe at or just below the soil surface (**Figure 2**; Root personal communication 2003). Before its circumscription, *B. montanum* was known as *B. simplex* in Montana (Vanderhorst 1997). *Botrychium simplex* also looks like *B. mormo* (Wagner 1998). In Iowa, Michigan, and Nebraska, *B. simplex* has been found associated with *B. campestre*, with which it is the putative parent species of *B. gallicomontanum* (Farrar and Johnson-Groh 1991, Farrar personal communication 2003).

Table 6. Diagnostic characteristics presented in Wagner and Wagner (1993) for the determination of the "eastern" and "western" types of *Botrychium simplex*.

Characteristic	*B. simplex sensu lato*	"eastern" *B. simplex*	"western" *B. simplex*
Sporophore	1-pinnate; one to eight times the length of the trophophore	One to four times the length of the trophophore; arises from common stalk below middle to near the top, well above the leaf sheath	Three to eight times the length of the trophophore, arises directly from the top of the leaf sheath
Common stalk	Absent or well developed	Well developed	Much reduced or absent
Trophophore	Stalk 0 to 3cm, 0 to 1.5 times the length of the trophophore rachis, blade dull to bright green, linear to ovate-oblong to fully triangular with ternately arranged pinnae	Nonternate or if subternate, the lateral pinnae are smaller than the central pinnae and simple to merely lobed, tip undivided, texture papery to herbaceous	Ternate with three equal segments, or rarely non-ternate but resembling a single segment of a ternate blade, tip divided usually into three parts, texture thin and herbaceous
Pinnae	Up to seven pairs of pinnae or well-developed lobes, spreading to ascending, closely or widely separated, distance between 1st and 2nd pinnae pairs is frequently greater than between 2nd and 3rd pairs, basal pinna pair larger and more complex than adjacent pair, cuneate to fan-shaped, strongly asymmetric, venation pinnate or like ribs of fan, with midrib	Lateral pinnae are smaller than central pinnae, simple to merely lobed or rarely pinnate, pinnae adnate to rachis, rounded and ovate to spatulate, segment sides at angles mostly less than 90 degrees	Pinnae usually strongly contracted at the base to stalked, angular to fan-shaped, segment sides at angles mostly more than 90 degrees as in Botrychium lunaria
Habitat	Dry fields, marshes, bogs, swamps, roadside ditches	Often upland fields	Along marshy margins and in meadows

Figure 2. *Botrychium simplex* sporophytes at Dugout Gulch, Wyoming, Black Hills National Forest (BOSI-12) after emergence, and before separation of the trophophore and sporophore. Over 200 individuals were seen at this location in 2004. Photo by Katherine Zacharkevics, provided by Beth Burkhart.

Figure 3. *Botrychium simplex* var. *compositum* (“western” *simplex*) collected on Boreas Pass, Colorado.

The gametophytes of *Botrychium* species remain poorly understood. They are achlorophyllous and are wholly dependent on mycorrhizal fungi for their 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 1972, Whittier 1973, Whittier 1981, Whittier 1984, Melan and Whittier 1989, Thomas and Whittier 1993).

The gametophyte of *Botrychium simplex* was studied in detail by Campbell (1922). Like all other *Botrychium* species studied, the gametophyte of *B. simplex* has a dorsal ridge that bears the gamete-producing structures. Antheridia are borne at the top of the dorsal ridge and archegonia on either side of the dorsal ridge. The gametophytes of *B. simplex* are monoecious, with both male and female gamete-producing structures. 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. *Botrychium simplex* gametophytes have been found with young sporophytes attached (Clausen 1938).

Sources for keys photographs, illustrations, and descriptions

There are numerous sources of keys, photographs, illustrations, and descriptions that are of great value in identification of *Botrychium simplex*. The best source currently available for use in Region 2 is Farrar (2005), which includes keys, descriptions, photographs, and a discussion of the diagnosis, distribution, and habitats of all western North American moonworts. This source includes details for each of the currently recognized varieties of *B. simplex* (vars. *simplex*, *tenebrosum*, *compositum*, and *fontanum*), but unfortunately it is unpublished and is not yet widely available. Root (2003) is another useful but unpublished source of information on moonworts in Colorado that includes keys, silhouettes, and diagnostic information. Wagner and Wagner (1993) include a description of *B. simplex*. Lellinger (1985) includes a good description, key, and photograph. Internet sources including Wisconsin State Herbarium (2003) and Rook (2002) contain photographs, habitat information, and links to other sources. Wagner and Wagner (1983) include a figure showing the varieties of *B. simplex*.

Figure 4. *Botrychium simplex* var. *tenebrosum* from Park County, Wyoming (*Kirkpatrick #5317*).

Figure 5. *Botrychium simplex* var. *simplex* (“eastern” *simplex*) from East Inlet, Rocky Mountain National Park, Colorado (*Douglass #62-25*).

There are also several sources of illustrations of *Botrychium simplex*. Hitchcock (1823) includes the first illustration of *B. simplex* (**Figure 1**). Huxley (1972) contains an illustration of *B. simplex* in Europe. Hitchcock et al. (1969) and Cronquist et al. (1972) include an illustration of "western" *B. simplex* (var. *compositum*). This illustration is also included in Lackschewitz (1991). Another illustration of "western" *B. simplex* is found in Dorn and Dorn (1972). Gray (1908) includes an illustration of var. *compositum*. Chadde and Kudray (2001a) provide an illustration of "eastern simplex." A description and illustration are included in Britton and Brown (1913) for both *B. simplex* (**Figure 6**) and *B. tenebrosum* (**Figure 7**). Polunin (1959) includes an illustration of var. *tenebrosum*. Weber and Wittmann (2001a, 2001b) provide a brief description, an illustration, and a key for *Botrychium* in Colorado. Campbell (1922) includes detailed descriptions and numerous detailed illustrations of the gametophyte and embryo of *B. simplex*.

Silhouettes can be helpful in identifying moonworts. Farrar (2001) includes the silhouettes of many moonwort species, including *Botrychium simplex*. Mantas and Wirt (1995) also include silhouettes, illustrations, and a description of *B. simplex* in Montana.

Because of its wide distribution, many floras and field guides describe *Botrychium simplex* (e.g., Coulter and Nelson 1909, Rydberg 1922, Davis 1952, Harrington 1954, Peck 1961, Gleason and Cronquist 1963, Munz and Keck 1968, Huxley 1972, Great Plains Flora Association 1986, Lackschewitz 1991, Welsh et al. 1993). Lorain (1990) includes a key for moonworts found in the Idaho Panhandle and a description of *B. simplex* (var. *compositum*). Full technical descriptions for *B. simplex* and its varieties are in Clausen (1938). Kolb and Spribille (2000) and Farrar (2005) include useful tables comparing the diagnostic characteristics of western moonwort species including *B. simplex*. There is no type specimen for *B. simplex* (Zika et al. 1995).

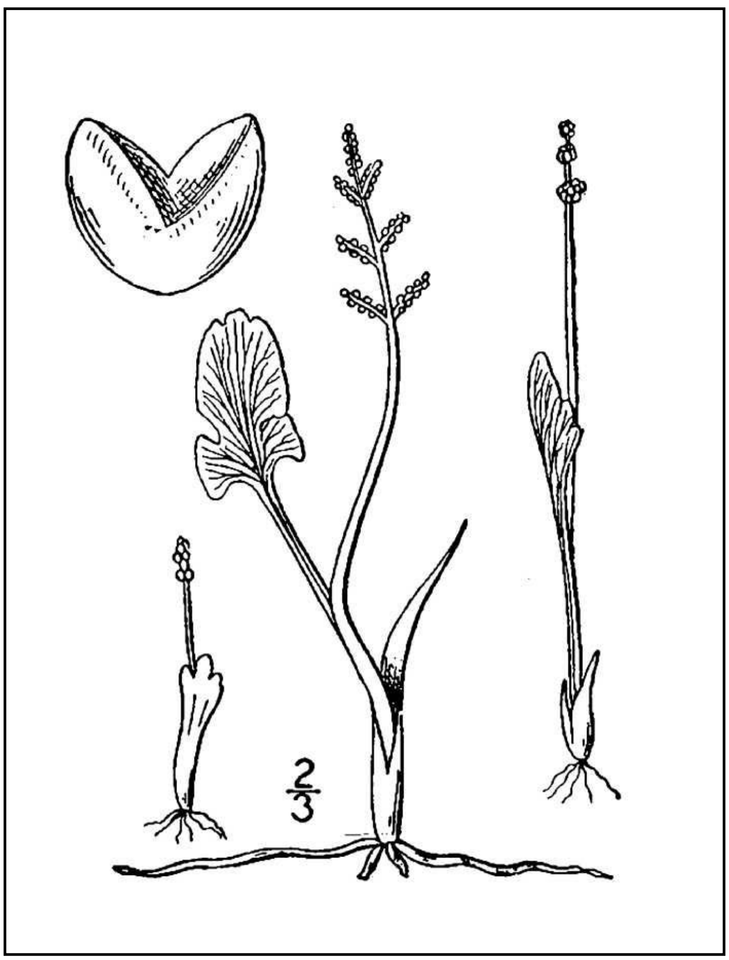

Figure 6. Illustration of *Botrychium simplex* (Britton and Brown 1913).

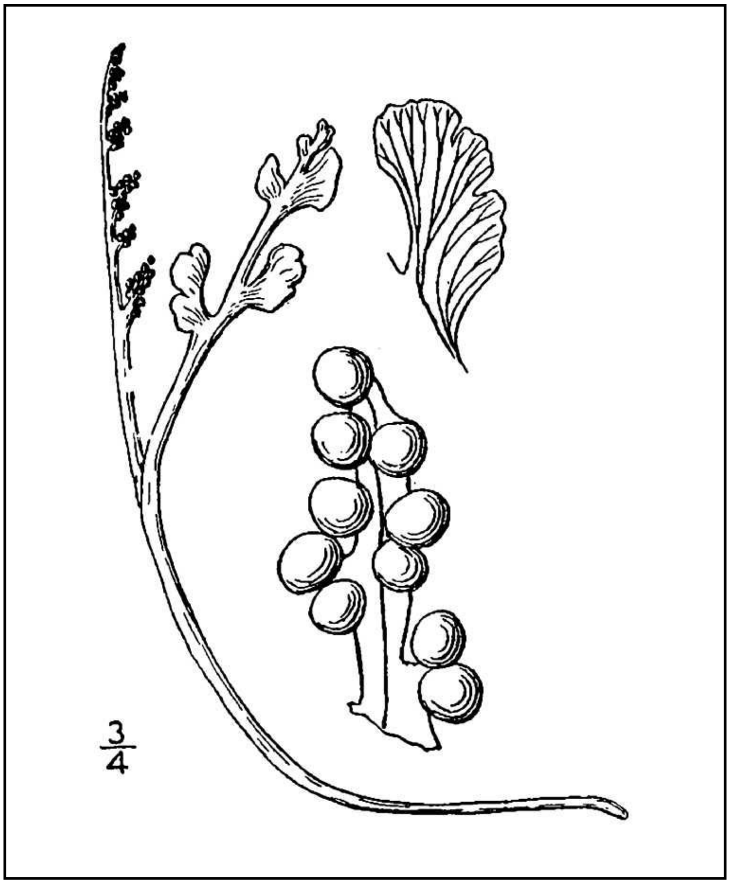

Figure 7. Illustration of *Botrychium tenebrosum* (Britton and Brown 1913).

Distribution and abundance

Members of subgenus *Botrychium* are distributed worldwide, predominantly in temperate and northern temperate habitats, but there are also representatives in temperate South America, New Zealand, and Australia (Clausen 1938, Wagner and Wagner 1993). In general, western North America and the Great Lakes region are recognized as centers of diversity and abundance for this subgenus.

Botrychium simplex is a circumboreal species, known from North America, Greenland, Iceland, Europe, Scandinavia, Corsica, and Japan (**Figure \8**; Clausen 1938, Wagner and Wagner 1986, Ollgaard 1971, Farrar 2001, Anderberg 2003). In the western hemisphere, it is known from Greenland across the Canadian arctic and subarctic to Alaska, south to California, Colorado, Mississippi, and North Carolina (**Figure 9**; Kartesz 1999, Farrar 2005). It is known from 10 provinces in Canada, 36 U.S. states and the District of Columbia. In some parts of its range, *B. simplex* is more common than other *Botrychium* species, and it is ranked G5 (globally secure) accordingly. Along with *B. matricariifolium*, *B. simplex* is the most common moonwort in eastern North America. However, in many parts of its range, including Region 2, *B. simplex* is locally quite rare. It has apparently been extirpated in Connecticut, Maryland, Virginia, and possibly Ohio (Kartesz 1999). See **Table 7** for a list of states and provinces where *B. simplex* is found, including subnational (S) ranks and special state designations.

It appears that three of the four currently recognized varieties of *Botrychium simplex* (*compositum*, *simplex*, and *tenebrosum*) occur in Region 2. Dr. H. Wagner annotated many Colorado specimens housed at the University of Colorado Herbarium and noted whether they were "western" or "eastern" *simplex*. From Wagner's annotations, it appears that both "eastern" (either var. *simplex* or an undescribed variety) and "western" (probably mostly var. *compositum*) *B. simplex* have been documented in Colorado (Farrar personal communication 2003, Root personal communication 2003). However, Wagner noted only one specimen (*Douglass #62-25*; from Grand County, Colorado) to be "eastern" *simplex*, and from photographs, Farrar has confirmed that this

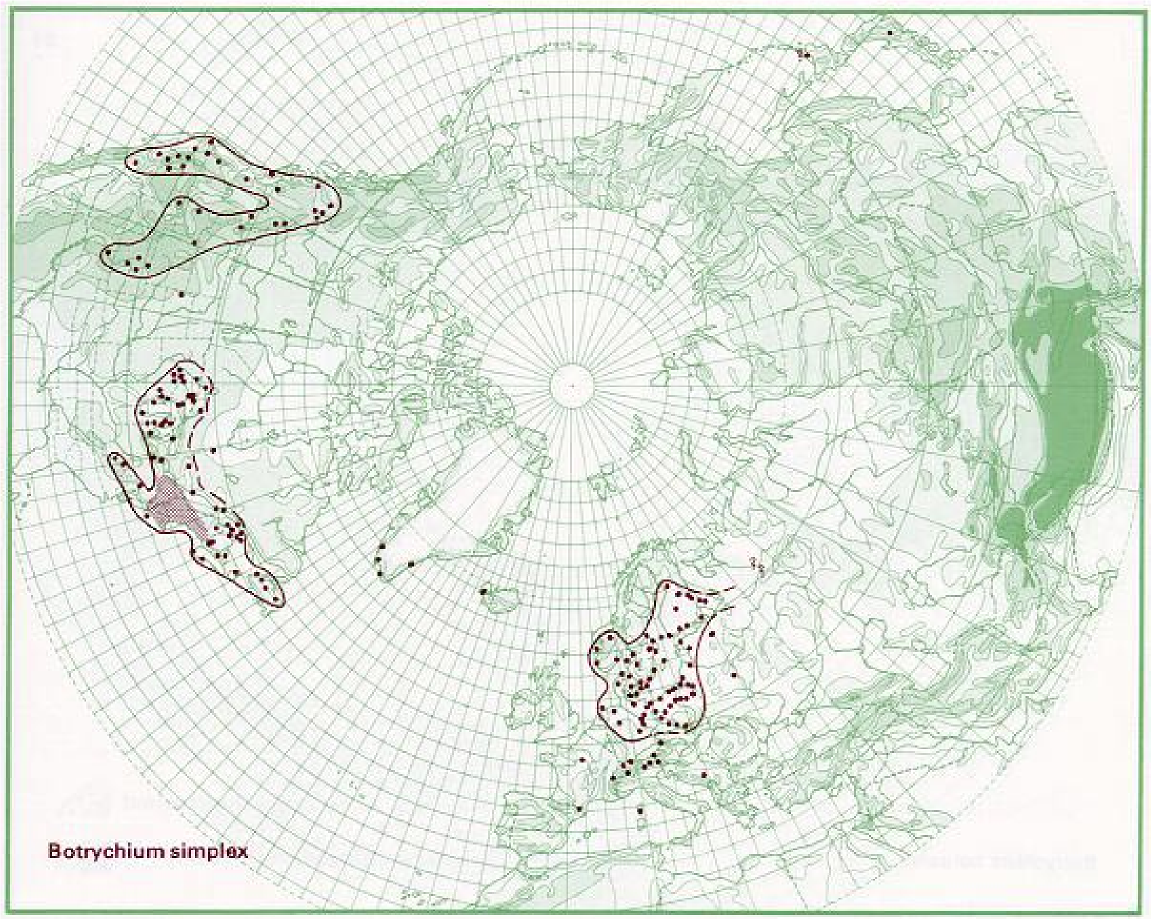

Figure 8. Global distribution map of *Botrychium simplex* (Anderberg 2003).

is either "eastern" *simplex* (var. *simplex*) or the new undescribed variety from the Southwest. Farrar may employ molecular techniques to confirm the taxonomic status of these plants (Farrar personal communication 2003). This occurrence has not been seen since 1962, despite attempts by Peter Root to find it (Root personal communication 2003). At least five Wyoming specimens housed at the Rocky Mountain Herbarium are labeled var. *tenebrosum*, and Wyoming material falls into this variety, according to Hartman and Nelson (2001). Most Colorado and Wyoming specimens have not been re-examined by Farrar in light of the contemporary species concept.

Two erroneous sources of distribution information were found in the literature. The distribution map in Wagner and Wagner (1993) does not show occurrences in the Black Hills, which contain one occurrence in Lawrence County, South Dakota and several, newly discovered occurrences in Crook County, Wyoming. These occurrences are important to note since they add continuity to the distribution of *Botrychium simplex* across North America. Wherry (1938) noted that Rydberg had misleadingly attributed this species to the prairies of Colorado, but the source of Rydberg's erroneous report was not found. Rydberg (1906) and Rydberg (1922) do not include the plains of Colorado within the distribution of *B. simplex*.

Many surveys for *Botrychium simplex* and other moonwort species have been completed in the past two decades (e.g., Lorain 1990, Vanderhorst 1997). Recent surveys in Region 2 have led to the discovery of new moonwort occurrences (e.g., Fertig 2000, Kolb and Spribille 2000, Thompson 2000, Buell 2001, Steinmann 2001a, Steinmann 2001b, Thompson 2001, Abbott 2003, Crook personal communication 2003, Farrar personal communication 2003, Fertig 2003, Root personal communication 2003, Farrar 2005, Burkhart personal communication 2006). The known distribution of this species in the states of Region 2 consists of disjunct clusters of occurrences (**Figure 10**, **Table 8**). This apparent pattern may be an artifact of the intensity of searching that has been conducted in particular areas rather than an ecologically meaningful pattern. For example, extensive searches in Yellowstone

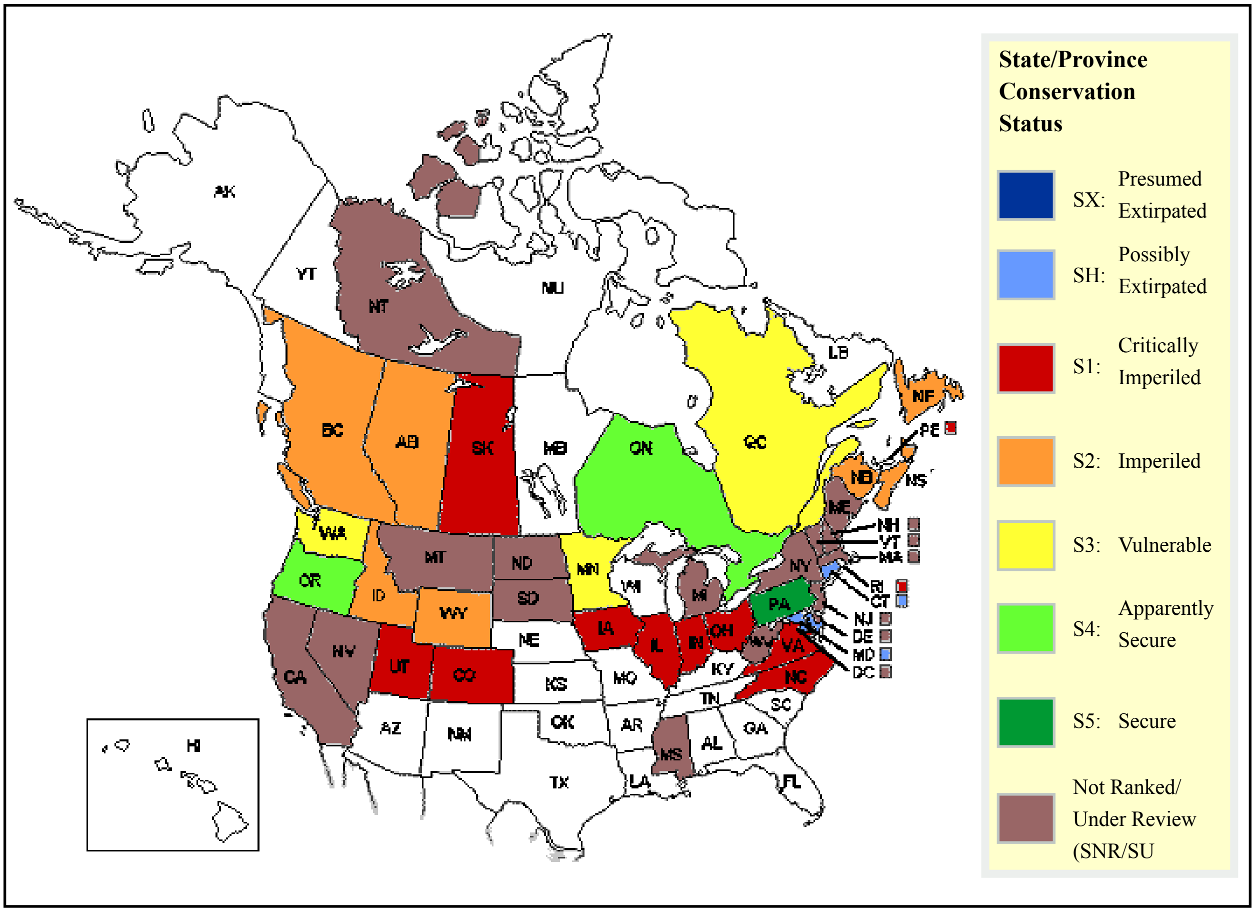

Figure 9. Distribution of *Botrychium simplex* in North America, color-coded by S rank (from NatureServe 2005). *Botrychium simplex* is now ranked S2 in Colorado.

Table 7. Known distribution of *Botrychium simplex* in the western hemisphere (from Ollgaard 1971, Kartesz 1999, USDA Natural Resources Conservation Service 2002, and NatureServe 2005). USDA Forest Service Region 2 states are in bold.

Nation	State/Province/District	S rank	State Designation	Status
Canada	Alberta	S2		
Canada	British Columbia	S2S3		
Canada	New Brunswick	S4		
Canada	Newfoundland	S2		
Canada	Northwest Territories	SNR		
Canada	Nova Scotia	S2S3		
Canada	Ontario	S4?		
Canada	Prince Edward Island	S1		
Canada	Quebec	S3S4		
Canada	Saskatchewan	S1		
Greenland	Julianehaab	none		
USA	California	SNR		
USA	**Colorado**	**S2**		
USA	Connecticut	SH	Special Concern	Extirpated
USA	Delaware	SNR		

Table 7 (concluded).

Nation	State/Province/District	S rank	State Designation	Status
USA	District of Columbia	SNR		
USA	Idaho	S2		
USA	Illinois	S1	Endangered	
USA	Indiana	S1	Endangered	
USA	Iowa	S1	Threatened	
USA	Maine	SNR		
USA	Maryland	SH	Endangered	Extirpated
USA	Massachusetts	SNR		
USA	Michigan	SNR		
USA	Minnesota	S3	Special Concern	
USA	Mississippi	SNR		
USA	Montana	SU		
USA	**Nebraska**	**none**		
USA	Nevada	SNR		
USA	New Hampshire	SNR		
USA	New Jersey	SNR		
USA	New York	SNR	Exploitably Vulnerable	
USA	North Carolina	S1		
USA	North Dakota	SU		
USA	Ohio	S1	Endangered	Possibly Extirpated
USA	Oregon	S4		
USA	Pennsylvania	S5		
USA	Rhode Island	S1		
USA	**South Dakota**	**SNR**		
USA	Utah	S1		
USA	Vermont	SNR		
USA	Virginia	S1		Extirpated
USA	Washington	S3	Sensitive	
USA	West Virginia	SNR		
USA	Wisconsin	unknown		
USA	**Wyoming**	**S2**		

National Park (outside of Region 2) have located numerous occurrences, but adjacent areas within Region 2 have been less intensively searched. Intensive searches on the Black Hills National Forest occurred in 2004 and 2005 and resulted in the discovery of 14 new occurrences, whereas it was previously known from only three occurrences. In fact, *B. simplex* was the most commonly encountered moonwort species (Burkhart personal communication 2006). On the other hand, Hollis Marriott and Walter Fertig conducted *Botrychium* surveys in Wyoming in 1989, 1993, and 1999 (Fertig 2000). While many occurrences of other species were found, these surveys resulted in relatively few discoveries of *B. simplex* plants. Forested habitats have not been extensively searched for *B. simplex* in Region 2 (Root personal communication 2003). Also, because of the tendency to find *B. simplex* in wetter, shadier habitats than other moonworts in Region 2, this species may not be encountered as often and therefore be underreported.

The majority of the known occurrences of *Botrychium simplex* in Region 2 are on National Forest System land (**Table 9**). These are distributed across 11 national forests, with most occurrences reported from the Black Hills, Shoshone, and White River national

Table 8. Summary information for the known occurrences of *Botrychium simplex* in the states of USDA Forest Service Region 2, including occurrences in Wyoming that are outside of the Region 2 administrative boundary.

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
1	Colorado	Boulder	Caribou Flats	9,750	7/28/1949	7/28/1949	**USDA Forest Service (USFS) Roosevelt National Forest**	Unknown	Prettyman (s.n.)	COLO	Wagner 1989: "western simplex"	Wet soil of peat bog
2	Colorado	Boulder	4th of July Trailhead	10,100	7/27/2001	7/27/2001	**USFS Roosevelt National Forest**	Unknown	David Steinmann (s.n.)	COLO	Verified by Peter Root	In the open hillside meadow on the south facing slope to the east of the trailhead and on the north side of the road, along the edges of willows and near rocks.
3	Colorado	Clear Creek	South of Echo Lake	10,600	8/4 or 8/5/1984	8/4 or 8/5/1984	City of Denver Echo Lake Park	1	Florence Wagner	N/A	Root personal communication 2003; note in CNHP files from unknown source	Not reported
4	Colorado	Conejos	Terrace Reservoir	9,020	7/1/1995	7/1/1995	**USFS Rio Grande National Forest**	"Abundant"	*M. Aitken and E. Vanwie (RG30)*	COLO	Originally identified as *Botrychium hesperium*	On flat, southeast facing, dry, open area in bottom of canyon; with *Antennaria* spp. Sporulating
5	Colorado	Conejos	Between Fish Lake and Blue Lake	11,490	8/8/2001	8/8/2001	**USFS San Juan National Forest**	Unknown	*S. O'Kane (5872)*, Arnold Clifford, Dave Jamieson	SJNM		Rolling tundra with tree islands of *Picea* with many ponds; bare spots in grassy matrix on south-facing slope
6	Colorado	El Paso	Pikes Peak	~10,940	before 1937	before 1937	**Probably USFS Pike National Forest**	Unknown	*I.M. Johnston (2415)*	GH	Wherry (1938), Clausen (1938)	Not reported
7	Colorado	Gilpin	Teller Lake	9,600	8/1/1919	8/1/1919	**USFS Roosevelt National Forest**	Unknown	*H.M. Schmoll (11)*	COLO	Wagner 1989: "western simplex" (orig. labeled *Botrychium lunaria*)	Near border of lake
8	Colorado	Grand	North Inlet Trail	8,700	6/29/1962	6/29/1962	National Park Service (NPS): Rocky Mountain National Park	Unknown	*MM. Douglass (62-31)*	COLO	T. Spribille: one plant may be *Botrychium paradoxum* - sent to F. Wagner. Specimen originally ident. as *B. lunaria*. Annotated by P. Root.	Not reported

Table 8 (cont.).

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
9	Colorado	Grand	East Inlet	8,500	6/27/1962	6/27/1962	NPS Rocky Mountain National Park	Unknown	*M.M. Douglass (62-25)*	COLO	Annotated as *Botrychium simplex* by W. Wagner (1989); originally ident. as *B. lunaria*; P. Root noted that Wagner considers this specimen to be "eastern simplex." This was confirmed by Farrar (personal communication 2003)	Near the falls, in wet marshy area of spruce-fir forest right beside stream
10	Colorado	Gunnison	No information	9,200?	No data	No data	No data	No data	Arnett (2002)	RM?	Mentioned in Arnett (2002), no specimen at RM	Spruce/fir forest and/or subalpine meadow
11	Colorado	Hinsdale	Northeast of Gravel Mountain	10,600	8/24/1999	8/24/1999	Bureau of Land Management (BLM)	Unknown	*M. Arnett (7417)*	RM	Sporulating	Avalanche meadow
12	Colorado	Hinsdale	East fork of Alpine Gulch	12,200	8/30/1999	8/30/1999	BLM	Unknown	*M. Arnett (7902)*	RM	Sporulating	Spruce forest to krummholz; along gulch
13	Colorado	Hinsdale	Upper West Fork of Mineral Creek	12,680	8/11/1999	8/11/1999	**USFS Gunnison National Forest**	Unknown	*M. Arnett (6836)*	RM	Sporulating	Alpine tundra; basin of an unnamed lake
14	Colorado	Hinsdale	Thirty Mile Campground	9,000	7/27/1995	7/27/1995	**USFS Rio Grande National Forest**	12	*M. Aitken and E. Vanwie (RG 174 & 175)*	COLO	Sporulating	On dry, open northwest slope of tuff
15	Colorado	Lake	Fremont Pass	11,100	9/12/2000	9/12/2000	**USFS San Isabel National Forest**	5 to 10	A. Kolb & T. Spribille	N/A	Late in season; many dried-up plants	Along railroad tracks; open, dry site
16	Colorado	Saguache	No information	No data	No data	No data	No data	No data	University of Colorado Herbarium (2003)	COLO?		Not reported
17	Colorado	Summit	Breckenridge Ski Area	11,155	809/2000	8/9/2000	**USFS White River National Forest**	more than 10	*A. Kolb & T. Spribille (8)*	COLO		Open, clearcut, subalpine forest

Table 8 (cont.).

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
18	Colorado	Summit	Boreas Pass	11,483	8/9/2000	8/9/2000	**USFS White River National Forest**	20 to 50	*A. Kolb & T. Spribille (11)*	COLO		Open, eroded, grassy slopes at timberline; area of historical burn; on gravelly soil among young spruce trees; also on roadbeds; aspect: west; moisture: dry
19	Colorado	Summit	Vail Pass Rest Area	11,400	7/22/1999	7/22/1999	**USFS White River National Forest**	~50 or more	Nancy Redner	KH		Subalpine meadow; downslope of subalpine forest of lodgepole and spruce-fir, on a 15 to 35 percent slope; organic and granite soils, cobbly, seasonally wet but becoming dry; hillside is used by elk
20	Colorado	Summit	Copper Mountain	~10,500	7/14/2000	7/14/2000	**USFS White River National Forest**	1	*P. Root (1258)*	KH	Three-part trophophore partly eaten away, with vehicle tracks on the plant	In a narrow road between ski trails, in shade
21	Colorado	Summit	Keystone	~10,500	2000	2000	**USFS White River National Forest**	less than 10	A. Kolb & T. Spribille (2000)	N/A		Not reported
22	Colorado	Summit/ Eagle	Shrine Pass	11,155	8/15/2000	8/15/2000	**USFS White River National Forest**	5	*A. Kolb & T. Spribille (22)*	COLO		Open, eroded, grassy slopes, subalpine; area of historical burn; red sandstone; west-facing slope
23	Colorado	Teller	*Mueller Ranch, Reservoir #1*	~9,000	7/02/1980	9/28/1983	State of Colorado Dome Rock State Wildlife Area	Unknown	*Darrow and Sigstedt (9340)* (1980); W.L. Baker and D. Kuntz (1983)	COCO	Baker and Kuntz (1983). COCO Specimen was verified by D. Farrar	Marshy ground; in a marshy area among *Carex aquatilis*
24	Colorado	Teller	Mount Baldy	10,300	6/28/1942	6/28/1942	**USFS Pike National Forest**	Unknown	*J.B. Hartwell, C.W.T. Penland, and G.R. Marriage (1251)*	KH	Wagner considers all but one of the plants on this sheet to be “western simplex”	Not reported
25	Nebraska	Brown	Niobrara Valley Preserve	~2,400	Unknown	~6/2/1986	The Nature Conservancy	“fairly common”	Farrar and Johnson-Groh (1986), D.R. Farrar (86-6-2-2)	ISC	Not found at this location in attempts to find it in 2004 by Farrar (personal communication 2006)	In the sandy floodplain in swales under cottonwood and juniper along the Niobrara River below the narrow canyon area; on river floodplain in shaded opening between large *Juniperus virginiana*

Table 8 (cont.).

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
26	South Dakota	Custer	Lightning Creek	5,630	6/18/2004	7/8/2004	**USFS Black Hills National Forest**	1	C. Skelton, D. King, and T. Price (BOSI-10)	N/A	Nearing sporulation on July 8	In thick moss cover, near a faint game trail (which has caused disturbance on the slope) and a young downed spruce; in partial shade near a drainage bottom, in dry-mesic soil
27	South Dakota	Lawrence	Steamboat Rock	~4,500	6/20/1953	6/20/1953	**USFS Black Hills National Forest**	Unknown	Unknown	Unknown	from SDNHP	In grass with *Agoseris glauca*
28	South Dakota	Lawrence	Tinton Road	6,426	6/12/2004	7/9/2004	**USFS Black Hills National Forest**	14	C. Skelton, D. Farrar, T. Price, and D. King (BOSI-8, BOTR-11)	N/A	Not yet sporulating on June 22, yellowing stalk and brown sporangia on July 9; *Botrychium minganense* is also found at this location	In a large open meadow with high forb cover at the edge of an old stock pond and near a small stand of young aspen and an old roadbed; moisture: dry/mesic
29	South Dakota	Lawrence	Long Draw	6,160	6/17/2004	6/17/2004	**USFS Black Hills National Forest**	1	C. Skelton, D. King, and T. Price (BOSI-9)	N/A		On the toe of a north-northeast facing slope above a southeast facing draw, with high cover of moss and forbs. In partial shade in lower slope of the draw. Moisture: mesic. The edges of the draw have scattered spruce and aspen growing in patches.
30	South Dakota	Lawrence	Meadow Creek	4,600	6/23/2004	6/23/2004	**USFS Black Hills National Forest**	8	C. Skelton, D. King, and T. Price (BOSI-13)	N/A	All individuals had already sporulated	In an open grass/forb area on the edge of a shallow depression; near a small ponderosa pine in an area with other small ponderosa pine saplings; area is grazed; moisture: dry-mesic
31	South Dakota	Lawrence	Higgens Gulch	5,480 to 5,520	6/9/2004	7/13/2004	**USFS Black Hills National Forest**	13	B. Burkhart, C. Mayer, C. Skelton, D. Reyher, D. Farrar, D. King, R. Crook, S. Popovich, and T. Price (BOTR-4)	N/A	Possibly two varieties of *Botrychium simplex* present; *B. pallidum* also occurs at this location	At the base of an open slope in a grass/forb dominated area; there was a skid trail on the slope above this occurrence; the slope is fairly steep with small shrubs starting to emerge

Table 8 (cont.).

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
32	South Dakota	Lawrence	Citadel Rock	5,200 to 5,240	6/10/2004	6/22/2004	**USFS Black Hills National Forest**	1	C. Mayer, C. Skelton, D. Farrar, D. King, and T. Price (BOTR-5)	N/A	Sporangia were still green on June 10, but were yellowing by June 22; *Botrychium pallidum* and *B. michiganense* are also found at this location	In partial shade in an open grassy meadow; an open old road bed passes through the meadow where some weeds (*Taraxacum sp., Trifolium*) occur
33	South Dakota	Pennington	Four Corners	6,560 to 6,600	5/26/2005	6/7/2005	**USFS Black Hills National Forest**	26	B. Burkhart, C. Mayer, S. Corey, I. Drieling, C. Buckert, and K. Zacharkevics (BOTR-31)	N/A	Other *Botrychium* species were seen at this location but the identification is not yet verified	Open meadow of Black Hills Montane Grassland with native species composition; area is very flat; minor landforms seem important; area has been heavily grazed; limestone parent material
34	South Dakota	Pennington	Redbank Spring	6,600	6/8/2005	6/8/2005	**USFS Black Hills National Forest**	~75	B. Burkhart, C. Mayer, S. Corey, I. Drieling, C. Buckert, and K. Zacharkevics (BOTR-30)	N/A	Likely that many more individuals are in the vicinity. Other *Botrychium* species are present at this site but isozyme analysis is required to identify them. This is a very rich site for *Botrychium* species.	Open rolling grassland with fairly heavy grazing. High forb diversity. Shrubby cinquefoil scattered frequently on the hillside. Limestone parent material. Moisture: mesic. Topographic position: lower slope.
35	South Dakota	Pennington	Coulsen Hughes Draw	6,600	7/21/2004	7/21/2004	**USFS Black Hills National Forest**	1	D. King (BOTR-19)	N/A	Not yet sporulating	On an old mossy skid trail at the base of a northeast facing slope; spruce dominate the edges of the skid trail; in partial shade of a young spruce tree upslope of the plant
36	Wyoming	Carbon	Slash Ridge	~9,600	8/4/1990	8/4/1990	**USFS Medicine Bow National Forest**	Unknown	*P. Root (90-70)*	KH	Western type. Trophophores slightly concave but basal segments strongly folded; two parts of leaf separated at or just below soil surface	In *Sibbaldia* clumps and bare soil between road and willow carr edge

Table 8 (cont.).

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
37	Wyoming	Crook	Bearlodge Campground	4,670 to 4,680	6/30/2003	7/2/2004	**USFS Black Hills National Forest**	3	C. Mayer, R. Crook, D. King, D. Farrar, M. Gabel, K. Zacharkevics, S. Corey, A. Kratz, C. Skelton, D. Rarrar, D. King, S. Popovich, and T. Price (BOTR-1, BOTR-2)	N/A	*Botrychium simplex* has been found in three areas at this location; *B. michiganense* is also present at this location	Mossy, grassy area under a thin cover of bur oak and *Pinus ponderosa*; moist to dry-mesic, sandy soil; plants are found among a stand of 1 to 3 foot tall aspen trees; forbs are scattered, grasses small but nearly continuous in places; bare ground is covered occasionally by pine needles
38	Wyoming	Crook	FS Road 830	4,730	6/2/2003	6/2/2003	**USFS Black Hills National Forest**	1	R. Crook, D. Farrar, C. Mayer, and D. King	N/A		Open sunny roadside meadow with *Pinus ponderosa* at margins
39	Wyoming	Crook	Beaver Creek	4,900 to 4,940	6/11/2004	7/2/2004	**USFS Black Hills National Forest**	60	B. Burkhart, C. Skelton, D. Rarrar, D. King, R. Crook, and T. Price (BOTR-6)	N/A	In early sporulation on June 11; one plant sporulating on July 2; *Botrychium pallidum* is also found at this location	Near and on the remnants of an old road bed; in an open site in grass/forb vegetation near a stand of medium sized ponderosa pines; in a flatter area on the upper slope of a north-facing hill; the site is currently grazed
40	Wyoming	Crook	Warren Peak	6,500 to 6,600	6/25/2004	6/25/2004	**USFS Black Hills National Forest**	7	D. King, C. Skelton, and T. Price (BOSI-14)	N/A	Sporangia are immature	On a steep, north-facing slope dominated by native forbs; the ground is covered in moss and rocks where vegetation cover is sparse; plants are in an open area with a little shade provided by a large ponderosa pine; area is grazed

Table 8 (cont.).

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
41	Wyoming	Crook	Dugout Gulch	4,400 to 4,500	6/21/2004	7/8/2004	**USFS Black Hills National Forest**	207	C. Skelton, D. King, T. Price, and P. Sweanor (BOSI-12)	N/A	Large variety in stage of maturation seen on June 21; plants had sporulated on July 8; both "eastern" and "western" varieties were seen; specimens are being verified by D. Farrar	On a toe slope between two different drainages; there is a vague native surface old road bed (currently unused) going along the toe slope where the plants are growing; with grasses and small ponderosa pines; area is grazed.
42	Wyoming	Crook	Fawn Creek	4,920	6/11/2004	7/2/2004	**USFS Black Hills National Forest**	1	B. Burkhart, C. Skelton, D. Farrar, D. King, R. Crook, and T. Price (BOSI-6)	N/A	Plant could not be found again on July 2	In an old open unused road bed with very little tree cover; plant is growing in an area covered by needle litter; area is grazed
43	Wyoming	Fremont	Vicinity of Deacon Lake	9,400 to 10,000	8/17/1984	8/17/1984	**USFS Shoshone National Forest**	Unknown	*R.L. Hartman (18845)*	RM	Var. *tenebrosum*; sporulating	Coniferous forest, grassy ridge, and high basin with internal drainage; grassy slopes
44	Wyoming	Johnson	French Creek Swamp	8,100	6/25/2001	6/25/2001	**USFS Bighorn National Forest**	Unknown	*W.Fertig (19618)*	RM	Var. *tenebrosum*; sporulating	Grass and moss-rich hummocks along banks of small stream in marsh at edge of *Pinus contorta/ Picea engelmannii* woods
45	Wyoming	Lincoln	Corral Creek	9,400	9/17/1980	9/17/1980	**USFS Bridger-Teton NF**	"Rare"	*R.W. Lichvar (3923)*	RM	Var. *tenebrosum*; sporulating	Among talus boulders with *Arnica* and *Carex*
46	Wyoming	Park	Yellowstone Lake, Mouth of Pelican Creek	~7,740	1873	1873	NPS Yellowstone National Park	Unknown	*C.C. Parry (306)*	GH, UC, NY, PH, RM, MO	Cited in Clausen (1938)	Mouth of creek
47	Wyoming	Park	Kirwin	9,300	8/13/1984	8/13/1984	**USFS Shoshone National Forest**	Unknown	*R.S. Kirkpatrick (5317a)* and R.E.B. Kirkpatrick	RM	Var. *tenebrosum*; sporulating	Riparian areas adjacent to *Picea engelmannii* and *Abies lasiocarpa* forest; moist creek banks
48	Wyoming	Park	Hayden Creek	8,600 to 9,400	7/14/1985	7/14/1985	**USFS Shoshone National Forest**	Unknown	*R.L. Hartman and B.E. Nelson (20707)*	RM		Rocky outcrops

Table 8 (cont.).

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
49	Wyoming	Park	Little Bald Ridge	6,900 to 7,800	7/18/1985	7/18/1985	**USFS Shoshone National Forest**	Unknown	*R.L. Hartman (21051)*	RM		Moist north exposure at edge of Douglas-fir forest
50	Wyoming	Park	Ridge Northeast of Windy Mountain	8,600 to 9,600	8/17/1985	8/17/1985	**USFS Shoshone National Forest**	Unknown	*R.L. Hartman (21880)*	RM		Limestone outcrops and adjacent slopes
51	Wyoming	Park	Along West Blackwater Creek Trail	7,600	8/3/1982	8/3/1982	**USFS Shoshone National Forest**	Unknown	*E.F. Evert (4619)*	RM	Determined as *Botrychium lunaria*	In moss in montane forest
52	Wyoming	Park	Kitty Creek	7,200	8/1/1982	8/1/1982	**USFS Shoshone National Forest**	Unknown	*E.F. Evert (4594)*	RM	Determined as *Botrychium lunaria*	Not reported
53	Wyoming	Teton	Heart Lake Geyser Basin	7,480	6/27/1995	6/27/1995	NPS Yellowstone National Park	125	*J.J. Whipple (4474, 4478)*	YELLO		About 30 meters above Columbia Pool; east aspect; 10 percent slope; adjacent to hot ground that is too hot to support vascular plants; also on north aspect; 5 percent slope on edge of barren sinter sheet and *Botrychium lunaria*wetland west of Rustic Geyser
54	Wyoming	Teton	Lower Geyser Basin	7,240	6/24/1994	6/5/2003	NPS Yellowstone National Park	Several hundred	*J.J. Whipple (4263, 4264)*	YELLO		In a meadow near "bobby sox trees" (see **Definitions** for explanation) in areas that are less saturated than other areas in the meadow
55	Wyoming	Teton	Madison Plateau	~8,000	8/10/1978	8/10/1978	NPS Yellowstone National Park	Unknown	*W. Gernon (7)*	RM, YELLO	ID is dubious; annotated by Don Despain in 1978 but consists of one misshapen lamina (Whipple personal communication 2003)	Wetland

Table 8 (concluded).

	State	County	Location	Elevation (ft.)	Date first observed	Date last observed	Land ownership/ management	Abundance	Collector / Observer	Herbarium	Notes	Habitat
56	Wyoming	Teton	Northwest side of Mariposa Lake on Two Ocean Plat	~8,950	8/20/1979	8/20/1979	NPS Yellowstone National Park	Unknown	T. Caprio (s.n.)	YELLO	At least one of the three plants on this sheet is *Botrychium simplex* (Whipple personal communication 2003)	Not reported
57	Wyoming	Teton	Upper Geyser Basin near Old Faithful	7,300	6/7/1994	6/7/1994	NPS Yellowstone National Park	3	J.J. Whipple	N/A	No collection made due to small population size; not seen again at this site despite repeated visits	Along abandoned road in roadbed where asphalt has been removed
58	Wyoming	Teton	West slope of Corner Mountain	10,000	8/7/1997	8/7/1997	Unknown	Unknown	*W.Fertig (17953)*	RM	Var. *tenebrosum*	Open, moist clay soil below white limestone boulders at timberline; community of *Antennaria media/ Phlox pulvinata* with 60 percent vegetative cover; sporulating

Herbarium acronyms:

COCO: Carter Herbarium, Colorado College

COLO: University of Colorado Museum Herbarium

GH: Gray Herbarium, New York

ISC: Ada Hayden Herbarium

KHD: Kalmbach Herbarium, Denver Botanic Gardens

MO: Missouri Botanical Garden

PH: Academy of Natural Sciences, Philadelphia

RM: Rocky Mountain Herbarium

SJNM: San Juan College Herbarium, Farmington, NM

UC: University of California Berkeley

YELLO: Yellowstone National Park Herbarium

Table 9. Land ownership / management status summary for the 50 known occurrences of *Botrychium simplex* within the administrative boundary of USDA Forest Service Region 2.

Land Ownership Status	Number of Populations	Subtotals
USDA Forest Service	**41**	
Bighorn National Forest		1
Black Hills National Forest		16
Gunnison National Forest		1
Medicine Bow National Forest		1
Pike National Forest		2
Rio Grande National Forest		2
Roosevelt National Forest		3
San Isabel National Forest		1
San Juan National Forest		1
Shoshone National Forest		7
White River National Forest		6
National Park Service	**2**	
Rocky Mountain National Park		2
Bureau of Land Management	**2**	
State of Colorado	**1**	
Dome Rock State Wildlife Area		1
City of Denver	**1**	
Echo Lake Park		1
The Nature Conservancy	**1**	
Niobrara Valley Preserve		1
Unknown	**2**	

forests. Yellowstone National Park (outside Region 2) and Rocky Mountain National Park include six and two occurrences, respectively.

In Colorado, *Botrychium simplex* is known from 24 locations in 14 counties, but data are sparse for most occurrences. Almost no data are available for occurrences in Gunnison, Jackson, and Saguache counties; these locations have not been verified. Arnett (2002) reported a collection of *B. simplex* from Gunnison County, but a specimen or other confirmation has not been found. A report from Jackson County ("on the west side of Cameron Pass") is mentioned in a species abstract draft for *B. simplex* (Schwab 1992), but no specimen or other ancillary information exists for this report. Popovich (personal communication 2006) and Proctor (personal communication 2006) searched suitable habitats near Cameron Pass but did not find *B. simplex*. It now appears that this report arose from a 1986 observation initially identified as *B. simplex*, but later determined to be *B. hesperium* (Farrar personal communication 2006). Saguache County is included in the range for *B. simplex* by the University of Colorado Herbarium (2002), but a corresponding specimen for this occurrence has not been found. Two other occurrences lacking vouchers are in Clear Creek and Lake counties.

Occurrences in Colorado are patchy, with clusters in the vicinity of Pikes Peak, Breckenridge, and Granby. Two clusters are in the San Juan Mountains. The patchy nature of the Colorado distribution of *Botrychium simplex* is likely to be the result of survey intensity because in two cases (around Breckenridge (#17 in **Table 8**) and Granby (#8 and 9 in **Table 8**)) the occurrences were documented during one survey or by one person. In areas such as Pikes Peak, numerous collectors were involved, but most used known locations as the start of their search.

Three occurrences of *Botrychium simplex* have been documented in the Pikes Peak area in El Paso and Teller counties (#6, 23, and 24 in **Table 8**). The occurrence on Pikes Peak that is documented in Wherry (1937) was found on the south side of the peak (Root personal communication 2003). A moonwort survey was conducted on the north side of Pikes Peak in 2001; numerous moonworts were found, but

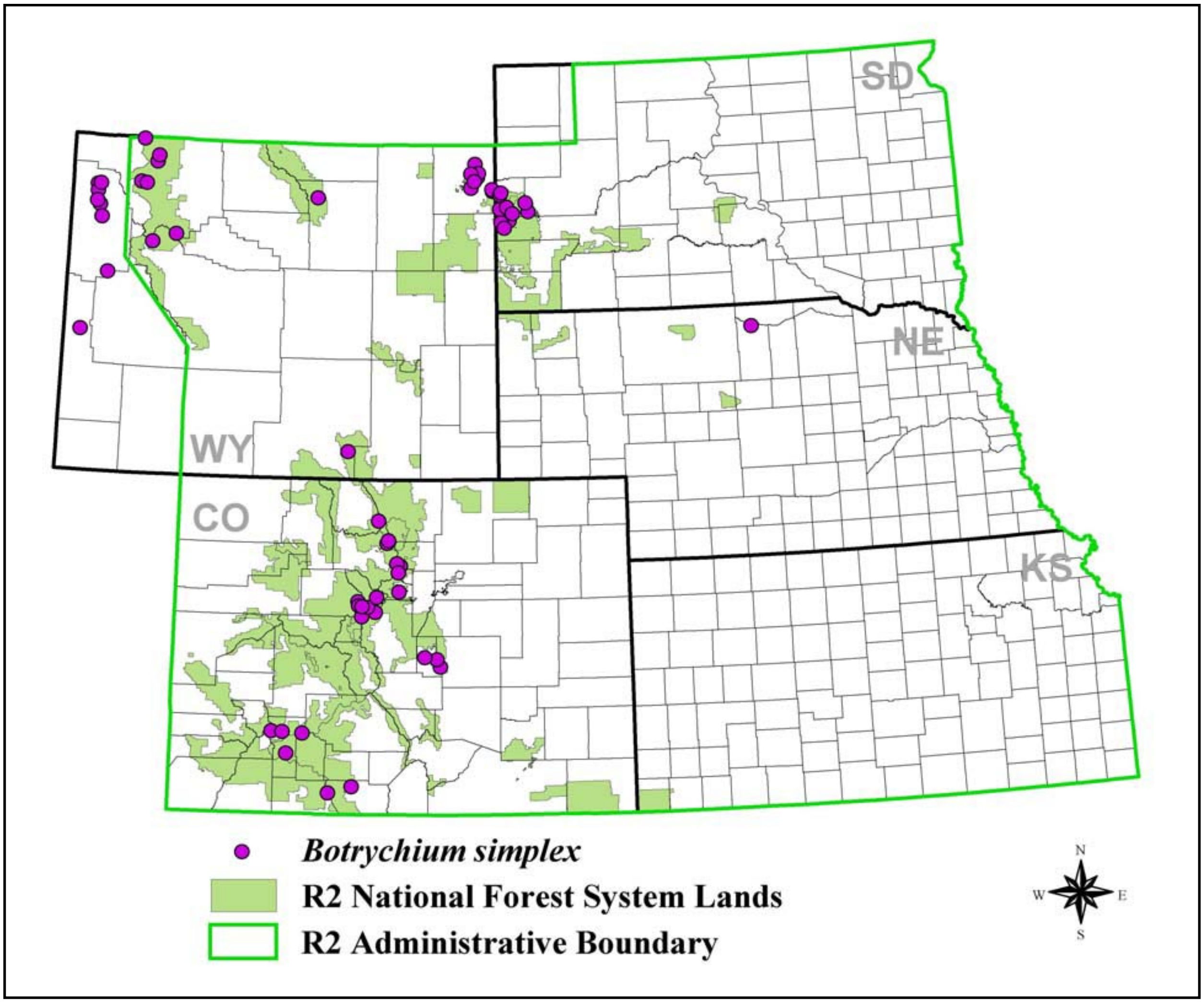

Figure 10. The distribution of *Botrychium simplex* in the states of USDA Forest Service Region 2. **Table 8** is a complete summary of the known occurrences in Region 2.

no *B. simplex* (Steinmann 2001a, Steinmann personal communication 2003).

Recent work by Dave Steinmann and Peter Root (Steinmann 2001b, Root personal communication 2003, Steinmann personal communication 2003) identified numerous moonwort occurrences in the subalpine zone in the Indian Peaks Wilderness Area of Colorado. Two occurrences of *Botrychium simplex* are known from Boulder County, one of which was discovered in 2001 as a result of this work (#2 in **Table 8**). *Botrychium simplex* was much less commonly found than other species including *B. lunaria*, *B. echo*, *B. hesperium*, and *B. minganense* (Steinmann personal communication 2003).

Annette Kolb, Nancy Redner, Peter Root, Tony Spribille, and others conducted extensive surveys for *Botrychium* species at Copper Mountain, Breckenridge, and Keystone ski resorts in Summit County, Colorado. Their work identified five new occurrences of *B. simplex* in which Kolb and Spribille (2000) estimate a total of 85 stems (#15, 17, 18, 21, and 22 in **Table 8**). Thompson (2001) presents data on moonwort aggregations in Summit County. Aggregations were observed to range in size from a single plant to 1.43 acres, with up to 590 individuals in single species aggregations and as many as 1,717 individuals in aggregations containing multiple moonwort species. *Botrychium simplex* was the rarest moonwort observed in these surveys relative to *B. lanceolatum*, *B. echo*, *B. minganense*, *B. hesperium*, *B. lunaria*, *B. pallidum*, and *B. pinnatum*.

No population estimates exist for 14 of the 24 occurrences in Colorado, and based on the 10 occurrences where there is some indication of abundance, the total population for Colorado is between 100 and 200 individuals (**Table 8**).

In Wyoming, *Botrychium simplex* is known from 23 occurrences in seven counties (**Table 8**). Wyoming Natural Diversity Database has not tracked *B. simplex* since 1986, so they have not maintained information for this species (Heidel personal communication 2003). Six of the Wyoming occurrences are from Yellowstone National Park, and another is on the Bridger-Teton National Forest in Region 4. The other 16 occurrences fall within the administrative boundaries of USFS Region 2. Seven occurrences are located on the Shoshone National Forest, east of Yellowstone National Park; these combined with the six on Yellowstone National Park represent the largest concentration of occurrences in Wyoming. Walt Fertig (2003) discovered *B. simplex* at French Creek Swamp on the Bighorn National Forest in 2001. One occurrence is also known from the Medicine Bow National Forest (#36 in **Table 8**).

In the Black Hills National Forest in Wyoming, two occurrences (#37 and 38 in **Table 8**) of *Botrychium simplex* were discovered in the Bearlodge Mountains of Crook County in 2003. Only one plant was found at each site. One of these locations, the Bearlodge Campground, also contained *B. campestre* and *B. "michiganese"* and has become notorious for moonwort species (Crook personal communication 2003). In 2004 and 2005, Farrar, his graduate students, and USFS botanists conducted intensive surveys of the Black Hills National Forest in Wyoming and discovered four more occurrences (Burkhart personal communication 2006).

The largest occurrence of *Botrychium simplex* in Region 2 was one of those discovered in 2004. Located at Dugout Gulch in Crook County, this population was estimated to contain 207 individuals (Burkhart personal communication 2006). Of the 15 Wyoming occurrences in Region 2, populations have been estimated for only 6 and these total 279 individuals.

Before 2004 *Botrychium simplex* was known from only a single occurrence in South Dakota, a population in Lawrence County that had not been seen since 1953 (#27 in **Table 8**). This is probably the population that is reported for the Black Hills of South Dakota by Dorn and Dorn (1972). Ode (2001) mentions the existence of another historic occurrence in northeastern South Dakota, but no detailed information is available regarding this occurrence. As in Wyoming, intensive surveys of the Black Hills National Forest in 2004 and 2005 resulted in the discovery of nine new occurrences of *B. simplex* in South Dakota. The estimated abundance for occurrences in South Dakota is 140 individuals.

In Nebraska, *Botrychium simplex* is known from a single report from Brown County at the Niobrara Valley Preserve owned by The Nature Conservancy (Farrar and Johnson-Groh 1986, Farrar personal communication 2003; #25 in **Table 8**). Here it is described as being "fairly common" (Farrar personal communication 2003), but there are no data on the size of this occurrence. The Ada Hayden Herbarium (ISC) houses a collection from this occurrence, but no other reports of *B. simplex* are known from Nebraska (Bolick personal communication 2003, Farrar personal communication 2003, Lewis personal communication 2003, Rolfsmeier personal communication 2003). There are no specimens of *B. simplex* at the University of Nebraska State Museum Herbarium (NEB) (Bolick personal communication 2003). Farrar attempted unsuccessfully to find *B. simplex* in the Niobrara Valley Preserve in 2004 (Farrar personal communication 2006).

Botrychium simplex has not been documented in Kansas, and it is doubtful that it will be found there (Farrar personal communication 2003).

Population trend

There are no rigorous quantitative data on population trend for *Botrychium simplex* in Region 2, and the available data are too sparse to determine whether populations have increased or decreased. For species such as *B. simplex* where the proportion of dormant plants varies among years, it is difficult to accurately monitor population trends (Lesica and Steele 1994).

The recent discovery of many occurrences in Region 2 is probably the result of an increase in interest in this species and its congeners rather than an increase in population. Moonworts are now known to be much more widespread and abundant on the Black Hills National Forest (Burkhart personal communication 2006) and in Summit County, Colorado (Thompson 2001) than previously thought. Additional searches are likely to discover more occurrences.

Many locations of *Botrychium simplex* in Region 2 are known only from very old herbarium specimens. Because of the uncertain location of these collections, it is difficult to determine whether they are extant or whether protective management should be implemented on their behalf. Seventeen of the 50 known occurrences were last visited more than 20 years ago. The failure to observe any occurrence in Region 2 more than once may herald a decline of this species, but it is probably more indicative of the difficulties in finding it.

It is not known how *Botrychium simplex* responds to and recovers from drought. A severe regional drought beginning in 2001 and extending into 2003 may have reduced populations of *B. simplex* in Region 2. The degree to which *B. simplex* depends on years that are particularly favorable (perhaps with respect to precipitation) for successful reproduction is not known.

There is evidence to suggest that *Botrychium simplex* has declined elsewhere in its range. *Botrychium simplex* is believed to have been extirpated from Connecticut, Virginia, Maryland, and possibly Ohio (Kartesz 1999, USDA Natural Resources Conservation Service 2002). *Botrychium simplex* is apparently increasing within Region 9 (Chadde and Kudray 2001b).

The quality and amount of wetland habitat for *Botrychium simplex* has certainly decreased in portions of the states of Region 2, but this decline in habitat has been less severe at higher elevations where most Region 2 *B. simplex* occurrences are found. Since 1986, wetlands have been lost at a rate of 58,500 acres per year in the continental United States (Dahl 2000). In Colorado alone, an estimated one million acres of wetlands (50 percent of the total for the state) were lost before 1980 (Dahl 1990). In total, estimated losses from all Region 2 states is approximately 39 percent of the original wetland acreage (Dahl 1990). Not included in these numbers is the loss and degradation of non-jurisdictional wetlands (i.e., those wetlands not regulated by Section 404 of the Clean Water Act), such as riparian areas. It is unknown what percentage of the wetlands lost in Region 2 would be considered *B. simplex* habitat. In some cases, anthropogenic disturbance may have even created habitat for *B. simplex* (Thompson 2001). This does not, however, suggest that human disturbance can be relied upon for the conservation of this species. See the following Habitat section for information on wetland and other habitats where *B. simplex* occurs.

Habitat

Habitat descriptions and characterization

Botrychium simplex grows in a variety of habitats and conditions and has been noted for its broad ecological amplitude (Chadde and Kudray 2001b). Hitchcock (1823) originally described *B. simplex* from plants found in "dry hilly pastures," but it has since been found in a wide range of other habitats. Lellinger (1985) describes its habitat as "Terrestrial in meadows, barrens, and woods, usually in subacid soil." Wagner and Wagner (1993) describe the habitat as "dry fields, marshes, bogs, swamps, and roadside ditches." Rydberg (1922) broadly defines the habitat as grassy places and open woods. In general, moonworts tend to grow in places that are unpromising to botanists (Wagner and Wagner 1983, Root personal communication 2003).

In Michigan, Minnesota, and Wisconsin, *Botrychium simplex* has been reported from both open and closed canopy settings. These include rich black ash (*Fraxinus nigra*) and cedar (*Thuja occidentalis*) swamps, jack pine (*Pinus banksiana*) woods, prairies, an open area dominated by reed canary grass (*Phalaris arundinacea*), an open field with non-native grasses, stands of northern hardwood forest, and on glacial till and outwash. It is also reported from sites that have been disturbed by humans, including borrow pits, tailings ponds, road shoulders, and old roadbeds (Chadde and Kudray 2001b, Burkhart personal communication 2006). *Botrychium simplex* is known from prairie habitats in Iowa and Michigan (Johnson-Groh 1999). Only three species of moonworts (*B. simplex*, *B. campestre*, and *B. gallicomontanum*) are restricted to prairie habitats. In Idaho, Davis (1952) reports *B. simplex* from dry woods and meadows while Lorain (1990) describes its habitat as the shaded understory of western redcedar/oakfern forests. In California, it is known from open meadows and damp places (Munz and Keck 1968), and it is "always in open grassy areas, often more or less marshy" and "in damp meadows with sedges (including *Carex aurea*), grasses, and *Mimulus primuloides*" (Wagner and Devine 1989). In Utah, it is known from "moist to somewhat dry woods and open slopes at 2,300 to 3,500 m" (Welsh et al. 1993). In Montana, *B. simplex* is known from disturbed seral lodgepole pine forests (Mantas and Wirt 1995). In Ohio, *B. simplex* has been documented from moist, shaded situations including weedy thickets and mesophytic woods (Ohio Department of Natural Resources 2003). Herbarium specimens describe other habitats, including cold barren knolls in old pastures (Vermont); upper lake beach in shade (Ontario); cold, damp woods (Maine); and alpine meadows and meadow-forest edge (Oregon).

Region 2 habitat descriptions

The variability of habitat for *Botrychium simplex* in Region 2 is as great as its morphological variability, making it very difficult to characterize its habitat. It is found not only in typical moonwort habitat, but also in shaded sites and in wetlands, where no other moonworts that have been documented in Region 2 are found. Thus, the ecological amplitude of *B. simplex* is probably broader than any other moonwort in Region 2. Habitats documented in herbarium specimens range from "dry, open northwest slope of tuff" to "wet soil of peat bog" to railroad right-of-way. Farrar (2005) describes *B. simplex* as "a plant of open habitats, occurring in pastures, meadows, orchards, prairies, wetlands, fens, sand dunes, and in lake and stream edge vegetation," and further states that "most of its habitats are at least temporarily wet and some (fens) are permanently saturated." Harrington (1954) reports *B. simplex* from pastures, meadows, and gravelly slopes of open places. Schwab (1992) notes that it is usually found in moist meadows, gravel slopes, marshy areas, streamsides, and open places. Arnett (2002) reports finding *B. simplex* in spruce fir forest and subalpine meadows. Cronquist et al. (1972) report it from "moist to rather dry meadows in the mountains, apparently not above timberline." *Botrychium simplex* was found in 2005 in open shrublands dominated by *Dasiphora floribunda* (shrubby cinquefoil) in South Dakota (**Figure 11**). **Table 8** has a summary of habitat descriptions documented for Region 2 occurrences.

Recent surveys have shed light on local variation in the kinds of habitats where *Botrychium simplex* is found. In the Black Hills, it was most often found on toeslopes adjacent to riparian greenbelts in deep soils, and also on abandoned roadbeds (Burkhart personal communication 2006, Popovich personal communication 2006). In Colorado, it is more often reported from wetter sites near streams, but it has also been found in seasonally dry sites. Popovich (personal communication 2006) speculates that the greater annual precipitation in the Black Hills permits *B. simplex* to persist in upland settings (though still typically in swales or proximal to a riparian area or floodplain) while it is more tightly constrained by water availability in Colorado.

Varietal differences in habitat

The varieties described for *Botrychium simplex* have significant differences in their habitat affinities. The distribution of these varieties in Region 2 may account for the diversity of habitats reported for *B. simplex*. For example, *B. tenebrosum* was described from plants growing in deep shade in maple swamps (Eaton 1899). Similarly, Wagner and Wagner (1983) characterize the habitat for var. *tenebrosum* as "shaded bog edges." This habitat description does not fit plants in Wyoming that fit the physical description of var. *tenebrosum*. Var. *typicum* (reported in Colorado by Harrington (1954)) is known from dry upland fields

Figure 11. Habitat of *Botrychium simplex* at Redbank Spring, South Dakota on the Black Hills National Forest (BOTR-30). This area was a rich site for *Botrychium* species in 2005. The shrubs are *Dasiphora floribunda*. Photo by Black Hills National Forest, provided by Beth Burkhart.

(Wagner and Wagner 1983). Wagner and Wagner (1983) characterize the habitat for var. *compositum* as moist low meadows.

Farrar (2005) notes that varietal habitat segregation exists and describes general habitat affinities for the four varieties he circumscribed. Var. *compositum* is frequently found in meadow and roadside habitats in Colorado and is "often the most common, and frequently the only species of moonwort present in mountain meadow sites throughout the western mountains" (Farrar 2005). Farrar (2005) notes that var. *compositum* may become especially common in alluvial meadows derived from granitic substrates. Var. *simplex* is known from "meadow woodlands" throughout its range. Var. *tenebrosum* is found in forests, swamp margins, and in dune complexes of the northeastern U.S., Greenland, and Canada. In northeastern North America it is most commonly present in permanently wet, often deeply shaded habitats. Var. *fontanum* occurs in fens and in calcareous seeps, but it is not yet known from within Region 2. **Table 3** is a summary of the distinguishing characteristics of these varieties.

Elevation, slope, and aspect

Wagner and Wagner (1993) reported a maximum elevation of 7,200 ft. for *Botrychium simplex*. However, most occurrences within Region 2 are found at higher altitudes. The elevation of the known occurrences of *B. simplex* in Region 2 ranges from approximately 2,400 ft. along the Niobrara River, where it occurs in a floodplain with cottonwood (*Populus* c.f. *deltoides* ssp. *monilifera*) and red cedar (*Juniperus virginiana*) (Farrar and Johnson-Groh 1986, Farrar personal communication 2003), to 12,680 ft. in tundra. Occurrences in Colorado are found at higher elevations (8,500 to 12,680 ft.) than those in Wyoming (4,670 to 10,000 ft.) and South Dakota (approximately 4,500 ft.).

Botrychium simplex has been documented from flat sites as well as from slopes as steep as 35 percent in Region 2. It has been found on wasting scree and talus slopes and sloping open soil in the alpine (Colorado Natural Heritage Program 2006). Most alpine species cannot tolerate the chronic disturbance regime in these sites. *Botrychium simplex* is also commonly found on flat sites that may or may not be disturbed.

Botrychium simplex does not appear to favor any particular aspect. In two instances, *B. simplex* occurs on south-facing exposures in both alpine and arctic habitats. One site is in the alpine of Colorado in Conejos County. 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) and these may allow *B. simplex* to persist at elevations and latitudes outside its normal range. This phenomenon has often been observed in the alpine and arctic (e.g., Bliss 1987).

Soil

Botrychium species are often found in alkaline, calcium-rich soils (Root personal communication 2003). Occurrences of *B. simplex* within Region 2 have been reported in calcareous soils at a variety of elevations. *Botrychium simplex* is also known from subacid or acid soils that are high in organic matter in Region 2 and elsewhere (Lellinger 1985, Steinmann personal communication 2003, Colorado Natural Heritage Program 2006). *Botrychium simplex* has been found in acidic soils associated with mines outside Region 2 (Chadde and Kudray 2001b).

Botrychium simplex also occurs on a range of soil textures in Region 2, including cobbly, gravelly, sandy, and clay loam. It is documented from riparian and wetland sites where soils tend to be silty (e.g., marshy areas, willow carr edge). In Montana, plants occur in open areas with shallow soils, which apparently caused stunted growth (Mantas and Wirt 1995). The suitability of germination sites varies with microscale heterogeneity of soil conditions, which probably causes the patchy distribution of *Botrychium* species (Johnson-Groh et al. 2002).

Moisture

Habitat descriptions for Region 2 occurrences range from "dry slope" and "along railroad tracks in dry site" to "in marshy area" and "wetland." This suggests that while habitat for *Botrychium simplex* in Region 2 might be constrained to some extent by water availability, the species has a broad tolerance of soil moisture conditions. *Botrychium simplex* is often found in wetlands and is a wetland indicator species in Region 2 (**Table 10**; U.S. Fish and Wildlife Service 1988), but its fidelity to wetland habitats is much lower than that of *B. multifidum*, with which it often grows. *Botrychium simplex* is more common in eastern North America where soils tend to be wetter. Although numerous herbarium specimen labels and other reports note dry soil conditions, most sites are at least seasonally wet. Kolb and Spribille (2000) report that sites in Summit County where *B. simplex* was

Table 10. Wetland Indicator Status for *Botrychium simplex* (U.S. Fish and Wildlife Service 1988).

Region	Geographic Areas in Region	Wetland Indicator Status
North Plains	MT (Eastern), ND, SD, WY (Eastern)	FAC
Central Plains	CO(Eastern), NE, KS	FAC
Intermountain	CO (Western), NV, UT	FACU
Northwest	ID, OR, MT (Western), WA, WY (Western)	FACU

Facultative (FAC): Equally likely to occur in wetlands or non-wetlands (estimated probability 34 to 66 percent).

Facultative Upland (FACU): Usually occurs in non-wetlands (estimated probability 67 to 99 percent), but occasionally found on wetlands (estimated probability 1 to 33 percent).

found were dry at the time. There are no soil moisture data available from which the physiological tolerances of *B. simplex* to desiccation could be determined.

Disturbance as a habitat attribute

Although information on the disturbance regime in Region 2 occurrences of *Botrychium simplex* is sparse, some observations (within and outside of Region 2) suggest that disturbance assists in the creation and maintenance of suitable habitat. Some disturbed sites in which *B. simplex* has been found in Region 2 include scree slopes, subalpine forest clearcuts, "Open eroded grassy slopes at timberline in historic burn," ski slopes, roads, abandoned roadbeds, floodplains and riparian areas, and railroad right-of-ways (**Table 8**). Five of the sites where it was found on the Black Hills National Forest in 2004 and 2005 are abandoned, unused roadbeds (Burkhart personal communication 2006). It has also been reported from disturbed sites outside of Region 2, including pastures, tailings ponds, borrow pits, and road shoulders (Chadde and Kudray 2001b). There is a general tendency to find *Botrychium* species in sites that were disturbed approximately 10 years previously (Johnson-Groh and Farrar 2003). Buell (2001) noted that *Botrychium* species were most commonly found in Summit County on ski runs that had been created more than 30 years previously, and seldom on newer ski runs.

In Yellowstone National Park, *Botrychium simplex* appears to be strongly associated with geothermally-influenced meadows (Whipple personal communication 2003). These meadows usually support interesting and atypical plant assemblages. They are probably maintained by a mild disturbance regime imposed by geothermal activity (Whipple personal communication 2003). They tend to be slightly disturbed but not recently disturbed, and competitive species are not common. It is interesting to note that *B. simplex* also shows an affinity for hot springs in Greenland, where it was documented from a site where the soil is warmed by the spring (Ollgaard 1971). This suggests a general affinity for geothermal features. The Reproductive biology and autecology section of this document provides a more detailed treatment of the ecological role of disturbance for *B. simplex*.

Fire

Botrychium simplex was documented in forests dominated by *Pinus contorta* (lodgepole pine) and *P. ponderosa* (ponderosa pine) in Region 2. Both are fire-adapted species, and fire is a natural and relatively frequent part of forests dominated by these species. Stand ages of subalpine lodgepole forests south of Yellowstone suggest a 200 to 400 year fire 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. simplex* is unclear, but there is no evidence that it has an affinity for newly burned areas. Although *B. simplex* is associated generally with these forest types, it typically grows in streamsides or meadow edge habitats where burning may be less frequent or intense. Most occurrences of *B. simplex* known from Region 2 are found in or near subalpine forest dominated by *Picea engelmannii* (Engelmann spruce) and *Abies lasiocarpa* (subalpine fir), which have much longer fire-return intervals than the aforementioned forest types (Veblen personal communication 2003). The Community ecology section contains more details on the vegetation associated with *B. simplex*.

Meadows

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

Three occurrences of *Botrychium simplex* are in geothermally influenced meadows in Yellowstone National Park. These meadows are maintained by a mild disturbance regime imposed by geothermal activity (Whipple personal communication 2003). Like *B. multifidum* (subgenus *Sceptridium*), *B. simplex* shows an affinity for these habitats, but it is absent from many *B. multifidum* locations. The Community ecology section of this document describes the plants associated with *B. simplex*.

Mycorrhizae as a habitat attribute

Most of the life cycle of *Botrychium* species occurs underground, and scientists understand very little about this part of its 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 establishment and persistence of *Botrychium* occurrences. 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*. The Reproductive biology and autecology section of this document contains a discussion of mycorrhizal interactions with *Botrychium*.

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), equivalent to ruderal species in the CSR model. The perennial life history and slow growth exhibited by *Botrychium* species are characteristic of stress-tolerant species in the CSR model (Grime 2001).

Moderate to light disturbance may be a critical part of the autecology of *Botrychium* species, including *B. simplex* (Clausen 1938, Lellinger 1985, Wagner and Wagner 1993, Barker and Hauk 2003, Johnson-Groh and Farrar 2003). The disturbance regime required by *B. simplex* has not been studied and is not well understood. Habitat attributes for most moonwort species suggest that they depend on a natural disturbance regime imposed by wildfires, floods, landslides, or avalanches (Alverson and Zika 1996). While these forces are clearly at work in most occurrences of *B. simplex*, some occurrences grow in relatively undisturbed sites (e.g., "in moss in montane forest"). The role of disturbance in the autecology of *B. simplex* is less clear than for other members of subgenus *Botrychium*.

In at least three locations in Region 2, *Botrychium simplex* has been documented from sites that burned historically. Reed Crook's speculations (personal communication 2003) on the role of fire in the reproductive biology of *B. multifidum* may be relevant for *B. simplex* as well. There are many similarities between the life histories of *Botrychium* species and the club mosses (*Lycopodium*). Both have subterranean, mycoparasitic gametophytes that persist for years underground 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 underground. Because the gametophytes require years to mature, sporophytes are not observed until years after the fire and require about 80 years before population levels return to normal (Crook personal communication 2003).

The wetter habitats where *Botrychium simplex* is found are often dominated by competitive species such as *Carex aquatilis*. Observations suggest that *B. simplex* is not particularly competitive. Whipple (personal communication 2003) has noted that it is often found with typical competitive wetland species, but that it is usually found 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, and this is maladaptive in stressed or disturbed habitats (Grime 2001).

Reproduction

The reproductive biology of ferns is very different in many respects from that of the flowering plants. Like all Pteridophytes, but unlike angiosperms and gymnosperms, *Botrychium* spores develop into gametophytes that live independently of the sporophyte, and the two often have different ecological requirements. The gametophyte is haploid and produces male and female sex cells in the antheridia and archegonia respectively. Male sex cells are motile and 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).

Upon fertilization, the diploid phase of the life cycle begins, and this produces the sporophyte that is the life stage most familiar and visible in ferns, particularly in *Botrychium* since the gametophyte is subterranean and difficult to observe. In *B. simplex*, several archegonia may be fertilized at the same time, but only one embryo develops per gametophyte.

Several species of *Botrychium* reproduce asexually via gemmae, which are vegetative propagules produced by the sporophyte. These bud-like structures are borne on the underground portion of the sporophyte among its roots and are abscised at maturity (Farrar and Johnson-Groh 1990). Reproduction via gemmae omits the haploid phase of the life cycle, and it may be an adaptation to dry environments, where it is difficult for the male gamete to move through the soil (Farrar and Johnson-Groh 1990). Reproduction via gemmae has been documented in *B. pumicola*, a close relative of *B. simplex* (Camacho 1996, Camacho and Liston 2001), and in *B. gallicomontanum*, for which *B. simplex* is a putative parent species (Farrar and Johnson-Groh 1991), but it apparently does not occur in *B. simplex* (Farrar and Johnson-Groh 1986).

Botrychium simplex typically takes approximately five years to produce its first emergent leaf, but it may be capable of producing a small, aboveground, fertile frond in one year (Campbell 1922). This is in contrast to *B. matricariifolium*, which takes 10 years to develop an adult sporophyte (Muller 1993, Zika et al. 1995), and *B. lunaria*, which has a relatively small cotyledon and may require up to seven years to produce a sporophore (Campbell 1922). When given sucrose supplements, *B. dissectum* plants in culture were observed to sporulate in one year, but it is doubtful if this ever occurs in nature (Farrar and Johnson-Groh 1990, Whittier 1996). The gametophyte and its mycobiont are thought to nourish the embryonic sporophyte (Camacho 1996).

Spores are produced by the sporophyte in sporangia that are borne on the sporophore. Members of subgenus *Sceptridium* and *Osmundopteris* are capable of producing a trophophore but no sporophore. Moonworts, on the other hand, always produce both structures when emergent (Vanderhorst 1997). Spores are produced by reduction division (meiosis) and are capable of producing a gametophyte. They provide the only plausible means of long-distance dispersal for *Botrychium* species.

Phenology

Johnson-Groh and Lee (2002) divide the aboveground portion of the moonwort life cycle into four stages: emergence; separation, when the trophophore and sporophore separate from each other and open; spore release, or sporulation; and senescence. The emergence of *Botrychium simplex* occurs in the first week of June in Yellowstone National Park (Whipple personal communication 2003) and in the Black Hills (Burkhart personal communication 2006) (**Figure 2**). Separation probably occurs shortly after emergence, as all herbarium specimens had already separated. As observed by Johnson-Groh and Lee (2002) for *B. gallicomontanum*, the separation stage is brief in *B. simplex* while that of *B. mormo* is more protracted. Herbarium specimens from Colorado and Wyoming suggest that *B. simplex* typically sporulates from late June (as noted by Hitchcock 1823) through July and August, and sometimes into mid-September. *Botrychium simplex* sporulates as early as late May in Ohio (Ohio Department of Natural Resources 2003). Most plants have senesced by September to early October (Whipple personal communication 2003), but some may senesce as early as July (Burkhart personal communication 2006). Observations of Black Hills National Forest occurrences suggest that phenological stages vary considerably in their timing even within an occurrence.

Fertility

Botrychium species produce as many as 20 to 100 or more sporangia per sporophore, and each sporophyte may produce thousands of spores, 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. simplex*. Spores of *B. virginianum* germinated on agar showed a 90 percent germination rate (Peck et al. 1990).

Dispersal

Dispersal frequency and distance are not known for *Botrychium simplex*, but some inferences can be drawn from studies of *Botrychium* and other fern genera. Researchers have hypothesized that the dispersal distances for some *Botrychium* spores range 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 taken immediately below ferns and were considerably smaller only 2 m away from spore sources. While most spores land close to the parent plant, they occasionally are a means of long-distance dispersal because some are carried considerable distances by the wind (Briggs and Walters 1997).

In addition to wind dispersal, animals may disperse *Botrychium* spores (Wagner and Wagner 1993, Wagner personal communication 2002). The spores have thick walls that may help to retain their viability as they pass through an animal's digestive tract (Johnson-Groh 1998, Wagner personal communication 2002). It has been thought that deer and small mammals may disperse the spores of forest species such as *B. dissectum* along trails and roads. After feeding the spores of *B. virginianum* to a vole, J.D. Montgomery was able to recover them intact from the vole's droppings (Root personal communication 2003). Elk and other ungulates that eat the sporophores of *B. simplex* could act as effective dispersal vectors that would selectively move spores to other potentially suitable habitats. Gifford and Brandon (1978) suggested that soil movement by soil insects, worms, or larger animals may break up and disperse gametophytes. Buell (2001) observed that distribution patterns of *Botrychium* in Summit County, Colorado often followed swales, heavy equipment tracks, and erosion rills, suggesting that surface runoff may play a role in the dispersal of spores.

Recent genetic studies of *Botrychium simplex* suggest, as seen in studies of other ferns cited above, that its migration is extremely limited (Farrar 2005). Populations of *B. simplex* in the Sierra Nevada range of California showed great variability in allelic composition, even among samples taken from single 2 by 0.5 mile meadow. Differentiation was observed even between occurrences 100 m apart that would not be possible if unrestricted inter-occurrence migration was occurring. Thus, Farrar (2005) concludes that occurrences more than a few miles apart are effectively isolated and that suitable, uncolonized habitats at these distances have a low probability of receiving a sufficient number of spores to assure colonization.

Cryptic phases

Like other species of *Botrychium*, *B. simplex* is probably capable of remaining dormant for at least one year and perhaps more. Long periods of dormancy are well documented for many species in subgenus *Botrychium* (Muller 1993, Kelly 1994, Lesica and Ahlenslager 1995, Johnson-Groh 1998, Johnson-Groh 1999, Johnson-Groh and Farrar 2003). Dormancy is relatively brief for *B. mormo*. Only 24 percent of *B. mormo* plants remaining dormant one year return in the following year, and only 4 percent return after a second year of dormancy (Johnson-Groh 1998).

The dominance of subterranean life stages of *Botrychium* species was highlighted by the recent work of Johnson-Groh et al. (2002) who observed very high ratios of underground (gametophytes and juvenile sporophytes) to aboveground structures.

The importance of spore banks for *Botrychium simplex* 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 simplex* is unknown. Measurements of non-chlorophyll-bearing spores of other fern families have an average viability length of 1045 days (Lloyd and Klekowski 1970), and the spores of some fern genera may remain viable for long 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).

Mycorrhizae

Botrychium species rely upon mycorrhizae in both the sporophytic (Bower 1926, Gifford and Foster 1989) and gametophytic (Campbell 1922, Bower 1926, Scagel et al. 1966, Gifford and Foster 1989, Schmid and Oberwinkler 1994) stages. Germination can occur without mycorrhizal infection; however, the gametophyte will not mature without an arbuscular mycorrhizal symbiont (Campbell 1911, Whittier 1972, Whittier 1973). Many observations suggest that the fungi forming symbioses with *Botrychium* species are zygomycetes that provide resources to gametophytes via a "Paris-type" arbuscular mycorrhizal association

(Read et al. 2000). 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 the mycorrhizal interaction (Schmid and Oberwinkler 1994). Daigobo (1979) observed infection of the gametophyte of *B. multifidum* (subgenus *Sceptridium*) with an endophytic (arbuscular mycorrhizal) fungus in all gametophytes examined at all developmental stages. Dormancy in *Botrychium* species appears to be related to the health of mycorrhizae (Johnson-Groh 1998).

It is unknown how or if the mycorrhizal interaction changes when 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 not wholly dependent on a mycobiont for carbohydrates. *Botrychium* sporophytes have reduced, non-proliferous 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 a 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* occurrences is the presence of mycorrhizae. Little is known about the nature of this interaction. Farrar (1998) noted that mycorrhizal fungi are low in species diversity, ubiquitous in disturbed and undisturbed sites, and generalists in what plant species they infect (Smith and Read 1997). Recent studies have measured high species diversity of arbuscular mycorrhizal (AM) fungi in a single hectare (Bever et al. 2001). A single plant root was observed to host up to 49 species of AM fungi (Vandenkoornhuyse et al. 2002). These observations, coupled with the ubiquity and low host specificity of AM fungi, suggest that mycorrhizae may not be a limiting factor in the distribution of *B. simplex*. Future studies will be able to identify fungal symbionts of *Botrychium* species through experimentation with gametophytes in axenic culture (Read et al. 2000).

Mycorrhizae can affect 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 *Festuca idahoensis* (Idaho fescue) when grown together in the presence of mycorrhizal fungi. With 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). At least 10 records of sterile hybrid combinations have been documented (Wagner 1980, Wagner et al. 1984, Wagner et al. 1985, Wagner and Wagner 1988, Wagner 1991, Wagner 1993, Ahlenslager and Lesica 1996). There are no records of hybrids in Region 2, but it is possible that hybrids may exist where *B. simplex* occurs with *B. lunaria* in several locations in Region 2. Two sterile hybrids have been documented between *B. simplex* and other *Botrychium* species. *Botrychium lunaria* (Wagner and Wagner 1988) and *B. matricariifolium* (Wagner 1980, Wagner 1991) are both capable of hybridizing with *B. simplex*. The resulting offspring have intermediate characteristics and sterile, abortive spores (Wagner 1993).

Evidence from cytological and molecular research on many moonwort species strongly suggests an allopolyploid origin through historic hybridization events (Wagner 1993, Hauk and Haufler 1999, Wagner and Grant 2002). Three polyploid moonworts, *Botrychium hesperium*, *B. pseudopinnatum*, and *B. gallicomontanum*, are believed to have arisen through ancient hybridization events involving *B. simplex*. As such, these species are referred to as "nothospecies." **Table 5** describes the purported parentage of these nothospecies. Other moonworts also have a suspected hybrid parentage, including the recently described *B. alaskense*, which is an allotetraploid of *B. lunaria* and *B. lanceolatum* (Wagner and Grant 2002).

The anatomy of the gametophyte (Bower 1926) and the difficulty that gametes encounter when traveling through dry soil (McCauley et al. 1985, Soltis and Soltis 1986) serve to limit outcrossing and 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). This demonstrates the presence of multiple species in these genus communities rather than intraspecific variants.

Demography

Members of the genus *Botrychium* appear to have naturally low rates of outcrossing, resulting in low levels of genetic variability (Farrar 1998, Farrar 2005). *Botrychium* species have coped with this for thousands, if not millions, of years, and it is therefore not a concern in assessing species or population viability. They are not subject to inbreeding depression because they do not carry a genetic load of deleterious alleles typical among outcrossing species (Farrar 2005).

As in other *Botrychium* species, the gametophyte of *B. simplex* appears to be designed for self-fertilization since the antheridia are positioned just above the archegonia (Campbell 1922). Water moving through the soil is likely to bring the male sex cells to the archegonia on the same plant (Bower 1926). Hauk and Haufler (1999) found genetic evidence confirming that there are extremely high levels of inbreeding in *B. simplex*, and this has been observed in other *Botrychium* species (Soltis and Soltis 1986, Swartz and Brunsfeld 2002, Farrar 2005). Moonwort species consistently show very low intraspecific allelic variability when compared with other ferns and seed plants (Farrar 1998). Watano and Sahashi (1992) observed that heterozygous genotypes were very rare in four species in subgenus *Sceptridium* 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. Camacho and Liston (2001) found high genetic diversity in *B. pumicola*, suggesting that outcrossing is occurring or that *B. pumicola* has some other mechanism for maintaining high genetic diversity. The rare presence of interspecific hybrids in natural settings indicates the ability for cross-fertilization hybridization to occur (Wagner 1980, Wagner et al. 1984, Wagner et al. 1985, Wagner and Wagner 1988, Wagner 1991, Ahlenslager and Lesica 1996).

Given the breeding biology of *Botrychium simplex*, it is possible that they may not be sensitive to the effects of inbreeding depression since opportunities for sexual recombination are rare. The spatial distribution of *B. simplex* occurrences suggests that gene flow (via spore or, less frequently, gamete movement) between occurrences is probably very low.

The life span of the *Botrychium simplex* sporophyte has not been measured. Johnson-Groh and Farrar (2003) estimate that moonworts live approximately 10 years. The aboveground longevity of *B. campestre* is approximately four years while *B. mormo* rarely live longer than two years (Johnson-Groh 1998). *Botrychium australe* plants live an average of 11.2 years (Kelly 1994), but *B. dissectum* (subgenus *Sceptridium*) individuals can live at least a few decades (Montgomery 1990, Kelly 1994), and *B. multifidum* (subgenus *Sceptridium*) may live for as long as 100 years (Stevenson 1975).

Botrychium gametophytes are reported to persist underground for up to five years (Winther personal communication 2002) and grow very slowly from an embryo into an adult gametophyte that produces gametes (Wagner 1998). The longevity and the fate of the gametophyte after the production of a sporophyte have not been reported (Vanderhorst 1997). 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). **Figure 12** is a diagrammatic representation of the life cycle of *B. simplex*, and **Figure 13** presents a lifecycle graph (after Caswell 2001). The Reproductive biology and autecology section of this document provides a discussion of the life cycle of *B. simplex*.

No population habitat viability analysis (PHVA) has been done for *Botrychium simplex*. The only *Botrychium* species for which a PHVA has been conducted is *B. mormo* (Berlin et al. 1998). Although genetic studies confirm its close relationship with *B. simplex*, *B. mormo* differs in many significant ways from *B. simplex* and most other moonworts as well. Nonetheless, some of the conclusions drawn from the model are relevant to most members of the genus. Three factors have the most influence in the model: the number of viable spores set per sporophyte, the nature and extent of a spore bank, and spore germination rate. Unfortunately, these are the factors about which the least is known.

Impacts to *Botrychium simplex* occurrences resulting from environmental stochasticity are possible (Johnson-Groh et al. 1998). Environmental stochasticity includes variation in fecundity and survival as a consequence of environmental conditions and catastrophic local events. It may lead to local extinction (Lande 1998, Oostermeijer et al. 2003). Environmental stochasticity can operate at many scales and may affect a species across part or all of its range. Maintaining the largest occurrences possible is most likely to reduce potential negative consequences. Extinction probability models show that belowground stages buffer local

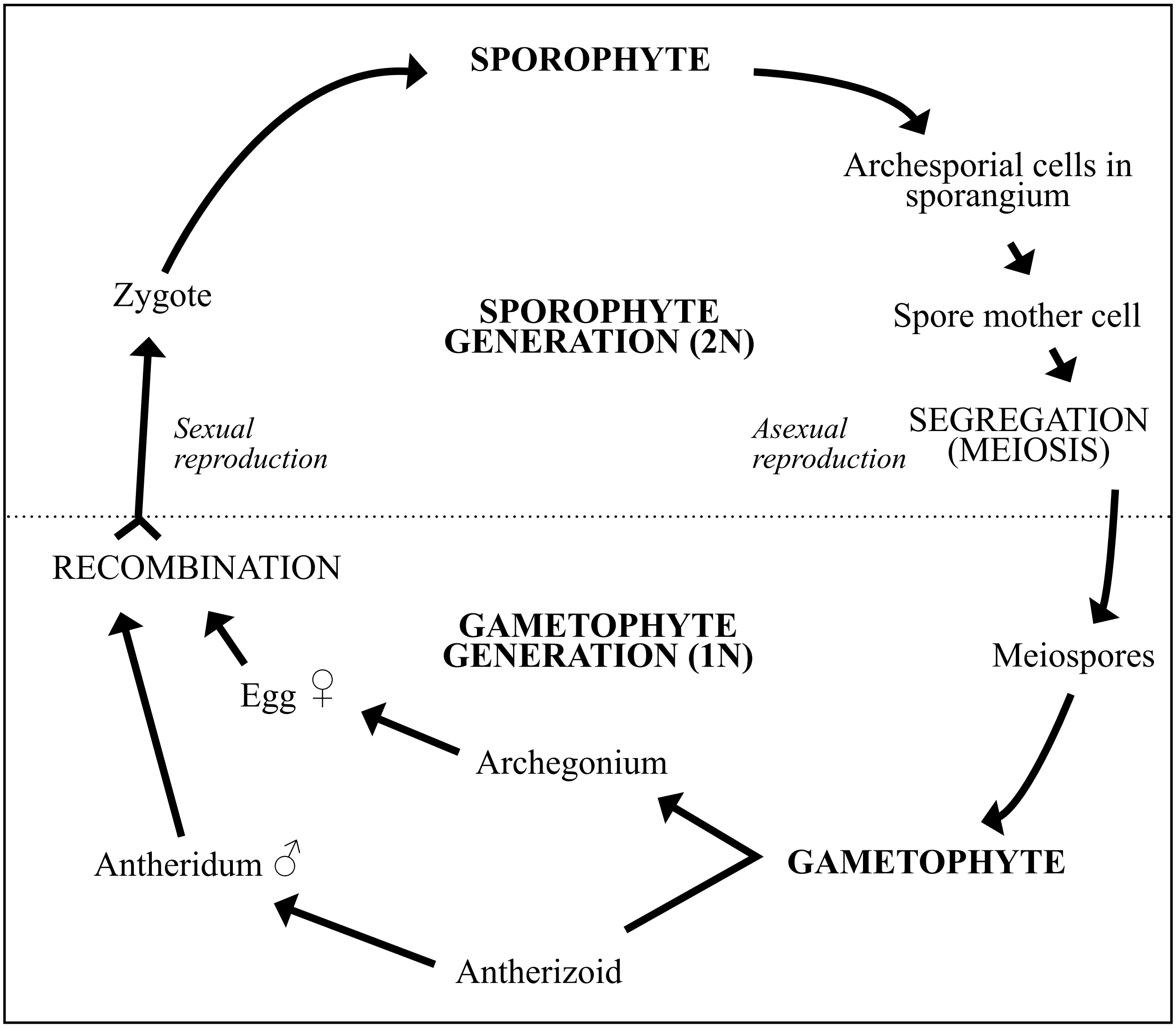

Figure 12. Lifecycle diagram for *Botrychium simplex* (after Lellinger 1985), illustrating the alternation of generations.

Botrychium occurrences against extinction (Johnson-Groh et al. 1998), but recovery may not be possible after major disturbances.

Botrychium occurrences appear to have a metapopulation structure that may be an important component of their viability. Johnson-Groh and Farrar (2003) provide an overview of the metapopulation structure of subgenus *Botrychium*. Using metapopulation classes defined by Hanski and Simberloff (1997), they note that *Botrychium* populations do not conform to a single metapopulation class. Rather, complex spatial interactions in multiple metapopulation classes probably best characterize the structure of *Botrychium* populations. *Botrychium simplex* and other species of *Botrychium* may depend on a shifting mosaic of suitable habitats for their long-term persistence (Clausen 1938, Chadde and Kudray 2001b), as does *Pedicularis furbishiae* (Pickett and Thompson 1978, Menges and Gawler 1986). If this is the case, then spores are the only means by which *B. simplex* 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 to conditions unsuitable to them. Succession is of particular concern for meadow species, including *B. simplex* (Johnson-Groh and Farrar 2003). Evidence of limited dispersal of *B. simplex* suggests that the probability of successful long-distance dispersal is low (Farrar 2005).

Demographic studies of *Botrychium simplex* are lacking, and basic parameters circumscribing its life-history characteristics are unknown. Sporophytes of *Botrychium* species can remain dormant for one

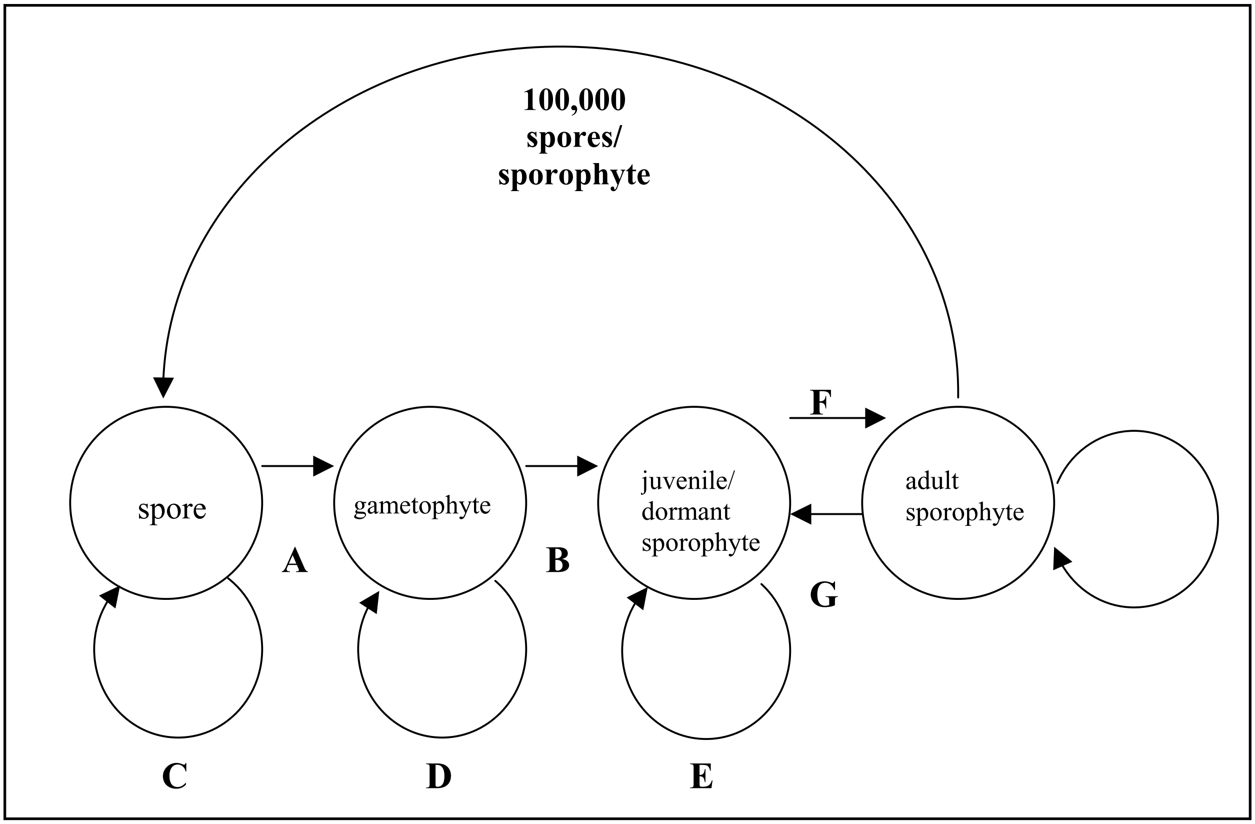

Figure 13. Hypothetical life cycle graph (after Caswell 2001) for *Botrychium simplex*. Transition probabilities are not known and are difficult to quantify since important stages of the lifecycle occur underground (A-G). See Johnson-Groh et al. (1998) for the best information currently available regarding these parameters for subgenus *Botrychium*. 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. simplex*.

or more years, and most of the population at a given site probably resides underground (Johnson-Groh et al. 2002). Johnson-Groh et al. (2002) found that the average ratio of belowground to aboveground plants for moonwort species was 332:1. *Botrychium mormo*, the closest relative of *B. simplex* studied, had among the lowest ratios observed, at 65:1. An average of 728 gametophytes per square meter was documented for *B. mormo*. 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 observations indicate that long-term studies are needed to assess the true population size (Johnson-Groh and Farrar 2003), and that the observation of a single emergent sporophyte may indicate the presence of a viable occurrence (Casson et al. 1998), or an early stage of colonization.

Studying how plants become established is problematic because important events in the life cycle of *Botrychium* occur underground. The requirement of darkness for spore germination (Whittier 1973) is not surprising, given the gametophyte's need to establish a mycorrhizal symbiosis within a few cell divisions (Campbell 1911). The mechanism by which spores get under ground is not known, but they somehow get from the soil surface to a depth of 1 to 2 inches. Water and 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.

Community ecology

Botrychium simplex is known from a range of plant communities in Region 2, and the list of associated species documented with it is long (**Table 11**). Kolb and Spribille (2000) present associated species in plot data from Shrine Pass (White River National Forest) and Boreas Pass (White River and Pike national forests), both in Colorado.

Table 11. Associated species reported with *Botrychium simplex* in USDA Forest Service Region 2.

Associated Species	Colorado	Nebraska	South Dakota	Wyoming	Exotic?
Abies lasiocarpa	X			X	
Achillea millefolium			X	X	
Aconitum columbianum			X		
Agoseris glauca			X		
Agrestis thurberiana	X				
Agrimonia sp.			X		
Allium sp.				X	
Amelanchier alnifolia				X	
Amelanchier sp.				X	
Androsace septentrionalis			X		
Anemone sp.			X		
Antennaria media				X	
Antennaria neglecta			X		
Antennaria sp.	X		X	X	
Aquilegia sp.	X				
Arctostaphylos uva-ursi			X	X	
Arnica c.f. *fulgens*				X	
Arnica cordifolia	X				
Arnica sp.				X	
Artemisia ludoviciana				X	
Artemisia sp.				X	
Astragalus sp.			X		
Betula papyrifera			X		
Botrychium campestre		X		X	
Botrychium echo	X				
Botrychium hesperium	X				
Botrychium lanceolatum	X				
Botrychium lunaria	X			X	
Botrychium michiganense				X	
Botrychium minganense	X		X		
Botrychium multifidum	X			X	
Botrychium pinnatum	X			X	
Campanula rotundifolia			X	X	
Carex aquatilis	X			X	
Carex aurea				X	
Carex parryana				X	
Carex rossii	X				
Carex sp.			X	X	
Castilleja sulphurea			X		
Cerastium arvense			X	X	
Cirsium eatonii	X				
Cirsium sp.	X				
Clematis tenuiloba			X		

Table 11 (cont.).

Associated Species	Colorado	Nebraska	South Dakota	Wyoming	Exotic?
Collinsia parviflora				X	
Collomia linearis				X	
Comandra umbellata				X	
Corylus cornuta			X	X	
Crataegus chrysocarpa				X	
Crepis runcinata				X	
Cryptogramma sp.	X				
Cynoglossum officinale			X	X	X
Dasiphora floribunda			X		
Delphinium bicolor			X	X	
Deschampsia caespitosa	X			X	
Dodecatheon pulchellum			X	X	
Elymus trachycaulus			X		
Erigeron spp.	X				
Festuca spp.	X				
Fragaria sp.		X		X	
Fragaria virginiana	X		X		
Gallium boreale			X	X	
Gallium sp.			X		
Geranium richardsonii			X	X	
Geum sp.			X		
Geum triflorum			X	X	
Halenia deflexa			X		
Heterotheca depressa				X	
Heterotheca pumila	X				
Hieracium umbellatum				X	
Iris missouriensis			X		
Juncus balticus				X	
Juniperus communis			X	X	
Juniperus virginiana		X			
Koeleria macrantha			X		
Leucanthemum vulgare			X		X
Lewisia pygmaea			X		
Linum perenne				X	X
Lithophragma parviflora			X	X	
Lupinus sp.			X		
Mahonia repens			X	X	
Maianthemum stellatum			X	X	
Mertensia lanceolata			X	X	
Mimulus guttatus				X	
Monarda fistulosa			X	X	
Muhlenbergia cuspidata			X		
Myosotis verna				X	

Table 11 (cont.).

Associated Species	Colorado	Nebraska	South Dakota	Wyoming	Exotic?
Orthilia secunda	X				
Oxalis dillenii				X	
Oxalis sp.			X		
Oxytropis campestris			X		
Panicum acuminatum				X	
Pentaphylloides floribunda				X	
Phleum pratense			X		X
Phlox hoodii			X		
Phlox pulvinata				X	
Picea engelmannii	X			X	
Picea glauca			X		
Pinus contorta	X			X	
Pinus ponderosa			X	X	
Poa bulbosa				X	
Poa compressa				X	
Poa nemoralis	X				
Poa pratensis				X	X
Poa sp.			X	X	
Polygala senega			X		
Polygonum viviparum				X	
Polytrichum piliferum	X				
Populus c.f. *deltoides* ssp. *monilifera*		X			
Populus tremuloides			X		
Potentilla sp.			X	X	
Prunella vulgaris			X		
Pseudotsuga menziesii				X	
Pyrola chlorantha	X				
Quercus macrocarpa				X	
Ranunculus sp.			X		
Rhacomitrium canescens				X	
Ribes sp.				X	
Rosa sp.			X		
Rubus idaeus				X	
Salix bebbiana			X		
Salix planifolia				X	
Sanicula marilandica			X		
Sedum lanceolatum			X		
Sedum sp.				X	
Selaginella densa	X		X		
Senecio canus			X		
Senecio sp.	X				
Shepherdia argentea	X				
Shepherdia canadensis			X		

Table 11 (concluded).

Associated Species	Colorado	Nebraska	South Dakota	Wyoming	Exotic?
Sisyrinchium idahoense				X	
Solidago multiradiata var. *scopulorum*	X				
Solidago sp.			X	X	
Spiraea betulifolia				X	
Sporobolus heterolepis			X		
Stipa richardsonii			X		
Symphoricarpos albus				X	
Symphoricarpos sp.			X	X	
Taraxacum officinale				X	X
Taraxacum sp.			X	X	
Thalictrum alpinum	X				
Thalictrum dasycarpum			X		
Thalictrum sp.			X		
Thermopsis rhombifolia				X	
Trifolium repens			X	X	X
Urtica dioica			X		
Vaccinium caespitosum	X				
Vaccinium myrtillus	X				
Vaccinium sp.	X		X		
Viola adunca			X		
Viola sp.			X		
Zigadenus sp.				X	
Zigadenus venosus				X	

Some species and species groups commonly associated with *Botrychium simplex* in Region 2 reflect its habitat affinities. Coniferous trees are common associates with *B. simplex*, particularly in Region 2. Conifers reported with *B. simplex* include *Picea engelmannii*, (Engelmann Spruce) *Picea glauca* (white spruce), *Pseudotsuga menziesii* (Douglas fir), *Pinus ponderosa* (ponderosa pine), *P. contorta* (lodgepole pine), and *Abies lasiocarpa* (subalpine fir). Sedges (*Carex* species) are also frequently documented with *B. simplex* range-wide and with many occurrences in Region 2. The sedge most commonly associated with *B. simplex* in Region 2 is *C. aquatilis*. Strawberry species (*Fragaria vesca* and *F. virginiana*) are common associates of many *Botrychium* species including *B. simplex* (Root personal communication 2003).

Rigorous work on the community ecology of *Botrychium simplex* is lacking. Using phytosociological methods, Kolb and Spribille (2000) described the community in which *Botrychium* species were found in Summit County, Colorado as "*Festuco – Heterothecetum pumilae*," (the Fescue- Dwarf Aster Community), named for the dominant plants (*Festuca brachyphylla* and *Heterotheca pumila*) in the community. This community is characterized by ruderal taxa, including *Fragaria virginiana*. *Botrychium simplex* is not noted as a characteristic species of this community type, but occurrences of *B. simplex* were found in this community type at Shrine Pass and Boreas Pass. Detailed community description and plot data can be found in Kolb and Spribille (2000).

Species in subgenus *Botrychium* often occur 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 two or more *Botrychium* species to be found in close association with one another. *Botrychium simplex* has been documented with numerous other moonwort species in Region 2 and elsewhere. Wagner and Wagner (1983) document the co-occurrence of *B. simplex* with nine other moonwort species in genus communities (*B. minganense*, *B. crenulatum*, *B. lunaria*, *B. pinnatum*, *B. lanceolatum*, *B. echo*, *B. hesperium*, *B. matricariifolium*, and *B. paradoxum*). *Botrychium simplex* has also been documented with *B. ascendens* (Wagner and Wagner 1986, Vanderhorst

1997), *B. pedunculosum* (Wagner and Wagner 1986), *B. mormo* (Wagner and Wagner 1981, Chadde and Kudray 2001a), *B. multifidum* (Wagner and Devine 1989), and *B. campestre* and *B. gallicomontanum* (Farrar and Johnson-Groh 1986). Ollgaard (1971) reports the presence of *B. lanceolatum*, *B. lunaria*, and *B. multifidum* with *B. simplex* in Greenland.

Within Region 2, *Botrychium simplex* is also frequently documented in genus communities. Colorado element occurrence records note the presence of *B. echo*, *B. hesperium*, *B. lanceolatum*, *B. lunaria*, and *B. minganense* with *B. simplex* (Colorado Natural Heritage Program 2006). *Botrychium simplex* has been found with *B. campestre* in Brown County, Nebraska (Farrar and Johnson-Groh 1986, Farrar personal communication 2003). Both *B. campestre* and *B. 'michiganense'* have been documented from the Bearlodge Campground in Crook County, Wyoming, but they have not been found in close association with *B. simplex* at this location. *Botrychium simplex* has been found with *B. pallidum* at three locations on the Black Hills National Forest, and with other unidentified taxa at one location (Burkhart personal communication 2006). Outside Region 2, *B. simplex* has been found with *B. multifidum* at two sites in Yellowstone National Park (Whipple personal communication 2003), and these species are often found together (Crook personal communication 2003, Farrar 2005).

The coexistence of 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 interspecific competition. Competition may be for access to the mycorrhizae if it is occurring at all. No research has been done on *Botrychium* species with respect to these issues. Wagner and Wagner (1983) offer an interesting discussion of this issue from a population biology standpoint.

Moonworts clearly tolerate some degree of grazing, but there is little information on which to base management decisions regarding livestock and wildlife grazing (Johnson-Groh and Farrar 2003). Montgomery (1990) found that even repeated removal of the leaf of *Botrychium dissectum* (subgenus *Sceptridium*) for three years did not kill the plants, and on this he commented (p. 178) "It is certainly remarkable that these plants persist." Observations of *B. minganense* and *B. montanum* by George Wooten on the Okanogan National Forest in Oregon may offer some insights into possible impacts of grazing on *B. simplex*. These two species were monitored in a trampled spring area, and while they appeared to benefit from disturbance in lightly trampled areas, they were destroyed in heavily trampled areas. Moderately trampled areas were not affected (Roche 2004). The sensitivity of *B. simplex* to grazing is not known, but some observations suggest that it tolerates a moderate level of grazing. It is documented from pastures in numerous locations outside of Region 2.

There is some speculation that spores of at least some *Botrychium* species are dispersed by mammals, based primarily on observations of herbivory on the sporophores of *B. mormo* (Casson et al. 1998, Wagner personal communication 2002). Although dispersal by animals has not been demonstrated for *B. simplex*, it is possible that elk, deer, and other grazing ungulates act as dispersal agents by eating the ripe sporophores in the fall. This would be advantageous to *B. simplex*, since elk and deer use *B. simplex* habitats and would tend to deposit the spores in sites suitable for germination. Small mammals may also function as short-distance spore dispersal agents. The large, thick walled spores of *Botrychium* species may be an adaptation to dispersal by herbivores (Wagner 1998).

Plants on Vail Pass in the White River National Forest have been subjected to small mammal predation (Colorado Natural Heritage Program 2006). The plant found at Copper Mountain also appeared to have been partially eaten or damaged, but it also had been driven over by a vehicle (Root personal communication 2003). There are no reports of parasitism or disease in the literature for any *Botrychium* species in Region 2.

CONSERVATION

Threats

Observations suggest that there are several threats to the persistence of *Botrychium simplex*. In Ohio, soil compaction, drying of habitat, and removal of vegetation are threats to *B. simplex* (Ohio Department of Natural Resources 2003). Chadde and Kudray (2001b) noted that major threats to *B. simplex* in Wisconsin, Michigan, and Minnesota include exotic earthworms, exotic plants, succession to closed canopy forest, major disturbance (ranked medium for degree of impact), and canopy thinning and minor disturbance (ranked low for degree of impact). Lorain

(1990) noted no anthropogenic threats to *B. simplex* in Idaho but included windthrow or trampling by large game animals as potential natural threats. Threats to *Botrychium* occurrences in the Wallowa Mountains of Oregon include natural succession, recreational use, and impacts from pack animals and domestic livestock (Alverson and Zika 1996). Berlin et al. (1998) described and ranked numerous threats to *B. mormo*. These were sorted into four general categories: exotics, forestry practices, development and land use, and other. While many of these threats are specific to *B. mormo*, some are relevant for *B. simplex* as well: earthworms, clearcutting, salvage sales, road right-of-way management, lakeshore development, recreation site development, anthropogenic alteration of hydrology, human population increase, natural windthrow, and flooding. While these factors pose potential threats for *B. simplex*, the nature and magnitude of the threat is different for *B. simplex* due to ecological and geographic differences between these species.

In approximate order of decreasing priority, threats to *Botrychium simplex* in Region 2 include ski area development and maintenance, road construction and maintenance, timber harvest, recreation, fire, grazing, effects of small population size, woody plant encroachment, exotic species invasion, succession, global climate change, and pollution. More complete information on the biology and ecology of this species may elucidate other threats specific to Region 2. Assessment of threats to this species will be an important component of future inventory and monitoring work. See the sections below for specific discussion of the known threats to *B. simplex*.

Influence of management activities and natural disturbances on individuals and habitat

Ski area development and maintenance

Construction of facilities to support recreational skiing presents potential threats to specific moonwort occurrence, primarily those on the White River National Forest. Because of a lack of baseline data, it is not know to what extent the creation of ski runs and ski areas has benefited or negatively impacted occurrences of *Botrychium* species, including *B. simplex*. For species that can occur in forested sites such as *B. simplex* and *B. multifidum*, the potential negative impacts from ski run creation are greater than for most other moonwort species that are found almost exclusively in open sites.

Creation of ski runs and facilities for recreational skiers has altered habitats in the mountains of Region 2, and it is not known if occurrences in ski runs remain viable for long periods. Their viability could easily be compromised by management activities needed to maintain ski runs. Snow-making activities can change the hydrology of an area by increasing surface runoff and snow depth. The impacts of these factors to *Botrychium simplex*, if any, are not known. Construction of a ski hut near the Vail Pass occurrences presents a potential threat due to disturbance associated with construction and increased use of the hut. Because the presence of *B. simplex* was known before construction, the hut was located in a site where impacts to the occurrence would be reduced. Summer use of the hut could still result in trampling of individuals.

Road and trail construction and maintenance

Road construction and maintenance threaten known occurrences of *Botrychium simplex* in Region 2. New road construction and road widening or modification can directly impact occurrences of *B. simplex* in the area. Potential secondary effects of roads include changes in hydrology, an increase in erosion, and the introduction of weed species.

One occurrence at Copper Mountain in Colorado was reported in an infrequently used road where renewed use, repair, and/or maintenance may compromise its viability. Four other occurrences in Region 2 are known to be within or adjacent to active roads in Region 2, and others may be as well. In 2004 and 2005, five additional occurrences were discovered within or adjacent to old unused roadbeds in the Black Hills National Forest. The threats to these occurrences appear minimal at present, but they could increase if the roads were reopened. One *Botrychium simplex* occurrence is located along active railroad tracks where similar threats may exist.

Timber harvest

Direct impacts to *Botrychium* species from timber harvest include soil compaction, disruption of the organic surface horizon, changes in light, and loss of soil nutrients and moisture (Johnson-Groh and Farrar 2003). Sedimentation is another documented threat. Secondary impacts of timber harvest such as road building or other activities may also represent potential threats to *B. simplex* occurrences. It might be expected that occurrences of *B. simplex* within a timber harvest area would be negatively impacted. Outside of ski areas on the White River National Forest, there are no occurrences of *B. simplex* in Region 2 known to be currently or historically impacted by timber harvest.

Recreation

Recreational use of *Botrychium simplex* habitat presents a direct threat to individuals, which may be killed or damaged. Off-road vehicle use (both motorized and non-motorized) represents the greatest recreational threat to *B. simplex*. Off-road vehicle use has the potential to be highly detrimental to *B. simplex* habitat by disturbing soil and plant community integrity, altering hydrology and hydrologic processes, increasing erosion, and enhancing the spread of invasive plant species. Off-road use of motorized vehicles has affected the Caribou Flats area of Boulder County, Colorado where one occurrence has been documented. Off-road vehicle use could threaten occurrences in unused roadbeds on the Black Hills National Forest if these areas begin to be used as trails. Use of mountain bikes and "mountainboards" (similar to snowboards but equipped with wheels for use on ski slopes in the summer) has the potential to impact individuals on ski slopes.

Fire

Fire affects individual plants directly by burning their aerial portions, but *Botrychium* species, including *B. simplex*, appear to suffer no ill consequences from this (Johnson-Groh and Farrar 2003). Particularly hot fires or fires when the soil is desiccated could result in plant mortality, but due to the strong dependence of the species on mycorrhizae, removal of leaf tissue via burning or other means is probably inconsequential to the plant's survival (Montgomery 1990, Wagner and Wagner 1993, Johnson-Groh and Farrar 1996a, Johnson-Groh and Farrar 1996b, Johnson-Groh 1999). Fires that occur in the summer or fall (when forest fires are most common) would preclude any reproductive output for that year and might kill spores lying near the surface (Root personal communication 2003).

While fire itself may not have much effect on *Botrychium simplex*, the secondary effects of fire can threaten individuals. Sedimentation is one such secondary threat. Burial by sediment has resulted in the apparent mortality of buried individuals of other *Botrychium* species (Johnson-Groh and Farrar 2003). Part of a permanent plot at Frenchman's Bluff, Minnesota was buried by soil excavated by gophers. While this resulted in the temporary loss of *B. gallicomontanum* individuals, after 11 years of monitoring at this site the population had rebounded (Johnson-Groh 1999, Johnson-Groh and Farrar 2003).

Grazing

Grazing offers both potential benefits and detriments with regard to *Botrychium simplex*. This species is found in pastures outside of Region 2, suggesting that it is tolerant of some level of grazing. However, it is most often reported from "old pastures," suggesting that *B. simplex* is less compatible with an intensive grazing regime. Elk and other ungulates frequent the meadow habitats of *B. simplex* and may occasionally graze it. Grazing can eliminate a season's contribution to the spore bank (Johnson-Groh and Farrar 2003). Livestock tend to prefer grasses, permitting the competitive release of broad-leaved plants, which may benefit *B. simplex* in some situations. However, disturbance of the surface by cattle may injure some individuals (potentially above and below ground). Livestock grazing may indirectly affect *B. simplex* by increasing erosion, altering the plant community composition, damaging the soil structure (particularly in wet conditions), and introducing invasive plants. Therefore, the use of cattle grazing as a management tool for the enhancement of habitat is risky for a plant as rare as *B. simplex*.

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 occurrences. Because there are limited data on occurrence sizes in Region 2, the degree to which many occurrences are threatened by the effects of demographic stochasticity is unknown. Reported numbers of individuals at most occurrences of *Botrychium simplex* in Region 2 fall below the generally accepted minimum effective population size of 50 reproductive individuals (Soulé 1980) needed to buffer against the probability that a fluctuation in vital rates will take the occurrence to the extinction threshold. *Botrychium* species have extremely low levels of heterozygosity because unmasked deleterious alleles have been purged from populations. This has led Farrar (2005) to suggest that the lack of genetic variability in *Botrychium* species should not be a concern in assessing species or population viability.

Environmental stochasticity refers to random variations in the physical and biological environment. For a single occurrence, this includes natural events happening at random intervals that cause the deaths of a large proportion of individuals in the occurrence. Such events may occur very rarely, yet still have a significant impact on the occurrence (Menges 1991). Maintaining multiple occurrences well-distributed throughout Region 2 can mitigate the effects of environmental stochasticity. Studies of *Botrychium simplex* and other species of *Botrychium* suggest that there is little genetic connectivity among occurrences of *B. simplex* in Region 2, so these occurrences are more vulnerable to extirpation by catastrophic events such as fires, landslides, and disease.

Woody plant encroachment

Encroachment of trees and other woody plants is considered a threat to many *Botrychium* species, which are not typically found in shaded or heavily forested sites; it is a particular threat to meadow species (Johnson-Groh and Farrar 2003). The degree to which *B. simplex* is threatened by succession is less clear since it is often documented in forested sites in the shade.

Exotic species

Six exotic species have been documented with *Botrychium simplex* in Region 2 (**Table 11**): houndstongue (*Cynoglossum officinale*), oxeye daisy (*Leucanthemum vulgare*), timothy (*Phleum pratense*), Kentucky bluegrass (*Poa pratensis*), dandelion (*Taraxacum officinale*), and white clover (*Trifolium repens*). Of these species, it is likely that Kentucky bluegrass represents the greatest threat since it can be highly invasive and competitive in riparian areas and meadows. Its presence in wet sites in Rocky Mountain National Park is a concern for land managers (Connor personal communication 2003). Oxeye daisy is known to occur near *B. simplex* at one location in South Dakota, but it is also spreading in the mountains of Colorado. There are no data suggesting a direct impact of noxious weeds on *Botrychium* species (Johnson-Groh and Farrar 2003), but their mutual affinity for disturbance may cause *Botrychium* species and their habitat to be vulnerable.

The management of weed species may also be a cause for concern with respect to *Botrychium* species. Johnson-Groh (1999) observed the effects of herbicide and fire on prairie moonworts (*B. simplex*, *B. campestre*, and *B. gallicomontanum*). Plants that had been hit directly with the herbicide Roundup were apparently killed (Johnson-Groh 1999, Johnson-Groh and Farrar 2003). Efforts to control noxious weeds will probably affect moonwort occurrences in right-of-ways or other sites targeted for weed management. At the time this report was written, it was not possible to determine if *B. simplex* was more or less affected by herbicide than the other moonwort species.

In the deciduous, hardwood forest habitats of *Botrychium mormo*, which is a close relative of *B. simplex*, 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 interface of the O and A horizons (Smith and Read 1997). The activity of earthworms has resulted in the elimination of the duff layer and a shift in species composition in *B. mormo* habitat (Berlin et al. 1998, Gundale 2002). Although earthworms present a possible threat to *B. simplex*, no research has shown that they are affecting species of *Botrychium* other than *B. mormo*. Earthworms are a diverse group of over 3,500 species worldwide, and the expansion of global commerce may be increasing the likelihood of exotic earthworm invasions with potential adverse effects on soil processes and plant species (Hendrix and Bohlen 2002).

Global climate change

Global climate change is likely to have wide-ranging effects in the near future for all habitats. However, the direction of projected trends is 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 West. Decreased precipitation could have dire consequences for occurrences of *Botrychium simplex* in Region 2 if it results in drying of its moist habitats. Giorgi et al. (1998) showed that temperature and precipitation increased under simulated doubling of atmospheric carbon dioxide levels. Either scenario could 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 affect occurrences at the southern edge of their natural range, pushing them northward and upward. With extensive habitat disturbance in the path of potential migration, many organisms will not be able to move. Preserves may provide short-term protection, but these may be ineffectual if organisms are trapped without suitable routes for migration.

Pollution

Atmospheric nitrogen deposition has become one of the most important agents of vegetation change in densely populated regions (Köchy and Wilson 2001). Nitrogen loading and the associated vegetation change have been observed to be greatest near large metropolitan areas (Schwartz and Brigham 2003). Thus, measurable impacts from nitrogen pollution might be expected in many locations in Region 2, especially in Colorado. Nitrogen enrichment experiments show universally that nitrogen is limited (Gross et al. 2000). This is likely to cause a few species to increase in abundance while many others decline (Schwartz and Brigham 2003). Acid deposition, which has increased markedly in Colorado through the 20th century, may have already caused changes to the soil chemistry that threaten the viability of *Botrychium simplex*. High elevation watersheds of the Front Range have already reached an advanced stage of nitrogen saturation (Burns 2002).

Over-utilization

Voucher specimens do assist with taxonomic research on *Botrychium simplex*. However, caution should be exercised in collection. Weber and Wittmann (2001a, 2001b) recommend not collecting the roots because they contain no diagnostic characteristics, and collecting them kills the plant. To minimize the risks 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 it with the fingers. Although evidence suggests that leaf removal does not have a significant, long-term effect on *Botrychium* species (Johnson-Groh and Farrar 1996a), collection of the species in Region 2 is only advisable in larger occurrences. Johnson-Groh and Farrar (2003) suggest that no collections be made in occurrences of fewer than 20 plants. Instead, they recommend that photographs be taken and deposited at an herbarium in lieu of a specimen. If collections are made of *B. simplex*, Johnson-Groh and Farrar (2003) recommend that no more than 10 percent of an occurrence be collected. Also, it is important to collect mature specimens since immature sporophytes are difficult to identify (Ohio Department of Natural Resources 2003). Where occurrences are already of questionable viability, collection of material from them, even if the plants survive, is a risky endeavor. For example, in an occurrence of three sporophytes, there is almost no margin of error, and accidentally removing the apical bud could easily contribute to the extirpation of the species at this site. This is a difficult issue for some *Botrychium* species, and particularly for *B. simplex*, since collection and verification by an expert is the only way to be absolutely sure of proper identification.

There are no known commercial uses for *Botrychium simplex*. 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." *Botrychium* species are not widely sold in the herb trade, but they are mentioned as ingredients in tinctures and poultices for the treatment of external or internal injuries. There is potential for over-utilization of *Botrychium* species if their popularity increases in the herb trade. Because they cannot be readily cultivated, any commercial use would require the harvest of wild plants.

Conservation Status of __Botrychium simplex__ in Region 2

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

There are no data available from which meaningful inferences can be made regarding population trend or changes in distribution of *Botrychium simplex* in Region 2. Some occurrences that were last documented 20 or more years ago have been searched for and not relocated, but this does not necessarily indicate that they are extirpated. Vague locations of older collections and the difficulty of *Botrychium* hunting create uncertainty as to whether some occurrences can ever be relocated. A precise location was known for the occurrence at Old Faithful in Yellowstone National Park; however, this occurrence has not been seen since 1994 despite several searches at the right time of year (Whipple personal communication 2003). Apparently, the only occurrence in the states of Region 2 that has been successfully revisited is at Lower Geyser Basin in Yellowstone National Park, but this occurrences falls outside the administrative boundary of Region 2. The Population trend section has more information on population status in Region 2.

Do habitats vary in their capacity to support this species?

In the absence of a disturbance regime, ecological succession may lead to unsuitable conditions for *Botrychium* species. This may be less of a concern, however, for *B. simplex* than for other moonwort species in Region 2 since it is often found in shaded, forested areas. Variables such as fire regime, amount of litter, soil moisture variation, and soil texture may affect habitat suitability. Some habitats may be suitable for one variety of *B. simplex* but unsuitable for others.

Vulnerability due to life history and ecology

The vulnerability of *Botrychium simplex* due to its life history and ecology remains uncertain since these factors are poorly understood. Chadde and Kudray (2001b) note that the intrinsic vulnerability of *B. simplex* is low; occurrences are generally resilient and/or resistant to change. However, the reliance of most *Botrychium* species on disturbance to create and maintain appropriate habitat may make them vulnerable to woody plant encroachment and interspecific competition.

The wetland and riparian habitats of *Botrychium simplex* are the most critically threatened habitats in the semi-arid West. These sites are often sought after for human uses, and some are heavily used. Consequently, they are subject to hydrologic alterations that could render them unsuitable for *B. simplex*.

Evidence of populations in Region 2 at risk

The rarity and small occurrence sizes of *Botrychium simplex* in Region 2 suggest that it is vulnerable to extirpation locally or even regionally, and that all of the Region 2 occurrences are at risk. The number of individuals documented thus far from the 50 occurrences in Region 2 falls between 500 and 600 plants. Of these 50 occurrences, 17 have not been seen again in more than 20 years (**Table 8**), and the current status of these occurrences is uncertain. Stochastic events and normal environmental variation could result in extirpation of many of the small and localized occurrences in Region 2. The quality and availability of habitat in Region 2 has probably declined due to fragmentation, exotic species invasion, hydrologic alteration, and edge effects that decrease the quality of small patches of natural vegetation. The majority of occurrences and potential habitat are known from public lands managed by the USFS and the National Park Service, where they receive some degree of protection. Chadde and Kudray (2001b) note that the habitat integrity is good in Region 9, with most habitats and sites protected and not commonly impacted by management. This may be true in much of Region 2 as well, but the creation of ski runs on USFS land in Region 2 has resulted in extensive habitat modification in areas known to be occupied by *B. simplex*. Because the ecology of this species is poorly understood, current management may be placing unsustainable demands on the species despite good intentions.

Current data suggest a high degree of imperilment for this species in Region 2, due largely to small occurrences, but these data are sparse. More occurrences are likely to be found in Region 2 by targeted surveys, but the known patterns of distribution and abundance in Region 2 suggest that large, robust occurrences are unlikely to be found. This underscores the need to conduct additional surveys for this species and to monitor known occurrences rigorously.

Management of Botrychium simplex in Region 2

Implications and potential conservation elements

The most current data available suggest that *Botrychium simplex* is imperiled in most of Region 2 due to the low number of small, isolated occurrences. The loss of any occurrence is significant and may result in the loss of important components of the genetic diversity of the species. Within Region 2, most reports of *B. simplex*, as well as most areas of suitable habitat, are on National Forest System land. Thus the responsibility of maintaining viable populations within the administrative boundary of Region 2 falls largely on the USFS. Further research is needed, however, before meaningful inference can be offered regarding restoration policy.

Desired environmental conditions for *Botrychium simplex* include sufficiently large areas where the natural processes on which the species depends can occur, permitting it to persist unimpeded by human activities and their secondary effects, such as weeds. This includes a satisfactory degree of ecological connectivity between occurrences and apparently suitable but unoccupied habitat. Given the current paucity of detailed information on 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. simplex* depends are functioning properly at many or most of the occurrences of this species. Again, further research on this species' ecology and distribution will help to develop effective approaches to its management and conservation. Until a more complete picture of the distribution and ecology of this species is obtained, however, priorities lie with conserving the known occurrences and locating additional occurrences.

Conservation elements essential to maintaining viable occurrences of *Botrychium simplex* in Region 2 include early- to mid-seral stage or other suitable habitats and the maintenance of processes that create and support these habitats. Unfortunately, these are poorly understood at present. Given the rate at which new data are becoming available and the incompleteness of our current knowledge of *B. simplex* in Region 2, it is difficult to formulate conservation strategies at present. More complete knowledge of its distribution will permit the identification of the occurrence in Region 2 that are the most suitable for conservation management. Surveys are needed to better understand how the recognized subspecies of *B. simplex* differ in habitat affinities, distribution, and autecology in Region 2. Demographic studies designed to determine the impacts of grazing, succession, fire, and exotic species on population viability are also high priorities for research on *B. simplex* in Region 2. Research is needed to investigate the subterranean life history, ecology, reproductive biology, role of mycorrhizae, and role of disturbance in the autecology of *B. simplex* so that conservation efforts can be most effective. Restoration of occurrences of members of *Botrychium* subgenus *Botrychium* is probably precluded by the difficulties in propagating them.

Tools and practices

Species inventory

Botrychium simplex is seldom seen because it often grows in wetter sites than other moonworts (Root personal communication 2003), and it is cryptic and small. This species can be found from approximately mid-June through August and possibly into September, but it may remain dormant in less favorable (dry) years. Given the difficulty in finding moonworts, inventory and monitoring studies are difficult, and dormant occurrences have been reported to be extirpated (Lesica and Steele 1994). Success in finding *B. simplex* requires training, time, and persistence (Wagner 1946, Wagner and Wagner 1976). New occurrences continue to be found within Region 2 (e.g., Kolb and Spribille 2000, Fertig 2003) and elsewhere Repeated surveys may be needed to identify all the *Botrychium* species present. The Bearlodge Campground in Crook County, Wyoming, where three moonwort species have been found over 30 years, is a classic example of the need to revisit a location to understand its moonwort flora.

Johnson-Groh and Farrar (2003) offer suggestions for conducting surveys for *Botrychium* species. Suggested techniques include systematic search methods, marking plants, collection protocols, documentation, and other concerns. The protocols they defined are based on the assumption that one week can be spent searching approximately 25 high priority sites per year, for about one hour each. Four or five people are recommended to walk transects at each area selected so that better coverage is achieved in the time of the visit. When plants are discovered, they are marked with pin flags and the surrounding area searched intensively but carefully on hands and knees.

These methods have certain limitations that need to be considered. Johnson-Groh and Farrar (2003) note four limitations:

- ❖ low confidence in distribution and abundance due to population variability
- ❖ difficulty in finding the plants
- ❖ predominance of the belowground lifecycle stages
- ❖ the occurrence of many species in genus communities that complicates identification.

Popovich (personal communication 2003) implemented this protocol and noted that the area searched appeared to have been heavily impacted by the intensiveness of the search efforts. It can also be cost-prohibitive to have four or five surveyors on site to implement these protocols. Johnson-Groh and Farrar (2003) also recommend using the "timed meander search procedure" described by Goff et al. (1982) for *Botrychium* searches.

Due to limitations in time and funding, attempts to search for rare plants often involve looking for multiple species over large areas. While this approach has been effective in finding occurrences of many rare plant species, it may not be effective for *Botrychium simplex* since it is difficult to find and identify. Searching for *B. simplex* requires one's full attention, so attempts to search for this species are more likely to be successful if it is being sought along with other *Botrychium* species rather than during a general sensitive species inventory. Having experts (e.g., contractors, agency botanists, or others trained and experienced with searching for *Botrychium* species) 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 broad range of habitats that *Botrychium simplex* occupies, it is difficult to recommend specific areas where searches are likely to locate new occurrences. *Botrychium simplex* is found over a wide elevation range and is erratically distributed, with less habitat fidelity than any other *Botrychium* species in Region 2. Searches that focus on areas that contain habitat types in which *B. simplex* has been previously found are most likely to yield new discoveries. Given its tendency to occur with other moonwort species, sites where other moonwort species have been documented should also be searched for *B. simplex*. Known locations for *B. multifidum*, in particular, warrant searching for *B. simplex* since these species are often found together (Crook personal communication 2003). This can be difficult, however, because *B. multifidum* is best seen in the fall after the leaves of *B. simplex* have already senesced.

Recent surveys in Region 2 have been successful in locating new moonwort occurrences. Searches in the Bearlodge Ranger District of Wyoming and neighboring South Dakota identified numerous occurrences of *Botrychium* species (Crook personal communication 2003, Burkhart personal communication 2006, Farrar personal communication 2006). The White River and Shoshone national forests are now known to support many occurrences of *B. simplex*, but additional surveys will likely find more. Despite recent discoveries of moonwort occurrences in Summit County, Colorado, more inventories are needed to document the distribution of *B. simplex* there since all potential habitat was not visited (Kolb and Spribille 2000). Zimmerman Lake and other sites in the Cameron Pass area are high priority search areas on the Arapaho-Roosevelt National Forest (Schwab 1992).

Searches in apparently suitable habitats in Colorado have failed to identify new discoveries of *Botrychium simplex* (Popovich personal communication 2006). Near the toll booth along the road to Mount Evans at least seven species of moonworts have been found, and there is abundant riparian habitat in the area that appears suitable for *B. simplex*; however, in repeated searches of this area by experts, no *B. simplex* has yet been found. Near the east portal of the Moffat Tunnel, there is also suitable habitat that has been searched without finding any *B. simplex* (Popovich personal communication 2006). These observations contrast sharply with moonwort survey efforts on the Black Hills National Forest in 2004 and 2005, where *B. simplex* was the most commonly found *Botrychium* species (Burkhart personal communication 2006).

Botrychium simplex has a known affinity for plains habitats, and it is probably more abundant in plains riparian areas than we realize. Given the difficulty in finding this species, it has likely just evaded discovery. Portions of major rivers on the plains of Region 2 that still have an active floodplain (e.g., Platte, Republican, Arikaree) should be included in inventory plans for *B. simplex*.

Habitat inventory

Aerial photographs, topographic maps, soil maps, and geology maps can be used to refine surveys of large areas. Use of these tools is most effective for species for which we have basic knowledge of its substrate and habitat specificity, such as *Botrychium simplex*, and from which distribution patterns and potential search areas can be deduced.

The use of deductive and inductive techniques to model the distribution of *Botrychium simplex* would also 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) has been used to model the distribution of other sensitive plant species in Wyoming (e.g., Fertig and Thurston 2003) and Colorado (Decker et al. 2005). Combining CART with envelope models such as DOMAIN, BIOCLIM, or MaxEnt can help to refine a potential distribution map further by adding inference on the likelihood of the presence of *B. simplex* (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. simplex* in Region 2, but without a geospatially explicit dataset of these practices, this possibility cannot be accounted for in the model.

In general, the best search areas for all *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, Johnson-Groh and Farrar 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 on a list of good areas to search. Wagner and Wagner (1976) noted that areas within 10 to 20 feet of the edges of woods are particularly lucrative sites to search, and specimen label data from Region 2 and elsewhere support this statement. Wetlands, riparian areas, floodplains, hot springs, streamsides, peaty sites, wet slopes, avalanche meadows, wet meadows, colluvial slopes, and sparsely vegetated sites above treeline are all potential search areas for *B. simplex*. The Community ecology and Habitat sections of this document contain discussions of other environmental correlates and commonly associated species.

Soil moisture and texture are not useful for identifying potential habitat for *Botrychium simplex*. However, soil nutrient levels may be a useful filter tool in the selection of potential search areas. In four states outside of Region 2, soils that support moonwort communities averaged 916 ppm of calcium and 140 ppm of magnesium (Hansen and Johnson-Groh 2003). The potential value of these and other edaphic factors in identifying habitat for *B. simplex* in Region 2 has not been assessed.

Population monitoring

There are several monitoring studies of *Botrychium simplex* from which a monitoring protocol could be developed for Region 2. Permanent plot monitoring of *B. simplex* in Iowa and Minnesota is described in Johnson-Groh and Farrar (1996b) and Johnson-Groh (1999). Many techniques that have been used to monitor occurrences of other *Botrychium* species (e.g., Montgomery 1990, Muller 1992, Kelly 1994, Johnson-Groh and Lee 2002) could also be suitable for monitoring *B. simplex*, depending on the situation and the question to be answered by monitoring. The Pike-San Isabel National Forest (Carpenter 1996) has drafted protocols for monitoring *B. lineare*, and these may be useful for *B. simplex*. Johnson-Groh and Lee (2002) have studied the phenology and demography of *B. gallicomontanum* and *B. mormo*, using methods that could be applied to *B. simplex*. These methods are presented in the *Botrychium* Inventory and Monitoring Technical Guide (Johnson-Groh and Farrar 2003), which has been designed for members of subgenus *Botrychium*. Using the protocols described by Johnson-Groh and Farrar (2003) would facilitate the comparison of monitoring data from sites across the United States.

The sampling strategy recommended by Johnson-Groh and Farrar (2003) involves the deployment of permanently-marked 1 square meter plots within a moonwort occurrence. The number of plots needed will depend on a power analysis completed after at least two years' of data have been recorded. These plots are established non-randomly to capture the variation in aspect, drainage, canopy cover, and other environmental variation present at the site, and they are oriented north-south. The plots may contain one or numerous species of moonworts. Within each plot, a numbered tag is installed 2.5 cm north of each moonwort. Searching for plants within the plot may require litter removal because small plants are often unable to emerge through litter. All plants are measured from the base of the plant to the top of the tropophore. The developmental stage of each sporophyte is recorded (e.g., just emerging, releasing spores). Disturbances such as herbivory are also documented for each plant or plot. Johnson-Groh (1999) notes that it can be difficult to be assured that an individual that had been marked in a previous year is the same individual in subsequent years. See Johnson-Groh and Farrar (2003) for details regarding these protocols.

For species such as *Botrychium simplex* where the proportion of dormant plants may vary among years, it is difficult to monitor population trends (Lesica and Steele 1994). However, recent advances have been made in mark-recapture methods for species that exhibit prolonged dormancy or are difficult to find in concealing vegetation (Alexander et al. 1997). These methods, which have been applied to plants, may be useful for estimating *B. simplex* populations.

Annual monitoring of selected occurrences of *Botrychium simplex* in Region 2 could provide data that will improve our understanding of its ecology and population trends. Population trend monitoring protocols pertaining to members of subgenus *Botrychium* are defined in Johnson-Groh and Farrar (2003). Counting the emergent sporophytes at each occurrence every year would provide valuable data on the status of the species. In small occurrences, the entire population should be counted; in large occurrences, a randomized permanent plot technique could be used to monitor a representative sample of the occurrence (Chadde and Kudray 2001b). Tracking individuals by marking or mapping them within each sampling unit could provide information on the life span, dormancy, recruitment success, and population trends of this species (Kolb and Spribille 2000). It is important to note that monitoring *Botrychium* occurrences is difficult due to their curious life history attributes and small size, and tens of years of data will be needed to draw meaningful inferences (Johnson-Groh and Farrar 2003). Monitoring occurrences in Region 2, with return intervals of not more than 10 years, is a high conservation priority (Kolb and Spribille 2000).

Adding a photopoint and photoplot component to this work, following recommendations presented in Elzinga et al. (1998) and Hall (2002), will facilitate the tracking of individuals and add valuable qualitative information. These techniques can be done quickly in the field, and although they do not provide detailed cover or abundance data, they can help to elucidate patterns observed in quantitative data.

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

Beneficial management actions

Because many additional occurrences of *Botrychium simplex* have likely not been found in Region 2, surveys prior to management actions within potential habitat will help to identify new occurrences and to alleviate anthropogenic threats to this species (Lorain 1990). The value of surveys before construction is evinced by the discovery of *B. simplex* on Vail Pass at a site where a ski hut was to be built. The pre-project survey probably reduced the impacts to this occurrence. Identifying large, high quality occurrences through surveys and monitoring will also help managers to prioritize conservation actions. 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. simplex*.

There is no evidence of any direct impacts to occurrences of *Botrychium simplex* from recreation, but impacts on this species are difficult to document, as shown by the fact that no occurrence of *B. simplex* in Region 2 has been seen more than once. Occurrences in ski areas are most likely to incur the loss of individuals due to summer recreational use of ski slopes. Off-road motorized vehicle use also has the potential to affect *B. simplex*. Hiking may result in trampling where *B. simplex* occurs near trails or backcountry huts. Signage or temporary fences that could be removed in the winter could help dissuade recreational use in known occurrences of *B. simplex*. Mitigating recreation impacts to *B. simplex* may be important at some occurrences.

Preventing the alteration of the hydrology in *Botrychium simplex* occurrences is an important management consideration. This is probably most important in wetland sites such as seepy or wet meadows, streamsides, floodplains, and wet slopes (e.g., #1, 9, 13, 23, 25, 29, and 49 in **Table 8**).

The observations of Muller (1999) suggest that maintaining occasional perturbations by humans or animals recreates pioneer habitats that benefit *Botrychium matricariifolium*. Thompson (2001) noted that the clearing of ski runs created a great deal of suitable habitat for moonworts, which are not found under tree canopies. While this may be true, ski runs cannot be depended upon for the long-term conservation of moonworts since they are managed for the benefit of skiers, not moonworts. For example, installing pipelines in ski runs for the production of artificial snow will have negative impacts on plants in the ski runs. It is not known if the autecological parameters needed by moonworts (e.g., appropriate mycorrhizal hosts, suitable disturbance regime) can persist indefinitely in ski runs. Kolb and Spribille (2000) recommend that *B. simplex* occurrences should be protected from ground-disturbing activities. More research is needed on the practical application of management protocols for the maintenance of moonwort occurrences.

The utility of fire as a habitat management tool for *Botrychium simplex* is not known. Evidence suggests that the direct impacts of fire are not detrimental to *Botrychium* (Johnson-Groh and Farrar 2003). Fire can slow succession to closed canopy and create open habitats for *B. simplex*. It can also alter soil characteristics in ways that may favor *B. simplex*. Burning actually appears to have had positive effects on *B. campestre*, *B. gallicomontanum*, and *B. simplex* occurrences in Iowa, but fire combined with erosion and desiccation, both natural results of fire, may be deleterious. There were no significant differences in plant size in burned and unburned plots at these locations, but fires occurring after drought have resulted in population decline (Johnson-Groh and Farrar 1996b, 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. simplex* since they have similar life histories.

Beneficial management actions regarding grazing are unclear, but there is evidence to suggest that *Botrychium simplex* may tolerate some 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." The Community ecology and Threats sections of this document have more information on grazing.

Any management strategies that protect occurrences of *Botrychium simplex* from invasion by exotic species will confer the greatest benefits. Because herbicides are known to kill this species, their use within occurrences of *B. simplex* should be limited to direct application to the target species. Aggressive management of noxious weeds before they become dominant in *B. simplex* occurrences could avert costly and risky eradication efforts. Where possible, hand-pulling should be the favored method of managing weeds within *B. simplex* occurrences.

Mitigating threats to occurrences of *Botrychium simplex* from intensive land use practices (i.e., off-road vehicle use) will confer benefits to the species. Controlling motorized access to habitat and providing appropriate signage at access points may decrease impacts to *B. simplex*.

Populations of *Botrychium* are inherently variable (Johnson-Groh 1999). Many occurrences are small, increasing the likelihood of local extirpation. *Botrychium simplex* may depend on a shifting mosaic of suitable habitats in appropriate successional stages that can be colonized (as described by Pickett and Thompson 1978). If this is the case, then the metapopulation dynamics of *B. simplex* become crucial to its management and conservation, and underscore the need to conserve nearby areas of suitable habitat that are not currently inhabited by *B. simplex* (Chadde and Kudray 2001b).

Restoration

Growing any *Botrychium* species in the greenhouse or lab is extremely difficult (Whittier 1972, Gifford and Brandon 1978). *Botrychium simplex* is hardy to USDA Zone 3 (average minimum annual temperature –40 ºF) and it can be propagated from spores with difficulty, but it is not generally cultivated (Rook 2002). No spores of *B. simplex* 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 future restoration work. Herbarium specimens and wild populations (within limits) may also provide propagules for restoration. While *Botrychium* species are generally very difficult to transplant, *B. virginianum* transplants were relatively successful (Wagner and Wagner 1976). Moonworts have been transplanted in Summit County, Colorado in sites where pipelines passed through occurrences, but it is not known if these efforts were successful (Kolb and Spribille 2000, Buell 2001). Buell (2001) details moonwort transplant protocols that may be useful for *B. simplex*.

Information Needs and Research Priorities

Kolb and Spribille (2000) offer suggestions for research on *Botrychium simplex*. Vanderhorst (1997) also offers suggestions for research and management for occurrences on the Kootenai National Forest. Research needs for other moonworts 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. simplex* as well.

Surveys are among the greatest research needs for *Botrychium simplex* in Region 2. As concern and awareness of *Botrychium* species has increased, more inventories have focused on finding these species. Consequently, there are many recent discoveries of *B. simplex* and other *Botrychium* species in Region 2. It is likely that occurrences of this and other moonwort species remain to be discovered, and many are likely to be on USFS land. Better habitat data may help to evaluate the environmental tolerances of *B. simplex* (Farrar 2001). While current data portray the range of habitats in which *Botrychium simplex* is found in Region 2, research is needed to develop an understanding of the ecosystem processes on which it depends. Some habitat attributes are commonly seen in Region 2 occurrences, but the relative importance of these attributes to the establishment and persistence of *B. simplex* is unknown. Habitat data will also support the development of potential habitat maps, which would be a valuable tool in locating new occurrences (Kolb and Spribille 2000).

The occurrence of *Botrychium simplex* in saturated fen habitats is not well documented, and research is needed to assess the importance of this habitat (Farrar 2001, Root personal communication 2003). Saturated fen habitats also need to be searched for other moonworts, including *B. montanum*, *B. minganense*, *B. crenulatum*, and *B. pinnatum* (Farrar 2001, Root personal communication 2003). *Botrychium crenulatum* has not been found in Colorado but it may grow in fens (Root personal communication 2003). Searches of calcareous fens and seeps for var. *fontanum* within Region 2 are also needed. Other potential habitats for *B. simplex* such as avalanche chutes (ecologically similar to ski runs) have not been adequately searched.

A replicated demographic monitoring study that compares vital rates of *Botrychium simplex* in burned vs. unburned, grazed vs. ungrazed, shaded vs. unshaded, and weedy vs. unweedy sites would answer many questions about this species. This is a high priority for determining appropriate management practices with respect to *B. simplex*. Monitoring of occurrences is needed to better understand life history characteristics including age, dormancy, growth rates, and reproductive rates in Region 2. In particular, the subterranean portion of this species' life cycle remains poorly understood, despite the fact that much of its lifespan occurs underground. More information regarding this species' reproductive rates and its ability to colonize new sites would improve our understanding of this species' response to change, but these remain highly speculative.

There are no data in Region 2 from which population trend can be determined. Johnson-Groh and Farrar (2003) have developed and successfully used standard population trend monitoring protocols for *Botrychium* species. Other sampling designs have been used in the study of *Botrychium* species as well (e.g., Lesica and Steele 1994, Berlin et al. 1998, Johnson-Groh 1999). The problem with any of these methodologies is that it is very difficult to determine the total number of plants in any *Botrychium* occurrence due to the high proportion of plants residing under ground as gametophytes and juvenile sporophytes, and due to the annual variation in the aboveground sporophyte population (Lesica and Steele 1994). There are apparently no published data on the prevalence of dormancy in *B. simplex*. Due to the inherent difficulties in monitoring *Botrychium* occurrences, determination of population trend requires decades of study (Johnson-Groh and Farrar 2003). Periodic monitoring is needed to assess population trends at significant moonwort occurrences (Kolb and Spribille 2000).

Although current research suggests that *Botrychium simplex sensu lato* is a valid taxon, better circumscription of the varietal taxa in Region 2 is needed to understand their distribution, relative abundance, and habitat affinities (Farrar 1998, Farrar 2001, Farrar personal communication 2003). Recent and ongoing research has resulted in a clearer picture of the taxonomically relevant variability within *B. simplex*. Analyzing isozymes, as in Farrar (1998) and Farrar (2001), and using DNA sequences, as employed by Hauk (1995) and Hauk et al. (2003), is one approach to sorting out the distribution and habitat affinities of the varieties in Region 2. The distribution of varieties of *B. simplex* is very poorly understood but is important from a management and conservation perspective.

Developing a better understanding of the ecological requirements of *Botrychium simplex* is important for its conservation and management. Research on the autecology of *B. simplex*, particularly its responses to fire, grazing, disturbance, and succession, is needed. The species' response to water table fluctuation, inundation, and drought are particularly relevant throughout Region 2. The effects of herbivores and exotic species on the viability of *B. simplex* occurrences have not been investigated. More research is needed to determine the types, intensities, and periodicity of disturbance that create and maintain suitable habitat for *Botrychium* species, including *B. simplex*. Habitat manipulation studies (i.e., addition of artificial snow to plots, soil scarification) in robust moonwort occurrences could help to identify those ecological factors with the greatest influence on the distribution and persistence of *B. simplex* (Kolb and Spribille 2000). The amount of disturbance that *B. simplex* can tolerate is not known but is important to managers. Current observations of its response to both natural and anthropogenic disturbance in Region 2 are informal, and quantitative data are needed to understand the role of disturbance in the life history, establishment, and persistence of *B. simplex*.

A clearer understanding of the relationship between *Botrychium simplex* and its mycorrhizal symbionts is important. An assessment of the effects of disturbance type and periodicity, especially for fire, and grazing, on *B. simplex*'s mycorrhizal symbionts will assist managers in developing appropriate management protocols. Research is also needed to assess the effect of different mycorrhizal species and infection levels on spore output. Studies of the role of mammals and other potential spore dispersal agents will assist with the management of *B. simplex*.

Metapopulation studies are very difficult to conduct for *Botrychium* species, but it is likely that the metapopulation structure is important (Johnson-Groh and Farrar 2003). Investigation of migration, extinction, and colonization rates could yield valuable data for the conservation of *B. simplex* and other *Botrychium* species. Gene flow among and within populations of *B. simplex* could be investigated by analyzing the frequency of molecular-genetic markers.

Identifying habitat characteristics through vegetation plot sampling would add analytical power to the assessment of *Botrychium simplex* habitat. Kolb and Spribille (2000) used releves, but other quantitative methods would apply as well. Estimating cover and/or abundance of associated species is needed to begin the investigation of interspecific relationships through ordination or other statistical techniques.

Understanding environmental constraints on *Botrychium simplex* could facilitate the conservation of this species. Gathering data on edaphic characteristics (e.g., moisture, texture, chemistry, particularly pH) from the vegetation or demographic monitoring plots described above would permit the canonical analysis of species-environment relationships. These data would facilitate hypothesis generation for further studies of the ecology of this species.

There are many barriers to habitat and population restoration for *Botrychium simplex*. *Botrychium* species are extremely difficult to propagate (Whittier 1972, Gifford and Brandon 1978, Wagner and Wagner 1983), and propagating them for reintroduction to the wild is probably not feasible. The subterranean ecology of these species is crucial to understanding their dynamics, yet it is very poorly understood. As obligate mycorrhizal hosts, they cannot survive without fungal partners, but very little is known about the specifics of this relationship. The difficulties in growing *Botrychium* species are presumably the result of their delicately attuned mycorrhizal relationships (Wagner and Wagner 1983). The mycobionts of *B. simplex* have not been identified. Restoration or maintenance of native vegetation will be a crucial part of any restoration of *B. simplex*.

Buell (2001, page 11) describes a method for transplanting *Botrychium* species that was used by Nancy Redner of the USFS. This method was used to mitigate pipeline and road construction impacts on *Botrychium* occurrences at the Copper Mountain Ski Resort in Colorado. No data on survivorship of the transplanted plants are available, but Buell (2001) described this method as “reasonably successful.” Several moonwort species, including *B. echo*, were transplanted in 2003 to mitigate construction impacts along the Guanella Pass Road (ERO Resources Corporation 2003). Their methods involved excavating a large area of soil around each plant or cluster of plants and not allowing the soil clump to break. Plants and their soil were transported using spring-form baking pans with removable bottoms. Popovich (personal communication 2005) estimated that up to 50 percent of the transplants survived into 2004. Cody and Britton (1989) note that transplanting *Botrychium* species is usually fatal. Because there has not been any long-term assessment of the success of transplants, the value of this practice for conservation is questionable and cannot be relied upon as a mitigation tool. Monitoring transplants could determine whether these techniques can be successful (Kolb and Spribille 2000).

Additional research and data resources

Farrar, Ahlenslager, and Johnson-Groh are currently assessing a suite of *Botrychium* species for Washington and Oregon. Johnson-Groh and Farrar (2003) are drafting monitoring protocols for *Botrychium* species. Dr. Reed Crook is planning to conduct habitat modeling for *B. multifidum* in the Black Hills (Crook personal communication 2003); this will apply to *B. simplex* since they are often found together.

Dr. Farrar plans to assess specimens from Region 2 using molecular phylogenetic techniques. This will result in a clearer picture of the distribution of *Botrychium simplex* varieties in Region 2. It will also determine whether the undescribed variety collected in the southwestern United States is also found in Colorado. The habitat differences among the varieties in Region 2 will be apparent with more inventories.

DEFINITIONS

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

Allopolyploid – A polyploid formed from the union of genetically distinct chromosome sets, usually two different species (Allaby 1998).

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).

Bobby sox trees – Lodgepole pine trees that, having died due to flooding, act as wicks that absorb geothermal water. As this water evaporates, it leaves silicon dioxide behind, which colors the base of the trunks white (hence the moniker "bobby sox trees") (Whipple personal communication 2003).

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; 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 simplex*, 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 do 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 (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.

Nothospecies – A species of hybrid origin (International Association for Plant Systematics 2001).

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 life cycle of plants. Haploid spores are produced by meiosis that gives rise to gametophytes (Allaby 1998).

Sympatry – The occurrence of species (or varieties in the case of *Botrychium simplex*) together in the same area (Allaby 1998).

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

References

Abbott, R.E. 2003. Survey for selected Orchidaceae, *Botrychium* spp., and other species of concern within a portion of the Crimson Vegetation Management Project Area. Unpublished report to the USDA Forest Service, Arapaho-Roosevelt National Forest by the Colorado Natural Heritage Program, Ft. Collins, CO.

Ahlenslager, K. and P. Lesica. 1996. Observations of *Botrychium* x *watertonense* and its putative parent species, *B. hesperium* and *B. paradoxum*. American Fern Journal 86:1-7.

Alexander, H.M., N.A. Slade, and W.D. Kettle. 1997. Application of mark-recapture models to estimation of the population size of plants. Ecology 78(4):1230-1237.

Allaby, M. 1998. A Dictionary of Plant Sciences. Oxford University Press, New York, NY.

Alverson, E.R. and P.F. Zika. 1996. *Botrychium* diversity in the Wallowa Mountains, Oregon. American Journal of Botany 83:123.

Anderberg, A. 2003. Den Virtuella Floran, Naturhistoriska Riksmuseet (In Swedish). Accessed via the Internet at http://linnaeus.nrm.se/flora/.

Arnett, M. 2002. A floristic inventory of the southern Gunnison Basin and the southeastern Uncompahgre Basin, Colorado. Master's Thesis. University of Wyoming, Laramie, WY.

Barbour, M.G., J.H. Burk, and W.D. Pitts. 1987. Terrestrial Plant Ecology. Benjamin/Cummings Publishing Company, Inc., Menlo Park, CA.

Barker, M.S. and W.D. Hauk. 2003. An evaluation of *Sceptridium dissectum* (Ophioglossaceae) with ISSR markers: implications for *Sceptridium* systematics. American Fern Journal 93:1-19.

Beauvais, G.P., D.A. Keinath, P. Hernandez, L. Master, and R. Thurston. 2004. Element Distribution Modeling: A Primer (Version 1.0). Wyoming Natural Diversity Database, University of Wyoming, Laramie, WY.

Berch, S.M. and B. Kendrick. 1982. Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern-like allies. Mycologia 74:769-776.

Berlin, N., P. Miller, J. Borovansky, U.S. Seal, and O. Byers. 1998. Population and Habitat Viability Assessment Workshop for the Goblin Fern (*Botrychium mormo*): Final Report. CBSG, Apple Valley, MN.

Bever, J.D., P.A. Schultz, A. Pringle, and J.B. Morton. 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. Bioscience 51:923-931.

Bierhorst, D.W. 1958. Observations on the gametophytes of *Botrychium virginianum* and *B. dissectum*. American Journal of Botany 451-9.

Bliss, L.C. 1987. Introduction. *In*: L.C. Bliss, editor. Truelove Lowland, Devon Island, Canada: A High Arctic Ecosystem. University of Alberta Press, Edmonton, Alberta, Canada.

Bolick, M. 2003. Personal communication with Curator of the University of Nebraska State Museum Herbarium regarding *Botrychium simplex* in Nebraska.

Bower, F.O. 1926. The ferns (Filicales). Volume 2. Cambridge University Press, Cambridge, UK. 344 pp.

Briggs, D. and S.M. Walters. 1997. Plant variation and evolution. Cambridge University Press, Cambridge, UK.

Britton, N.L. and H.A. Brown. 1913. An Illustrated Flora of the Northern United States, Canada, and the British Possessions. Volume 1. Charles Scribner's Sons, New York, NY.

Buell, K.H. 2001. Moonwort (*Botrychium* subg. *Botrychium*) survey report-Breckenridge Ski Resort. Prepared for Breckenridge Ski Resort, Breckenridge, CO. Submitted to USDA-Forest Service, White River National Forest, Silverthorne, CO. Habitat Concepts, Inc., Yampa, CO.

Bureau of Land Management Colorado. 2000. Colorado BLM State Director's Sensitive Species List. Accessed via the Internet at http://www.co.blm.gov/botany/sens_species.htm.

Bureau of Land Management Wyoming. 2002. BLM Wyoming Sensitive Species Policy and List. Accessed via the Internet at http://www.blm.gov/nhp/efoia/wy/2003ib/Wy2003-001atch1.pdf.

Burkhart, B. 2002. Region 2 Sensitive Species Evaluation Form for *Botrychium simplex*. USDA Forest Service, Region 2, Lakewood, CO.

Burkhart, B. 2006. Personal communication with Botanist of the Black Hills National Forest regarding *Botrychium simplex*.

Burns, D.A. 2002. The effects of atmospheric nitrogen deposition in the Rocky Mountains of Colorado and southern Wyoming - a synthesis and critical assessment of published results. U.S. Geological Survey Water Resources Investigations Report 02-4066.

Camacho, F.J. 1996. New report of subterranean sporophytic gemmae on *Botrychium pumicola*. American Fern Journal 86:27-28.

Camacho, F.J. and A. Liston. 2001. Population structure and genetic diversity of *Botrychium pumicola* based on inter-simple sequence repeats. American Journal of Botany 88:1065-1070.

Campbell, D.H. 1911. The Eusporangiatae: The comparative morphology of the Ophioglossaceae and Marattiaceae. Wash Publ. No. 140. Carnegie Institute.

Campbell, D.H. 1922. The gametophyte and embryo of *Botrychium simplex*, Hitchcock. Annals of Botany 36:441-456.

Carpenter, A. 1996. Monitoring plan for the rare fern *Botrychium lineare*. Pike-San Isabel National Forest, El Paso County, Colorado. Unpublished report produced for the USDA Forest Service.

Casson, J., J. Dobberpuhl, D. Farrar, A. Hoefferle, C. Johnson-Groh, H. Peters, H. Wagner, F. Wagner, C. Westfield, and P. Miller. 1998. Population life history and viability working group report. Population and Habitat Viability Assessment Workshop for the Goblin Fern (*Botrychium mormo*). Final Report. CBSG, Apple Valley, MN.

Caswell, H. 2001. Matrix Population Models. Second Edition. Sinauer Associates, Inc., Sunderland, MA.

Chadde, S. and G. Kudray. 2001a. Conservation assessment for goblin fern/ little goblin moonwort (*Botrychium mormo*). Prepared for the USDA Forest Service, Eastern Region.

Chadde, S. and G. Kudray. 2001b. Conservation assessment for least grape-fern/least moonwort (*Botrychium simplex*). Prepared for the USDA Forest Service, Eastern Region.

Clausen, R.T. 1938. A monograph of the Ophioglossaceae. Memoirs of the Torrey Botanical Club 19:1-177.

Clute, W.N. 1901. Our Ferns in Their Haunts: A Guide to all the Native Species.

Colorado Natural Heritage Program. 2006. Biodiversity Tracking and Conservation System. Colorado State University, Ft. Collins, CO.

Connor, J. 2003. Personal communication with Threatened and Endangered Species Biologist at Rocky Mountain National Park regarding invasive species.

Cothrel, S.R., J.P. Vimmerstedt, and D.A. Kost. 1997. *In situ* recycling of urban deciduous litter. Soil Biology & Biochemistry 29:295-298.

Coulter, J.M. and A. Nelson. 1909. New Manual of Botany of the Central Rocky Mountains (Vascular Plants). American Book Company, Chicago, IL.

Coville, F.V. 1900. Description of *Botrychium pumicola*. Page 69 *in* L.M. Underwood. Our Native Ferns and Their Allies with Synoptical Descriptions of the American Pteridophyta North of Mexico. Sixth edition. Henry Holt and Company, New York, NY.

Cronquist, A., A.H. Holmgren, N.H. Holmgren, and J.L. Reveal. 1972. Intermountain Flora-Vascular Plants of the Intermountain West, U.S.A. Volume 1. The New York Botanical Garden, New York, NY.

Crook, R.W. 2003. Personal communication USDA Forest Service botanist regarding *Botrychium simplex* in Wyoming and South Dakota.

Dahl, T.E. 1990. Wetland losses in the United States 1780s to 1980s. U.S. Department of the Interior, Fish and Wildlife Service. St. Petersburg, FL.

Dahl, T.E. 2000. Status and trends of wetlands in the conterminous United States 1986-1997. U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C.

Daigobo, S. 1979. Observations on the gametophytes of *Botrychium multifidum* from nature. Journal of Japanese Botany 54:169-177.

Davis, R.J. 1952. Flora of Idaho. WM. C. Brown Company, IA.

Decker, K., A. Lavender, and D.G. Anderson. 2005. Modeling the potential distribution of *Phacelia scopulina* var. *submutica* (Debeque Phacelia) and *Astragalus debequaeus* (Debeque Milkvetch) in western Colorado. Prepared by the Colorado Natural Heritage Program for U.S. Fish and Wildlife Service and Colorado State Parks.

Dorn, R.D. and J.L. Dorn. 1972. The ferns and other Pteridophytes of Montana, Wyoming, and the Black Hills of South Dakota. Published by the authors, Laramie, WY.

Dyer, A.F. 1994. Natural soil spore banks - can they be used to retrieve lost ferns? Biodiversity and Conservation 3: 160-175.

Dyer, A.F. and S. Lindsay. 1992. Soil spore banks of temperate ferns. American Fern Journal 82:89-122.

Eaton, A.A. 1899. A new species of *Botrychium*. Fern Bulletin 7:7-8.

Elzinga, C.L., D.W. Salzer, and J.W. Willoughby. 1998. Measuring and monitoring plant populations. BLM Technical Reference 1730-1.

ERO Resources Corporation. 2003. Moonwort Transplantation- Colorado Forest Highway 80, Guanella Pass Road, Park and Clear Creek Counties, Colorado. Unpublished report prepared for the Federal Highways Administration.

Farrar, D.R. 1998. Population genetics of moonwort *Botrychiums*. Population and Habitat Viability Assessment Workshop for the Goblin Fern (*Botrychium mormo*). Final Report. CBSG, Apple Valley, MN.

Farrar, D.R. 2000. A genetic assessment of the systematic relationships of western moonwort species (*Botrychium* subgenus *Botrychium*). Final report submitted to Kathy Ahlenslager, Colville National Forest / Mary Stensvold, Tongass National Forest. Unpublished.

Farrar, D.R. 2001. Isozyme characterization of moonwort ferns (*Botrychium* subgenus *Botrychium*) and their interspecific relationships in northwestern North America. Final report submitted to Kathy Ahlenslager, Colville National Forest.

Farrar, D.R. 2002. Personal communication with *Botrychium* expert regarding the demography of *Botrychium* species.

Farrar, D.R. 2003. Personal communication with *Botrychium* expert regarding *Botrychium simplex*.

Farrar, D.R. 2005. Systematics of western moonworts - *Botrychium* subgenus *Botrychium*. Produced for the USDA Forest Service Region 2.

Farrar, D.R. 2006. Personal communication with *Botrychium* expert regarding *Botrychium simplex*.

Farrar, D.R. and C.L. Johnson-Groh. 1986. Distribution, systematics, and ecology of *Botrychium campestre*, the prairie moonwort. Missouriensis 7:51-58.

Farrar, D.R. and C.L. Johnson-Groh. 1991. A new prairie moonwort (*Botrychium* subgenus *Botrychium*) from northwestern Minnesota. American Fern Journal 81:1-6.

Farrar, D.R. and C.L. Johnson-Groh. 1990. Subterranean sporophytic gemmae in moonwort fern *Botrychium* subgenus *Botrychium*. American Journal of Botany 77:1168-1175.

Farrar, D.R. and J.F. Wendel. 1996. Eastern moonworts: genetics and relationships (Abstract). American Journal of Botany 83:124.

Fernald, M.L. 1949. Botrychiaceae *Botrychium simplex* var. *laxifolium*. Rhodora 51:103.

Fertig, W. 2000. State species abstract for *Botrychium campestre*. Wyoming Natural Diversity Database, Laramie, WY.

Fertig, W. 2003. Confessions of a moonwort stalker. Castilleja 22:9-11.

Fertig, W. and R. Thurston. 2003. Modeling the potential distribution of BLM sensitive and USFWS threatened and endangered plant species. Wyoming Natural Diversity Database, Laramie, WY.

Forman, R.T.T. and L.E. Alexander. 1998. Roads and their major ecological effects. Annual Reviews of Ecological Systems 29:207-231.

Gerard, J. 1633. The Herbal or General History of Plants. 1633 Edition as Revised and Enlarged by T. Johnson. Dover Publications Inc., New York, NY.

Gifford, E.M. and D.D. Brandon. 1978. Gametophytes of *Botrychium multifidum* as grown in Axenic Culture. American Fern Journal 68:71-75.

Gifford, E.M. and A.S. Foster. 1989. Morphology and Evolution of Vascular Plants. Third edition. W.H. Freeman and Company, New York, NY.

Giorgi, F., L.O. Mearns, C. Shields, and L. McDaniel. 1998. Regional nested model simulations of present day and 2 x CO_2 climate over the central plains of the U.S. Climatic Change 40:457-493.

Gleason, H.A. and A. Cronquist. 1963. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. Van Nostrand Reinhold Company, New York, NY.

Goff, F.G., G.A. Dawson, and J.J. Rochow. 1982. Site examination for threatened and endangered plant species. Environmental Management 6:307-316.

Gray, A. 1908. Gray's New Manual of Botany. Seventh Edition. A Handbook of the Flowering Plants and Fern of the Central and Northeastern United States and Adjacent Canada. Revised and enlarged by B.L. Robinson and M.L. Fernald. American Book Company, New York, NY.

Great Plains Flora Association. 1986. Flora of the Great Plains. University Press of Kansas, Lawrence, KS.

Grime, J.P. 2001. Plant Strategies, Vegetation Processes, and Ecosystem Properties. Second edition. John Wiley & Sons, Chichester, West Sussex, England.

Gross, K.L., M.R. Willig, and R. Gough. 2000. Patterns of species density and productivity at different spatial scales in herbaceous plant communities. Oikos 89:417-427.

Gundale, M.J. 2002. Influence of exotic earthworms on the soil organic horizon and the rare fern *Botrychium mormo*. Conservation Biology 16:1555-1561.

Hall, F.C. 2002. Photo Point Monitoring Handbook - Parts A and B. General Technical Report PNW-GTR 526. USDA Forest Service Pacific Northwest Research Station, Portland, OR.

Hansen, K. and C.L. Johnson-Groh. 2003. Soil Properties of *Botrychium* Habitats. Sigma Xi Research Symposium, Gustavus Adolphus College, St. Peter, MN.

Hanski, I.A. and D. Simberloff. 1997. The metapopulation approach, its history, conceptual domain, and application to conservation. Pages 5-26 *in* I. Hanski and M.E. Gilpin, editors. Metapopulation Biology. Ecology, Genetics, and Evolution.

Harrington, H.D. 1954. Manual of the Plants of Colorado. Sage Books, Denver, CO.

Hartman, R.L. and B.E. Nelson. 2001. A Checklist of the Vascular Plants of Colorado. Rocky Mountain Herbarium, University of Wyoming. Unpublished.

Hauk, W.D. 1995. A molecular assessment of relationships among cryptic species of *Botrychium* subgenus *Botrychium* (Ophioglossaceae). American Fern Journal 85:375-394.

Hauk, W.D., C.R. Parks, and M.W. Chase. 2003. Phylogenetic studies of Ophioglossaceae: Evidence from rbcL and trnL-F plastid DNA sequences and morphology. Molecular Phylogenetics and Evolution 28:131-151.

Hauk, W.D. and C.H. Haufler. 1999. Isozyme variability among cryptic species of *Botrychium* subgenus *Botrychium* (Ophioglossaceae). American Journal of Botany 86:614-633.

Heidel, B. 2003. Personal communication with Botanist with Wyoming Natural Diversity Database regarding *Botrychium simplex*.

Hendrix, P.F. and P.J. Bohlen. 2002. Exotic earthworms invasions in North America: ecological and policy implications. BioScience 52:801-811.

Hitchcock, C.L., A. Cronquist, M. Ownbey, and J.W. Thompson. 1969. Vascular Plants of the Pacific Northwest. Part 1: Vascular Cryptogams, Gymnosperms, and Monocotyledons. University of Washington Press, Seattle, WA.

Hitchcock, E. 1823. *Botrychium simplex*. American Journal of Science and Arts 6:103.

Hoefferle, A.M. 1999. Impacts of aerial leaf removal on leaf size of the daisy leaf moonwort (*Botrychium matricariifolium*) and the triangle moonwort (*Botrychium lanceolatum* var. *angustisegmentum*) in the subsequent year. Master's Thesis. Michigan Technological University, Houghton, MI.

Hopkins, C.O., R.L. Wooley, K. Grenier, C. Levack, C. Close, S. Malaby, K. Cushman, and R. Halvorson. 2001. Conservation strategy for *Botrychium pumicola* (pumice grapefern) on the Deschutes, Fremont, and Winema National Forests, and Prineville, District, Bureau of Land Management, Oregon. Produced by the USDA Forest Service and Bureau of Land Management.

Huxley, A. 1972. Mountain Flowers in Color. The MacMillan Company, New York, NY.

International Association for Plant Taxonomy. 2001. International Code of Botanical Nomenclature (Saint Louis Code), Electronic Version. Accessed via the Internet at http://www.bgbm.fu-berlin.de/iapt/nomenclature/code/SaintLouis/.

International Union for Conservation of Nature and Natural Resources. 1978. The IUCN Plant Red Data Book. Compiled by G. Lucas and H. Synge for the Threatened Plants Committee of the Survival Service Commission of the International Union for Conservation of Nature and Natural Resources. The World Wildlife Fund, Morges, Switzerland.

Johnson-Groh, C.L. 1998. Population demographics, underground ecology and phenology of *Botrychium mormo*. Population and Habitat Viability Assessment Workshop for the Goblin Fern (*Botrychium mormo*). Final Report. CBSG, Apple Valley, MN.

Johnson-Groh, C.L. 1999. Population ecology of *Botrychium* (moonworts): Status report on Minnesota *Botrychium*. Permanent plot monitoring. Gustavus Adolphus College, St. Peter, MN.

Johnson-Groh, C.L. and D.R. Farrar. 1996a. Effects of leaf loss on moonwort fern, *Botrychium* subg. *Botrychium*. American Journal of Botany 83:127.

Johnson-Groh, C.L. and D.R. Farrar. 1996b. The effects of fire on prairie moonworts (*Botrychium* subg. *Botrychium*). American Journal of Botany 83:134.

Johnson-Groh, C.L. and D.R. Farrar. 2003. *Botrychium* inventory and monitoring technical guide. Unpublished report for the USDA Forest Service.

Johnson-Groh, C.L. and J.M. Lee. 2002. Phenology and demography of two species of *Botrychium* (Ophioglossaceae). Journal of Botany 89:1624-1633.

Johnson-Groh, C.L., D.R. Farrar, and P. Miller. 1998. Modeling extinction probabilities for moonwort (*Botrychium*) populations (Abstract). Botanical Society of America Annual Meeting.

Johnson-Groh, C.L., C. Reidel, L. Schoessler, and K. Skogen. 2002. Belowground distribution and abundance of *Botrychium* gametophytes and juvenile sporophytes. American Fern Journal 92:80-92.

Kartesz, J.T. 1999. A synonymized checklist and atlas with biological attributes for the vascular flora of the United States, Canada, and Greenland. First edition. *In*: J.T. Kartesz and C.A. Meacham. Synthesis of the North American Flora [computer program]. Version 1.0. North Carolina Botanical Garden, Chapel Hill, NC.

Kelly, D. 1994. Demography and conservation of *Botrychium australe*, a peculiar, sparse mycorrhizal fern. New Zealand Journal of Botany 32:393-400.

Kempema, L., L. Smart, and C.L. Johnson-Groh. 2003. Investigations of mycorrhizal symbiosis in the genus *Botrychium*. Sigma Xi Research Symposium, Gustavus Adolphus College, St. Peter, MN.

Köchy, M. and S.D. Wilson. 2001. Nitrogen deposition and forest expansion in the northern Great Plains. The Journal of Ecology 89:807-817.

Kolb, A. and T. Spribille. 2000. New populations and habitat characteristics of rare moonworts (*Botrychium* subg. *Botrychium*) in Summit County, Colorado. Unpublished report. USDA Forest Service, White River National Forest, Dillon Ranger District, Silverthorne, CO.37 pp.

Krebs, C.J. 1972. Ecology: The Experimental Analysis of Distribution and Abundance. Harper and Row, New York, NY.

Lackschewitz, K. 1991. Vascular Plants of West-Central Montana - Identification Guidebook. USDA Forest Service, Intermountain Research Station.

Lande, R. 1998. Anthropogenic, ecological and genetic factors in extinction and conservation. Researches on Population Ecology 40:259-269.

Langmaid, L.L. 1964. Some effects of earthworm invasion in virgin podzols. Canadian Journal of Soil Science 44: 34-37.

Lellinger, D.B. 1985. A field manual of the ferns and fern allies of the United States and Canada. Smithsonian Institution Press, Washington, D.C.

Lesica, P. and K. Ahlenslager. 1995. Demography and life history of three sympatric species of *Botrychium* subg. *Botrychium* in Waterton Lakes National Park, Alberta, Canada. Draft manuscript prepared in cooperation with Waterton National Park, U.S. Fish and Wildlife Service, and Montana Natural Heritage Program. 22 pp.

Lesica, P. and B.M. Steele. 1994. Prolonged dormancy in vascular plants and implications for monitoring studies. Natural Areas Journal 14:209-212.

Lewis, D. 2003. Personal communication with Curator of the Ada Hayden Herbarium regarding *Botrychium simplex*.

Lloyd, R.M. and E.J. Klekowski. 1970. Spore germination and viability in Pteridophyta: evolutionary significance of chlorophyllous spores. Biotropica 2:129-137.

Lorain, C.C. 1990. Field investigations of *Botrychium* subgenus *Botrychium* (Moonworts), on the Idaho Panhandle National Forests. Unpublished for the Idaho Panhandle National Forests.

MacArthur, R.H. and E.O. Wilson. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, NJ.

Manabe, S. and R.T. Wetherald. 1986. Reduction in summer soil wetness induced by an increase in atmospheric carbon dioxide. Science 232:626-628.

Mantas, M. and R.S. Wirt. 1995. Moonworts of western Montana (*Botrychium* subgenus *Botrychium*). Unpublished report prepared for the Flathead National Forest.

Marler, M.J., C.A. Zabinski, and R.M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology 80:1180-1186.

McCauley, D.E., D.P. Whittier, and L.M. Reilly. 1985. Inbreeding and the rate of self-fertilization in a grape fern, *Botrychium dissectum*. American Journal of Botany 72:1978-1981.

Melan, M.A. and D.P. Whittier. 1989. Characterization of mucilage on the proximal cells of young gametophytes of *Botrychium dissectum* (Ophioglossaceae). American Journal of Botany 76:1006-1014.

Menges, E.S. 1991. The application of minimum viable population theory to plants. Chapter 3 *in* D.A. Falk and K.E. Holsinger, editors. Genetics and Conservation of Rare Plants. Oxford University Press, New York, NY.

Menges, E.S. and S.C. Gawler. 1986. Four-year changes in population size of the endemic Furbish's lousewort: implications for endangerment and management. Natural Areas Journal 6:6-17.

Milberg, P. 1991. Fern spores in grassland soil. Canadian Journal of Botany 69:831-834.

Miller, J.H. 1968. Fern gametophytes as experimental material. Botanical Review 34:361-440.

Montgomery, J.D. 1990. Survivorship and predation changes in five populations of *Botrychium dissectum* in eastern Pennsylvania. American Fern Journal 80:173-182.

Muller, S. 1992. The impact of a drought in spring on the sporulation of *Botrychium matricariifolium* (Retz) A. Br. in the Bitcherland (Northern Vosges, France). Acta Ecologia 13:335-343.

Muller, S. 1999. Plant communities and conservation of *Botrychium*-rich grasslands in the Bitcherland. Biodiversity and Conservation 8:1519-1532.

Muller, S. 1993. Population dynamics in *Botrychium matricariifolium* in Bitcherland (northern Vosges Mountains, France). Belgian Journal of Botany 126:13-19.

Munz, P.A. and D.D. Keck. 1968. A California Flora with Supplement. University of California Press, Berkeley, CA.

NatureServe. 2005. NatureServe Explorer: An Online Encyclopedia of Life [web application]. Version 4.5. NatureServe, Arlington, VA. Available http://www.natureserve.org/explorer.

Nielsen, G.A. and F.D. Hole. 1963. Earthworms and the development of coprogenous A1 horizons in forest soils of Wisconsin. Soil Science Society of America Proceedings 28:426-430.

Nilsson, O. 1981. Project Linnaeus: Assessing Swedish plants threatened with extinction. *In*: H. Synge, editor. The Biological Aspects of Rare Plant Conservation. John Wiley and Sons, New York, NY.

Ode, D. 2001. Region 2 Sensitive Species Evaluation Form for *Botrychium simplex*. USDA Forest Service, Region 2, Lakewood, CO.

Ohio Department of Natural Resources. 2003. Species abstract for *Botrychium simplex* E. Hitchc. least grape fern. Accessed via the Internet at http://www.dnr.state.oh.us/dnap/.

Ollgaard, B. 1971. *Botrychium multifidum* and *B. simplex* in Greenland. Botanisk Tidsskrift 66:357.

Oostermeijer, J.G.B., S.H. Luijten, and J.C.M. den Nijs. 2003. Integrating demographic and genetic approaches in plant conservation. Biological Conservation 113:389-398.

Paris, C.A., F.S. Wagner, and W.H. Wagner. 1989. Cryptic species, species delimitation, and taxonomic practice in the homosporous ferns. American Fern Journal 79:46-54.

Peck, J.H., C.J. Peck, and D.R. Farrar. 1990. Influences of life history attributes on formation of local and distant fern populations. American Fern Journal 80:126-142.

Peck, M.E. 1961. A Manual of the Higher Plants of Oregon. Binfords and Mort Publishers, in cooperation with the Oregon State University Press and the National Science Foundation.

Peet, R.K. 2000. Forests and meadows of the Rocky Mountains. Pages 75-122 *in* M.G. Barbour and W.D. Billings, editors. North American Terrestrial Vegetation. Cambridge University Press, New York, NY.

Pickett, S.T.A. and J.N. Thompson. 1978. Patch dynamics and design of nature reserves. Biological Conservation 13: 27-37.

Platt, J.R. 1964. Strong inference. Science 146:347-353.

Polunin, N. 1959. Circumpolar Arctic Flora. Oxford University Press, London, England.

Popovich, S.J. 2003. Personal communication with USFS Botanist regarding *Botrychium* survey protocols.

Popovich, S.J. 2005. Personal communication with USFS Botanist regarding *Botrychium* transplantings on Guanella Pass, Colorado.

Popovich, S.J. 2006. Personal communication with USFS Botanist regarding *Botrychium simplex*.

Proctor, J. 2006. Personal communication with USFS Botanist regarding *Botrychium simplex*.

Read, D.J. 1998. Biodiversity: plants on the web. Nature 396:22-23.

Read, D.J., J.G. Duckett, R. Francis, R. Ligrone, and A. Russell. 2000. Symbiotic fungal associations in “lower” land plants. Philosophical Transactions of the Royal Society of London B 355:815-831.

Roche, K. 2004. Letter of Instruction for *Botrychium simplex* (little grapefern): A Technical Conservation Assessment.

Rolfsmeier, S. 2003. Personal communication with Curator of the High Plains Herbarium regarding *Botrychium simplex*.

Rook, E.J.S. 2002. *Botrychium simplex* - Small grape fern. Accessed via the Internet at http://www.rook.org/earl/bwca/nature/ferns/botrysim.html.

Root, P. 2003. Personal communications regarding *Botrychium* species and *Botrychium* Training Workshop Notes (July 10, 2003).

Root, P. 2003. Guide to Colorado Moonworts. Unpublished guide distributed at a workshop hosted by the USDA Forest Service.

Rydberg, P.A. 1906. Flora of Colorado. Agricultural Experiment Station, Ft. Collins, CO.

Rydberg, P.A. 1922. Flora of the Rocky Mountains and Adjacent Plains. Hafner Publishing Co., New York, NY.

Scagel, R.F., R.J. Bandoni, G.L. Rouse, W.B. Schofield, J.R. Stein, and T.M. Taylor. 1966. An Evolutionary Survey of the Plant Kingdom. Wadsworth Publishing Co., Belmont, CA.

Schmid, E. and F. Oberwinkler. 1994. Light and electron microscopy of the host-fungus interaction in the achlorophyllous gametophyte of *Botrychium lunaria*. Canadian Journal of Botany 72:182-188.

Schwab, E. 1992. *Botrychium simplex*. Unpublished Species Abstract (draft). Colorado Natural Heritage Program Element Manual Files.

Schwartz, M.W. and C.A. Brigham. 2003. Why plant population viability assessment? Chapter 1 *in* C.A. Brigham and M.W. Schwartz, editors. Population Viability in Plants. Springer-Verlag, Berlin, Germany.

Scott, M.J., P.J. Heglund, M.L. Morrison, J.B. Haufler, M.G. Raphael, W.A. Wall, and F.B. Samson. 2002. Predicting Species Occurrences- Issues of Accuracy and Scale. Island Press, Washington, D.C.

Smith, S.E. and D.J. Read. 1997. Mycorrhizal symbiosis. Second edition. Academic Press, Inc., San Diego, CA.

Soltis D.E. and P.S. Soltis. 1986. Electrophoretic evidence for inbreeding in the fern *Botrychium virginianum* (Ophioglossaceae). American Journal of Botany 73:588-592.

Soulé, M.E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pages 151-169 *in* M.E. Soulé and B.A. Wilcox, editors. Conservation Biology: an Evolutionary Perspective. Sinauer Associates, Sunderland, MA.

South Dakota Natural Heritage Program. 2003. Element occurrence records for *Botrychium simplex*.

Steinmann, D. 2001a. Moonwort survey for the Pikes Peak Highway Recreation Corridor. Unpublished report prepared for the City of Colorado Springs, CO.

Steinmann, D. 2001b. Moonworts in the Indian Peaks. Aquilegia 25:3.

Steinmann, D. 2003. Personal communication with *Botrychium* expert regarding *B. simplex*.

Stevenson, D.W. 1975. Taxonomic and morphological observations on *Botrychium multifidum* (Ophioglossaceae). Madrono 23:198-204.

Swartz, L.M. and S.J. Brunsfield. 2002. The morphological and genetic distinctness of *Botrychium minganense* and *B. crenulatum* as assessed by morphometric analysis and RAPD markers. American Fern Journal 92:249-269.

The Plant Names Project. 1999. International Plant Names Index. Published on the Internet: http://www.ipni.org [accessed 5 October 2003].

Thomas, R.D. and D.P. Whittier. 1993. Gameotophytes and young sporophytes of *Botrychium jenmanii* in Axenic culture. International Journal of Plant Science 154:68-74.

Thompson, R.W. 2000. Moonwort supplement to the Breckenridge Ski Area Peak 7 Upgrading Biological Assessment-Biological Evaluation for the proposed Peak 7 Road, Summit County, CO. Unpublished report produced for the USDA Forest Service. Western Ecosystems, Inc. Boulder, CO.

Thompson, R.W. 2001. Technical ecology report for Copper Mountain Resort's Trails and Facilities Improvement Plan, Summit County, Colorado. Western Ecosystems, Inc. Approved by J. Doerr, White River National Forest.

Thuiller, W., M.B. Araujo, and S. Lavorel. 2003. Generalized models vs. classification tree analysis: Predicting spatial distributions of plant species at different scales. Journal of Vegetation Science 14:669-680.

University of Colorado Herbarium. 2003. Online database accessed via the Internet at http://cumuseum.colorado.edu/Research/Botany/.

USDA Forest Service. 2003. Forest Service Manual Rocky Mountain Region. Chapter 2670. Threatened, Endangered, and Sensitive Plants and Animals. USDA Forest Service Region 2, Lakewood, CO.

USDA Natural Resources Conservation Service. 2002. The PLANTS Database, Version 3.5 (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA.

U.S. Environmental Protection Agency. 1997. Climate change and colorado. EPA 230-F-97-008f. Office of Policy, Planning, and Evaluation, Climate and Policy Assessment Division, Washington, D.C.

U.S. Fish and Wildlife Service. 1988. National list of vascular plant species that occur in wetlands. U.S. Fish and Wildlife Service Biological Report 88 (24).

Van Der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. Nature 396:69-72.

Vandenkoornhuyse, P., S.L. Baldauf, C. Leyval, J. Straczek, and J.P.W. Young. 2002. Extensive fungal diversity in plant roots. Science 295:2051-2052.

Vanderhorst, J. 1997. Conservation assessment of sensitive moonworts (Ophioglossaceae, *Botrychium* subgenus *Botrychium*) on the Kootenai National Forest. Montana Natural Heritage Program. 88 pp.

Veblen, T. 2003. Presentation on fire ecology presented at the Colorado Native Plant Society Annual Meeting.

Wagner, F.S. 1993. Chromosomes of North American grapeferns and moonworts (Ophioglossaceae: *Botrychium*). Contributions to the University of Michigan Herbarium 19:83-92.

Wagner, F.S. 2002. Personal communication with *Botrychium* expert regarding *Botrychium* spores and dispersal.

Wagner, W.H. 1946. *Botrychium multifidum* in Virginia. American Fern Journal 36:117-121.

Wagner, W.H. 1980. A probable new hybrid grapefern, *Botrychium matricariifolium* x *simplex*, from central Michigan. Michigan Botanist 19:31-36.

Wagner, W.H. 1991. New examples of the moonwort hybrid, *Botrychium matricariifolium* x *simplex* (Ophioglossaceae). Canadian Field Naturalist 105:91-94.

Wagner, W.H. 1998. A background for the study of moonworts. Population and Habitat Viability Assessment Workshop for the Goblin Fern (*Botrychium mormo*): Final Report. CBSG, Apple Valley, MN.

Wagner, W.H. and T.B. Devine. 1989. Moonworts (*Botrychium*: Ophioglossaceae) in the Jonesville Area, Butte and Tehama Counties, California. Madrono 36(2):131-136.

Wagner, W.H. and J.R. Grant. 2002. *Botrychium alaskense*, a new moonwort from the interior of Alaska. American Fern Journal 92:164-170.

Wagner, W.H. and F.S. Wagner. 1976. How to find the rare grapeferns and moonworts. Fiddlehead Forum 3:2-3.

Wagner, W.H. and F.S. Wagner. 1981. New species of moonworts, *Botrychium* subg. *Botrychium* (Ophioglossaceae), from North America. American Fern Journal 7:20-30.

Wagner, W.H. and F.S. Wagner. 1983. Genus communities as a systematic tool in the study of new world *Botrychium* (Ophioglossaceae). Taxon 32:51-63.

Wagner, W.H. and F.S. Wagner. 1986. Three new species of moonworts (*Botrychium* subgenus *Botrychium*) endemic in western North America. American Fern Journal 76:33-47.

Wagner, W.H. and F.S. Wagner. 1988. Detecting *Botrychium* hybrids in the Lake Superior region. Annals of the Michigan Botanical Garden 27:75-80.

Wagner, W.H. and F.S. Wagner. 1993. Ophioglossaceae C. Agardh. Oxford University Press, New York, NY.

Wagner, W.H., F.S. Wagner, and J. Beitel. 1985. Evidence for interspecific hybridization in pteridophytes with subterranean mycoparasitic gametophytes. Proceedings of the Royal Society of Edinburgh 86B:273-278.

Wagner, W.H., F.S. Wagner, C. Haufler, and J.K. Emerson. 1984. A new nothospecies of moonwort (Ophioglossaceae, *Botrychium*). Canadian Journal of Botany 62:629-634.

Warren, N. 2003. R2 Individual species recommendations for *Botrychium simplex*. USDA Forest Service, Region 2, Lakewood, CO.

Watano, Y. and N. Sahashi. 1992. Predominant inbreeding and its genetic consequences in a homosporous fern Genus, *Sceptridium* (Ophioglossaceae). Systematic Botany 17:486-502.

Weber, W.A. and R.C. Wittmann. 2001a. Colorado Flora: Eastern Slope. Third edition. University Press of Colorado, Boulder, CO.

Weber, W.A. and R.C. Wittmann. 2001b. Colorado Flora: Western Slope. Third edition. University Press of Colorado, Boulder, CO.

Welsh, S.L., N.D. Atwood, S. Goodrich, and L.C. Higgins. 1993. A Utah Flora. Second edition. Brigham Young University Print Services. Provo, UT.

Wherry, E.T. 1937. Botrychiaceae *Botrychium simplex* var. *cordatum*. American Fern Journal 27:58.

Wherry, E.T. 1938. Colorado ferns. American Fern Journal 28:125-141.

Whipple, J. 2003. Personal communication with Yellowstone National Park botanist regarding *Botrychium simplex* in Yellowstone National Park.

Whittier, D.P. 1972. Gametophytes of *Botrychium* as grown in sterile culture. Botanical Gazette 133:336-339.

Whittier, D.P. 1973. Effect of light and other factors on spore germination in *Botrychium dissectum.* Canadian Journal of Botany 51:1791-1794.

Whittier, D.P. 1981. Spore germination and young gametophyte development of *Botrychium* and *Ophioglossum* in axenic culture. American Fern Journal 71:13-19.

Whittier, D.P. 1984. The organic nutrition of *Botrychium* gametophytes. American Fern Journal 74:77-86.

Whittier, D.P. 1996. Delayed gametophyte growth in *Botrychium*. American Journal of Botany 83:133.

Wilson, H.C. 1969. Ecology and successional patterns of wet meadows. Rocky Mountain National Park, Colorado. Ph.D. Dissertation. University of Utah, Salt Lake City, UT.

Windham, M.D., P.G. Wolf, and T.A. Ranker. 1986. Factors affecting prolonged spore viability in herbarium collections of three species of *Pellaea*. American Fern Journal 76:141-148.

Winther, J. 2002. Personal communication with University of Colorado graduate student regarding *Botrychium* gametophytes.

Wisconsin State Herbarium. 2003. Wisconsin Vascular Plant Species. Accessed via the Internet at www.botany.wisc.edu/herbarium/.

Wyoming Natural Diversity Database. 2003. Element occurrence records for *Botrychium simplex* in Wyoming.

Zika, P.R., R. Brainerd, and B. Newhouse. 1995. Grapeferns and moonworts (*Botrychium*, Ophioglossaceae) in the Columbia Basin. USDA Forest Service, Walla Walla, WA.