

**Conservation Assessment**  
**for**  
**13 Species**  
**of**  
**Moonworts**  
**(*Botrychium* Swartz Subgenus *Botrychium*)**

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USDI Bureau of Land Management, Oregon and Washington

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## **Disclaimer**

This Conservation Assessment was prepared to compile information on taxa within *Botrychium* Swartz subgenus *Botrychium*. It does not represent a management decision by the USDA Forest Service (Region 6) or USDI Bureau of Land Management (Oregon/Washington BLM). Although the best scientific information available was used and subject experts were consulted in preparation of this document, it is expected that new information will arise. In the spirit of continuous learning and adaptive management, if you have information that will assist in conserving *Botrychium* taxa, please contact the interagency Special Status Species Conservation Planning Coordinator in the Portland, Oregon Forest Service Region 6 and Oregon/Washington (OR/WA) BLM offices or at: <http://www.fs.fed.us/r6/sfpnw/issssp/contactus/>.

## **Executive Summary**

### **Taxonomic Group and Species**

#### Vascular Plants

*Botrychium ascendens* W.H. Wager, Upward Lobed moonwort

*Botrychium campestre* W.H. Wagner and Farrar, Prairie moonwort

*Botrychium crenulatum* W. H. Wagner, Crenulate moonwort

*Botrychium hesperium* (Maxon & Clausen) W. H. Wagner & Lellinger, Western moonwort

*Botrychium lanceolatum* (S. G. Gmelin) Angstrom subsp. *lanceolatum*, Lanceleaf moonwort

*Botrychium lineare* W. H. Wagner, Slender moonwort

*Botrychium lunaria* (L.) Swartz, Common moonwort

*Botrychium minganense* Victorin, Mingan moonwort

*Botrychium montanum* W. H. Wagner, Mountain moonwort

*Botrychium paradoxum* W. H. Wagner, Peculiar moonwort

*Botrychium pedunculatum* W. H. Wagner, Stalked moonwort

*Botrychium pinnatum* H. St. John, Northern moonwort

*Botrychium pumicola* Colville, Pumice moonwort

### **Management Status**

Of these 13 species, the rarest one in Oregon and Washington is *Botrychium campestre*, which is known from a single plant in Oregon. Farrar (pers. com 2007) confirmed its identity. The next rarest is *Botrychium lineare*, a Candidate for federal listing under the federal Endangered Species Act (US Fish and Wildlife Service [US FWS] 2001, 2005a). Five species are U.S. Fish and Wildlife Species of Concern (*B. ascendens*, *B. crenulatum*, *B. paradoxum*, *B. pedunculatum*, and *B. pumicola*). *Botrychium minganense* and *B. montanum* are Survey and Manage species under the Northwest Forest Plan Survey and Manage Standards and Guidelines (USDA Forest Service [FS], USDI Bureau of Land Management [BLM] 2001).

Within the National Forest System, the 13 species in this assessment are included on the Region 6 Regional Forester's Sensitive Species List (USDA FS 2004). Although *B. fenestratum* is also included on the list, this undescribed entity is now recognized as *B. hesperium* and is addressed as such in this assessment. (*B. hesperium* is Region 6 Sensitive in Washington only, with *B. fenestratum* Region 6 Sensitive in Oregon only). Six of the 13 species are Region 6 sensitive species in Oregon only (*B. lanceolatum*, *B. lunaria*, *B.*

*minganense*, *B. montanum*, *B. pinnatum*, and *B. pumicola*). The remaining 6 species (*B. ascendens*, *B. campestre*, *B. crenulatum*, *B. lineare*, *B. paradoxum*, and *B. pedunculatum*) are sensitive in both Oregon and Washington.

The OR/WA BLM (USDI BLM 2005) State Director's Special Status Species List also includes these 13 species of *Botrychium*. The OR/WA BLM identifies *B. pumicola* as a Special Status Species in Oregon due to its rank as State Threatened. In addition, *B. lineare* is a Special Status Species in both Oregon and Washington due to its federal Candidate status.

Four species are Bureau Sensitive in Oregon and Bureau Assessment in Washington (*B. ascendens*, *B. crenulatum*, *B. paradoxum*, and *B. pedunculatum*). Two species are considered Bureau Assessment in both Oregon and Washington (*B. campestre*, *B. lunaria*). *Botrychium montanum* is Bureau Assessment in Oregon, but Bureau Tracking in Washington, while *B. hesperium* is Bureau Assessment in Washington but Tracking in Oregon. One species is Bureau Tracking for both states (*B. minganense*), while two species are considered Bureau Tracking in Oregon only (*B. lanceolatum* and *B. pinnatum*). Bureau Tracking Species are not considered Special Status Species for management purposes by the BLM.

Although *Botrychium lunaria* and *B. simplex* are on the OR/WA BLM (USDI BLM 2005) State Director's Special Status Species List as Bureau Assessment in Washington, they are not included on the August 2006 "List of Tracked Species" maintained by the Washington Natural Heritage Program. This indicates that these species are not of concern in Washington; however the BLM list has not been updated to reflect this. Due to this new information and ranking by the Heritage Program, *B. lunaria* and *B. simplex* were not addressed as Washington BLM Assessment species in this assessment.

### **Range & Habitat**

Ten of the 13 species are only known from North America. Of the three occurring outside North America, *Botrychium lunaria* is documented from South America, Eurasia, New Zealand and Australia; *B. lanceolatum* is found in Eurasia; and *B. minganense* is reported from Iceland.

In Oregon and Washington the geographic range of each of the 13 moonwort species varies over a total of 18 Oregon counties and 10 Washington counties. Four OR/WA BLM Districts and 14 Region 6 National Forests have at least one of these species. Habitats for these 13 range from undisturbed closed canopy western red-cedar forests and pumice landscapes to open formerly cultivated homestead meadows, plantations and roadsides.

*Botrychium campestre* and *B. pumicola* are not documented from Washington (Washington Natural Heritage Program [WNHP] 2006). Five of the species (*B. lanceolatum*, *B. lunaria*, *B. minganense*, *B. montanum*, and *B. pinnatum*) are not considered rare by the Washington Natural Heritage Program and are not FS sensitive species in Washington (USDA FS 2004).

The relative abundance of the six species considered rare in both Oregon and Washington varies widely (Oregon Natural Heritage Information Center [ORNHIC] 2002 and WNHP 2002). While *Botrychium lineare* is documented from three occurrences, all with less than

50 stems, *Botrychium crenulatum* is known from 145, some with hundreds of stems. *Botrychium hesperium*, *B. paradoxum*, *B. pedunculosum*, and *B. ascendens* are known from 15 to 30 occurrences with stems counts ranging from ten to several hundred. The number of stems per occurrence for each of the seven additional species tracked in Oregon ranges from less than ten to several hundred. *Botrychium campestre* is known from one plant. *Botrychium lunaria* is documented from less than 16 sites. *Botrychium montanum*, *B. lanceolatum*, and *B. pinnatum* are documented from less than 80 sites, while *B. minganense* and *B. pumicola* from 100-200 sites.

With the exception of *Botrychium pumicola* and *B. montanum*, there is an apparent association with older (10 to 30 years) disturbances. This includes abandoned roadbeds, roadsides and ditches, pastures, and meadows. Management activities, including grazing, that maintain these conditions maintain moonwort populations. With succession to dense, closed canopy conditions moonwort populations decline. There is also a positive correlation with calcareous soils. With a few exceptions a high (80%) predictability is gained by thinking of moonworts as species which follow disturbance on moist, but well-drained calcareous soil. At this point there is no clear correlation with habitat or environmental change, and population size or vigor. The population trends of these species are unknown in Oregon and Washington. It is suspected that changes affecting mycorrhizal fungi may affect moonworts.

Although 98% of the occurrences of these 13 species of moonworts in Oregon and Washington are on federal lands, this is probably a function of where surveys are conducted, and does not likely represent the actual distribution of these species. Occurrences on non-federal lands are largely unknown.

### **Threats**

Identification of threats is somewhat challenging for moonworts, since so much information is still needed on habitat requirements, environmental tolerances and the effects of management. For the purpose of this assessment, threats to moonworts in Oregon and Washington (ORNHIC 2002 and WNHP 2002) are actions that alter existing site characteristics, including actions that would change the microclimate, canopy coverage, hydrology, or mycorrhizal association on a site from the regime that has supported a given population over the past decade. Information on known occurrences indicate that off-road vehicle damage, camping and hiking; timber harvest and firewood cutting; exotic plants and herbicides; succession to closed canopy (fire suppression); and road widening and maintenance are threats. Livestock grazing may also be considered a threat to sites of these species, but the issue is complex. Farrar states that meadow populations of *Botrychiums* are maintained by current levels of grazing and that removal of grazing may be detrimental, especially if succession to woody vegetation occurs (Farrar 2006). The major threat from logging and other vehicular activities is the actual physical disturbance of the soil that breaks root and mycorrhizae connections or otherwise uproots the moonwort plants.

### **Management Considerations**

Even with our best efforts to conserve them, some, or even most, existing populations of moonworts may become extinct, as this is the nature of species dependent upon disturbance

and early seral stages of community succession. *Botrychium* species may always have existed in metapopulation dynamics where population extinction is balanced with founding of new populations. Management approaches for these species should include maintenance of suitable, but unoccupied habitat that will be available for colonization by spores and the development of new populations. It is also important to consider maintaining existing populations, as they are the source of spores that will create new populations.

Little is known about the maintenance and manipulation of moonwort populations. Even when statistically rigorous long-term monitoring is implemented, population trends for *Botrychium* are very difficult to interpret in any way that is meaningful for the agency land manager at the field level. These species require some degree of active management to maintain individual sites/populations. The overarching, likely most important management consideration for site/population management is to continue the level and type of disturbance that has supported the site/population over the last decade (Farrar 2006). For all but *Botrychium pumicola* and *B. montanum*, this includes maintaining and encouraging a 10-30 year disturbance cycle. Additional considerations may include:

- Maintaining light regime, hydrology (hydrologic flow and water table level), and habitat and microclimatic conditions, including existing canopy closures.
- Maintaining conditions which sustain mycorrhizal diversity.
- Avoiding disturbance of above ground plants and the substrate in the area, including the duff layer and the collection of special forest products (e.g. moss), to minimize impacts to below ground plants.
- Avoiding actions that would contribute towards establishment of competing exotic vegetation.
- Avoiding excessive siltation or deposition of soil.
- Providing early to mid-stages of plant succession.

### **Research, Inventory, and Monitoring Opportunities**

The following are information gaps for the species in this assessment:

- Population trends.
- Fungal associates, their habitat requirements, and the role they play in the life history of each of these 13 species.
- Effective management areas (sizes) and habitat characteristics necessary to maintain known occurrences in project areas
- Short-term and long-term effects of timber harvest, grazing, recreation, fire, fire suppression, and exotic plants on the maintenance of known occurrences.
- Identification of high likelihood habitat, to help prioritize surveys and ensure appropriate habitat conservation.
- Actual distribution and range of each of the 13 species.

Actions to consider to fill the information gaps:

- Develop and implement Inventory and Monitoring Protocols; establish priorities and inventory high likelihood habitats.

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## **I. Introduction**

### **A. Goal**

Management for these species follows U. S. Department of Agriculture Forest Service (FS) policy for sensitive species (SS) (FSM 2670), Species of Concern (SOC) and Species of Interest (SOI) (FSM 1921.76), and U. S. Department of the Interior Bureau of Land Management (BLM) Oregon and Washington Special Status Species (SSS) policy (BLM 6840) (USDI BLM 2005a).

For Oregon and Washington BLM administered lands, SSS policy details the need to manage for species conservation. For Region 6 of the FS, policy requires the agency to maintain viable populations of all native and desired non-native wildlife, fish, and plant species in habitats distributed throughout their geographic range on National Forest System lands and provide appropriate ecological conditions to help avoid the need to list SOC and SOI under the Endangered Species Act. Management of sensitive species “must not result in a loss of species viability or create significant trends toward federal listing” (FSM 2670.32).

This conservation assessment summarizes existing knowledge regarding the biology and ecology of thirteen species of moonworts, threats to these species, and management considerations to provide information to line managers to assist in the formulation of options for management activities. These species are of concern primarily because of the relatively low number of documented occurrences and plants per occurrence, as displayed in Table 1. Of the 743 occurrences in 2002, 52% had less than 10 plants per occurrence and 82% less than 50 plants per occurrence (Oregon Natural Heritage Information Center [ORNHIC] 2002, Washington Natural Heritage Program [WNHP] 2002).

### **B. Scope**

The geographic scope of this assessment includes lands within Region 6 of the FS and lands administered by the BLM in Oregon and Washington (*hereafter referred to as “the analysis area”*). For the most part knowledge of these species is from federal lands, although knowledge from non-federal lands is included in this Conservation Assessment, if the information can help provide for federal management and conservation of the species. This assessment summarizes existing knowledge of these relatively little known vascular plants.

A great deal of new information regarding these species has been generated in the last few years, especially with respect to distribution, habitat, and genetic structure. Information updates may be necessary to keep this assessment current with time. Threats named here summarize known or suspected existing threats, which also may change with time. Management considerations apply to localities, specifically; however some larger scale issues such as range-wide concerns are listed.

Table 1. Summary of the number of occurrences by range of individuals in an occurrence of rare *Botrychium* species in Oregon and Washington (WHNP 2002, ORNHIC 2002). Species are arranged in order of the least number of occurrences to most. Shading indicates that the species is not considered SS or SSS in Washington, so the tally for these represents Oregon populations only.

Species Name	# of occurrences with <10 plants	# of occurrences with 11-50 plants	# of occurrences with 51-100 plants	# of occurrences with >100 plants	Largest # of plants in a single occurrence	Total # of occurrences
<i>Botrychium campestre</i> *	1	0	0	0	1	1
<i>Botrychium lineare</i>	2	1	0	0	14	3
<i>Botrychium lunaria</i> **	11	3	1	1	300	16
<i>Botrychium hesperium</i>	6	6	3	4	464	19
<i>Botrychium paradoxum</i>	17	2	0	0	142	19
<i>Botrychium pedunculosum</i>	12	7	1	1	1918	21
<i>Botrychium ascendens</i>	16	5	3	2	213	26
<i>Botrychium montanum</i>	32	18	1	3	900	54
<i>Botrychium lanceolatum</i>	28	16	2	10	800	56
<i>Botrychium pinnatum</i>	41	23	1	15	1473	80
<i>Botrychium minganense</i>	63	38	5	7	166	113
<i>Botrychium crenulatum</i>	67	47	17	14	415	145
<i>Botrychium pumicola</i>	89	51	13	37	1700	190
<b>Total Occurrences</b>	384	227	48	94		743

\*Although this species is listed by both the BLM and FS as SSS in Washington, there are no sites in Washington; the sole location of this species is within Oregon.

\*\*Although this species is currently listed as Bureau Assessment in Washington by the BLM, new information and rankings by the Washington Natural Heritage Program indicate that this species is not of concern in Washington. When updated, the BLM SSS list will remove this species. Totals for this species reflect Oregon populations only.

### C. Management Status

Table 2 displays the conservation status of the 13 species in Oregon and Washington for the USDA FS (2004), USDI BLM (2005), USDI FWS (2005), NatureServe (2005), Oregon Natural Heritage Information Center (2005), and Washington Natural Heritage Program (2006). Of these 13 species, the rarest is *Botrychium campestre*, known from a single plant in Oregon (Zika and Alverson 1996). Farrar (pers. com. 2007) confirmed its identity. *Botrychium lineare*, a Candidate for federal listing as Threatened under the federal Endangered Species Act (FWS 2001, 2005a) is the next rarest. Five species are U.S. Fish and Wildlife Species of Concern (*B. ascendens*, *B. crenulatum*, *B. paradoxum*, *B. pedunculosum*, and *B. pumicola*). *Botrychium*

*minganense* and *B. montanum* are Survey and Manage species under the Northwest Forest Plan (USDA FS, USDI BLM 2001, as amended).

Also occurring in the analysis area is *Botrychium michiganense* (Gilman et al in press), a new species to science known from the Colville National Forest in Washington. Since its conservation status has not been evaluated, it is not further evaluated in this document.

Table 2. Conservation and management status of *Botrychium ascendens*, *B. campestre*, *B. crenulatum*, *B. hesperium*, *B. lanceolatum*, *B. lineare*, *B. lunaria*, *B. minganense*, *B. montanum*, *B. paradoxum*, *B. pedunculosum*, *B. pinnatum*, and *B. pumicola*, as ranked by the U.S. Forest Service (2002, 2004), U.S. Bureau of Land Management (2003 and 2005b), U.S. Fish and Wildlife Service (1993, 1996, and 2005), NatureServe (2005), Oregon Natural Heritage Information Center (2004 and 2005), and Washington Natural Heritage Program (2006).

Taxa	U.S. Forest Service Sensitive Species List for OR & WA <sup>1</sup>	U.S. BLM Special Status Species List for OR and WA <sup>2</sup>	U.S. Fish and Wildlife Service <sup>3</sup>	NatureServe Global Rankings <sup>4</sup>	Oregon State Rank/List <sup>4</sup>	Washington Rank/Status <sup>4, 5</sup>
<i>Botrychium ascendens</i>	Sensitive in OR & WA	Bureau Sensitive in OR & Bureau Assessment in WA	Species of Concern	Imperiled -- Vulnerable to Extirpation or Extinction (G2G3)	Imperiled (S2)/List 1	Imperiled to Vulnerable (S2S3) Sensitive
<i>Botrychium campestre</i>	Sensitive in OR & WA	Bureau Assessment in OR & WA		Vulnerable to Extirpation or Extinction -- Apparently Secure (G3G4)	Critically Imperiled (S1)/List 2	
<i>Botrychium crenulatum</i>	Sensitive in OR & WA	Bureau Sensitive in OR & Bureau Assessment in WA	Species of Concern	Vulnerable to Extirpation or Extinction (G3)	Imperiled (S2)/List 1	Vulnerable (S3)/Sensitive
<i>Botrychium hesperium</i>	Sensitive in WA ( <i>B. fenestratum</i> is Sensitive in OR)	Bureau Tracking in OR & Bureau Assessment in WA		Vulnerable to Extirpation or Extinction -- Apparently Secure (G3G4)	SNR List 3	Critically Imperiled (S1)/Threatened
<i>Botrychium lanceolatum</i>	Sensitive in OR	Bureau Tracking in OR		Demonstrably Widespread, Abundant, and Secure (G5)	Vulnerable (S3) List4	
<i>Botrychium lineare</i>	Sensitive in OR & WA	Federal Candidate: Special Status Species	Candidate	Critically Imperiled (G1)	Critically Imperiled (S1)/List 1	Critically Imperiled (S1)/Threatened
<i>Botrychium lunaria</i>	Sensitive in OR	Bureau Assessment in OR & WA*		Demonstrably Widespread, Abundant, and Secure (G5)	Imperiled (S2)/List 2	
<i>Botrychium minganense</i>	Sensitive in OR	Bureau Tracking in OR & WA		Apparently Secure (G4)	Vulnerable (S3)/List 4	
<i>Botrychium montanum</i>	Sensitive in OR	Bureau Assessment n Oregon & Bureau Tracking in WA		Vulnerable to extirpation or extinction (G3)	Imperiled (S2)/List 2	
<i>Botrychium paradoxum</i>	Sensitive in OR & WA	Bureau Sensitive in OR & Bureau Assessment in WA	Species of Concern	Imperiled (G2)	Critically Imperiled (S1)/List 1	Imperiled (S2)/Threatened
<i>Botrychium pedunculosum</i>	Sensitive in OR & WA	Bureau Sensitive in OR & Bureau Assessment in WA	Species of Concern	Imperiled to Vulnerable to Extirpation or Extinction (G2G3)	Critically Imperiled (S1)/List 1	Imperiled to Vulnerable (S2S3)/Sensitive
<i>Botrychium pinnatum</i>	Sensitive in OR	Bureau Tracking in OR		Apparently Secure (G4)?	Vulnerable (S3)/List 4	
<i>Botrychium pumicola</i>	Sensitive in OR	State Threatened in OR: Special Status Species	Species of Concern	Vulnerable to Extirpation or Extinction (G3)	Vulnerable (S3)/List 1	

<sup>1</sup>Designated by a USFS Regional Forester; a Sensitive Species is one in which population viability is a concern, as evidenced by significant current or predicted downward trends in population numbers or density, or significant current or predicted downward trends in habitat capability that would reduce a species' existing distribution.

<sup>2</sup>Designated by the Oregon and Washington BLM director. an Assessment Species is one not included as federally Threatened, Endangered Proposed, or Candidate; State Listed or BLM Bureau Sensitive and on List 2 of the Oregon Natural Heritage Database, or on the Sensitive Species List of the Washington Natural Heritage Program. Bureau sensitive species are those taxa which are eligible for federal listed, federal candidate, state listed or state candidate status.

<sup>3</sup>Designated by the U.S. Fish and Wildlife Service (FWS), a Candidate Species is any species for which the FWS has on file sufficient information on biological vulnerability and threats to support a proposal to list as endangered or threatened. Species of Concern is an informal federal term that refers to those species that might be in need of conservation actions.

<sup>4</sup>Key to rankings: G = Global rank based on range wide status, S = State rank based on status of a species in an individual state.

- G1 Critically imperiled globally because of extreme rarity (five or fewer occurrences or very few remaining individuals) or because of some factor making it especially vulnerable to extinction.
- G2 Imperiled globally because of rarity (six to 20 occurrences) or because of factors demonstrably making a species vulnerable to extinction.
- G3 Vulnerable to extirpation or extinction throughout its range or found locally in a restricted range (21 to 100 occurrences).
- G4 Apparently secure, though it may be quite rare in parts of its range, especially at the periphery.
- G5 Demonstrably secure, though it may be quite rare in parts of its range, especially at the periphery.
- S1 Critically imperiled in the state because of extreme rarity (five or fewer occurrences or very few remaining individuals) or because of some factor making it especially vulnerable to extinction.
- S2 Imperiled in the state because of rarity (six to 20 occurrences) or because of factors demonstrably making a species vulnerable to extinction.
- S3 Vulnerable throughout its statewide range or found locally in restricted statewide range (21 to 100 occurrences) or because of other factors making it vulnerable to extinction.
- S4 Apparently secure though it may be quite rare in parts of its statewide range, especially at the periphery (usually with more than 100 occurrences).
- S5 Demonstrably secure, through it may be quite rare in parts of its range, especially at the periphery.
- List 1 Taxa which are endangered or threatened throughout their range or which are presumed extinct.
- List 2 Taxa which are threatened, endangered or possibly extirpated from Oregon, but are stable or more common elsewhere.
- List 3 Taxa for which more information is needed before status can be determined, but which may be threatened or endangered in Oregon or throughout their range.
- List 4 Taxa of concern which are not currently threatened or endangered. This list includes taxa which are very rare but are currently secure, as well as taxa which are declining in numbers or habitat but are still too common to be proposed as threatened or endangered.

<sup>5</sup>Threatened applies to any taxon likely to become Endangered in Washington within the foreseeable future if factors contributing to its population decline or habitat degradation or loss continue. Sensitive describes any taxon that is vulnerable or declining and could become Endangered or Threatened in the state without active management or removal of threats.

\*Although this species is currently considered Bureau Assessment for the BLM in Washington, new information and rankings from the Washington Natural Heritage Program indicate no concern for this species; the species will be removed from the BLM list due to this updated information.

Within the National Forest System, the 13 species in this assessment are included on the Regional Forester's Sensitive Species List (USDA FS 2004). Although *B. fenestratum* is also included on the Sensitive Species List, this undescribed entity is now recognized as *B. hesperium* and is discussed as *B. hesperium* in this assessment (Farrar 2005). Table 3, an excerpt of the Regional Forester's List, displays the distribution of 13 moonworts species by National Forest (USDA FS 2004). In addition, *B. hesperium* is now documented from the Wallowa-Whitman National Forest (Farrar 2006). Table 4, an excerpt from the Oregon and Washington BLM State Director's Special Status Species List (2005) for lands administered by BLM, shows distributions of the 13 species by BLM District.

Table 3. Excerpt from Regional Forester's Sensitive Species List, showing distribution by FS unit (USDA FS 2004). For abbreviations under "Regional Forester's Sensitive Species List," O = Oregon only, W = Washington only, W/O = both states. For abbreviations under National Forest names, D = Documented on the National Forest for which it is indicated and S = Suspected.

SENSITIVE SPECIES PLANT LIST REGION 6 FOREST SERVICE July 2004	Regional Forester's Sensitive Species List	Columbia River Gorge	Colville	Deschutes	Fremont	Gifford Pinchot	Malheur	Mt. Baker-Snoqualmie	Mt. Hood	Ochoco	Okanogan	Olympic	Rogue River	Siskiyou	Umatilla	Umpqua	Wallowa-Whitman	Wenatchee	Willamette	Winema
<i>Botrychium ascendens</i>	W/O		D				D	D		D	D	S					D			
<i>Botrychium campestre</i>	W/O		*								S						D			
<i>Botrychium crenulatum</i>	W/O		D				D			D	D		S	S	D		D			
<i>Botrychium hesperium</i>	W		D								S						D***			
<i>Botrychium lanceolatum</i>	O					**	D		D				S		D	S	D			S
<i>Botrychium lineare</i>	W/O		D								S						D			
<i>Botrychium lunaria</i>	O							S							D		D			
<i>Botrychium minganense</i>	O						D	D	D						D	S	D		D	
<i>Botrychium montanum</i>	O						D	D	D						D		D		D	
<i>Botrychium paradoxum</i>	W/O		D							D	D				D		D	D		
<i>Botrychium pedunculatum</i>	W/O		D					D			S				D		D			
<i>Botrychium pinnatum</i>	O						D	D	D						D		D			
<i>Botrychium pumicola</i>	O			D	D								S			S			S	D

\* The 2004 List shows this species as documented on the Colville NF. However recent confirmation of the population indicates that the plants are not *B. campestre*; and based on new information *B. campestre* is not suspected on the unit either (Farrar 2006).

\*\* Although the 2004 List indicated that *B. lanceolatum* was documented from the Gifford Pinchot NF, the species is not considered sensitive in Washington (Swartz pers.comm. 2007).

\*\*\* *Botrychium hesperium* is documented from the Wallowa-Whitman National Forest (Farrar 2006).

Table 4. Excerpt from Oregon (OR) and Washington (WA) BLM Special Status Species List (USDI BLM 2005b), showing distribution by BLM unit. Columns on the top right are BLM District names. For abbreviations under BLM District names, D = Documented on the BLM District for which it is indicated and S = Suspected.

<b>SPECIAL STATUS SPECIES LIST for the BLM in OR/WA (May 2005)</b>	<b>BLM Status in OR<sup>1</sup></b>	<b>BLM Status in WA<sup>1</sup></b>	<b>Burns</b>	<b>Coos Bay</b>	<b>Eugene</b>	<b>Lakeview</b>	<b>Medford</b>	<b>Prineville</b>	<b>Roseburg</b>	<b>Salem</b>	<b>Spokane</b>	<b>Vale</b>
<b>Species Name</b>												
<i>Botrychium ascendens</i>	BSO	BAW						S			S	S
<i>Botrychium campestre</i>	BA	BA									S	S
<i>Botrychium crenulatum</i>	BSO	BAW	D			D		S			S	D
<i>Botrychium hesperium</i>	BTO	BAW									S	S
<i>Botrychium lanceolatum</i>	BTO		D									S
<i>Botrychium lineare</i>	FC	FC									S	S
<i>Botrychium lunaria</i> <sup>2</sup>	BA	BA	D								D	S
<i>Botrychium minganense</i>	BT	BT	D		D			S	S		D	D
<i>Botrychium montanum</i>	BAO	BTW			S			S		S	D	S
<i>Botrychium paradoxum</i>	BSO	BAW									S	S
<i>Botrychium pedunculatum</i>	BSO	BAW									S	S
<i>Botrychium pinnatum</i>	BTO		D									S
<i>Botrychium pumicola</i>	STO					S		D				

<sup>1</sup>BLM Status (USDI BLM 2003). BA = Bureau Assessment in Oregon and Washington pertains only to OR/WA BLM, and includes species that are not presently eligible for official federal or state status, but are of concern in Oregon and Washington and may, at a minimum, need protection or mitigation in BLM activities. BAO = Bureau Assessment in Oregon only. BAW = Bureau Assessment in Washington only. FC = Federal Candidate includes taxa proposed for listing under the Endangered Species Act. BS = Bureau Sensitive species are eligible for federal listing, federal candidate, state listed, or state candidate status. BSO or BSW = Bureau Sensitive in Oregon (BSO) or Washington (BSW) are species that could easily become endangered or extinct in the state. They are restricted in range and have natural or human-caused threats to survival. BT = Bureau Tracking are species not otherwise listed in the categories above. BTO = Bureau Tracking in Oregon. STO = State Threatened in Oregon species are officially listed in Oregon Administrative Rules (OAR): Oregon Department of Fish and Wildlife OAR 635-100-125; Oregon Department of Agriculture OAR 603-73-070. Bureau policy applies to these species within the State of Oregon.

<sup>2</sup>Although *Botrychium lunaria* is on the OR/WA BLM (USDI BLM 2005) State Director's Special Status Species List as Bureau Assessment in Washington, it is not included on the August 2006 "List of Tracked Species" maintained by the Washington Natural Heritage Program. This indicates that this species is not of concern in Washington. The BLM list will be updated to reflect this new information.

There are five relevant documents that address management issues for the 13 species in the analysis area. A "Conservation Strategy for *Botrychium pumicola* (Pumice Grape Fern) on the Deschutes, Fremont, and Winema National Forests, and Prineville District, BLM, Oregon" (Hopkins et al. 2001) identified habitat conditions and/or activities that posed threats to the long-term viability of *B. pumicola*. The goal of the conservation strategy was to provide management direction that would ensure viable populations of *B. pumicola* are maintained throughout the

range and that listing under the Endangered Species Act would not become necessary. Specific strategies included the selection of protected and managed populations, establishment of monitoring criteria, and provisions for acquiring additional information on the disturbance ecology, threats, habitat needs of the species, and responses to forest practices to ensure viability on multiple use lands.

A 1995 (Zika) report summarized what was known about the range, habitat, and ecology of 17 moonworts in the Columbia Basin. Conservation measures for the highest priority and rarest species (*Botrychium ascendens*, *B. crenulatum*, *B. lineare*, *B. paradoxum*, *B. pedunculatum*, and *B. pumicola*) included the use of watersheds to define functional populations, and the recommendation to protect the maximum amount of habitat available within the watershed. Additional inventories for *B. lineare* and *B. pedunculatum* were suggested. For more widespread species, such an approach was not warranted (*B. lanceolatum*, *B. lunaria*, *B. minganense*, and *B. pinnatum*). The report recommended protection of the largest and most vigorous sites on each National Forest.

A “Draft Management Plan for the Moonworts, *Botrychium ascendens*, *B. crenulatum*, *B. paradoxum*, and *B. pedunculatum* in the Wallowa-Whitman, Umatilla, and Ochoco National Forests” (Zika 1994) proposed several guidelines to address management of these species. The plan recommended that all activities that may affect known populations or potential habitat should be preceded with 1-2 years of botanical inventory and documentation of sites should include specimen vouchers. Other guidelines included establishing reserves at type localities and in pristine habitats, eliminating competing and destructive resource and recreational use from these areas, completely censusing all type localities, documenting land use history in moonwort habitats in historical time, and quantifying recreational use and potential impacts on known habitats.

“A Draft Management Guide for Rare *Botrychium* Species (Moonworts and Grapeferns) on the Mt. Hood National Forest” (Zika 1992b) proposed management recommendations for three *Botrychium* species, *B. minganense*, *B. montanum*, and *B. pinnatum*. On the Mt. Hood National Forest, these species are largely confined to riparian corridors, where management practices at the time were not expected to sustain either the habitats or the populations of moonworts. Logging in riparian zones and inadequate buffer strips along riparian zones were the primary conflicts. The Guide recommended monitoring to evaluate long-term consequences of management activities, such as grazing and logging.

This study found that most populations of these species on the Forest were small with less than 50 individuals. Populations with more than 40 individuals were considered significant. Locating moonworts was recognized as time-consuming and difficult. More surveys were recommended to understand the status and distribution of moonworts on the Forest. It was also suggested that grazing allotments with riparian zones be systematically inventoried for rare moonworts, as these habitats support a number of them elsewhere.

The Northwest Forest Plan Survey and Manage Standards and Guidelines included the development of management recommendations for two species, *B. minganense* and *B. montanum* (Potash 1998a and 1998b). The Management Recommendations focused on several key suggestions, including the following: maintain the light regime, hydrology (hydrologic flow and

water table level), habitat and microclimatic conditions, including existing canopy closures and hydrologic flow; avoid disturbance of aboveground plants and the substrate in the area, including the duff layer; and avoid excessive siltation or deposition of soil.

In 1998 Region 6 contracted with Iowa State University for Dr. Donald Farrar to determine isozyme patterns for fourteen species of moonworts on National Forest System lands in Oregon and Washington, and to provide a reference collection to aid in the identification of the species (Farrar 2001). In 1998 Region 6 also contracted with Gustavus Adolphus College for Dr. Cindy Johnson-Groh to determine which species of *Botrychium* reproduce vegetatively by means of underground structures (Johnson-Groh 2001).

In 2001 both professors through their institutions contracted with Region 6 (to provide information on nomenclature, taxonomy, life history, biology, habitat, threats, research, and monitoring) in preparation for conservation assessments for moonwort species in Oregon and Washington. (Please see Appendices 3-16 for additional information on each of the 13 species). This document is based primarily on their work, in collaboration with Kathy Ahlenslager, Colville National Forest Botanist.

## II. Classification and Description

The following historical view of taxonomy and species recognition is based on a summary by Farrar (2005).

### A. Systematics and Synonymy

**Family:** Ophioglossaceae  
**Genus:** *Botrychium*  
**Subgenus:** *Botrychium* (syn. *Eubotrychium*)

The first description of a *Botrychium* species was of *B. lunaria*, described in 1542 by Fuchs as *Lunaria minor*. Linnaeus recognized two species of *Botrychium* in his 1753 *Species Plantarum*, *B. lunaria* and *B. virginiana*. He placed both in the genus *Osmunda*. Presl (1845) was the first to use the name *Botrychium*, recognizing 17 species in his treatment of the genus. The first modern comprehensive treatment of the family and the first treatment to recognize the current subgenera was that of Clausen in his 1938 *Monograph of the Ophioglossaceae*. This publication provides the best reference point from which to discuss more recent taxonomic assessments and recognition of new species.

Clausen (1938) recognized three genera within the Ophioglossaceae: *Botrychium*, *Ophioglossum*, and *Helminthostachys*. These three genera plus *Cheiroglossa*, a segregate from *Ophioglossum*, continue to be recognized by most botanists as constituting the family Ophioglossaceae and the order Ophioglossales (Wagner 1993). This order of plants has no close relatives among the ferns. Cladistic analyses based on DNA sequences consistently place the Ophioglossales as sister to the Psilotales (*Psilotum* and *Tmesipteris*) (Manhart 1995, Pryer et al. 2001).

Within the genus *Botrychium*, Clausen (1938) described three subgenera: *Eubotrychium* (= *Botrychium*), *Sceptridium*, and *Osmundopteris*. The first two groups continue to be recognized as the moonworts (subgenus *Botrychium*) and the grapeferns (subgenus *Sceptridium*). The third of Clausen's subgenera remains controversial. Wagner (1993) continued to recognize

*Osmundopteris* as the subgenus containing the North American rattlesnake fern, *B. virginianum*. Kato (1987) split *Osmundopteris* into two subgenera, *Botrypus* (containing *B. virginianum*) and *Japanobotrychium*. Using two molecular data sets plus morphological/anatomical characters, Hauk (2000) reported *Botrychium* and *Sceptridium* to be well supported entities, but found *Botrypus* to be paraphyletic.

In 1938 Clausen recognized only six species of moonworts: *Botrychium lunaria*, *B. simplex*, *B. pumicola*, *B. boreale*, *B. matricariifolium*, and *B. virginianum*. All of these except *B. pumicola* were known from Europe as well as North America. While this seems over simplified compared to the current list of species, we must also credit Clausen with recognizing some varieties and subspecies that would later be defined as species. He recognized *B. minganense* as a variety of *B. lunaria*, *B. pinnatum* as *B. boreale* subspecies *obtusilobum*, and *B. hesperium* as a variety of *B. matricariifolium*. Clausen undoubtedly saw herbarium collections of other western U.S. moonworts but took a conservative approach in attributing these to variation within the species he recognized. His work was based on morphology without the knowledge of chromosome numbers and the role of allopolyploidy in speciation. He probably did not see some of the less common species now recognized.

Current recognition of North American species of subgenus *Botrychium* traces primarily to the work of W. H. and F. S. Wagner. Prior to Clausen's monograph, Victorin (1927) had described *B. minganense* as a new species. In 1956 Wagner and Lord confirmed the species status of that taxon listing a suite of morphological characters as well as chromosome number differentiating *B. minganense* from *B. lunaria*. Also prior to Clausen's (1938) description of *B. boreale* var. *obtusilobum*, Harold St. John (1929) had described this North American taxon as *B. pinnatum*. W. H. and F. S. Wagner (1983b) agreed that it was a species distinct from the European *B. boreale*. In the same publication they raised Clausen's *B. matricariifolium* var. *hesperium* to species level as *B. hesperium*.

From 1981 through 1998 the number of species recognized in subgenus *Botrychium* increased rapidly. Through extensive field studies and chromosome analyses the Wagners described five new diploid species (*B. campestre*, *B. crenulatum*, *B. lineare*, *B. montanum*, and *B. pallidum*) and seven polyploid species (*B. acuminatum*, *B. ascendens*, *B. echo*, *B. paradoxum*, *B. pedunculatum*, *B. pseudopinnatum*, and *B. spathulatum*). From their work *B. alaskense* was described in 2002 (Wagner and Grant). Two additional species recognized by them are in press (*B. adnatum* and *B. michiganense*) (Gilman et al.).

Recent work by Farrar, Johnson-Groh and Stensvold (Farrar and Johnson-Groh 1991, Farrar 2001, Stensvold et al. 2002) has resulted in recognition of three new species (*B. gallicomontanum*, *B. tunux*, and *B. yaaxudakeit*). Currently the North American species of subgenus *Botrychium* include 28 species, 11 diploids ( $n = 45$ ), 16 tetraploids ( $n = 90$ ), and 1 hexaploid ( $n = 135$ ). The morphology, range-wide distribution, and habitat for each of the 13 species in this assessment are described in Appendices 4-16. A key to the western species of *Botrychium* is displayed in Appendix 1 and characters to distinguish once-pinnate and twice-pinnate species are shown in Appendices 2 and 3.

## **B. Identification of *Botrychium*** (Farrar 2005)

Members of the Ophioglossaceae have a peculiar morphology, unlike any other ferns. They are described and differentiated using terms and concepts specific to the family, genus and subgenus as outlined below (see Figure 1).

Moonworts, like other members of the family, typically produce one leaf per year from an underground upright stem with a single apical meristem. The above-ground portion of a mature leaf is divided into two axes. One axis, bearing an expanded, usually photosynthetic lamina or blade, is called the trophophore or sterile segment. The other axis, bearing numerous globose sporangia, is called the sporophore or fertile segment. The trophophore and sporophore are joined into a common stalk or petiole, usually near the base of the expanded lamina. The common stalk extends underground to the stem apex where its base encloses the apical bud.

Species of *Botrychium* subgenus *Botrychium* are differentiated from species of the other subgenera in having trophophores that are at most twice pinnate and generally much smaller than the large, two or more times divided trophophores of subgenera *Sceptridium* and *Osmundopteris*.

Diagnostic characteristics of moonworts are present in both sporophore and trophophore, but more numerous in the latter (Figure 1a). Moonworts are of three basic forms, the once-pinnate, fan-leaflet form of most diploid species (Figure 1d), the triangular, twice-pinnate form of *B. lanceolatum* (Figure 1f), and the intermediate, pinnate-pinnatifid form of the allopolyploid species derived from ancestral hybridization between *B. lanceolatum* and species of the fan-leaflet group (Figure 1e). The last two are sometimes referred to as the midribbed species because their pinnae have strong central veins, whereas those of the fan-leaflet species have multiple parallel veins of equal size. Presence of a midrib in the basal pinnae is a good way to identify plants of the pinnate-pinnatifid group when they are too small to have developed pinna lobing.

Unusually large plants of the fan-leaved, once-pinnate species may have lower pinnae that become secondarily divided, more or less repeating the general morphology of the entire trophophore. This is especially true of *B. simplex*, but occasionally it happens in most species. However, this subdivision of pinnae is seldom repeated in non-basal pinnae as it is in the pinnate-pinnatifid species.

Initial segregation of species in the fan-leaflet group is usually made on the basis of pinna span. Pinna span refers to that portion of a circle that is “spanned” by the outer circumference of the pinna (Figure 1c). Convenient dividing points are: less than 60°, between 60° and 150°, and greater than 150°. Pinna bases may be sessile or short-stalked (Figure 1b). Pinna sides may be straight or concave, and converge at angles producing pinna bases that are acuminate (<30°), acute (30-90°), obtuse (>90°), truncate (180°) or cordate (>180°). The outer pinna margin may be entire, crenulate, dentate, lacerate or lobed. Unless noted otherwise, when used in a key or species description, pinna characters refer to the basal pinnae which are typically the largest and broadest.

The trophophore may be sessile or stalked (petioled) below the basal pair of pinnae. If stalked, the degree of trophophore stalk is best measured in relation to the distance between the first two

pair of pinnae, i.e., whether the trophophore stalk is longer or shorter than the distance between the first two pair of pinnae. A number of moonwort species have a glaucous surface giving them a gray or bluish cast that easily distinguishes them from species with a deep green color and lustrous surface.

Plant size varies considerably in most populations and is of limited usefulness in identifying species. Small plants often fail to fully develop the characters of full-sized plants, especially in pinna span and margin dissection. Extremely large plants often develop abnormalities (unusually large and highly divided basal pinnae, often with extra sporangia or small sporophores, and otherwise misshapen pinnae) uncharacteristic of the species.

Sporangia are occasionally produced on the basal trophophore pinnae of all species. Regular occurrence of these extra, or supernumerary, sporangia is limited to two species, *Botrychium ascendens* and *B. pedunculosum*, but not all plants of these species have supernumerary sporangia. *Botrychium paradoxum* is a special case in which no trophophore is produced. Instead, the trophophore has been converted to a second sporophore. *Botrychium X watertonense* is a sterile first-generation hybrid between *B. paradoxum* and *B. hesperium* in which all pinnae of the trophophore produce sporangia around their margins.

The sporophore of *B. lanceolatum* is usually divided into three main branches. This character may or may not be expressed in allopolyploid taxa having *B. lanceolatum* as one parent. When present, a distinctly three-parted sporophore is usually a good indicator of ancestral parentage by *B. lanceolatum*.

One of the most useful sporophore characters is the length of the sporophore stalk. This character must be used with caution because the sporophore stalk continues to lengthen until the time of spore release. The most useful comparison is the length of the sporophore stalk relative to the entire length of the trophophore, i.e., whether the sporangia-bearing portion of the sporophore is raised entirely above the trophophore at the time of spore release. The degree of sporophore branching and the length and angle of the branches may also be useful.

Spore size is a useful character, especially in distinguishing between diploid and polyploid species. Most diploid species have spores that are significantly smaller than those of tetraploids with which they might be confused. For example, the spores of *B. lunaria* range from 24 to 32 microns whereas those of *B. minganense* range from 32 to 40 microns (Wagner and Lord 1956).

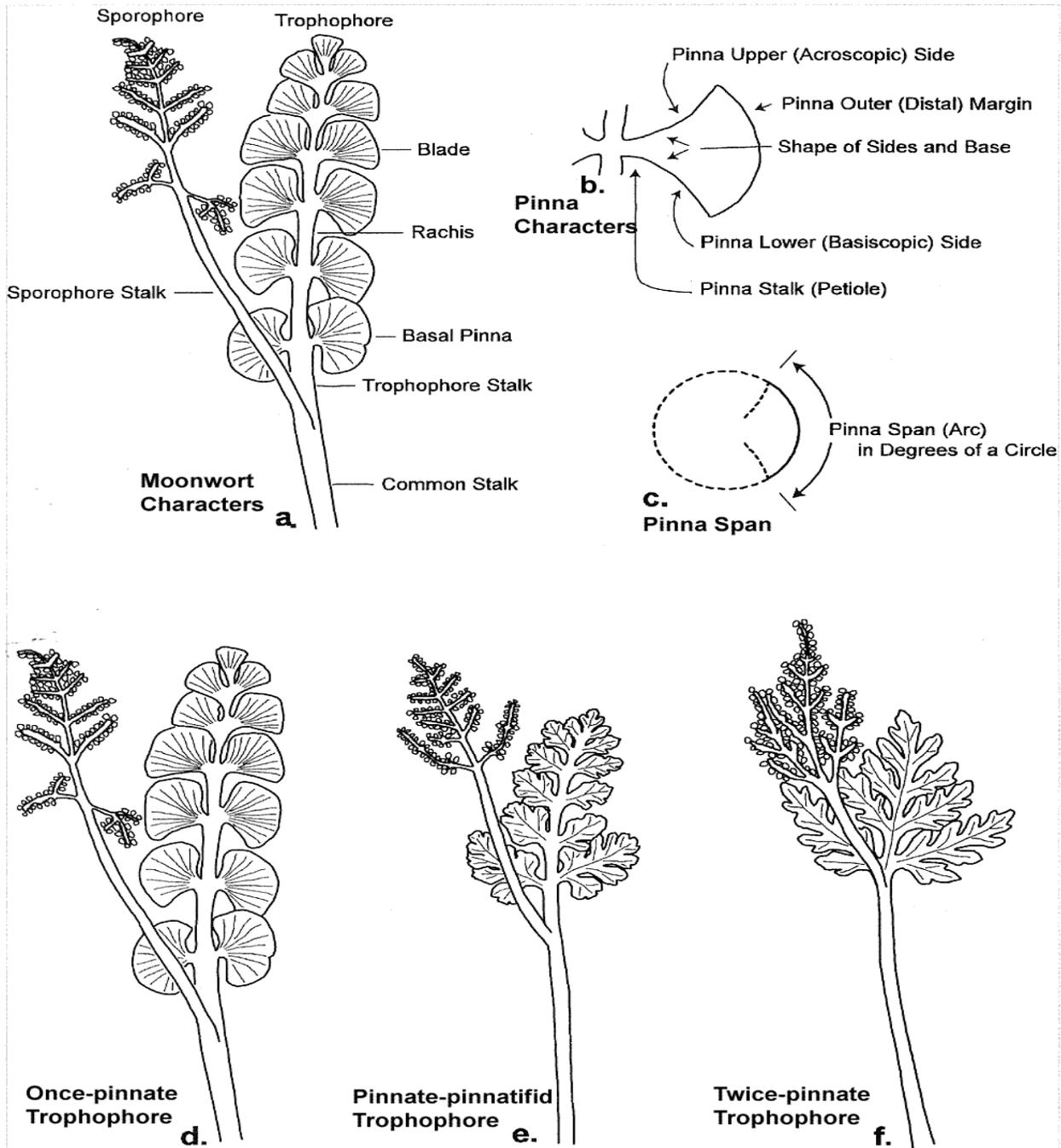


Figure 1. Morphology and terms used in moonwort identification (Farrar 2005).

### III. Biology and Ecology (Johnson-Groh 2001)

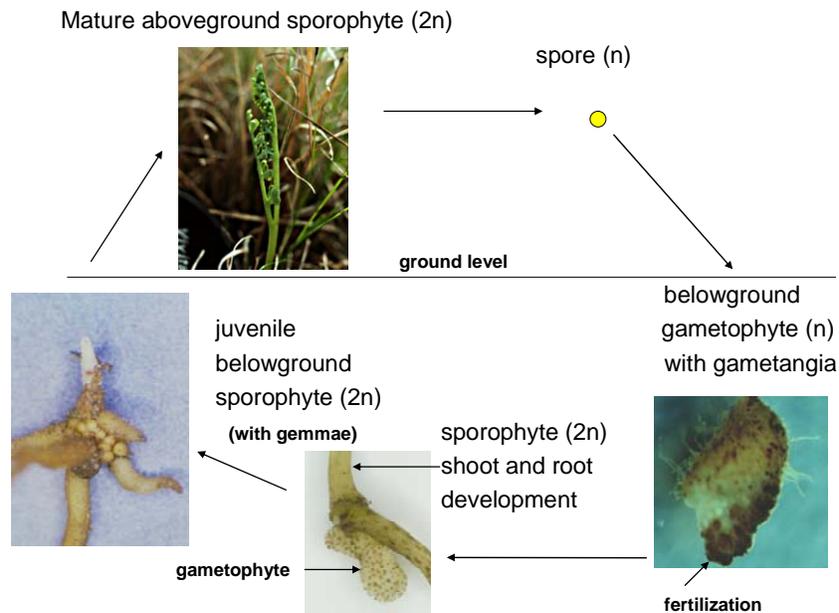
#### A. Life Cycle

Fern life cycles are composed of two stages, gametophyte and sporophyte (Figure 2). What follows is a brief summary of the fern life cycle followed by a detailed examination of the *Botrychium* life cycle. For all ferns, the leaf and the attached rhizome and roots below the soil surface constitute the diploid sporophyte generation of the life cycle. Sporangia occur on the fronds (typically lowerside) are where the spores (haploid) are formed following meiosis. These spores are passively released and dispersed by wind. In most ferns these spores will germinate on the soil surface (unlike *Botrychium* whose spores percolate and germinate underground) into a small (less than 1 cm) haploid gametophyte. The second stage, the gametophyte is photosynthetic and produces gametangia, male and female structures forming sperm and egg, respectively. Fertilization produces a diploid zygote that divides forming the new sporophyte (diploid).

Moonworts produce one leaf (including both segments, trophophore and sporophore) annually that is attached to a belowground rhizome that is upright and short (4-15 cm). Just below the soil surface is a single leaf-producing bud at the rhizome apex. The bud may contain up to six preformed leaves (Imaichi 1989). The rhizome and attached roots are off-white, stout, and succulent. The roots typically branch off at right angles to the rhizome and vary in length (up to 20 cm). The roots occasionally bifurcate but generally are unbranched, straight, and grow parallel to where they diverge from the plant (Johnson-Groh unpublished data). Roots at the top of the plant sometimes bend downward. Roots at the very bottom of the plant are often black, necrotic, and missing. The roots have no root hairs and are mycorrhizal. Stevenson (1975) observed contractile roots in *Botrychium multifidum*. Other than inferences to this report, contractile roots have not been reported in other species of moonworts and there is no evidence of contractile roots.

The sporophore produces spores in globose sporangia (ranging from ten sporangia per sporophore on small plants to over 100 on large plants). These spores filter into the soil and germinate in darkness (Whittier 1973). Following germination, a below ground achlorophyllous, fleshy gametophyte is produced. These gametophytes are small (usually less than 0.5 cm), irregularly shaped (often knobby), off-white with numerous rhizoids. The gametophyte produces gametangia (archegonia and antheridia) and sexual reproduction occurs resulting in a below ground juvenile sporophyte. (Moonworts are primarily self-fertilized; see population genetics section.)

It takes several years for this juvenile sporophyte to produce a leaf-bearing apex and emerge above ground (Johnson-Groh et al. 1998). The rhizome typically develops several (1-5) roots before it produces an apex. Johnson-Groh estimates that it takes 3-8 years for moonwort rhizomes to produce an emergent leaf. Prior to this the plant is totally dependent on mycorrhizae. Subsequently the plants produce one leaf annually, but it is common for moonwort plants to remain dormant belowground in a given year and produce no above ground leaf (Johnson-Groh 1998, Kelly 1994, Montgomery 1990).



**Figure 2. Generalized *Botrychium* life cycle** (Johnson-Groh et al. 2002).

In addition to these below ground stages, some species reproduce asexually via below ground gemmae, small (0.5-1mm) propagules that can independently give rise to a new plant once detached from the parent plant (Farrar and Johnson-Groh 1990). Gemmae form on the rhizome and abscise at maturity. Upon germination, gemmae develop 4 or 5 short roots prior to the differentiation of a shoot apex and production of leaves (Farrar and Johnson-Groh 1990). The first leaves formed are short and slender and do not reach the soil surface. The presence of vegetative reproduction greatly influences the population dynamics of these gemmiferous species. It is common in the field to see two or more leaves of gemmiferous moonworts emerging in close proximity. Excavation of these clusters usually reveals a large number of below ground sporophytes in various stages of development.

## **B. Population Genetics (Farrar 2005)**

### 1) Breeding system

In order to understand the distribution of genetic and morphological variation within and between species, it is necessary to understand the reproductive biology of moonwort ferns (see Life History section for a more complete description). Being pteridophytes, they have two separate life stages. The relatively large above-ground sporophyte produces spores that have half the number of chromosomes of the parent sporophyte. These spores germinate underground and grow into the gametophyte stage. Each gametophyte produces both male and female gametangia containing sperm and eggs, respectively.

When a sperm is released from a mature antheridium, it swims to an open archegonium, then down the archegonial neck to an egg with which it fuses to initiate the next sporophyte

generation. These acts of sexual reproduction take place underground. Travel through soil by swimming sperm must be considerably hindered relative to sperm swimming in liquid on the soil surface as is the case for most ferns. In the underground environment, sperm from one gametophyte plant may be unable to reach another gametophyte more than a few millimeters distant. They are quite capable though of swimming to archegonia and fertilizing eggs on the same gametophyte less than one millimeter away. This union of gametes from the same gametophyte constitutes intragametophytic self-fertilization.

Enzyme electrophoresis allows recognition of heterozygous individuals, those containing two different alleles at a given gene locus. Because heterozygous individuals of diploid species can be produced only by cross-fertilization between different gametophytes, electrophoretic determination of the number of heterozygous individuals in a population of a diploid species allows estimation of the amount of cross-fertilization that is occurring. Of thousands of individual *Botrychium* plants examined electrophoretically in several studies (Soltis and Soltis 1986, Hauk and Haufler 1999, Farrar 1998, 2001), less than 1% have shown heterozygosity from out-crossing. This observation provides strong support for the hypothesis that sexual reproduction in *Botrychium* is predominantly by intragametophytic self-fertilization.

Intragametophytic self-fertilization in pteridophytes has several important genetic consequences. Because all cells of an individual gametophyte are derived from a single initial cell, sperm and eggs produced by that gametophyte are genetically identical. Fertilization of an egg by sperm from the same gametophyte unites identical genotypes. The resulting sporophyte has exactly the genotype of the gametophyte from which it was produced. When that sporophyte produces spores, those too will be all be genetically identical and identical to the original gametophyte. Gametophytes growing from those spores will likewise be of the same genotype, and so on, as long as intragametophytic selfing occurs. With no means of generating genetic variability (except by rare mutations) sexual reproduction in *Botrychium*, through intragametophytic self-fertilization, becomes equivalent genetically to vegetative reproduction.

## 2) Genetic Vulnerability to Environmental Change

There is no reason to believe that historically plants of *Botrychium* have reproduced differently in the past than now. Underground bisexual gametophytes are characteristic of all Ophioglossaceae and of their closest relatives, the Psilotaceae. If low genetic variability is due to intragametophytic selfing which, in turn, is imposed by the underground environment, then it is reasonably to assume that *Botrychium* species have always maintained low genetic variability.

Two concerns are often raised regarding the vulnerability of species with low levels of genetic variability, especially those in small populations. First, it is inevitable that small populations of typically out-breeding species experience an increased rate of inbreeding. Such populations can suffer inbreeding depression caused by the expression of recessive deleterious alleles in the homozygous state. Second, low genetic variability can reduce a species' ability to adapt to a change in environment or to a range of environments.

Because of regular intragametophytic selfing, *Botrychium* species are not subject to inbreeding depression. They do not carry a genetic load of deleterious alleles sheltered in heterozygous individuals. All of their gene alleles have already been exposed to environmental selection, only

non-deleterious alleles remain in their genome. Because of their immunity to inbreeding depression, fitness is not a function of population size.

How *Botrychium* species cope with environmental variability and change is not clear. On the whole, *Botrychium* species do not seem to be any more habitat specific or any less widespread geographically than do other ferns or seed plants, despite their low genetic variability. A possible answer to this conundrum lies in the mycorrhizal association maintained by *Botrychium* species. A number of observations strongly suggest that moonwort *Botrychiums* rely heavily on their mycorrhizal partner for photosynthates, as well as mineral nutrients and water. With mycorrhizal fungi as an intermediary, *Botrychium* have greatly reduced direct interaction with their environment. They likely have less need for genetic tracking of environmental change than do most plants. Their greater need is for genetic stability in maintaining the mycorrhizal association.

Regardless of the means by which *Botrychium* species cope with reduced genetic variability, they have done so effectively for thousands, if not millions of years. This lack of genetic variability in *Botrychium* should not be a concern in assessing species or population viability.

### **C. Mycorrhizal Relationships**

Moonworts require endophytic mycorrhizae for gametophyte and sporophyte development (Berch and Kendrick 1982, Bower 1926, Campbell 1922, Schmid and Oberwinkler 1994). Germinating gametophytes are infected by vesicular arbuscular mycorrhizae (Schmid and Oberwinkler 1994). The mycorrhizae facilitate nutrient and water uptake. Little is known about how or when the gametophyte is infected or what are the fungal partners. Winther (pers. comm. 2002) is working on identifying *Botrychium* mycorrhizae and preliminary results have revealed two species of *Glomus* as fungal partners in *B. simplex*. Schmid and Oberwinkler (1994) studying the fungus interaction of the gametophyte of *B. lunaria* found no arbuscules in the gametophytes and they observed that the gametophytic hyphae did not infect the developing sporophyte. Studying the roots, Berch and Kendrick (1982) noted that between 80 and 100% of *B. oneidense* and *B. virginianum* root segments contained arbuscules.

Moonworts depend on mycorrhizae as a significant source of carbohydrate, minerals, and water. This observation is based on several ecological behaviors. First, similar to orchids, moonworts do not emerge every year (Johnson-Groh and Farrar 1993). They frequently fail to emerge for one to three consecutive years, with no subsequent decrease in size or other negative effects (Lesica and Ahlenslager 1996, Johnson-Groh 1997, Johnson-Groh and Farrar 1993). Second, "albino" moonworts have been observed (Johnson-Groh et al. 2002). Another indication that moonworts depend relatively little on their own leaves for photosynthesis is the observation that these leaves frequently do not emerge above the litter. In fact only a small proportion of the total population of *B. mormo* emerged from the litter (Johnson-Groh and Lee 2002, Johnson-Groh 1998). Herbivory and loss of leaves through fire do not affect the size and vigor of plants in the subsequent year (Hoefflerle 1999, Johnson-Groh 1998, Johnson-Groh and Farrar 1996b).

Finally, if leaves of juvenile plants are produced one per year, as in adults, 3-8 years may be required for development from gametophyte to a mature sporophyte with an emergent photosynthetic leaf (Johnson-Groh et al. 2002). Juvenile plants must rely on mycorrhizae for carbohydrates. Whittier (1984) noted that gametophytes may remain dormant (not actively

growing) for up to four months without an exogenous carbon source, resuming growth in the presence of sucrose. Thus, although there has been no physiological studies to confirm this, it seems certain that moonworts (*Botrychium* subg. *Botrychium*) may depend largely on mycorrhizae for carbon from other plants, in addition to that produced by their own photosynthesis.

If photosynthesis is not critical for this subgenus and mycorrhizae are primarily responsible for overall energy budget, then understanding the below ground biology of *Botrychium* is imperative. Indeed, assumptions made about the population biology of other ferns may be irrelevant to moonworts. Health of the mycorrhizal connection may determine juvenile recruitment and survivorship, and moonwort populations may appear or disappear in accordance with mycorrhizal health (Johnson-Groh et al. 2002).

Mycorrhizae play an important role in nutrient acquisition. This may be especially important for moonworts because of the inability of its roots to forage. Root-foraging has been observed in flowering plants (Caldwell 1994). It allows them to respond to small-scale nutrient patches. However, moonwort roots are relatively few (5-30/plant), do not have root hairs, and do not appear to have the morphological plasticity to forage for small-scale patches of soil nutrients. Typically roots extend almost perfectly horizontally for their entire length (3-20 cm). Only occasionally are roots observed to abruptly bend in another direction (Johnson-Groh unpublished data). Tibbet (2000) argued that mycorrhizae are especially important for roots that do not have the morphological plasticity to respond to small-scale nutrient patches. Mycelia rapidly colonize patches of soil nutrients, making them ideal foraging instruments of the autotroph. In moonworts it seems highly probable that its mycorrhizal mycelia are more important than root proliferation in nutrient acquisition (Johnson-Groh et al. 2002).

The role and ecological importance of mycorrhizae have been documented (Bever et al. 2001, Allen 1991). There is ample evidence that mycorrhizae alter plant communities by enhancing productivity, enhancing diversity, providing resistance to pathogens and differentially interacting with plants to alter the plant community structure. The ephemeral nature of moonworts is likely influenced by mycorrhizae. It also seems likely that different fungal partners elicit different responses (dormancy, competition, productivity, disease resistance) depending on the interaction between the species of moonworts, the fungal partners, and environmental parameters such as soil moisture, soil nutrition, competition, herbivory, etc. A complex interrelationship emerges and clearly more work is needed to understand the species, structure, and function of mycorrhizal partners in moonworts.

#### **D. Spores, Dispersal Mechanisms, Loss of Spores, Cryptic Phases**

Four stages of leaf development have been recognized: emergence, separation, spore release, and senescence (Johnson-Groh and Lee 2002). The emergent leaf lives for one to three months depending on the species. Though all plants produce sporophores, not all plants actually release spores. Johnson-Groh and Lee found that of 412 *Botrychium mormo* plants studied only 39% completed development moving through the first three stages of emergence, separation, and spore release; 55% of 219 *B. gallicomontanum* plants completed their development. Johnson-Groh and Lee observed senescing moonworts that appeared to produce viable spores, but that did not release the spores. These plants senesced, dropping the sporophore in the immediate vicinity of the parent plant, releasing spores passively. They noted that given the mycorrhizal germination

requirements, this could be an advantage, facilitating the mycorrhizal inoculation of spores and thereby maintaining the immediate population. Spore dispersal is probably risky for moonworts given the highly specific germination requirements. Moonwort spores are extremely difficult to cultivate (Whittier 1981) making it difficult to test the viability of these unreleased spores.

Other studies have reported variation in the number of plants that produce spores. Kelly (1994) demonstrated that only 9-20% of *B. australe* produce fertile spikes with sporangia in any given year. Kelly attributed this to light levels; plants in heavy shade were unlikely to be fertile. Muller (1992) found that some moonworts wilted prematurely and did not set spores due to a severe spring drought.

*Botrychium* sporangia dehisce and release spores passively. Wind sifts the spores out and disperses them. It is unknown how widespread moonwort spores disperse but based on the work of Peck et al. (1990) on *B. virginianum* we can conclude that most spores disperse within 5 m or less. Dyer (1994) found that the largest spore banks for other ferns occurred in samples taken immediately below ferns and that at a distance of 2 m away from the spore source, the spore bank was notably smaller. It seems likely that a few spores may become airborne and disperse farther. Because of the ability of moonwort gametophytes to self-fertilize, it is reasonable to expect that a single spore is capable of dispersing and establishing a new population (Farrar 1998).

Over time, a sizeable moonwort spore bank is established in the soil. Moonwort spores likely remain viable for long periods of time, as do those of many other ferns (Lloyd and Klekowski 1970, Miller 1968, Sussman 1965, Windham et al. 1986). These spores are probably dormant until conditions (moisture and mycorrhizae) are adequate, at which time many or all the spores in that localized area germinate and develop.

Johnson-Groh et al. (2002) predicted an average minimum spore density of approximately 6,000 spores per m<sup>2</sup> for several species of moonworts. Of the species studied, *Botrychium montanum* and *B. mormo* had the highest predicted maximum spore densities of 15,000 spores per m<sup>2</sup>; *B. virginianum* and *B. gallicomontanum* had the lowest at 100 per m<sup>2</sup>. This is considerably lower than 5,000,000 per m<sup>2</sup> estimated by Hamilton (1988) for two species of *Athyrium* or even 57,000 per m<sup>2</sup> estimated by Milberg (1991) for grassland soil (several species of ferns). This estimate is also lower than the estimate of 100,000 spores per m<sup>2</sup> made by Johnson-Groh et al. (1998) for *B. mormo*. Johnson-Groh et al. (2002) argue that given the need for mycorrhizal infection following germination, mortality at this stage is probably very high, and it is reasonable to expect high spore densities within moonwort populations.

Unlike most other flowering plants or ferns, the juvenile sporophyte stages of moonworts remain below ground for a number of years. The below ground recruitment of gametophytes and juvenile sporophytes therefore can be compared to seedling or sporeling recruitment above ground for flowering plants and most ferns. As with other plants, juvenile mortality is probably significant for *Botrychium*. Johnson-Groh et al. (2002) found that with one exception (*B. campestre*), the gametophyte density exceeds the juvenile sporophyte density for several species. Likewise, in most cases the below ground sporophyte density exceeds the density of above ground sporophytes. They found mortality (defined as the proportional change between stages) is greatest (93% for all species) between the juvenile sporophyte stage and emergent sporophytes

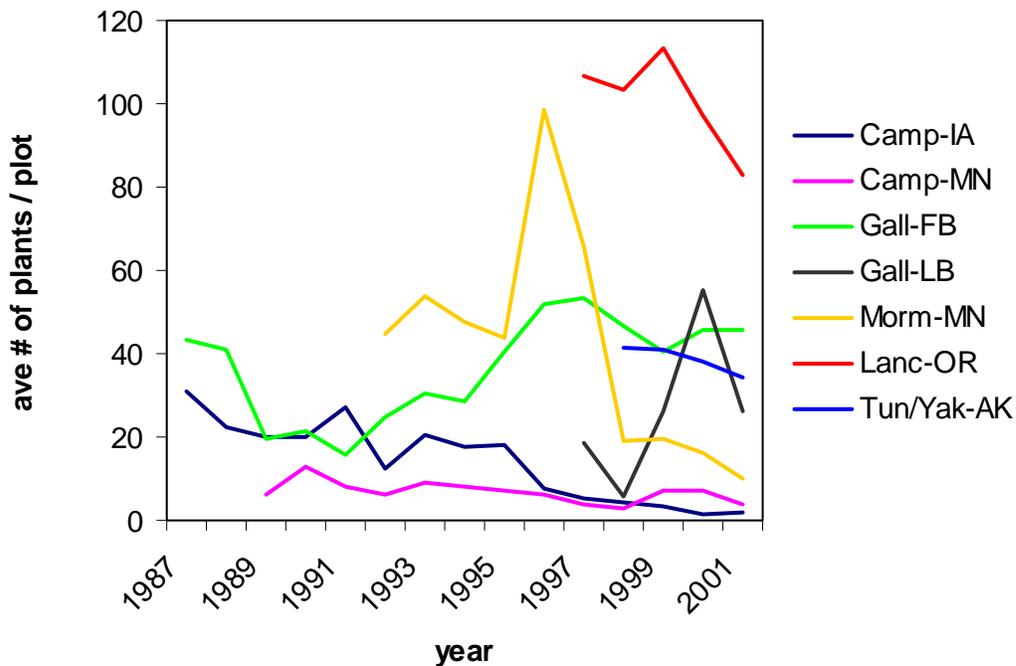
and an average of 73% mortality between the gametophyte and juvenile sporophyte stages. This high juvenile mortality is common among many plants.

Johnson-Groh and Lee (2002) found that species with gemmae (e.g., *B. campestre*, *B. gallicomontanum*) have a higher total below ground density than those without gemmae. Like spores, gemmae, once detached from the parent plant, require mycorrhizae for further development. Farrar and Johnson-Groh (1990) found relatively few gemmae that contained mycorrhizae, which could explain the low number of developing gemmae relative to the number of gemmae produced. (Gemmae obtain mycelia through their connection with the parent rhizome; if unsuccessful, they remain dormant.) Johnson-Groh and Lee (2002) note that the primary role of gemmae may be to maintain the population in a microsite that has already proven successful. The frequent occurrence of multiple stems within a small-localized area (1-4 cm<sup>2</sup>) suggests that gemmae are effective in local propagation. Dispersal beyond a short distance is limited, as evidenced by the low frequency of the highly gemmiferous species (*B. campestre*, *B. gallicomontanum*, Johnson-Groh et al. 2002).

Johnson-Groh and Lee (2002) also found that species that produced profuse gemmae produce the lowest number of gametophytes (*B. campestre*, *B. gallicomontanum*). Gemmae, a form of asexual reproduction, produce essentially the same genetic product that a selfing gametophyte produces. Johnson-Groh and Lee noted the advantage of gemma production is the positioning for immediate success (mycorrhizae present). A greater reliance on reproduction via spores and gametophytes by most species and the higher disperability of spores undoubtedly accounts for the higher frequencies in soil samples of the non-gemmiferous species. Johnson-Groh et al. (2002) note that the advantage of spore – gametophyte production allows dispersal to new sites, thereby insuring that “assets are diversified,” which may provide a long-term advantage to the species. They further draw from investment analogies, by noting that gemmae are short-term investments with immediate returns, whereas spores are long-term investments with greater evolutionary payback.

#### **E. Life History Characteristics (Recruitment, Survival, Lifespan, Population Dynamics)**

Long-term demographic studies (15 years) of moonworts reveal that population numbers are quite variable (Figure 3). Above ground moonwort populations fluctuate independently within and between populations, as well as between years and between different sites. Fire, herbivory, herbicides, and timber harvests may have an immediate impact on the above ground sporophytes (Johnson-Groh and Farrar 1996a and 1996b). However, the above ground populations are fairly resilient and rebound following perturbations, although recovery may take several years.



**Figure 3. Long term demographic study results showing population variability. Average number of plants per plot (5.7m<sup>2</sup>) by species and location. (Camp = *B. campestre*, Gall = *B. gallicomontanum*, Morm = *B. mormo*, Lance = *B. lanceolatum*, Tun/Yak = *B. tunux*, and *B. yaaxudakeit*, IA = Iowa, MN = Minnesota, OR = Oregon, and AK = Alaska., Johnson-Groh unpublished data)**

Several conclusions can be drawn regarding annual variation and monitoring. First, as others have shown, population sizes vary greatly from year to year (Montgomery 1990, Muller 1992, 1993; Johnson-Groh 1997, Johnson-Groh and Farrar 1993, Lesica and Ahlenslager 1996). This annual variation is due to many complex environmental and demographic factors. For example drought has a significant effect on the production of above ground stems as noted by Muller (1992) who found that *B. matricariifolium* is very sensitive to long periods of water deficits in May. Drought and earthworm invasion are the probable factors responsible for the large recent decline in *B. mormo* populations (Johnson-Groh 1998, Casson et al. 2001).

Second, numbers of individuals and trends vary greatly between plots. It is not unusual to have adjacent plots increase and decrease simultaneously in any given year (Johnson-Groh unpublished data). These differences reflect microsite differences such as soil moisture, herbivory, or mycorrhizae. Each individual population varies independently as a metapopulation and some may be declining and dying out while others are thriving. Populations occupy sites as long as the environmental parameters are suitable. However, the specific environmental parameters to a species are unknown.

Third, Johnson-Groh and Lee (2002) have shown that if populations are censused at a time when the population is senescing, a false estimate of the population size may be deduced. This was the case for *B. mormo*, which had previously been sampled late in the season after the population had declined. This late date had been selected because of the visibility of plants late in the season and literature reports (Wagner and Wagner 1981). Johnson-Groh and Lee (2002) found

that the largest plants, which emerge above the litter, are present late in the season; however the population size at the end of the season is approximately half the peak mid-season population size for *B. mormo*.

The results of fifteen years of monitoring by Johnson-Groh (Figure 3) reveal large differences between sites, species and between years. In addition to the variability at the population level, there is also a great deal of variability at the individual level. Individual plants may skip years, producing no above ground leaves in a given year, but remaining alive and producing leaves the following season (Lesica and Ahlenslager 1996, Johnson-Groh 1998, Montgomery 1990, Muller 1992 and 1993). While new plants are annually recruited into the population, older plants may disappear or reappear after absences of one to three years (Johnson-Groh 1998).

Hoefflerle (1999) and Johnson-Groh and Farrar (1996b) have assessed the impact of non-appearance of leaves in a given year by examining the impact of leaf loss. It was predicted that loss of leaf tissue would decrease the photosynthetic output of the plant and thereby decrease the total vigor. If this lack of photosynthetic tissue affects the plant then there should be a decline in the number or size of plants in the following year. Hoefflerle (1999) found that plants harvested in the spring did not show a significant reduction in size the subsequent year. However Hoefflerle did find a significant difference in plants collected in the fall with regard to trophophore width and lowest pinnae size, but not overall size. Johnson-Groh and Farrar (1996b) indicate that loss of the leaf either through herbivory, fire or collection has no effect on the subsequent return the following year. Damaged plants are as likely as undamaged plants to return and likewise plants are equally likely of returning after non-appearance for one year as they are for years following emergence. This is also true of the prairie moonworts where Johnson-Groh and Farrar (1996b) observed severely scorched or wilted plants following burns. Scorched plants emerge the following year and showed an increase in size (Johnson-Groh and Farrar 1996a and 1996b).

Because of this irregular appearance it is difficult to determine the longevity of individual plants. Working on *B. mormo*, Johnson-Groh (1998) found that almost half (47%) of the plants observed appeared for one year above ground and then did not emerge the following year. A few plants have appeared above ground continuously for up to six years. Of the 47% which fail to emerge in a given year, only 24% reappear in a subsequent year. This only addresses the probability of reappearance of individual plants and not how long each plant was in existence above ground prior to disappearing. Johnson-Groh (1998) found that most plants do not persist more than two years and only 24% of these return after a one year absence. Only 4% of these returned after two years of absence. Thus it seems that above ground longevity for most plants of *B. mormo* is relatively short (1-2 years) as compared with the prairie moonworts in which most plants have an above ground longevity of approximately four years (Johnson-Groh 1998).

Moonwort populations are highly buffered due to the below ground portion of the lifecycle. Below ground gametophytes and developing sporophytes may allow the population to rebound from infrequent catastrophic years. Diminished spore output will affect the population when this reduced cohort of spores filters down through the soil, germinates, and eventually produces emergent sporophytes, a process that may take several years or more (Johnson-Groh 1998).

Field evidence for this model may be found in the recovery of a population of *B. gallicomontanum* following a devastating fire (Johnson-Groh unpublished data). In 1987 a hot fire occurred concurrently with exceptionally dry conditions. This combination of drought and fire essentially killed all above and belowground structures, except the spores. Recovery has been slow, but the population has returned and grown at a steady growth rate of 4% per year.

Whereas it seems natural to become concerned with declines in moonwort populations, caution must be exercised. Long term monitoring (15 years) of midwestern species has revealed large variations in vigor of individual populations (Johnson-Groh unpublished data). Some midwestern populations appear to have declined to the point of extirpation while others have maintained stable populations for 15 years. Moonwort populations are best characterized as metapopulations in which small satellite populations are likely to be extirpated and stable source populations maintain a reserve of individuals capable of reestablishing new satellite populations.

This metapopulation model appears to fit moonworts, however consideration must be given to the time scale. Moonwort spores percolate underground and may lie dormant for many years before they germinate under suitable conditions. From germination to emergence above ground, it probably takes 3-8 years (Johnson-Groh unpublished data). The extirpation and recolonization of new moonwort populations likely is on a time scale of 10's of years rather than years.

Because of our inability to sample all populations over a large geographic area and because of the time scale, it is difficult to understand the ephemeral nature of these populations. Sampling shows that some populations have been stable for years, while others have declined to the point of extirpation. In these later sites the conditions are such that succession, lack of mycorrhizal or soil resources, herbivory, or some other environmental parameter is limiting the population. Overall species survival depends on the founding of new populations in other areas that have adequate resources to support a new population. With time, these new populations will flourish and then die out too.

#### **F. Range, Distribution and Abundance**

The geographic range of moonwort species varies greatly depending on the individual taxon. For example, *Botrychium pumicola* is restricted to central and south-central Oregon and northern California, but *B. lunaria* occurs world-wide (Farrar 2005). Refer to the appendices for each taxon for further descriptions of ranges.

As is often the case with inconspicuous species, the location of known sites/populations is probably more a function of where agency botanists have looked than a reflection of the true distribution. As a case in point, of the 1098 documented sightings in Oregon and Washington through January 2002, 98% occurred on federal land, 1% on state land, and 1% on private land. Table 5 displays the distribution of each taxon by county for those taxa that are included on the Region 6 sensitive plant list (USDA FS 2004) or the OR/WA BLM Special Status Species List (USDI BLM 2005).

In this assessment, relative abundance is described by the following set of variables, where those species at the top of the hierarchy have a high relative abundance and are therefore relatively more “common” than those species at the bottom of the hierarchy with a low relative abundance and are relatively “rare.”



Table 5. Distribution of 13 *Botrychium* species in Oregon and Washington by county and taxa (ORNHC 2002, WNHP 2002) where those taxa are included on the Region 6 sensitive plant list (USDA FS 2004) or the OR/WA BLM Special Status Species List (USDI BLM 2005), except for *B. lunaria*, which is no longer tracked in Washington (WNHP 2006).

TAXA	<i>B. ascendens</i>	<i>B. campestre</i>	<i>B. crenulatum</i>	<i>B. hesperium</i>	<i>B. lanceolatum</i>	<i>B. lineare</i>	<i>B. lunaria</i>	<i>B. manganense</i>	<i>B. montanum</i>	<i>B. paradoxum</i>	<i>B. pedunculatum</i>	<i>B. pinnatum</i>	<i>B. pumicola</i>
<b>OREGON COUNTIES</b>													
Baker	X		X		X			X	X	X		X	
Crook			X					X	X				
Deschutes													X
Douglas			X					X					
Grant	X		X		X		X	X	X	X	X	X	
Harney			X		X		X	X				X	
Hood River					X			X	X				
Jackson			X										
Klamath					X								X
Lake			X										X
Linn								X	X				
Marion									X				
Morrow								X					
Umatilla				X	X			X				X	
Union			X	X	X		X	X	X	X	X	X	
Wallowa	X	X	X	X	X	X	X	X	X	X	X	X	
Wasco								X	X			X	
Wheeler	X		X					X	X	X		X	
<b>WASHINGTON COUNTIES</b>													
Chelan										X			
Ferry	X		X	X		X				X	X		
King											X		
Mason	X												
Okanogan	X		X							X			
Pend Oreille	X		X	X						X	X		
Pierce	X												
Snohomish											X		
Stevens	X		X	X						X	X		
Whatcom	X										X		

## **G. Population Trends**

As discussed above in the section on life history characteristics, long-term demographic studies of moonworts reveal that population numbers are quite variable; above ground moonwort populations fluctuate independently within and between populations, as well as between years and between different sites. Agency botanists have inventoried and monitored individual populations of moonworts on the individual units within the analysis area since the early 1990s. Study methods, results, and findings vary greatly.

## **H. Habitat (Farrar 2006)**

Habitats for each taxon are described in the appendices. Although the information from sighting forms displayed on Table 6 indicates that most moonwort species occur over a wide range of habitats and landforms, Dr. Farrar states that “We have tended to describe habitats with too broad an approach. Many of the characters listed in Table 6 are not reflecting the important microhabitat characters.” Dr. Farrar has developed the following criteria to circumscribe moonwort habitats with at least 80% success. *Botrychium campestre*, *B. crenulatum*, *B. montanum* and *B. pumicola* are specialists and are even more predictable.

1. With the exception of *B. montanum* (and *B. pumicola*) moonworts tend to occur in areas of disturbance that are from 10 to 30 years old. This includes old roads and roadsides, picnic and camping grounds, pastured meadows, avalanche meadows, etc. We seldom find moonworts in abundance under mature old growth forests without recent disturbance.
2. Moonworts tend to occur in soil derived from calcareous bedrock and in hardwater seeps and fens. Moonworts seldom occur on soils derived from granites or other acid rocks excepts in areas of hardwater seeps.
3. Moonworts tend to occur in areas where some mineral soil is exposed or has been exposed within the last 10 -30 years. This probably has to do with the ability of arriving spores to percolate into the soil and perhaps also with the establishment and ecology of the appropriate mycorrhizal fungi.
4. As a result of 1, 2 & 3, moonworts tend to occur in disturbed habitats. Management activities, including grazing, that maintain these conditions maintain moonwort populations. With succession to dense, closed canopy conditions moonwort populations decline.

Table 6. Habitats of moonwort species in Oregon and Washington tallied from element occurrences maintained by the Washington Natural Heritage Program and Oregon Natural Heritage Information Center (WHNPS 2002, ORNHIC 2002).

TAXA	<i>B. ascendens</i>	<i>B. campestre</i>	<i>B. crenulatum</i>	<i>B. hesperium</i>	<i>B. lanceolatum</i>	<i>B. lineare</i>	<i>B. lunaria</i>	<i>B. manganense</i>	<i>B. montanum</i>	<i>B. paradoxum</i>	<i>B. pedunculosum</i>	<i>B. pinnatum</i>	<i>B. pumicola</i>
<b>HABITAT</b>													
Coniferous Forest	X		X	X	X	X	X	X	X	X	X	X	X
Shrubland	X			X						X			X
Dry Meadow	X			X	X			X	X	X	X	X	
Moist Meadow	X	X	X	X	X	X	X	X	X	X	X	X	
Bog													
Fen			X										
Intermittent Stream	X		X					X	X				
Perennial Stream	X		X	X	X	X		X	X	X	X	X	
Seep/Spring	X		X		X		X	X	X	X	X	X	
Alpine fellfield			X		X								X
Subalpine Mdw			X					X		X			
Roadside/Roadbed	X		X	X	X	X	X	X	X	X	X		X
<b>LANDFORM</b>													
Ridgetop													X
Upper Slope					X		X	X	X			X	
Mid Slope	X		X	X	X		X	X	X	X	X	X	X
Lower Slope	X	X	X	X	X	X	X	X	X	X	X	X	X
Alluvial Fan	X			X	X	X	X	X		X	X	X	X
Bench			X		X		X	X	X	X	X	X	
Saddle											X		
Basin	X		X	X				X	X	X	X	X	X
Draw	X		X		X			X	X			X	X
Ravine	X		X						X	X	X		X
Stream Terrace	X		X	X	X	X	X	X	X	X	X	X	
Floodplain	X		X	X	X	X		X	X	X	X	X	
Plateau					X			X	X				X
Moraine								X	X				
Glacial Cirque	X												

Using these criteria Farrar has had increasing success in detecting moonwort populations throughout the western mountains of the US and Canada, as well as the Great Lakes Region, for most species. Certain species are further restricted in their habitats:

*Botrychium campestre* occurs in undisturbed native bunchgrass prairies developed on calcareous bedrock or glacial till, in areas where mineral soil is exposed continuously or occasionally by fire.

*B. crenulatum* requires nearly permanent moisture, often occurring in saturated hardwater fens and seeps.

*B. montanum* occurs under mature old growth cedars, but these conditions also provide a calcareous substrate and a fine-textured litter similar to bare soil to which spores can percolate.

*B. pumicola* occurs in non-acidic volcanic ash and pumice that provides bare mineral soil and early successional plant communities.

So, if a different set of characteristics is used, the habitat becomes much more predictable than what is implied by using the characteristics in Table 6. With a few exceptions a high (80%) predictability is gained by thinking of moonworts as species which follow disturbance on moist, but well-drained calcareous soil.

### **I. Ecological Considerations** (Farrar 2006)

Disturbance plays a central role in the presence or absence of moonworts at a site. A few species of moonworts, e.g., *B. montanum*, are found primarily in mature or old-growth forests, and some, e.g., *B. campestre*, are found in native midgrass prairies. However, most field researchers familiar with moonworts report an affinity of most species to past (10 to 30-year-old) disturbances. These disturbances may be either natural, such as avalanche chutes, scree slopes, and back beaches, or anthropogenic, such as roadsides, old logging roads, campgrounds, and summer-grazed pastures.

The recently or periodically disturbed sites that support moonworts have several characteristics in common. They support vegetation that is in an early stage of succession, often composed of a rich mixture of native and non-native herbaceous perennials. They have a generous surface exposure of mineral soil (20% or more). They often have a compacted soil. They have a more or less perennially moist soil, but one well-drained due to slope position or soil type (e.g., high in sand or gravel). The best sites for most species (*B. simplex* is an exception) have a soil developed from limestone or other calcareous bedrock and with a near-neutral pH.

We do not know how these site characteristics interact to provide support for moonworts. *Botrychium* species have an erect subterranean stem that grows ever closer to the surface throughout the life of the plant. It is possible that spores of moonworts need exposed mineral soil in order to be transported sufficiently deep into the soil for optimal development of the gametophyte, for fertilization, and for subsequent development of the sporophyte. Development from spore to first production of above-ground leaves of *Botrychium* requires 5 to 10 years. If

colonization of a new site is by only a few individuals, it may take 10 to 20 years before the new colony attains a size sufficiently large to be detected.

*Botrychium* species are also dependent upon establishment and maintenance of a mycorrhizal relationship with an endophytic fungus. The fungus supplies water, minerals, and carbohydrates (obtained from its connection with other photosynthetic plants) to the moonwort. Although germination of moonwort spores occurs, gametophyte and subsequent sporophyte growth and development will not occur without the mycorrhizal connection. Therefore, germination and development requirements of the fungal partner must also be considered. Possibly exposure of mineral soil is important for fungal spores to percolate to the appropriate depth as well.

Johnson-Groh (2002) has summarized ecological considerations of moonworts regarding competition, herbivory, disease, and invasive worms. Although some of this information is from moonwort studies conducted outside Oregon and Washington, it is pertinent to the species in this assessment.

### 1) Competition

There are no documented reports of inter- or intraspecific *Botrychium* competition. The occurrence of moonworts in genus communities is common and has been documented (Wagner and Wagner 1983a). Mixed species assemblages of moonworts are common in a diversity of habitats. *Botrychium* are known to occur in densely vegetated grasslands and sparsely vegetated beaches or sand dunes. As such they are not easily classified as good or bad competitors, but because moonworts are basically parasites on other species via the mycorrhizae, it is not competition in the usual sense (Farrar 2006). As with many aspects of *Botrychium* life history competitive interactions are likely mediated by mycorrhizae and vary from habitat to habitat.

### 2) Herbivory

Herbivory is common in moonworts (Montgomery 1990, Johnson-Groh and Farrar 1993 and 1996b, Kelly 1994, Lesica and Ahlenslager 1996). Montgomery (1990) noted herbivory ranged from 12-91%. Damage recorded by Johnson-Groh and Lee (2002) varied from a small amount of trophophore or sporophore tissue removed to the extreme of being totally eaten, leaving only a short stump. They note that modest herbivory usually left the sporophore or trophophore apparently functional.

Insects may be a primary herbivore of moonworts. Johnson-Groh (unpublished data) has observed larvae “cocoons” embedded in the trophophore on a wide variety of species. Herbivory of the whole plant also could result from grazing by rodents or even ungulates. Farrar and Johnson-Groh have a lot of documentation of the whole plant having been eaten, but what hasn’t been documented is which critter did it. Wagner et al. (1985) and Zika (1995) have speculated that spores may benefit from ingestion by being dispersed through animal feces.

### 3) Invasive Worms

Because of glaciation northern landscapes have evolved in the absence of earthworms and subsequent colonization by native earthworms that survived south of glaciation has been slow (James 1998). European worms have arrived in northern landscapes following introductions through imported trees (soil balled around roots) and further spread through horticulture and fishing practices. Whereas there is extensive data on the effects of earthworms on soil properties

of agricultural settings there is little data on their impact in native forested ecosystems. Emerging data indicates that earthworms can significantly impact the duff and litter layers of forest soils (Groffman et al. 2000, Hale pers. comm.. 2001). Johnson-Groh has recorded the impacts of European earthworms on population of *Botrychium mormo* in Minnesota, where previously healthy populations have failed to return any individuals following an earthworm invasion.

#### 4) Disease

Few observations are recorded with regard to diseased *Botrychium* plants. Johnson-Groh and Lee (2002) noted that a few abnormally developed plants appeared to be diseased. They also observed some plants that appeared to be the result of mechanical damage and numerous plants with no visible damage that failed to complete development. They postulate that arrested development probably occurred due to inadequate resources (water, mycorrhizae, time), belowground herbivory or disease.

## IV. Conservation

### A. Threats

Identification of threats is somewhat challenging for moonworts since so much information is still needed on habitat requirements, environmental tolerances and the effects of management. For the purpose of this assessment, threats to moonworts in Oregon and Washington are actions that alter existing site characteristics (Hopkins et al. 2001; ORNHIC 2002; Potash 1998a and 1998b; WNHP 2002; Zika 1992a, 1992b, 1994, and 1995), including actions that would change the microclimate, canopy coverage, hydrology, or mycorrhizal association on a site. Since moonworts are tiny and inconspicuous, ground disturbance, such as trampling or burial due to surface erosion deposits, may negatively impact them (Potash 1998a and 1998b).

Six major actions that result in observed or suspected threats to *Botrychium* species in Oregon and Washington (Table 7) were compiled from element occurrences maintained by the Oregon Natural Heritage Information Center and Washington Natural Heritage Program (ORNHIC 2002, WHNPS 2002). These actions include livestock grazing and trampling; off-road vehicle use; camping and hiking; timber harvest and firewood cutting; exotic plant invasion and herbicide treatment; succession to closed canopy (fire suppression); and road widening and maintenance. Although fire (prescribed or wildland) was not listed as a threat by the Heritage Programs for any of the Oregon and Washington occurrences, it was added for discussion in this document, since fire is often perceived as a threat to vascular plants.

Although soil compaction was identified as a negative impact from element occurrence information, Farrar (2006) would replace ‘soil compaction’ with ‘physical destruction of plants:’ “Soil compaction is often listed as a threat, but I don’t think it is really a problem. Moonworts seem to have an amazing ability to push through the most compacted soils we can imagine, including parking lots, picnic and camping areas, road sides (in gravel and cracks in asphalt) old roads and trails. The only known site for *B. lineare* in Alaska is in an off-road vehicle trail. Rather than compaction, the major threat from logging and other vehicular activities is the actual physical disturbance of the soil that breaks root and mycorrhizae connections or otherwise uproots the moonwort plants.”

### 1) Livestock Grazing and Trampling

No formal studies have been conducted on the impact of grazing though there are numerous anecdotal observations. Most moonworts clearly tolerate and may benefit from some level of grazing, however it is unknown how much and how frequently they will tolerate it. Farrar (2006) states,

“It is important to note, that because moonworts are found in grazed sites, this does not imply that more or less grazing is better.

Removing grazing or increasing grazing cannot be expected to maintain populations. Cessation of grazing could also be listed as a threat, as moonworts are probably at a site because of the appropriate level of disturbance and the prevention of succession that the livestock provide.”

The removal of leaf tissue likely has little impact on the plant (see sections on herbivory and life history) on an annual basis. The impact of repeated annual harvest of the leaves is unknown. Timing of grazing will also have an impact. Grazing early will effectively remove the annual input of spores into the spore bank. The tolerance of moonworts to compaction associated with grazing or wildlife wallowing is also unknown. Grazing animals also have the potential to introduce exotic plants to moonwort sites (Zika 1992b, 1994, and 1995).

### 2) Off Road Vehicle Damage, Camping, and Hiking

Recreational activities in areas of rare moonworts have the potential to negatively impact them through ground disturbance, plant removal, sedimentation, and the introduction of exotic plants. Recreational activities by alpine hikers, mountain bikers, or off road vehicles have been observed to damage exposed fronds of *Botrychium pumicola* (Hopkins et al. 2001).

### 3) Timber Harvest and Firewood Cutting

The impacts of timber harvest on moonworts include disruption of the O-horizon, changes in light, and loss of soil nutrients and moisture, but it is not known if they negatively affect *Botrychiums*. Although soil compaction is often listed as an effect of timber harvest and other vehicle activities on sensitive plant sighting forms, consider that moonworts grow in old road beds. Thus, it's hard to think of compaction as a problem. Physical disruption of the soil is the greatest threat (Farrar 2006).

The magnitude of the impact depends on the harvest methods (clear-cutting, thinning, single tree selection, group selection, or salvage sales). No studies on the impact of timber harvest have been completed though Casson et al. (2001) reports several anecdotal observations of the negative response of plants following harvest. Zika (1992b) cites logging in riparian areas and firewood cutting as threats to rare moonworts on the Mt. Hood National Forest. In addition, changes to hydrology is another concern regarding timber harvesting.

Table 7. Perceived threats to *Botrychium* species in Oregon and Washington, as recorded from element occurrences maintained by the Oregon Natural Heritage Information Center and Washington Natural Heritage Program (ORNHC 2002, WHNPS 2002).

THREATS	Livestock Grazing & Trampling	Off Road Vehicle Use, Camping and Hiking	Timber Harvest and Firewood Cutting	Exotic Plant Invasion and Herbicide Treatments	Succession to Closed Canopy (Fire Suppression)	Road Widening & Maintenance
<b>TAXA</b>						
<i>B. ascendens</i>	X	X		X	X	X
<i>B. campestre</i>	X	X		X		
<i>B. crenulatum</i>	X	X	X	X	X	X
<i>B. hesperium</i>	X	X		X		
<i>B. lanceolatum</i>	X	X	X		X	X
<i>B. lineare</i>	X	X	X	X	X	
<i>B. lunaria</i>	X	X	X	X		
<i>B. minganense</i>	X	X	X	X	X	X
<i>B. montanum</i>	X		X		X	
<i>B. paradoxum</i>	X	X	X	X		
<i>B. pedunculosum</i>	X	X	X	X	X	X
<i>B. pinnatum</i>	X	X	X		X	X
<i>B. pumicola</i>	X	X	X			

4) Exotic Plant Invasion and Herbicide Treatment

Zika (1992 b and 1994) and Zika et al. (1995) include competition from exotic plant invasion as a threat to rare moonworts in the Columbia Basin. A population of *Botrychium gallicomontanum* in a native Minnesota prairie was sprayed with the herbicide Roundup. Thirteen days after the herbicide had been applied newly discovered moonworts were yellowed and deformed revealing obvious signs of damage. Two permanent plots were established in 1997 and have been monitored annually for the long-term effects of the herbicide (Johnson-Groh unpublished data). In 1998, very few of the plants tagged in 1997 reappeared, but there were 36 new plants that had not been present in 1997 when the plots were sprayed. Plants underground either as juvenile sporophytes, which have not yet emerged, or, as dormant adult sporophytes at the time of herbicide application, probably were not affected by the herbicide. These “new” recruits are typical of moonwort populations and will likely sustain the population despite one year of herbicide application.

5) Succession to Closed Canopy (Fire Suppression)

Succession to a closed canopy is likely to negatively impact moonwort species commonly found in meadows, roadsides or other open habitats. Based on the affinity of several species to occur in the open, it seems likely that succession to closed canopy will significantly alter the habitat

and thereby threaten extant populations. It is unknown whether the plants (and mycorrhizae) are responding to changes in light, competition or moisture that accompany a closed canopy. Succession to a closed canopy is a likely effect of taking meadows out of grazing.

The conservation strategy for *Botrychium pumicola* (Hopkins et al. 2001) identified habitat conditions and/or activities that posed threats to the long-term viability of *B. pumicola*. The absence of natural fire has created closed canopies at many sites. Heavy fuel accumulations, litter buildups, and vegetative competition and shade, particularly at montane sites, may be suppressing *B. pumicola*.

#### 6) Road Widening and Maintenance

Since almost all of the moonworts covered in this assessment are known to occur in roadbeds, maintenance and widening of roads may directly impact these populations. In both road widening and maintenance, direct mortality of plants may occur; however, these actions may also create additional habitat in the future by providing a periodic disturbance, particularly in areas adjacent to (but not within) populations.

#### 7) Fire

Fire in and of itself is not detrimental to moonworts (Johnson-Groh and Farrar 1996a). The leaves brown, but unless the fire is intense moonworts don't burn because of their succulent nature. Johnson-Groh and Farrar (1996a) compared burned and unburned prairie plots and found similar return in both treatments in subsequent years. Fires do not directly damage prairie moonwort populations. Severely scorched or wilted plants return the successive year and actually sometimes show an increase in size. The loss of the photosynthetic capacity the year of the fire is no different than non-emergence for a year. They concluded that normal (not excessively hot or dry) burns pose no serious threat to moonworts. However they noted that an exceptionally hot burn or one that comes when the soil is desiccated is harmful. Fires that are hot and stationary are likely to cause damage by killing the plant outright or indirectly by killing the mycorrhizae. Johnson-Groh and Farrar (1996a) note that leaf loss due to fire does little harm to the plant. Moreover the concurrent effects of fire such as damaged tissue, desiccation or sedimentation may be more important.

The indirect effects of fire, desiccation, and sedimentation negatively affect moonworts. Moonwort populations decline following droughts (Muller 1992) and it is probable that increased exposure and drying from fire result in declines. Decline due to fire desiccation is probably negligible unless compounded by drought, high amounts of herbivory, or other limiting factors.

Sedimentation resulting from burns, appears to have a significant effect on moonworts located down slope. Johnson-Groh and Farrar (1996a) found plants buried up to 4 cm deep following fire. Sedimentation in an Iowa prairie following fire buried 51% of the plot tags. Preliminary results from Amsberry and Meinke (2002) involving burial of *B. pumicola* in a manipulation study indicate a detrimental impact. For three years following the treatment no plants have returned in the buried plots.

Recovery from burial is slow and depends on the depth of burial (Johnson-Groh unpublished data). A Minnesota plot supporting 33 *B. gallicomontanum* was partially buried (10 cm) by gopher activity. Subsequently the population declined to a low of 8 individuals two years

following burial, but gradually increased in subsequent years. The population returned to its original size (34 plants) eleven years following the burial (Johnson-Groh unpublished data).

## **B. Conservation Status**

Ninety-eight percent of the occurrences of these 13 species of moonworts are on federal lands (ORNHIC 2002, WNHP 2002). Although this is probably a function of where surveys are conducted, rather than where plants actually occur, it is a consideration when addressing project effects, land exchanges with known rare *Botrychium* occurrences or potential habitat, or permitting the collection of Special Forest Products (Potash 1998b). The population trends of these species are unknown in Oregon and Washington.

In the absence of population trends for these species, we cannot determine if existing protections (land use allocations, Northwest Forest Plan Standard and Guidelines, agency best management practices, etc.) are in need of supplementation. With the existence of what appears to be unoccupied habitat, we would conclude that habitats vary greatly in their capacity to support these species. Through the work of Drs. Don Farrar and Cindy Johnson-Groh we are just beginning to understand the life history and ecology of these species. We do not know enough to suggest that populations are especially vulnerable to habitat change or other changes in the environment.

With the dependence on mycorrhiza, these species may be inherently vulnerable to habitat changes. Mapping of available habitat and occupied habitat is needed to evaluate whether the habitat used by these species is declining and whether current management is placing demands on these species. We do not know enough about the population trends of these species to say whether or not there is evidence that populations on federal lands in the analysis area or particular portions of it, are at risk. In the analysis area the FS and BLM manages sufficient habitat to influence conservation outcomes.

## **C. Known Management Approaches**

Little is known about the maintenance and manipulation of moonwort populations. Even when statistically rigorous long-term monitoring is implemented, population trends for *Botrychium* are very difficult to interpret in any way that is meaningful for the agency land manager at the field level. There are often such drastic variations in the vigor of individual populations and the number of individuals within that population, that a trend in one direction or another might be a temporary phenomenon.

Very little active manipulation to enhance sustainability of populations has been tried within the analysis area. Even when site manipulation is implemented in a rigorous scientific manner, interpretation of treatment effectiveness will be very difficult at best, since populations fluctuate so widely over time (Johnson-Groh 2001).

Chen et al. (1995) quantified distances of edge influence within forests for several microclimatic variables and assessed the influence of edge effects in relation to aspect, time of day, microorganisms, litter, and woody debris. They described gradients from a clear-cut edge to the interior for air temperature, soil temperature, relative humidity, and soil moisture.

Edge effects generally ranged from 180-240 m (540-720 ft.) into the forest for air temperature, 60-120 m (180-360 ft.) for soil temperature, 240 m (720 ft.) for relative humidity, and 0-90 m (0-270 ft. for soil moisture. These distances may provide land managers with the approximate area needed to maintain the appropriate habitat conditions for moonworts associated with forested habitats. Site conditions and timber harvest levels will have different influences on microclimatic conditions. For example, managed areas that have a natural break in the topography such as ridgeline could be smaller than those areas where an occurrence is adjacent to disturbed locations such as roads or clear-cuts. Slope and aspect are important considerations as well.

#### **D. Management Considerations**

Farrar (pers.comm. 2006) states,

From either anecdotal observations or from quantitative monitoring data, many researchers have noted the decline of specific populations of moonworts. Why these declines in observable (above-ground) plants occur and whether they are indicative of population extinction (plants could be dormant underground awaiting return of suitable conditions) are unknown. Until demonstrated otherwise, it is prudent to assume, for conservation management, that conditions supportive of *Botrychium* have permanently deteriorated. This could result from reproductive failure at any stage from spore production through germination, development and maturation. The failure could be due to changes in soil exposure, soil moisture, soil chemistry, and/or changes in the vegetation that affect either the moonworts or their mycorrhizal fungus. They could also result from introduction of pathogens or predators to either the *Botrychium* or the fungus.

Despite all of these unknown factors, we can assume that healthy, vigorous populations are so because of current and/or recently past (10 – 20 yr.) environmental conditions. For conservation management then, it is prudent to examine the current and recently past management that has supported the development of these populations and attempt to continue that management, including periodic or continuing disturbances. Managers should not assume that “protection” from such disturbances is appropriate. Change from recent management may indeed constitute a threat to the population.

Even with our best efforts to conserve them, some, or even most, existing populations of moonworts may become extinct. This is the nature of species dependent upon disturbance and seral stages of community succession. *Botrychium* species may always have existed in metapopulation dynamics where population extinction is balanced with founding of new populations. If this is the case, then conservation management must also include maintenance of suitable, but unoccupied habitat that will be available for colonization by spores and the development of new populations during the window of time that those sites are supportive of moonwort species. For this strategy to succeed, it is also critical to maintain existing populations to the best of our ability. They are the source of spores that will create new populations.

#### ***General considerations***

Applying Farrar’s comments above, the overarching management consideration in addressing the maintenance of known sites is to continue the level and type of disturbance that has supported the population over the last decade (Farrar 2006).

Continue to maintain habitat in the same way that allowed moonworts to establish. For all but *Botrychium pumicola* and *B. montanum*, this includes maintaining and encouraging a 10-30 year disturbance cycle.

In addition, the following general considerations may be considered when addressing the management of known sites/populations:

1. Maintain the light regime, hydrology (hydrologic flow and water table level), habitat, and microclimatic conditions, including existing amount of canopy closure.
2. Maintain conditions which enhance mycorrhizal diversity, such as above ground plant biomass, hydrology, and soil nutrition.
3. Avoid disturbance of above ground plants and the substrate in the area, including the duff layer and the collection of special forest products (e.g. moss), to minimize impacts to the below ground plants.
4. Maintain early to mid-successional plant communities.

Conservation approaches for moonworts should include the maintenance of stable populations, and address the maintenance of suitable unoccupied habitat for moonworts to inhabit.

Monitoring strategies should include periodic surveys for new populations.

Table 8 is a compilation of more specific management considerations addressing each of the threats identified in this Conservation Assessment. These considerations were previously identified in Region 6 FS draft management plans (Zika 1992b, 1994, and 1995), Survey and Manage management recommendations for *Botrychium montanum* and *B. minganense* (Potash 1998a and 1998b) and the conservation strategy for *Botrychium pumicola* (Hopkins et al. 2001).

Table 8. Threats, potential direct and indirect impacts to known sites, and management considerations for rare moonworts in Oregon and Washington (Farrar 2006<sup>1</sup>; Hopkins et al. 2001; ORNHIC 2002; Potash 1998a, 1998b; WNHP 2002; Zika 1992b, 1994, and 1995).

<i>Threats</i>	<i>Impacts</i>	<i>Management Considerations</i>
Livestock Grazing	<ul style="list-style-type: none"> <li>• Trampling</li> <li>• Introduction of noxious weeds</li> <li>• Displacement of soil</li> <li>• Burial by surface deposition</li> </ul>	<p>Consider fencing or other control measures to:</p> <ul style="list-style-type: none"> <li>• minimize or avoid direct impacts that crush plants, such as trampling or grazing</li> <li>• avoid excessive siltation or deposition of soil</li> </ul> <p>If supplemental feed is brought in, encourage the use of weed free feed.</p> <p>Remove noxious weeds at earliest notice; if possible, encourage stock to be brought in from weed free areas.</p> <p>Minimize variations in the number, timing, etc., of grazing animals.</p>
ORV Damage, Camping, and	<ul style="list-style-type: none"> <li>• Displacement of soil</li> <li>• Burial by surface deposition</li> </ul>	<p>Consider signage to deter use of areas with known sites. Rock and berm placement can also be considered to</p>

Recreational Trampling	<ul style="list-style-type: none"> <li>• Introduction of noxious weeds</li> </ul>	<ul style="list-style-type: none"> <li>• discourage or avoid excessive siltation or deposition of soil</li> <li>• minimize the establishment of competing exotic vegetation</li> </ul> <p>For OHV use, encourage tire and vehicle washing prior to being used near areas with known sites, to discourage weed invasion.</p> <p>Balance encouraging human use against carrying capacity and projected needs, costs, and the biological impacts on moonworts (Zika 1994 and 1995).</p>
Timber Harvest and Firewood Cutting	<ul style="list-style-type: none"> <li>• Changes to canopy cover</li> <li>• Changes to hydrology</li> <li>• Changes to mycorrhiza</li> <li>• Displacement of soil</li> <li>• Burial by surface deposition</li> <li>• Introduction of noxious weeds</li> </ul>	<p>Consider managing known sites during timber harvest activities in such a way so as to:</p> <ul style="list-style-type: none"> <li>• Avoid direct impacts that crush plants, such as road construction or reconstruction, yarding, moving of equipment or trampling</li> <li>• Maintain microclimate (air and soil temperature, soil moisture, and existing level of canopy cover)</li> <li>• Minimize disturbing the duff layer</li> <li>• Maintain existing hydrologic regime</li> <li>• Avoid excessive siltation or deposition of soil</li> <li>• Avoid actions contributing to the establishment of competing exotic vegetation</li> </ul>
Exotic Plants and Herbicides	<ul style="list-style-type: none"> <li>• Habitat degradation</li> <li>• Direct mortality of plants (herbicide application)</li> </ul>	<p>Actively remove exotic plants taking care to minimize potential impacts to moonworts</p>
<b><i>Threats</i></b>	<b><i>Impacts</i></b>	<b><i>Management Considerations</i></b>
Succession to Closed Canopy (Fire Suppression)	<ul style="list-style-type: none"> <li>• Changes in canopy cover</li> <li>• Heavy fuel accumulations</li> <li>• Litter build-up</li> <li>• Vegetative competition</li> </ul>	<p>Utilize active management (hand thinning, brush removal, piling and burning of fuels etc.) to maintain the habitat until more is known about the impact of succession to a closed canopy</p> <ul style="list-style-type: none"> <li>• Maintain vegetation in an early to mid-stage of plant succession</li> </ul>
Prescribed Fire	<ul style="list-style-type: none"> <li>• Changes to canopy cover</li> <li>• Changes to hydrology</li> </ul>	<ul style="list-style-type: none"> <li>• Avoid direct impacts that crush plants, such as moving of</li> </ul>

	<ul style="list-style-type: none"> <li>• Changes to mycorrhiza</li> <li>• Displacement of soil</li> <li>• Burial by surface deposition</li> <li>• Introduction of noxious weeds</li> </ul>	<p>equipment or trampling</p> <ul style="list-style-type: none"> <li>• Maintain existing level of canopy cover</li> <li>• Maintain microclimate (air and soil temperature, soil moisture)</li> <li>• Minimize disturbing duff layer</li> <li>• Maintain existing hydrologic regime</li> <li>• Avoid excessive siltation or deposition of soil</li> <li>• Avoid actions contributing to the establishment of competing exotic vegetation</li> </ul>
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## V. Research, Inventory, and Monitoring Opportunities

Inventories of potential habitat for moonworts on individual units within the analysis area have been conducted since the early 1990s. Population trend monitoring on National Forests has also been carried out using a variety of study methods (Ahrensleger pers. comm. 2005, Gehring and Potash 1996, Hafer pers. comm. 2004, Johnson-Groh pers. comm. 2004, Potash et al. 2004, Powers pers. comm. 2004, Raven 1997, Smith pers. comm. 2004, Stein pers. comm. 2004). Results have been highly variable and interpretation of these results to draw meaningful conclusions has been difficult for a number of reasons.

### A. Data and Information Gaps

Management questions for consideration by research and monitoring include:

- What specific site characteristics are necessary to maintain existing occurrences? What factors affect recruitment, plant growth, reproduction, and population structure? What microclimate/microsite conditions favor survival, growth, and reproduction of moonworts?
- How can we identify high likelihood habitats for these species in order to prioritize inventory efforts or to ensure habitat conservation? Moonworts may be common in one site and apparently absent from seemingly similar sites.
- What is the nature of the relationship between these species and their fungal symbiont? What are the habitat requirements for the mycorrhiza? What soil environment is needed to support the mycorrhiza? What factors impact this mycorrhizal relationship? How is the relationship between moonworts and mycorrhiza influenced by native and introduced fauna?
- Is there a correlation between the previous year's weather (e.g., dry winter/wet spring) and high or low population counts during the summer?
- What level of disturbance (overstory removal, grazing, fire, etc.) do these species need or tolerate?
  - Some sites are flat benches associated with old river or stream terraces where soils are alluvial in origin. Are periodic floods part of the natural disturbance regime at these sites and, if so, what is the effect this disturbance has on moonworts?

- How do populations vary in livestock grazed and ungrazed sites? How does native grazing (by meadow voles, rabbits, or elk) differ from livestock grazing? Do native grazers affect spore production and dispersal differently? Do native grazer population cycles exert an influence on moonwort reproduction? Large native ungulates are being managed at high levels for hunting in some areas. How is this affecting *Botrychium* microsites? How much livestock grazing is too much/enough?
- What is the fire ecology of moonworts?

## **B. Inventories and Monitoring**

Moonworts present several problems in applying conventional monitoring techniques. First is the difficulty of finding these small cryptic plants. Most moonworts are quite small and often overlooked. Crawling on hands and knees and parting the vegetation and/or litter is the best means of locating plants, but this is time consuming and doesn't allow accurate surveys of large areas. The ephemeral nature of the plants also makes it difficult to assess populations. Long-term demographic studies (15 years) of *Botrychium* reveal that population numbers are quite variable (Johnson-Groh 1997). Above ground *Botrychium* numbers fluctuate independently within and between sites, as well as between years.

Identification of moonworts is difficult (Zika 1995). Herbarium specimens are frequently misidentified, poorly prepared, and lack an adequate sample size. Each occurrence may have numerous young or depauperate forms, as well as natural variation. There are relatively few characters available to defining and recognizing species. Plants grow in mixed species groups, which leads to mixed collections on herbarium sheets.

Species ranges are also incompletely known. Despite these problems, with practice it is possible to identify nearly all plants. However, there will often be some plants in a population that are insufficiently developed to allow identifications from morphology alone (Farrar 2006).

Another significant problem relates to the life cycle of *Botrychium*, relatively little of which is visible above ground (Figure 1). Following emergence above ground, plants generally produce one leaf annually, but it is common for moonwort plants to remain dormant below ground in a given year and produce no above ground leaf (Johnson-Groh 1997).

In addition to these below ground stages, some species reproduce asexually via below ground gemmae. The presence of vegetative reproduction greatly influences the population dynamics of these gemmiferous species. It is common in the field to see two or more leaves of gemmiferous moonworts emerging in close proximity. Excavation of these clusters usually reveals a large number of below ground sporophytes in various stages of development.

*Botrychium* have a relatively short period of emergence annually. Permanent plots represent a population sample from which true population size estimates are made. If sampling was done at a time before all plants had emerged or after some had senesced, a false estimate of the population size is derived. Understanding how the population changes over the season allows more accurate estimates of population sizes, and thus more accurate assessments of the rarity of these species.

Status and trends can not be determined and evaluated for *Botrychium* species until unified protocols are developed that adequately address these unique problems. Because of the characteristics noted above (phenology, below ground ecology of plants, small size, and erratic appearance above ground) conventional monitoring techniques are less effective. The USFS Inventory and Monitoring Issue Team will establish inventory and monitoring protocols within the agency (Stensvold pers. comm. 2005). Their action plan will ensure that scientifically credible sampling, data collection, and analysis protocols are used in all inventory and monitoring activities. Expected products from this effort are species protocols, which will establish standardized inventory and monitoring approaches.

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