Botrychium hesperium (western moonwort)
A Technical Conservation Assessment

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

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COVER PHOTO CREDIT

Summary of Key Components for Conservation of *Botrychium hesperium*

Status

Botrychium hesperium (western moonwort) is known from approximately 33 locations in Region 2 of the USDA Forest Service (29 on USDA Forest Service lands). Three of these populations have not been seen within the last 20 years. Of the locations for which we have population size data, the total number of plants is estimated between 300 and 400. Other occurrences probably support significant numbers, but due to the lack of data for those sites, the total population size in Region 2 is not known. Some populations in Region 2 that have been identified as *B. hesperium* may be *B. 'michiganense'*, which has not been formally described but is known to occur in Wyoming and South Dakota, and possibly Colorado. *Botrychium hesperium* is not designated as a sensitive species in Region 2, but it is considered a sensitive species in Region 1. This species is ranked vulnerable (G3G4) by NatureServe. Within Region 2, it is known only from Colorado, where it is ranked imperiled (S2). *Botrychium hesperium* has no federal status.

Primary Threats

Observations and quantitative data have shown that there are several tangible threats to the persistence of *Botrychium hesperium*. The primary threats to *B. hesperium* are habitat loss, recreation, succession, overgrazing, the inherent effects of a small population size, sedimentation, timber harvest, exotic species invasion, global climate change, and pollution. However, these threats and their hierarchy are highly speculative because there is very little known about this species in Region 2. Because most of the known populations in Region 2 are small, they are threatened by stochastic processes.

Primary Conservation Elements, Management Implications and Considerations

Twenty-nine of the 33 known populations in Region 2 have been documented on land managed by the USDA Forest Service. The other four populations are known from Rocky Mountain National Park. At least one population is also partially located on private land. Three populations have not been seen in over 20 years, and 11 others were last seen in the 1980s. At present, conservation efforts focused on protecting the known populations of *Botrychium hesperium* in Region 2 are more likely to be effective than restoration efforts. Restoration of populations of members of *Botrychium* subgenus *Botrychium* is probably precluded by the great difficulties in propagating the species. Further species inventory efforts are needed to better understand the full range of *B. hesperium*. Research is needed to investigate the belowground life history, ecology, reproductive biology, the role of mycorrhizae, and the role of disturbance in the autecology of *B. hesperium* so that conservation efforts on its behalf can be most effective.

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Introduction

This assessment is one of many being produced to support the Species Conservation Project for the Rocky Mountain Region (Region 2) of the USDA Forest Service. *Botrychium hesperium* is the focus of an assessment because it is a species of concern in Region 2 due to its infrequent occurrence in localized areas and small population size. It is designated a sensitive species in Regions 1 and 6. Within the National Forest System, a sensitive species is a plant or animal whose population viability is identified as a concern by a Regional Forester because of significant current or predicted downward trends in abundance or in habitat capability that would reduce its distribution (FSM 2670.5(19)). Sensitive species and species of concern may require special management so knowledge of their biology and ecology is critical.

This assessment addresses the biology of *Botrychium hesperium* throughout its range in Region 2. This introduction defines the goal of the assessment, outlines its scope, and describes the process used in its production.

Goal of Assessment

Species assessments produced as part of the Species Conservation Project are designed to provide forest managers, research biologists, and the public with a thorough discussion of the biology, ecology, conservation status, and management of certain species, based on available scientific knowledge. The assessment goals limit the scope of the work to critical summaries of scientific knowledge, discussion of broad implications of that knowledge, and outlines of information needs. The assessment does not seek to develop or provide specific management recommendations. However, it does provide the ecological background upon which management must be based, and it focuses on the consequences of changes in the environment that result from management (i.e. management implications). Furthermore, it cites management recommendations proposed outside of Region 2 and examines the success of management plan implementations both within and outside of Region 2.

Scope of Assessment

This assessment examines the biology, ecology, conservation status, and management of *Botrychium hesperium* with specific reference to the geographic and ecological characteristics of the Rocky Mountain Region. Where supporting literature used to produce this assessment originated from investigations outside the region, this document places that literature in the ecological and social context of the central Rockies. Similarly, this assessment is concerned with reproductive behavior, population dynamics, and other characteristics of *B. hesperium* in the context of the current environment rather than under historical conditions. The evolutionary environment of the species is considered in conducting the synthesis, but in a current context.

In producing the assessment, refereed literature, non-refereed publications, research reports, and data accumulated by resource management agencies were reviewed. Not all material was considered equally reliable. The assessment emphasizes refereed literature because this is the accepted standard in science. Non-refereed publications or reports were used in the assessment only when information was unavailable elsewhere, and they were regarded with greater skepticism. Unpublished data (e.g. Natural Heritage Program [NHP] records) were important in assessing the geographic distribution, population size, and habitat of this species. These data required special attention because of the diversity of persons and methods used in collection. When no information was available regarding a particular issue, experts were contacted or information regarding closely related taxa was used to make inferences.

Treatment of Uncertainty in Assessment

Science represents a rigorous, systematic approach to obtaining knowledge. Competing ideas regarding how the world works are measured against observations. However, because our descriptions of the world are always incomplete and our observations are limited, science focuses on approaches for dealing with uncertainty. A commonly accepted approach to science is based on a progression of critical experiments to develop strong inference (Platt 1964). However, it is difficult to conduct experiments that produce clean results in the ecological sciences. Often, observations, inference, good thinking, and models must be relied on to guide our understanding of ecological relations. Confronting uncertainty then is not prescriptive. In this assessment, the strength of evidence for particular ideas is noted, and alternative explanations are described when appropriate.

At the time of this writing, *Botrychium hesperium* is in the process of being split into two taxa, *B. hesperium* and *B. 'michiganense'* (Farrar personal communication

2002). Much information currently cited as pertaining to *B. hesperium* may in fact pertain to *B. 'michiganense'*, and both of these taxa occur in Region 2 (Farrar personal communication 2002). Thus, we have distinguished between *B. hesperium* and *B. 'michiganense'* where possible in this report, but because of the overlapping ranges of these entities, difficulties involved in distinguishing them from one another, and the lack of certainty regarding the identity of most occurrences, we necessarily treat them collectively most of the time. At some time when the new taxon has been described and experts have determined the correct identity of plants in Region 2 and elsewhere, this assessment will need to be revised, probably as two documents (one for *B. hesperium* and one for *B. 'michiganense'*). The rarity of these taxa will also need to be reassessed, since the split will result in fewer occurrences of *B. hesperium*, and the number of *B. 'michiganense'* populations will be less than the currently known number of *B. hesperium* and *B. 'michiganense'* populations combined.

Publication of Assessment on the World Wide Web

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

Peer Review

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

MANAGEMENT STATUS AND NATURAL HISTORY

Management Status

Botrychium hesperium is not currently listed as a sensitive species in Region 2 of the USDA Forest Service (USDA Forest Service 2003), nor is it included on the Bureau of Land Management Colorado State Sensitive Species List. However, all *Botrychium* species have attracted much attention over the last decade from federal and state agencies (Johnson-Groh and Farrar 2003). *Botrychium hesperium* is considered a sensitive species in Regions 1 and 6 of the USDA Forest Service. *Botrychium 'michiganense'* is listed as sensitive in Region 9. *Botrychium 'fenestratum'*, a variant of *B. hesperium* that has not been formally described, is also considered sensitive in Region 6 (see Classification and Description section). *Botrychium hesperium* is not listed as Threatened or Endangered in accordance with the Endangered Species Act. It is not listed as endangered or vulnerable by the International Union for Conservation of Nature and Natural Resources (1978). *Botrychium hesperium* is considered threatened by the state heritage program in Michigan, where it is now recognized to be *B. 'michiganense'*; it is considered sensitive by the state heritage program in Washington (**Table 1**). NatureServe considers *B. hesperium* to be globally vulnerable (G3G4) because it occurs infrequently in localized areas, and it has small population sizes over a large geographical range (NatureServe 2003). In the United States it is ranked nationally imperiled (N2N3), and it is considered imperiled (N2?) in Canada. Within Region 2, *B. hesperium* is considered imperiled (S2) in Colorado. See the Distribution and Abundance section of this document for a summary of subnational ranks and status of *B. hesperium* outside of Region 2. When *B. 'michiganense'* is formally described, many state/province ranks, and perhaps the global rank of *B. hesperium* as well, will likely need revision. For explanations of NatureServe's ranking system, see the **Definitions** section of this document.

Table 1. Summary of the known global distribution and status of *Botrychium hesperium* and *B. 'michiganense'*. Region 2 states are in bold. See **Definitions** section for explanation of Rank codes.

Country	State/Province	S rank	State/Province Designation	Notes
Canada	Alberta	SU		*Botrychium hesperium* and *B. 'michiganense'* are ranked SU until the relative abundance of these taxa is resolved (Gould personal communication 2001).
Canada	British Columbia	S1S3		*Botrychium hesperium*
Canada	Ontario	S1		*Botrychium 'michiganense'*
Canada	Saskatchewan	S1		*Botrychium hesperium*
United States	Arizona	SR		*Botrychium hesperium*
United States	**Colorado**	**S2**		*Botrychium hesperium* **and possibly** *B. 'michiganense'*
United States	Idaho	no rank		*Botrychium 'michiganense'* (Reported in Farrar 2001)
United States	Michigan	S2	threatened	*Botrychium 'michiganense'*
United States	Montana	S2		*Botrychium hesperium*
United States	Oregon	S?		*Botrychium hesperium* (recently discovered by Zika et al. 2002)
United States	**South Dakota**	**no rank**		*Botrychium 'michiganense'*
United States	Utah	S1		*Botrychium hesperium*
United States	Washington	S1	sensitive	*Botrychium hesperium* and *B. 'michiganense'*
United States	**Wyoming**	**SR**		*Botrychium 'michiganense'*

Existing Regulatory Mechanisms, Management Plans, and Conservation Strategies

Adequacy of current laws and regulations

Botrychium hesperium has no legal protection unto itself that would prevent the destruction of habitat or individuals. *Botrychium hesperium* is not listed as threatened or endangered in accordance with the Endangered Species Act; therefore, there are no federal laws concerned specifically with its conservation. Because it is listed on Sensitive Species Lists in Regions 1 and 6, regional foresters must give consideration to this species so as to maintain its habitat and population persistence in those regions (see USDA Forest Service Document 2600). For example, biological evaluations are required for sensitive species in every project undergoing National Environmental Policy Act analysis. *Botrychium 'michiganense'* is a sensitive species in Region 9. Because *B. hesperium* is not a designated sensitive species in Region 2, it does not receive protection as such on USDA Forest Service lands there. As of this writing, a conservation strategy has not been written for this species at a national or regional level by the USDA Forest Service or any other federal agency. Given the rarity (small population size and small number of known populations) of *B. hesperium* in Region 2 and the ongoing human impacts to individuals and habitat, current laws and regulations are probably inadequate to conserve this species.

Adequacy of current enforcement of laws and regulations

There have been no known cases since this species was recognized in which an occurrence was extirpated due to human activities or the failure to enforce any existing regulations. However, this does not necessarily indicate that current regulations or their enforcement are adequate for its protection. Because there are no laws or regulations in Region 2 that offer any protection to *Botrychium hesperium*, the adequacy of enforcement cannot be assessed. See the Threats section of this document for an assessment of threats that may warrant consideration if laws or regulations are drafted in the future to address concerns for *B. hesperium*.

Biology and Ecology

Classification and description

Botrychium hesperium is a member of the adder's tongue family (Ophioglossaceae). The Ophioglossaceae family is comprised of three genera: *Ophioglossum*, *Cheiroglossa*, and *Botrychium* (Lellinger 1985, Wagner and Wagner 1993). *Botrychium* (grapeferns) is the most diverse of these genera with 50-60 species (Wagner and Wagner 1993). The genus *Botrychium* contains three subgenera: *Osmundopteris*, *Sceptridium*, and *Botrychium* (Wagner and Wagner 1993). Subgenus *Botrychium* (moonworts), which contains *Botrychium hesperium*, is the most diverse of the three subgenera with perhaps 25 to 30 species. See **Table 2** for an overview of the classification of *B. hesperium*.

Table 2. Classification of *Botrychium hesperium* after USDA Natural Resource Conservation Service (2002), with sources (not necessarily the original source) of particular portions cited below.

Kingdom	Plantae (Plants)
Subkingdom	Tracheobionta (Vascular Plants)
Division	Pteridophyta (Ferns)
Class	Filicopsida
Order	Ophioglossales
Family	Ophioglossaceae (Adder's Tongue Family)
Genus	*Botrychium* (Grapeferns)
Subgenus	*Botrychium*[1], *Eubotrychium*[2] (Moonworts)
Species	*Botrychium hesperium* Wagner and Wagner[1, 3]

[1]Wagner and Wagner 1993
[2]Clausen 1938
[3]Wagner and Wagner 1983b

Members of subgenus *Botrychium* are morphologically simple, and subtle interspecific differences make field identification difficult. In addition, members of this subgenus often grow together in genus communities (Wagner and Wagner 1983a). Morphological and genetic analyses of genus communities have demonstrated that hybridization rarely occurs and most hybrids have abortive spores (Wagner and Wagner 1983a, Wagner et al. 1984, Wagner and Wagner 1986) thus evincing the presence of multiple species in these genus communities rather than intraspecific variants. The cryptic nature of *Botrychium* species has made it difficult to circumscribe species (Paris et al. 1989, Wagner 1998, Hauk and Haufler 1999).

The diversity of the genus *Botrychium* in North America did not begin to be recognized until 1977 when Drs. Herb and Florence Wagner began work in earnest on *Botrychium* (Wagner 1993). Thirty-two species of *Botrychium* are currently described (Wagner and Wagner 1994) as compared to six in 1938 (Clausen 1938).

There has been much confusion regarding the recognition of *Botrychium hesperium* as a species. It was considered a subspecies of the widespread *B. matricariifolium* by early authors (e.g., Clausen 1938, Harrington 1954). It was first described by Wagner and Lellinger as a hybrid (*B.* x *hesperium*) in 1981 with *B. lanceolatum* and *B. simplex* as the purported parents (Lellinger 1981). Wagner and Wagner (1983b) then described *B. hesperium* and *B. echo* as "nothospecies" (species of hybrid origin).

The taxon currently recognized as *Botrychium hesperium* apparently includes two species, and it will soon be split by Drs. Florence Wagner and Don Farrar (Farrar personal communication 2002). Dr. Herb Wagner was in the process of splitting *B. hesperium* after recognizing consistent morphological differences in the taxon (Wagner and Wagner 1990). These differences were originally recognized in plants from the Great Lakes region, leading to the name *B. 'michiganense'* coined by Dr. Herb Wagner under which the new taxon will be described (Farrar personal communication 2002). *Botrychium* '*michiganense'* has been found in western North America in Wyoming, Washington, northern Idaho, Alberta, and possibly Colorado (Farrar 2001, Farrar personal communication 2002, Gould personal communication 2002, Heidel personal communication 2002, Farrar personal communication 2004, Root personal communication 2004). *Botrychium lanceolatum* is involved in the parentage of *B. 'michiganense'*, but the other parent is unclear (Farrar 2001).

Farrar considers the northern plants called *Botrychium 'fenestratum'* and *B. 'venulosum'* to be merely variants within *B. hesperium* (Farrar 2001, Farrar personal communication 2002). *Botrychium 'fenestratum'* is known from Oregon and Washington, while *B. 'venulosum'* has been documented from Montana (Farrar 2001). Farrar (2001) notes that *B. 'fenestratum'* grades morphologically into *B. hesperium*, and that no differences in allozyme patterns are evident between *B. 'fenestratum'*, *B. 'venulosum'*, and *B. hesperium* in enzyme electrophoretic analyses.

Botrychium species can be extremely difficult to identify, due to their subtle diagnostic characters, the frequent co-occurrence of multiple *Botrychium* species at a location, and the high morphological variability within populations. Positive identification requires comparison with silhouette outlines of verified specimens (such as those presented in Wagner and Wagner 1986) and use of dichotomous keys (see Weber and Wittmann 2001).

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

Botrychium hesperium averages 12 (5-20) cm in height. The features of the sterile portion of the leaf blade (trophophore) are valuable diagnostic characteristics for this and other *Botrychium* species. It has a short stalk, usually 0 to 3 mm long. The fertile blade (sporophore), on the other hand, is relatively tall, up to twice the length of the sterile blade. On live plants the trophophore is dull gray-green in color (Wagner and Wagner 1993, Chadde and Kudray 2001), which is a useful characteristic for field identification (Root personal communication 2002). The trophophore is oblong-linear to deltate, once or twice-pinnate, 2.5 (1-5) centimeters long, and firm (Wagner and Wagner

1984, Wagner and Wagner 1993). There may be up to six pairs of pinnae, usually crowded or overlapping, and broadly attached to a relatively wide rachis. The basal pair of pinnae are often much larger and more divided than the others and oblong-lanceolate with irregularly lobed margins. Often there is slightly more distance between the 1st and 2nd pinna pairs than between the 2nd and 3rd pinna pairs (Wagner and Wagner 1993). Northern *B. hesperium* plants are smaller and look like young plants from southern populations; this may be a response to colder growing season temperatures in the northern portion of its range (Wagner and Wagner 1983b). *Botrychium hesperium* is known to produce gemmae (Johnson-Groh et al. 2002), which are minute, spheric asexual propagules borne at the root bases of the sporophyte (Farrar and Johnson-Groh 1990, Wagner and Wagner 1993). *Botrychium hesperium* is an allotetraploid with a chromosome number of 4n=180 (Wagner 1993).

Botrychium 'michiganense' has several morphological differences from *B. hesperium* that are mentioned in Wagner and Wagner (1990). In general, *B. 'michiganense'* is larger and narrower than *B. hesperium* (Wagner and Wagner 1990). The pinnae are also more remote and have wedge-shaped bases (Farrar 2001). The trophophore segments in *B. hesperium* are relatively blunt, while those of *B. 'michiganense'* are sharper. The basal pinnae have a tendency to be strongly enlarged, and the sporophore has three main axes in *B. 'michiganense'* (Wagner and Wagner 1990). However, this is often true of *B. hesperium* as well and is not as valuable for diagnostic purposes. *Botrychium 'michiganense'* differs genetically from *B. hesperium* (Farrar personal communication 2002).

Botrychium hesperium and *B. 'michiganense'* are easily confused with *B. matricariifolium* (Higman and Penskar 1999). However, the range of *B. hesperium* sensu stricto will no longer overlap with *B. matricariifolium* after *B. 'michiganense'* is described (USDA Natural Resource Conservation Service 2003). *Botrychium hesperium* is also easily confused with *B. pinnatum* and *B. pedunculosum* and any member of the *matricariifolium* complex (Zika et al. 1995). *Botrychium hesperium* differs in chromosome number and other minor features from *B. pseudopinnatum* (n=135) (Wagner 1993, Zika et al. 1995). Farrar (personal communication 2002) also considers plants called *B. 'fenestratum'* and *B. 'venulosum'* to be conspecific with *B. hesperium*. These plants have a larger and more fully developed trophophore with more deeply-lobed pinnae than typical *B. hesperium* (Farrar 2001). Morphological intergradation and allozyme patterns indicate that plants thus described are best interpreted as variants within *B. hesperium* (Farrar 2001).

In Region 2, *Botrychium hesperium* is likely to be found with *B. echo*, from which it can be distinguished in the field by features of the trophophore as described in Wagner and Wagner (1983b). Distinguishing these two species can be difficult, as evinced by the fact that they were long considered to be the same species (Wagner and Wagner 1983b). *Botrychium hesperium* tends to have pinnae that are approximate or overlapping, while *B. echo* tends to have well-separated, non-overlapping pinnae. *Botrychium hesperium* also has rounded pinna tips and basal pinnae that are much larger than the adjacent pinnae, while *B. echo* tends to have pointed pinna tips and basal pinnae that are subequal to adjacent pinnae. *Botrychium echo* is shiny yellow-green when fresh, while *B. hesperium* is dull gray-green. This difference in color can be obscured late in the season when the leaves start fading and turning yellow (Root personal communication 2003). See Wagner and Wagner (1983b) for further details regarding the differences between *B. hesperium* and *B. echo*. **Figure 1** is reproduced from this paper and well illustrates the diagnostic differences in the trophophores of *B. hesperium* and *B. echo*.

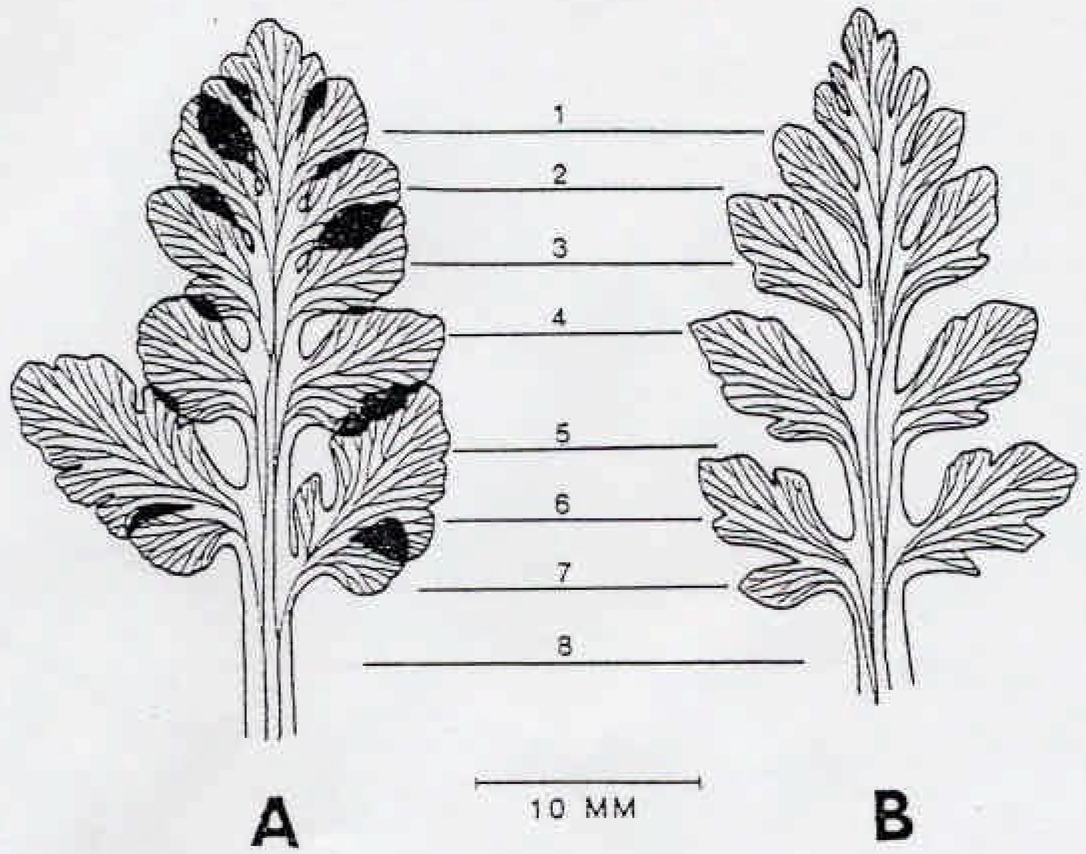

FIG. 4. Comparison of *B. hesperium* (A) and *B. echo* (B) from a mixed population at San Francisco Peaks, AZ. Tracings of cleared sterile blades. The characters are: 1 = ovate, broadly attached vs. linear-lanceolate, narrowly attached pinnae. 2 = Overlapping vs. separated pinnae. 3 = Repand vs. subentire margins. 4 = Rounded vs. pointed pinna tips. 5 = Basal pinnae much larger than vs. subequal to adjacent pinnae. 6 = Basal pinnae erect and subclasping vs. spreading and non-clasping. 7 = Basal pinnae with broad, overlapping basal lobe vs. with narrow separated basal lobe. 8 = Stalk long vs. short.

Figure 1. Comparison of diagnostic characteristics between *Botrychium hesperium* and *B. echo* from Wagner and Wagner (1983b).

Several sources are available for further technical information on *Botrychium hesperium*. See Kershaw et al. (2001) and Chadde and Kudray (2001) for photos, drawings, and descriptions. The original description can be found in Wagner and Wagner (1983b) with silhouettes of plants. Silhouettes are also presented for *B. hesperium*, *B. 'fenestratum'*, and *B. 'michiganense'* in Farrar (2001) and Mantas and Wirt (1995). The Flora of North America (Wagner and Wagner 1993) also offers a full description and a small range map of the species. For a description of differences between *B. hesperium* and *B. 'michiganense'* see Wagner and Wagner (1990). See **Figure 2** for a photo of *B. hesperium*.

Figure 2. *Botrychium hesperium*. Photo provided by Loraine Yeatts.

Distribution and abundance

While *Botrychium hesperium* is infrequent and localized in its occurrence, it has a large geographical range across North America. It is known from many states and provinces throughout northern North America (**Table 1**). The PLANTS Database lists Arizona, Montana, Michigan, Utah, Wyoming, Washington, and Colorado (USDA Natural Resource Conservation Service 2003). NatureServe (2003) lists the above states as well as Oregon, and the provinces of British Columbia, Ontario, and Saskatchewan. *Botrychium hesperium* (cited in the reference as *B. fenestratum*) was found in Washington in 1996 by Dr. Herb Wagner (Mehaffey 1996). It was also recently discovered in Oregon (Zika et al. 2002). Since it is found near the southern border of Colorado, this species probably also occurs in the mountains of northern New Mexico (Root personal communication 2003). Given the difficulties in finding occurrences of *B. hesperium*, it is likely that further range expansions will be discovered in future survey work.

Both *Botrychium hesperium* and *B. 'michiganense'* are present in Alberta (Gould personal communication 2002), Washington (Farrar 2001), and possibly Colorado (Farrar personal communication 2002). Farrar (personal communication 2002) has confirmed that all occurrences in Michigan and Ontario are *B. 'michiganense',* and thus these locations must be removed from range maps for *B. hesperium* such as the one in Flora of North America (Wagner and Wagner 1993). Region 9 of the USDA Forest Service has acknowledged this range revision and now lists *B. 'michiganense'* on its sensitive species list.

Botrychium hesperium is found mainly in mountainous areas of the west, with Montana and Colorado having relatively high numbers of known occurrences (NatureServe 2003). However, data on abundance are sparse, and the known populations are small (**Table 3**). The known populations in Region 2 range in size from one individual to somewhere between 50 and 100 individuals, with most reports documenting between four and 20 plants (**Table 3**). There are currently 33 populations known in Region 2 from Colorado Natural Heritage Program element occurrence records (Colorado Natural Heritage Program 2003), herbarium specimens, and other reports (**Figure 3**). Within Region 2, *B. hesperium* has thus far been found only in Colorado. Twenty-nine of the 33 occurrences are on USDA Forest Service lands (Arapaho-Roosevelt, Rio Grande, Routt, San Isabel, San Juan, and White River national forests). *Botrychium 'michiganense'* has been reported from a single location in Wyoming (Heider personal communication 2002, Wyoming Natural Diversity Database 2002) and from a single location in South Dakota (Crook personal communication 2003, Farrar personal communication 2004).

Table 3. Summary information for the known occurrences of *Botrychium hesperium* in Region 2 (all occurrences are in Colorado).

County	Element Occurrence Number	Location	Date of Last Recorded Observation	Estimated Abundance	Management Area/ Ownership	Elevation	Habitat	Collector/ Observer	Herbarium
Archuleta	29	Nipple Mountain	8/1/2001	less than 100	San Juan National Forest	10,360-10,480 ft.	Old clear-cut area, with young spruce.	P. Lyon	Not Available (N/A)
Boulder	3	Caribou Townsite	6/29/1989	not reported	Arapaho-Roosevelt National Forest, Boulder Ranger District	10,000 ft.	Gravelly hillside.	Root et al. (89-12)	Kalmbach Herbarium (Denver Botanic Garden)
	14	Glacier Lake	4/20/1921	not reported	Arapaho-Roosevelt National Forest, Boulder Ranger District and private	9,120 ft.	Dry, decomposed granite.	Bethel and Clokey (3987)	Oklahoma State University, University of Colorado Herbarium
	30	Mitchell Lake Trailhead	8/3/2000	3 to 5	Arapaho-Roosevelt National Forest, Boulder Ranger District	10,480 ft.	On berm in a parking lot. Tree cover: 5 to 7%; shrub cover: 0%; forb cover: 30%; graminoid cover: 10%; moss/ lichen cover: 0%; bare ground cover: 50%. Associated plant community: small spruce trees (10 to 15 years old). Subalpine, disturbed site. Aspect: East. Slope: 25%. Shape: Convex. Light exposure: open. Moisture: dry. Geomorphic land form: glaciated mountain slopes surrounding valley bottom. Soil texture: coarse, crubly, decomposing granite.	A. Peterson and Baker	N/A
	N/A	Coney Flats	8/11/1999	not reported	Arapaho-Roosevelt National Forest, Boulder Ranger District	9,900 ft.	Throughout the open moist meadow.	D. Steinmann (s.n.)	University of Colorado Herbarium
Clear Creek	2	Warrior Mountain	1980	20	Arapaho-Roosevelt National Forest, Clear Creek Ranger District	11,120 ft.	Not reported.	P. Root	N/A
	5	Juniper Pass Picnic Ground	7/14/1989	not reported	Arapaho-Roosevelt National Forest, Clear Creek Ranger District	11,020 ft.	Not reported.	P. Root (89-19)	Root Personal Collection
	6	Warrior Mountain Picnic Ground	8/30/1985	"abundant"	Arapaho-Roosevelt National Forest, Clear Creek Ranger District	11,270 ft.	In open area.	P. Root (85-61, 85-30, 85-35)	Kalmbach Herbarium (Denver Botanic Garden), Root Personal Collection
	7	Echo Lake Campground	8/3/1984	not reported	Arapaho-Roosevelt National Forest, Clear Creek Ranger District	10,560 ft.	Not reported.	Root (84-28)	Root Personal Collection

County	Element Occurrence Number	Location	Date of Last Recorded Observation	Estimated Abundance	Management Area/ Ownership	Elevation	Habitat	Collector/ Observer	Herbarium
Clear Creek	8	Warrior Mountain	8/3/1985	not reported	Arapaho-Roosevelt National Forest, Clear Creek Ranger District	11,080 ft.	Not reported.	Root (85-35)	Kalmbach Herbarium (Denver Botanic Garden)
	17	Devil's Canyon	8/15/1995	not reported	Arapaho-Roosevelt National Forest, Clear Creek Ranger District	10,040 ft.	Adjacent to a large meadow that was cleared with stumps still visible. Roadside areas of lodgepole regeneration stand. Light exposure: open. Topographic position: lower slope. Moisture: dry to moist.	S.T. Gill, K. Heiny, and K. Lindsay	N/A
Conejos	12	Spruce Hole Road	9/2/1985	not reported	Rio Grande National Forest, Conejo Peak Ranger District	10,400 ft.	Spruce-fir meadows.	Dixon (4691)	Adams State College
	13	Lookout Mountain	7/26/1987	not reported	Rio Grande National Forest, Conejo Peak Ranger District	10,500 ft.	Spruce-fir.	Dixon (5096)	Adams State College
	21	Platoro Reservoir	9/10/1998	3	Rio Grande National Forest, Conejo Peak Ranger District	10,400 ft.	On a roadcut. Habitat type: *Picea engelmannii/Vaccinium myrtillus*. Tree cover: trace; shrub cover: 0%; forb cover: 10%; graminoid cover: 2%; moss/lichen cover: trace; bare ground cover: 88%. Aspect: Southeast. Slope: 60%. Slope shape: straight. Light exposure: open. Topographic position: midslope. Moisture: dry. Parent material: tsl - summitville andesite (oligocene). Aphanitic to sparsely porphyritic dark andesite flows and breccias within and locally outside the platoro caldera. Geomorphic landform: glaciated mountain slopes. Soil texture: fine sandy loam.	D. Erhard	N/A
Huerfano	28	Blue Lake	7/22/1999	50 or more	San Isabel National Forest	11,280 ft.	Forb cover: 90%; bare ground cover: 10%. Habitat type: clearing in spruce forest. Aspect: North. Slope: level. Light exposure: open. Moisture: dry. Parent material: glacial till.	P. Root	N/A
Jackson	N/A	Cameron Pass	8/1/1986	not reported	Probably Routt National Forest	10,280 ft.	Disturbed soil along old vehicle tracks	P. Root (86-284) and J.D. Montgomery	University of Colorado Herbarium
Lake	26	Arkansas River Headwaters	9/12/2000	30 to 50	Probably San Isabel National Forest	11,100 ft.	Along railroad tracks. Light exposure: open. Moisture: dry.	A. Kolb and T. Spribille	N/A
	N/A	May Queen Campground	7/9/1998	40	San Isabel National Forest	9,900 ft.	On a boulder-strewn slope (~30 degrees) with grasses and forbs, underneath a powerline.	W. Hauk	N/A

County	Element Occurrence Number	Location	Date of Last Recorded Observation	Estimated Abundance	Management Area/ Ownership	Elevation	Habitat	Collector/ Observer	Herbarium
Larimer	1	Glacier Knobs	6/26/1987	10	Rocky Mountain National Park	9,850 ft.	Scattered on 10 square meters. Flat rock outcrop in thin soil cover. Geology: Gneiss outcrops and cliffs, decomposed gneiss and organic soil, flat	Root (87-11)	University of Colorado Herbarium, Kalmbach Herbarium (Denver Botanic Garden)
	4	Lawn Lake Trail	7/17/1987	20	Rocky Mountain National Park	10,680 ft.	Disturbed trailside through small meadow, scattered along disturbed trail edge ca. 30 spuare meters. Geology: granite. Soil: gravel and cobbles. Slope: flat.	Root (87-44)	Kalmbach Herbarium (Denver Botanic Garden), University of Colorado Herbarium, Rocky Mountain National Park
	9	Loch Vale Trail	8/27/1960	not reported	Rocky Mountain National Park	9,560 ft.	In small grassy plots on the dry cut bank of the horse trail. Always associated with Linnaea.	Weber (8650), Willard and Porsild (6062, 6063)	University of Colorado Herbarium
	N/A	Trail Ridge	7/17/1987	~20	Rocky Mountain National Park	10,800 ft.	On disturbed trailside through small meadow, among gravel and cobbles.	Root (87-37)	University of Colorado Herbarium
Mineral	19	Wolf Creek Pass	7/16/1998	4	San Juan National Forest, Divide Ranger District	11,000 ft.	Tree cover: trace; shrub cover: trace; forb cover: 15%; graminoid cover: 20%; moss/lichen cover: 0%; bare ground: 65%. Habitat type: *Picea engelmannii-Abies lasiocarpa/ Vaccinium myrtillus*. Aspect: NNE. Slope: 15%. Slope shape: concave. Light exposure: open. Topographic position: midslope. Moisture: dry. Parent material: unknown volcanics. Geomorphic landform: glaciated mountain slope. Soil texture: fine sandy loam. There is a perennial stream right next to this site which has an abundance of *Corydalis caseana* in it. In fact, this Corydalis species is quite common all over the ski area.	D. Erhard and S. Hartvigsen	N/A

County	Element Occurrence Number	Location	Date of Last Recorded Observation	Estimated Abundance	Management Area/ Ownership	Elevation	Habitat	Collector/ Observer	Herbarium
Mineral	22	Alberta Park	5/28/1999	14	San Juan National Forest, Divide Ranger District	10,280 ft.	Habitat type: *Picea engelmannii/ Vaccinium myrtillus*. Tree cover: trace; shrub cover: trace; forb cover: 15%; graminoid cover: 5%; moss/ lichen cover: 0%; bare ground cover: 80% (bare ground, gravel cobble). Aspect: Southeast. Slope: 40%. Slope shape: straight. Light exposure: open. Topographic position: lowerslope. Moisture: dry. Parent material: San Juan volcanics. Geomorphic landform: glaciated mountain slopes. Soil texture: fine sandy loam.	D. Erhard and S. Hartvigsen	N/A
Rio Grande	20	Fivemile Park	9/16/1998	4	San Juan National Forest, Divide Ranger District	11,000 ft.	Habitat type: Engelmann spruce/*H11*. Tree cover: 1%; shrub cover: 0%; forb cover: 30%; graminoid cover: 10%; moss/lichen cover: 4%; bare ground cover: 55%. Aspect: Southeast. Slope: 10%. Slope shape: straight. Light exposure: open. Topographic position: midslope. Moisture: dry. Parent material: tlp – Los Pinos formation (pliocene and oligocene). Mostly reworked bedded conglomerates, sandstones, and mud flow breccias containing rhyodacite and quartz latite clasts derived from volcanics of green ridge and rhyodacite of park creek. Geomorphic landform: glaciated mountain slope. Soil texture: fine sandy loam.	D. Erhard	N/A
Saguache	23	Lookout Mountain	9/18/1998	1	Rio Grande National Forest, Saguache Ranger District	10,600 ft.	On old logging spur road. Habitat type: *Picea engelmannii/Juniperus communis*. Tree cover: 2%; shrub cover: trace; forb cover: 20%; graminoid cover: 20%; moss/lichen cover: 10%; bare ground cover: 48%. Aspect: North. Slope: 10%. Slope shape: straight. Light exposure: open. Topographic position: midslope. Moisture: dry. Parent material: tcv - conejos formation (oligocene). Vent facies; flows and flow breccias of mostly porphyritic andesite and rhyodacite. Geomorphic land form: glaciated mountain slope. Soil texture: fine sandy loam.	D. Erhard	N/A

County	Element Occurrence Number	Location	Date of Last Recorded Observation	Estimated Abundance	Management Area/ Ownership	Elevation	Habitat	Collector/ Observer	Herbarium
San Juan	15	Molas Pass	8/4/1994	unknown (est. 100 or more plants of five species)	San Juan National Forest	10,845 ft.	Tree cover: 1% *Picea engelmannii*. Shrub cover: 1% Salix. Forb cover: 15%. Graminoid cover: 2%. Moss/ lichen cover: 1%. Total bare ground cover: 95%. Litter: 4%. Associated plant community: Engelmann spruce about 3 feet tall. Aspect: SW. Slope: 5%. Slope shape: Straight. Light exposure: open. Topographic position: midslope. Moisture: dry. Parent Material: sandstone. Geomorphic landform: glaciated mountain slope. Soil texture: sandy-silt.	L. Stewart (s.n.)	Dolores District Herbarium
	16	Lime Creek	July or Aug. 1994	1	San Juan National Forest, Columbine Ranger District	10,360 ft.	On pulloffs on both sides of highway. Habitat type: roadcut. Aspect: SW. Slope: 2%. Slope shape: straight. Light exposure: open. Tobographic position: flat roadcut- pulloff. Total forb cover: 30%. Moss/lichen cover: 2%. Total graminoid cover: 10%. Bare ground cover: 60%. Associated plant community: Heterotheca villosa (dominant) and other species.	L. Stewart	N/A
Summit	25	Copper Mountain	7/10/1998	10	White River National Forest	unknown	Across the highway from a parking area, under a telephone line. At the top of a steep east-facing slope in grass and forbs.	W. Hauk	N/A
	27	Vail Pass	7/22/1999	~50 or more	White River National Forest	11,400 ft.	Subalpine meadow with rivulet marked with willows and grasses. Downslope of subalpine forest of lodgepole and spruce -fir, on a southeast facing 15 to 35% slope. Organic and granite soils, cobbly, seasonally wet but becoming dry. Hillside is used by elk.	N. Redner	N/A
Summit/ Eagle	24	Shrine Pass	8/14/2000	~10	White River National Forest	11,120 ft.	Along road near ridge top, open gravelly slopes. Aspect: 224 degrees. Slope: 23%. Slope shape: convex. Light exposure: open. Moisture: dry.	Kolb and Spribille (2000)	N/A
Teller	18	Glen Cove Creek	7/7/1998	6	Pike National Forest	9,780 ft.	In a small opening in a lodgepole forest with scattered aspen in the drainage. NE facing aspect of a 30 to 45% slope. An ant hill is creating a more gravelly microhabitat. Gravel/moss/litter cover: 80%.	W. Hauk and K. Fayette	N/A

County	Element Occurrence Number	Location	Date of Last Recorded Observation	Estimated Abundance	Management Area/ Ownership	Elevation	Habitat	Collector/ Observer	Herbarium
Teller	Not Available (N/A)	Pikes Peak Highway	2000	~50 or more	Pike National Forest	11,085 to 11,275 ft.	Roadside areas that appear to have recovered from disturbance, in open meadows and near wetlands in the subalpine zone.	D. Steinmann (2000)	N/A

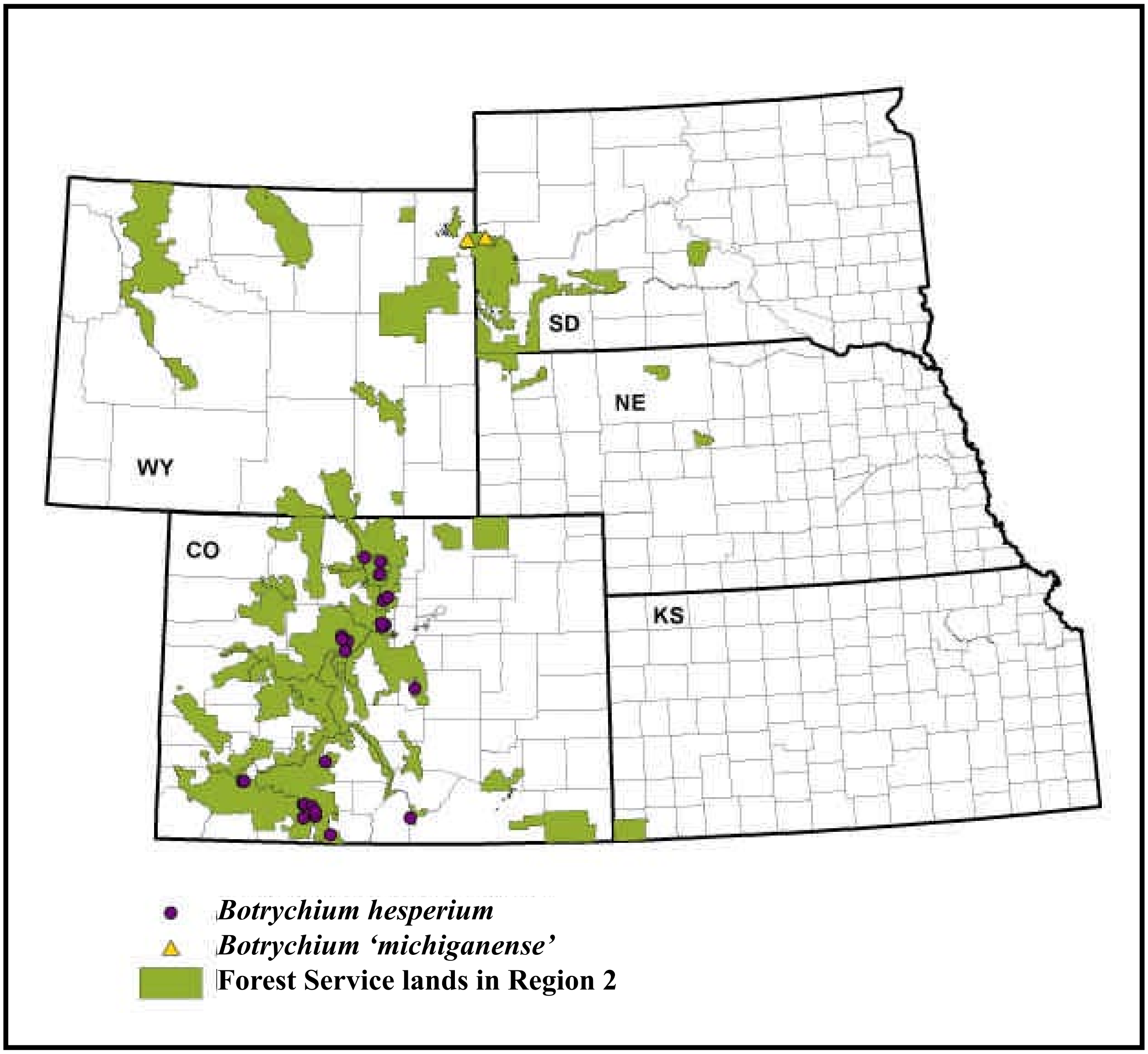

Figure 3. The known *Botrychium hesperium* and *B. 'michiganense'* populations in the states of Region 2.

Recent surveys have identified many new occurrences of *Botrychium hesperium* in Colorado. Large numbers of *B. hesperium* individuals have been found in recent work in Summit County, Colorado by Kolb and Spribille (2000), Buell (2001), and Thompson (2000 and 2001). Kolb and Spribille (2000) estimated that 26,000 *Botrychium* plants are present in Summit County, with 19,000 occurring in primarily anthropogenic habitats. The vast majority of these were the relatively common *B. lunaria*, but *B. echo* and *B. hesperium* comprised 4.15 percent (1079) and 2.18 percent (567), respectively, of the total *Botrychium* population in Summit County. Most of these data were derived from surveys done in and for ski areas on land leased from the USDA Forest Service. Dave Steinmann, Deborah Edwards, and Peter Root have found two populations (within ¼ mile of each other) of at least 50 individuals of *B. hesperium* and *B. echo* in a recent survey of the Pikes Peak Highway Recreation Corridor (Steinmann 2001a, Steinmann personal communication 2001) and one or more in the Indian Peaks Wilderness (Steinmann 2001b). Erhard and Hartvigsen of the USDA Forest Service have recently identified four populations from Mineral, Rio Grande, and Saguache counties. It thus appears likely that future surveys in Region 2 will yield further discoveries of *B. hesperium* populations.

Wagner and Wagner (1990) noted that eastern plants tend to differ significantly from western plants, and indeed all of the plants in Michigan and Ontario appear to be *Botrychium 'michiganense'*. The presence of *B. 'michiganense'* in Region 2 has also been confirmed (Farrar personal communication 2002, 2004). Many specimens collected in Region 2 remain to be annotated, so we are uncertain about the habitat, status, and ecological differences between these species. The only known occurrences of *B. 'michiganense'* in Region 2 are in Crook County, Wyoming at the Bearlodge Campground and in the Black Hills of South Dakota at Higgins Spring Gulch (Farrar personal communication 2002, 2004, Heidel personal communication 2002, Crook personal communication 2003). It may be in Colorado as well, although most Colorado material appears to be *B. hesperium* (Farrar personal communication 2004). Farrar has examined specimens in Root's personal collection and did not find any that appear to be *B. 'michiganense'* (Root personal communication 2004). Specimens collected at Glacier Lake in Boulder County (element occurrence record 14) by Bethel and Clokey (at University of Colorado) are somewhat suggestive of *B. 'michiganense'*. Further verification is needed, but this population is on private land and is apparently not accessible (Farrar personal communication 2004, Root personal communication 2004). Farrar (personal communication 2004) is planning to use isozyme data to help confirm the taxonomic status of material collected in Colorado.

In general, *Botrychium* populations in Region 2 tend to be highly disjunct, and this is certainly the case for *B. hesperium*. The known populations are scattered and are often separated by many miles.

Population trend

There are no data available from which Region 2 population trends for *Botrychium hesperium* can be determined. Very little work has been done following population trends in *Botrychium* species (but see Johnson-Groh 1998 and 1999). Populations show high variation in the number of emergent stalks among years (Johnson-Groh 1999, Root personal communication 2002, Johnson-Groh and Farrar 2003). Some plants and entire populations may not produce stalks every year (Johnson-Groh 1999, Root personal communication 2002). Drought may be the most significant factor determining stalk emergence for *Botrychium* species including *B. hesperium* (Lesica and Ahlenslager 1996, Johnson-Groh 1999).

Kolb and Spribille (2000) hypothesize that the abundance of *Botrychium* has increased in post-settlement times due to increased anthropogenic disturbance (associated with ski runs, roads, clear cuts, trails, mine sites, etc.). However, *Botrychium* habitat may have also decreased due to fire suppression and grazing of western grasslands and meadows. It should be stressed, however, that there are no data on the effects of fire or grazing on *B. hesperium*.

The only population trend data available from Colorado element occurrence records (Colorado Natural Heritage Program 2002) is a 1998 report that more individuals had been seen during a visit in 1987 to a site at Copper Mountain. However, the nature of this observation does not rule out the possibility that dormancy or search intensity is responsible for the observed difference.

Botrychium hesperium numbers in sample populations increased from 1990 to 1993 at Waterton Lakes National Park in Alberta, Canada (Lesica and Ahlenslager 1996). In this study, *B. hesperium* populations tended to be more stable than those of *B. paradoxum* and *B.* x *watertonense*.

Habitat

Botrychium hesperium occurs widely in western North America and is known from a wide range of habitat types. Little is known about its exact habitat associations and environmental tolerances, but its habitats tend to be early successional and subject to periodic disturbance (NatureServe 2003). Records of *B. hesperium* occurrences commonly cite the presence of coarse, gravelly soil and little or no tree cover. Grassy mountain slopes, snow fields, and road ditches with willows are listed as habitat types for *B. hesperium* in the Flora of North America (Wagner and Wagner 1993). Sand dunes are also included among its habitats in Wagner and Wagner (1993), but this probably refers to the Grand Sable and Sleeping Bear dunes in Michigan where plants then thought to be *B. hesperium* are now known to be *B. 'michiganense'* (Root personal communication 2003). Lellinger (1985) lists exposed areas, dry fields, and roadsides at high elevations among the habitats for *B. hesperium*. Some habitats listed on herbarium specimens collected in Colorado include gravelly hillsides, disturbed decomposing granite soil, and disturbed trailsides through meadows among gravel and cobbles. Element occurrence records from Colorado document populations on gneiss outcrops and cliffs, decomposed gneiss and organic soil slope, small openings in lodgepole forest, road cuts, next to a horse trail, and near an old fire ring (Colorado Natural Heritage Program 2002). Steinmann (2001a) found *B. hesperium* in open subalpine meadows. Natural habitats identified by Kolb and Spribille (2000), Thompson (2000), Thompson (2001), and Buell (2001) include areas where catastrophic fire has occurred, and persistent sites such as grassy or stony exposures near treeline in the krummholz zone and avalanche chutes. See **Table 3** for an overview of habitat descriptions for the populations in Region 2.

Botrychium hesperium occurs at high elevations in western North America. Wagner and Wagner (1993) indicate an elevation range of 200 to 2800 meters, but at the low end this is probably mostly *B. 'michiganense'*. Colorado element occurrence records document populations between 3002 and 3435 meters in elevation (Colorado Natural Heritage Program 2002). In Wyoming, *B. 'michiganense'* is found at 1524 meters (Wyoming Natural Diversity Database 2002). In South Dakota, *B. 'michiganense'* is found between 1500 and 1640 meters (Crook personal communication 2004).

There appear to be many associated species with which *Botrychium hesperium* is found. These species probably share affinities for habitats as well as mycorrhizal symbionts with *Botrychium* species. Zika et al. (1995) cite strawberry (*Fragaria* spp.) as a common associate of *Botrychium* in the Columbia River Basin, and they are common associates in Colorado as well (Kolb and Spribille 2001, Colorado Natural Heritage Program 2002). Peter Root (personal communication 2002) often finds *Botrychium* species with *Corydalis aurea* and *Solidago simplex* var. *nana* in Colorado, and Peggy Lyon (personal communication 2002) often finds them with *Senecio atratus* in Colorado. These species can be used to some extent as indicator species suggesting a high probability of the presence of *Botrychium* species in Colorado. Wagner and Wagner (1983b) suggest searching for *B. hesperium* and *B. echo* in the Rocky Mountains by looking in flat roadside ditches where there is gravelly soil dominated by *Picea* saplings and *Salix* shrubs. Associated species documented in Colorado element occurrence records include *Corydalis caseana*, *Fragaria virginiana*, *Senecio atratus*, *Thermopsis divaricarpa*, *Achillea lanulosa*, *Rosa woodsii*, *Heterotheca villosa*, and others. These are widespread taxa that tend to be found in sunny, open upland sites.

Botrychium hesperium is often found in genus communities with any of several other *Botrychium* species (Wagner and Wagner 1983a, Wagner and Wagner 1983b), such as *B. lanceolatum*, *B. echo*, and *B. minganense* (Wagner and Wagner 1983b, Colorado Natural Heritage Program 2002, Root personal communication 2002); *B. simplex*, *B. pallidum*, *B. pinnatum*, and *B. lunaria* are also noted with *B. hesperium* in surveys (Kolb and Spribille 2000) and Colorado element occurrence records (Colorado Natural Heritage Program 2002).

Habitats documented in Minnesota for *Botrychium 'michiganense'* include tailings ponds, gravel pits, ditches, an old log landing, and along a weedy roadside (Chadde and Kudray 2001) and herbaceous openings such as old homesteads and moist to dry brush fields (Wilfahrt 2001). In the Lake Superior area *B. 'michiganense'* often grows with *B. acuminatum* and *B. matricariifolium* (Wagner and Wagner 1986). In Wyoming, *B. 'michiganense'* is found in a *Pinus ponderosa* forest with *Populus tremuloides*.

Buell (2001) notes that *Botrychium* species have decidedly patchy, within-site distributions. The causes for this pattern are unknown, but it could be random or the result of patchy distributions of mycorrhizae or of other critical biotic or abiotic resources. The nature of *Botrychium* dispersal may also be random, resulting in a patchy distribution. Spores may be dispersed when mammals eat the fertile sporophytes (Wagner et al. 1985, Wagner 1998, F. Wagner personal communication 2002). Animal-mediated spore dispersal could account for concentrations of *Botrychium* species within a patch of suitable habitat. However, no studies have empirically demonstrated that this mode of dispersal effectively disperses any species of *Botrychium*. Buell (2001) noted many populations in Summit County, Colorado where it appeared as though water flowing downslope had dispersed spores to other locations along fall lines. More information pertaining to underground factors in conjunction with information on dispersal mechanisms will help elucidate the causes of patchy distribution patterns in *B. hesperium*.

Several habitat attributes are found commonly in occurrences of *Botrychium hesperium* and other *Botrychium* species in the mountains of Region 2. These are well summarized by Kolb and Spribille (2000) in their description of the *Festuco - Heterothecetum* community, in which *Botrychium* species were typically found in Summit County, Colorado. Sites were typically open, with much direct sunlight; well-drained; with 10 to 40 percent bare soil; with rock cover frequently 5 to 15 percent; on 20 to 30 percent non-southern slopes; historically disturbed; previously forested areas with a coniferous forest potential; often on calcareous substrates; usually at 3,210 to 3,510 meters elevation; and on compacted and eroded soils.

The periodicity of disturbance that is required for *Botrychium hesperium* and other *Botrychium* species is not known. To a large extent this probably controls the suitability of habitats for *B. hesperium*, as well as its metapopulation structure. Natural disturbance events that can create habitat for *B. hesperium* include flood, frost, landslide, and fire, while anthropogenic disturbances include bulldozer use, asphalt, clearcutting, ski run maintenance, and road maintenance (Colorado Natural Heritage Program 2002, Zika et al. 2002). Clearly the tempo and intensity of these disturbances varies greatly. Some, such as frost and ski run maintenance, have a shorter periodicity, while others such as clearcutting and floods are more catastrophic with a much longer periodicity. Clearly one unifying theme behind these disturbances is that they can create or maintain open conditions, which are apparently required by *B. hesperium*.

Johnson-Groh and Farrar (2003) suggest that sites that were disturbed approximately 10 years ago and then rested are most likely to support *Botrychium* populations. Buell (2001) found that *Botrychium* species were far more plentiful in areas that experienced disturbance (conversion to ski runs in most cases) more than 30 years ago but were held in a state of arrested succession by tree removal for ski run maintenance. Very few *Botrychium* individuals were found in apparently identical habitat if the site had been disturbed more recently. There are several possible explanations for this pattern. Poor spore dispersal ability could explain these observations, although it is likely that spores of *Botrychium* provide an excellent means of long-distance dispersal. Fungal species composition and abundance may change with succession (Allen and Allen 1990, Allen et al. 1999). Thus, successful establishment of *Botrychium* species may be delayed until suitable mycorrhizal symbionts are present after conversion to an early successional stage. The length of time required for spore germination, reproduction, and the maturation of adult sporophytes is also a factor determining the time elapsed between a disturbance event and the appearance of *Botrychium* sporophytes (Root personal communication 2003).

Botrychium species in the mountains of Region 2 are often found on fairly steep slopes (up to 40%). Slopes and roadcuts may provide an appropriate level of chronic disturbance due to periodic mass wasting events and erosion for maintaining suitable habitat. In general, *Botrychium* species are not often found on south-facing slopes in Colorado, suggesting that these sites are too xeric for *B. hesperium* (Root personal communication 2003).

There appears to be a great deal of unoccupied, but seemingly suitable, habitat for *Botrychium* species throughout the West that has resulted from both natural and human-induced disturbance (Root personal communication 2002, Johnson-Groh and Farrar 2003). Although much habitat appears suitable for *B. hesperium*, it may lack certain crucial attributes required for the establishment and persistence of the species. These attributes probably include timing and tempo of disturbance regime and edaphic factors (pH, texture, moisture), but further research is needed to determine the specific autecological requirements of *B. hesperium*.

Reproductive biology and autecology

In the Competitive/Stress-Tolerant/Ruderal model of Grime (2001), characteristics of *Botrychium* species most closely approximate those of stress-tolerant ruderals. Like many orchid and bryophyte species, *Botrychium* species are characterized by small stature, slow relative growth rates, and small propagules. A distinguishing characteristic of plants in this category is that stressful conditions are experienced during growth. *Botrychium* species have high reproductive outputs and possibly produce more spores per sporangium than any other vascular plant (Wagner 1998). This likens them to other "r" selected species, although their longevity and slow growth do not (Grime 2001).

Moderate to light disturbance is a critical part of the autecology of *Botrychium* species including *B. hesperium* (Lellinger 1985, Wagner and Wagner 1993). Locations in which *B. hesperium* is found throughout its range as documented in records from herbaria, heritage programs, and survey reports (e.g., Kolb and Spribille 2000) generally have been affected by some form of disturbance. Openings in the forest that support *B. hesperium* in Waterton Lakes National Park are maintained by insect and disease epidemics and fire, and they tend to have a thick layer of duff, suggesting a low to moderate disturbance regime (Lesica and Ahlenslager 1996). Because *B. hesperium* depends on open sites, disturbances that create and maintain these openings are a key component of its autecology.

Botrychium hesperium is a perennial plant. The root and stem are underground, and the leaf may not emerge every season (Lesica and Ahlenslager 1996). However, when the leaf emerges, a multitude of spores are produced by all members of subgenus *Botrychium*. *Botrychium* species have between 20 and 100 sporangia per sporophore (Wagner 1998).

Like all Pteridophytes and unlike angiosperms and gymnosperms, *Botrychium* spores develop into gametophytes that live independently of the sporophyte. The gametophyte produces male and female sex cells in the antheridia and archegonia respectively. Male sex cells must move through a fluid environment to fertilize a female egg cell. The subterranean nature of *Botrychium* gametophytes probably restricts many *Botrychium* species to self-fertilization (McCauley et al. 1985, Soltis and Soltis 1986). Cross-fertilization may occur (Wagner et al. 1985); however, the antheridia and archegonia are near each other and inbreeding is prevalent (McCauley et al. 1985, Soltis and Soltis 1986, Farrar and Wendel 1996). See **Figure 4** for a diagrammatic representation of the life cycle (after Lellinger 1985) of *B. hesperium*, and **Figure 5** for a life cycle graph (after Caswell 2001) for *B. hesperium*.

Reproductive output is variable in *Botrychium* and may be affected by many factors. Health of the plants and fungi, climate, plant age, predators, and other factors may influence spore production (Casson et al. 1998). It is unknown how long the spores of *B. hesperium* remain viable (Lesica and Ahlenslager 1996). Germination may take up to five years or begin immediately (Casson et al. 1998).

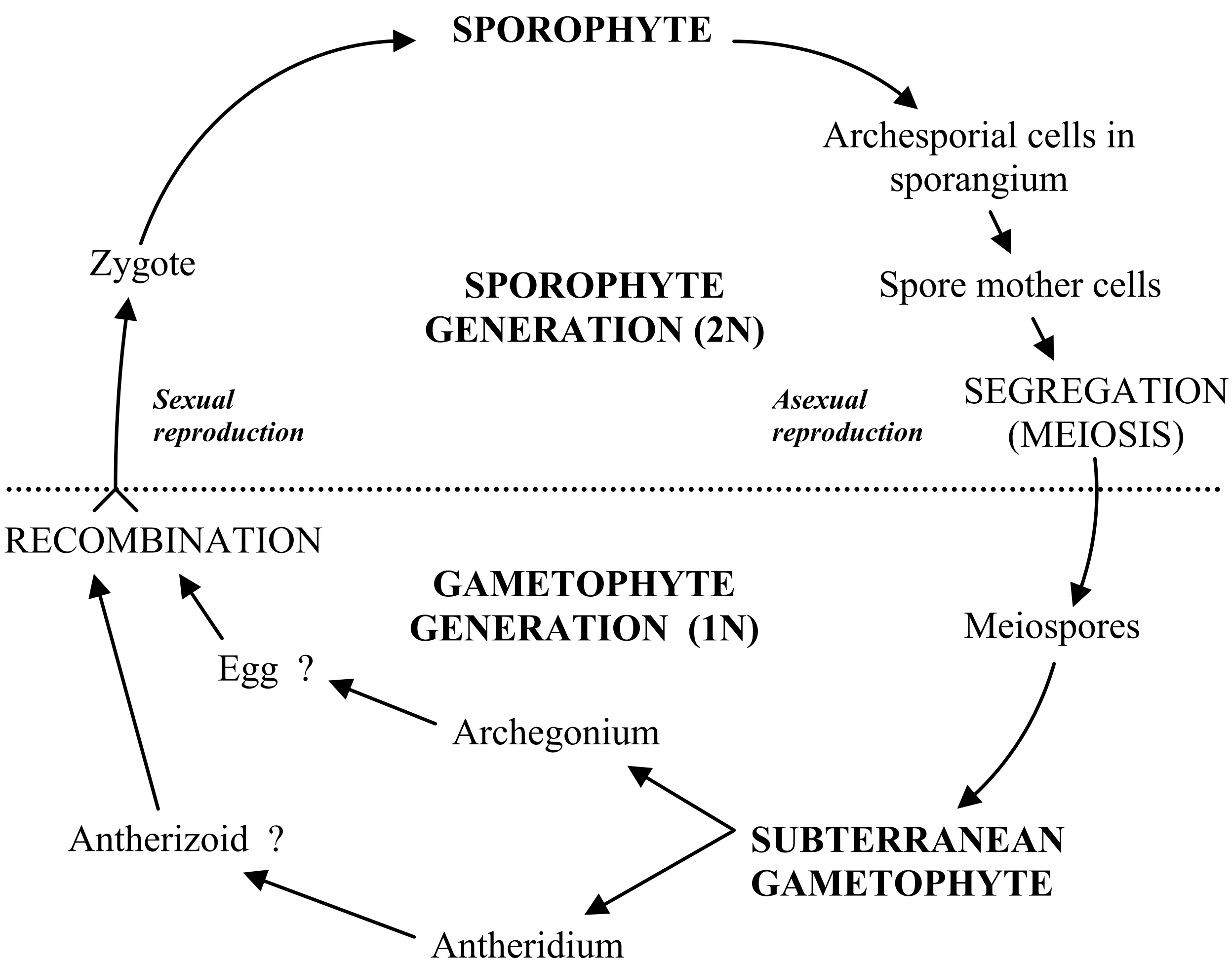

Figure 4. Life Cycle Diagram for *Botrychium hesperium* (after Lellinger 1985), illustrating the alternation of generations.

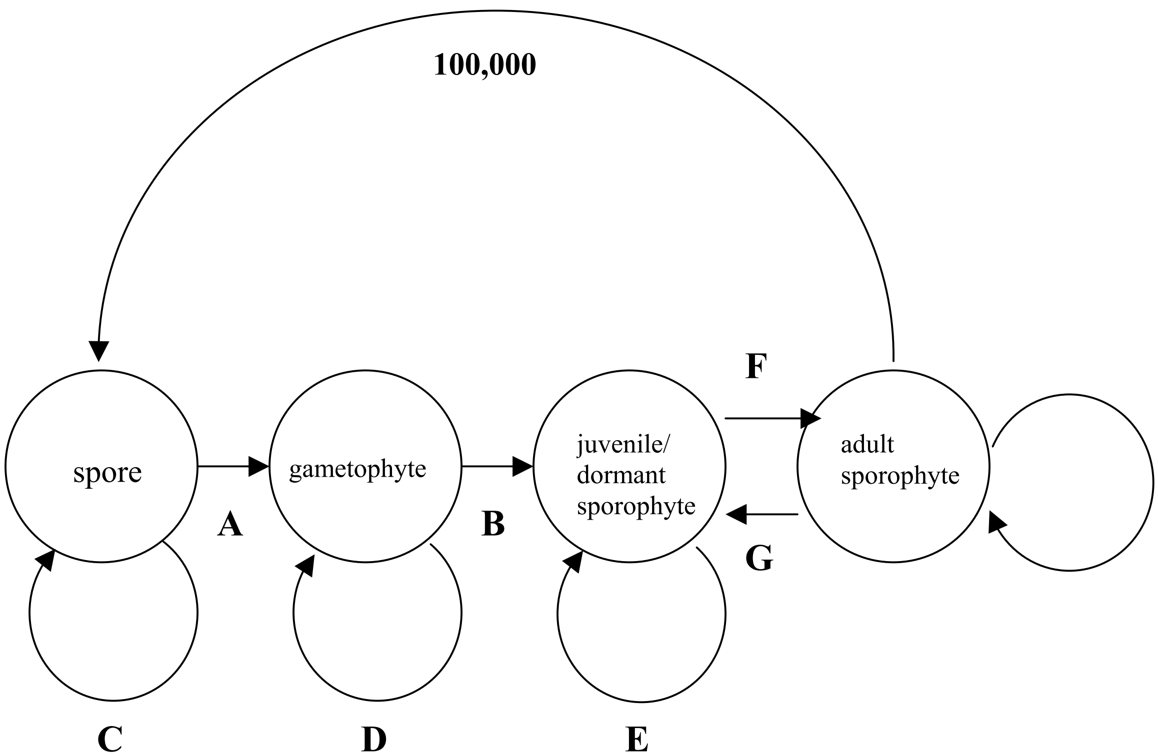

Figure 5. Hypothetical life cycle graph (after Caswell 2001) for *Botrychium hesperium*. Transition probabilities are not known and are difficult to quantify since important stages of the lifecycle occur underground (A-G). Please see Johnson-Groh et al. (1998) for the best information currently available regarding these parameters for subgenus *Botrychium*. The number of years needed for a juvenile sporophyte to reach adulthood and emerge from the ground is not known. Spore production is estimated from Wagner (1998). No transition probabilities are known for *B. hesperium*.

Botrychium spores are small and light and are likely carried by winds. Researchers have hypothesized that the average dispersal distance for some *Botrychium* species ranges from a few centimeters (Casson et al. 1998, Hoefferle 1999) up to three meters (Peck et al. 1990). If the probability of successful long-distance migration to suitable sites is low, then it may take a long time for some *Botrychium* populations to become established. However, spores certainly can travel great distances (Wagner and Smith 1993, Briggs and Walters 1997, Chadde and Kudray 2001). In addition to wind dispersal, small mammals may disperse *Botrychium* spores (Wagner and Wagner 1990, Wagner and Wagner 1993, F. Wagner personal communication 2002). *Botrychium* spores have thick walls that may help to retain their viability as they pass through an animal's digestive tract (Wagner and Wagner 1990, F. Wagner personal communication 2002). The flow of rainwater down slopes along fall lines may also effectively disperse spores to other locations (Buell 2001).

Mycorrhizae may be the most important factor for establishment, distribution, and abundance of *Botrychium* species (Johnson-Groh 1998, Johnson-Groh 1999). *Botrychium* species rely upon mycorrhizae in both the sporophytic (Bower 1926, Wagner and Wagner 1981, Foster and Gifford 1989) and gametophytic stages (Campbell 1911, Campbell 1922, Bower 1926, Scagel et al. 1966, Whittier 1973, Wagner et al. 1985, Foster and Gifford 1989, Schmid and Oberwinkler 1994). *Botrychium* spores need three to four weeks of darkness before they can germinate, with longer periods of darkness increasing the probability of germination (Whittier 1973). Germination can occur without mycorrhizal infection; however, the gametophyte will not mature without an arbuscular mycorrhizal symbiont (Campbell 1911, Whittier 1972, Whittier 1973). The subterranean, achlorophyllous gametophyte may live underground for up to five years (Winther personal communication 2002). The *Botrychium* gametophyte is a mycoparasite using carbohydrates and minerals gained from the mycorrhizal interaction (Schmid and Oberwinkler 1994).

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

Arbuscular (also referred to in the literature as vesicular-arbuscular) mycorrhizae are the known fungal symbiont with *Botrychium* species (Berch and Kendrick 1982, Schmid and Oberwinkler 1994). Little is known about the specific nature of mycorrhizal interactions in *Botrychium* species. In a study of the ultrastructure of the mycorrhiza of *B. virginianum* sporophytes, distinctive arbuscules were observed that are similar to those seen in Triassic fossils (Kovács et al. 2003). Johnson-Groh (1999) found that water relations were extremely important for mycorrhizae and *Botrychium*. Farrar (1998) notes that mycorrhizal fungi are low in species diversity, ubiquitous in disturbed and undisturbed sites, and generalist in whom they infect (Smith and Read 1997). Recent studies have measured surprisingly high species diversity of arbuscular mycorrhizal (AM) fungi in a single hectare (Bever et al. 2001). A single plant root has been observed to host up to 49 species of AM fungi simultaneously (Vandenkoornhuyse et al. 2002). These observations, coupled with the ubiquity and low host specificity of AM fungi, suggest that mycorrhizae may not be a limiting factor in the distribution of *B. hesperium*. However, changes in the mycoflora occur during succession (Allen and Allen 1990), and given the importance of mycorrhizal relationships to *Botrychium* species, it is likely that these changes affect the quality of habitat. Mycorrhizae can have large impacts on the composition of a plant community by shifting the intensity of competitive interactions (Read 1998, Van Der Heijden et al. 1998). Marler et al. (1999) found that the exotic *Centaurea maculosa* had more intense competitive effects on *Festuca idahoensis* when grown together in the presence of mycorrhizal fungi.

Hybrids between *Botrychium* species are rare (Wagner and Wagner 1993, Wagner 1998). However, at least ten records of sterile hybrid combinations have been documented (Wagner et al. 1984, Wagner et al. 1985, Wagner 1993). Sterile hybrids between *B. hesperium* and *B. echo* have been observed in sites where these species occur together (Wagner and Wagner 1983b). The nothospecies *B.* x *watertonense* (n=90) is the result of a cross between *B. paradoxum* and *B. hesperium*, between which it is intermediate (Wagner et al. 1984). It was discovered in Waterton Lakes National Park, where it co-occurs with its putative parents. It is highly distinctive morphologically in that it bears some sporangia on its trophophore, whereas *B. paradoxum* bears two sporophores and no sterile lamina, and *B. hesperium* has the typical leaf morphology of *Botrychium* species with a single sporophore and a single trophophore. Though spores are apparently abortive in *B.* x *watertonense*, this species may be capable of some reproduction through the apogamous production of spores (Wagner et al. 1984). Allopolyploidy may also have resulted in new species of *Botrychium* historically (Wagner 1993).

Demography

Members of the genus *Botrychium* appear to have naturally low rates of outcrossing (Farrar 1998). The anatomy of the gametophyte of *B. virginianum* appears to be designed for self-fertilization, since the antheridia are positioned above the archegonia. Water moving through the soil is likely to bring the male sex cells to the archegonia on the same plant (Bower 1926). Soltis and Soltis (1986) used electrophoretic techniques to confirm that there are extremely high levels of inbreeding in this species. Allelic variability within each moonwort species consistently shows very low intraspecific variation, when compared with other ferns and seed plants (Farrar 1998). McCauley et al. (1985) found *B. dissectum* (subgenus *Sceptridium*) to have an outcrossing rate of less than 5 percent. However, the presence of interspecific hybrids in natural settings indicates the ability for cross-fertilization hybridization to occur (Wagner et al. 1985). Due to their apparent predisposal to selfing, *B. hesperium* and other *Botrychium* species may not be particularly sensitive to the effects of inbreeding depression. Farrar (1998, personal communication 2002) hypothesizes that low genetic diversity would lead to high genetic stability, which might benefit *Botrychium* species by assuring that they remain attractive hosts to the mycorrhizal fungus. As obligate mycorrhizal hosts that obtain their mineral nutrition and some carbohydrates from their fungal symbionts, the establishment and maintenance of this relationship is of paramount importance to *Botrychium* species. As such, in theory genetic diversity would be more useful to *Botrychium* when present in their fungal symbionts, since they are the intermediaries between the roots and the rhizosphere and must adapt to environmental change.

As with all *Botrychium* species, basic parameters circumscribing life history characteristics are unknown. This is particularly true of the underground portion of the lifecycle (Berlin et al. 1998, Johnson-Groh 1999). The most thorough demographic studies of *Botrychium* species are of *B. campestre* (Johnson-Groh 1999) and *B. mormo* (Johnson-Groh 1998).

High ratios of underground to aboveground structures have been reported in several *Botrychium* species, including *B. hesperium* (Bierhorst 1958, Mason and Farrar 1989, Johnson-Groh 1998, Johnson-Groh 1999, Johnson-Groh et al. 2002). Of eight *Botrychium* species observed in this study (*B. campestre*, *B. hesperium*, *B. gallicomontanum*, *B. lanceolatum*, *B. montanum*, *B. mormo*, *B. yaaxudakeit*, and *B. virginianum*), *B. hesperium* has the highest ratio of belowground to aboveground structures (1950:1). Data for *B. hesperium* from this study are summarized in **Table 4**. The number of aboveground sporophytes observed in a given year may be a poor indicator of population size and viability, since much of the population is not visible and sporophytes can remain dormant for one or more years (Muller 1992, Johnson-Groh and Farrar 1996a, Lesica and Ahlenslager 1996, Johnson-Groh 1998, Johnson-Groh 1999). Because large numbers of gametophytes and non-emergent sporophytes may occur in the soil undetected, a single emergent sporophyte may indicate the presence of a viable population (Casson et al. 1998), or a recent colonist. The number of emergent sporophytes in a given year is highly variable and is an incomplete indicator of total population numbers in moonworts, including *B. hesperium*.

Table 4. Summary of density of belowground structures and other data presented in Johnson-Groh et al. (2002) for *Botrychium hesperium*.

Density of aboveground sporophytes (m^{-2})	0.4
Frequency of belowground structures (%)	65
Density of gametophytes (m^{-2})	478
Density of belowground juvenile sporophytes (m^{-2})	281
Density of gemmae (m^{-2})	21
Total density of belowground structures (m^{-2})	780
Ratio of belowground to aboveground plants	1950

The demography and life history of *Botrychium hesperium* were studied by Lesica and Ahlenslager (1996) in Waterton Lakes National Park. Only aboveground portions of the sporophyte phase of the life cycle were studied. As with *B. campestre*, prolonged dormancy of one or more years was observed, with 12 to 38 percent of the sample populations remaining dormant at any given time. Prolonged dormancy was strongly correlated with drought in the previous year. Recruitment rates varied between 25 and 40 percent from 1991 through 1993. Mortality rates were approximately 25 percent, with a half-life of 3.1 years for the 1990 cohort. Populations of *B. hesperium* in this study were highly variable, as is common among *Botrychium* species (Johnson-Groh 1999), but *B. hesperium* populations were more stable than those of *B. paradoxum* and *B.* x *watertonense*, which were also monitored.

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

Botrychium gametophytes are reported to persist underground for up to five years (Winther personal communication 2002), and they grow very slowly from embryo to sexually reproductive adult (Wagner 1998). Sporophytes also may live heterotrophically underground for several years before producing aboveground structures (Kelly 1994). Upon emergence aboveground, the sporophytes begin spore production on their fertile lamina (sporophore). Lesica and Ahlenslager (1996) determined a half-life of approximately three years or less for *B. hesperium* sporophytes, which is long compared to *B. mormo* (Johnson-Groh 1998), but short compared to other species, such as *B. australe* (11.2 years) (Kelly 1994) and *B. dissectum* (at least a few decades) (Montgomery 1990, Kelly 1994). Thus, *B. hesperium* is a relatively short-lived species, as are most members of subgenus *Botrychium* (Lesica and Ahlenslager 1996).

No population habitat viability analysis has been done for *Botrychium hesperium* as of this writing. The only *Botrychium* species for which such an analysis has been conducted is *B. mormo* (Berlin et al. 1998), a species that is similar to *B. montanum*, which differs in many significant ways from *B. hesperium* and from most other moonworts as well. Three factors were cited that have the most control in the model, although these are also the factors about which the least is known. These are viable spore set per sporophyte, the nature and extent of a spore bank, and spore germination rate. These factors are likely to vary significantly between taxa and under different ecological conditions.

Prolonged dormancy is often associated with environmentally induced stress, especially drought (Lesica and Steele 1994). Lesica and Ahlenslager (1996) observed higher rates of prolonged dormancy in 1992 following low levels of winter and spring precipitation in the previous year.

Botrychium species are often found in areas with light to moderate disturbance (Lellinger 1985, Wagner and Wagner 1993). Many moonworts are early to mid-seral species, and as succession proceeds to conditions unsuitable to them the viability of some populations may be compromised (Johnson-Groh and Farrar 2003). The typically small, highly variable populations of *Botrychium* species are vulnerable to local extirpation (Johnson-Groh et al. 1998). Thus, *B. hesperium* and other species of *Botrychium* may depend on a shifting mosaic of suitable habitats for their long-term persistence, as does *Pedicularis furbishiae* (Furbish's lousewort) (Pickett and Thompson 1978, Parsons and Browne 1982, Menges and Gawler 1986, Lesica and Ahlenslager 1996, Chadde and Kudray 2001). Spores would necessarily be the means by which *B. hesperium* migrated to new locations. The metapopulation dynamics of *B. hesperium* will be important to consider for conservation purposes (Pickett and Thompson 1978).

Populations of many *Botrychium* species tend to be small and localized (Colorado Natural Heritage Program 2002). This is probably due to the patchy nature of their habitat, which is a direct result of the nature of the natural disturbance that creates it. Nonetheless, apparently suitable habitat, which is plentiful, is often not occupied by *Botrychium*. This may be due to limitations in successful migration to the site, or the result of other unknown ecological parameters, such as insufficient time elapsed since a disturbance event. The observations of Buell (2001) are interesting in this regard: *Botrychium* species were found only on ski slopes that had been cleared for more than 30 years. A lack of appropriate mycorrhizal symbionts may be one factor limiting population growth in *Botrychium* populations. Early successional sites usually have low levels of mycorrhizae (Allen and Allen 1990, Allen et al. 1999).

Community ecology

Rigorous work on the plant community ecology of *Botrychium hesperium* is lacking. Using phytosociological methods, Kolb and Spribille (2000) described the community in which *Botrychium* species (possibly including *B. hesperium*) were found in Summit County, Colorado as "*Festuco – Heterothecetum pumilae*," named for the dominant genera (represented by *Festuca brachyphylla* and *Heterotheca pumila*) in the community. This community is characterized by ruderal taxa including *Fragaria virginiana*.

There have been many observations of herbivory on *Botrychium* species including *B. hesperium*. Sporophytes are often found to have been browsed, probably by deer or rabbits (Wagner and Wagner 1990). In some cases, as many as 80 percent of the plants have been completely browsed (Wagner et al. 1985). *Botrychium mormo* appears incapable of dispersing spores on its own, since the sporangia do not fully open (Casson et al. 1998). The spores of *Botrychium* species also have relatively thick walls, which may enhance their ability to survive a trip through the gut of an animal (Wagner et al. 1985). These observations have led to the hypothesis that animals may disperse the spores of *Botrychium* species (Wagner and Wagner 1990, Wagner and Wagner 1993, Wagner 1998, F. Wagner personal communication 2002). J.D. Montgomery recovered the spores of grape fern (*B. virginianum*) from the droppings of a vole after feeding them to it, after which the spores appeared intact (Root personal communication Root 2003). However, the viability of these spores was not assessed. See **Figure 6** and **Figure 7** for diagrammatic representations of the interrelationships of *B. hesperium* with herbivores and other factors in its environment.

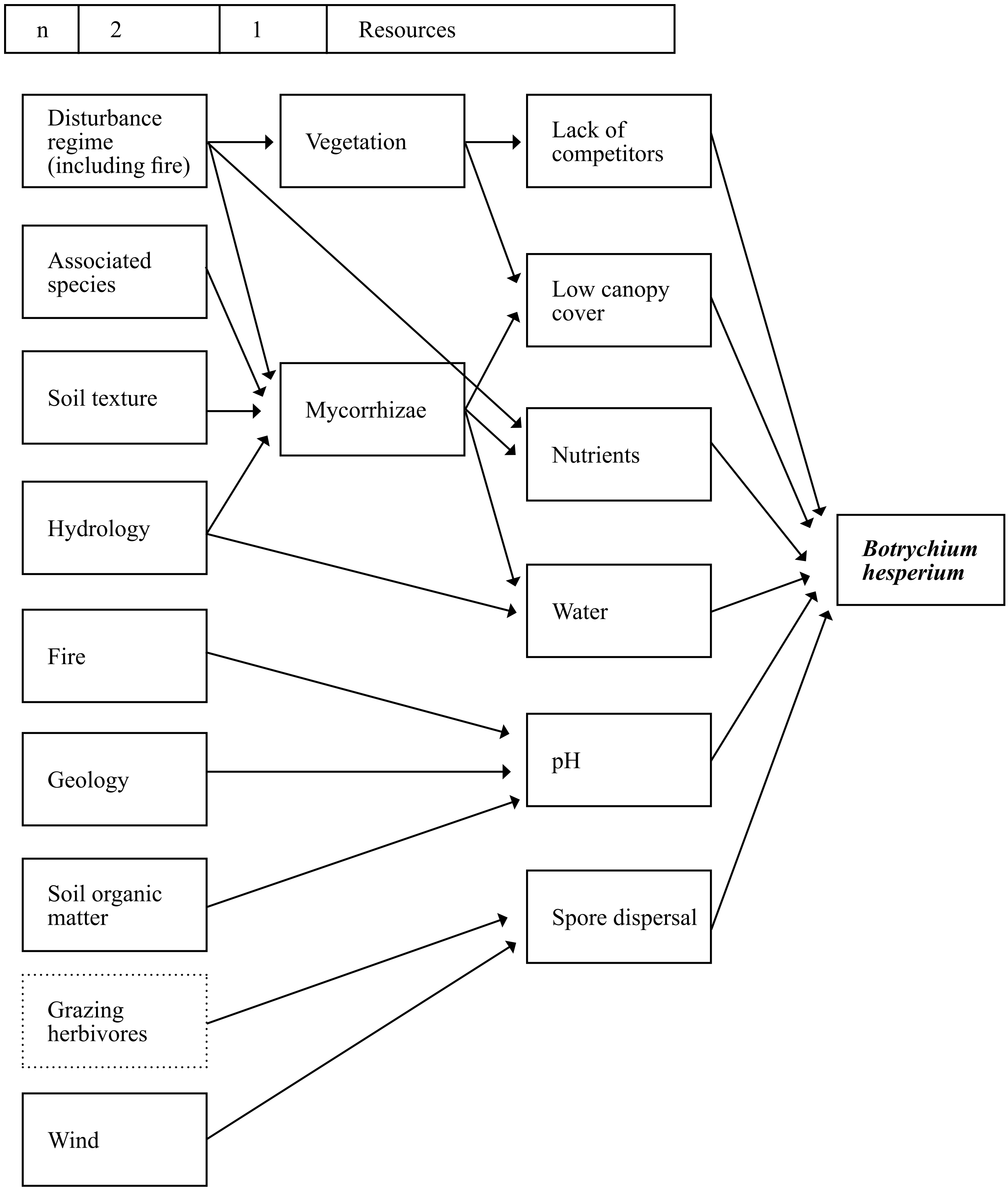

Figure 6. Envirogram outlining the resources of *Botrychium hesperium*. Cells with dotted borders are speculative.

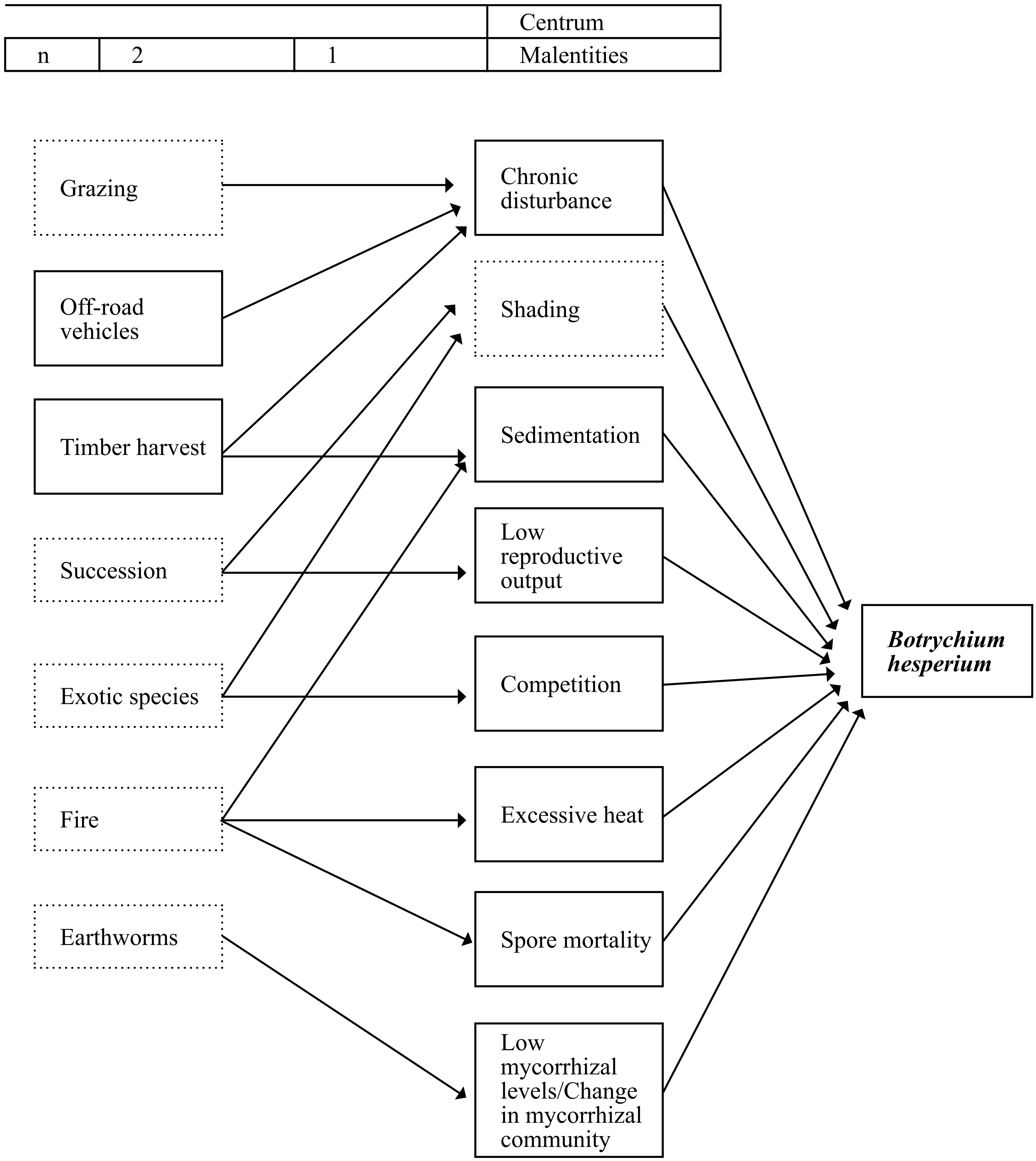

Figure 7. Envirogram outlining the malentities to *Botrychium hesperium*. Cells with dotted borders are speculative.

The coexistence of many species of *Botrychium* in genus communities is interesting from a community ecology standpoint. If the members of genus communities occupy the same niche, then they coexist in violation of Gause's competitive exclusion principle (Krebs 1972). Because water, nutrient, and some carbohydrate uptake are mediated by mycorrhizae, it is possible that even if genus community members depend on the same resources, coexisting plants are not engaged in direct intraspecific competition. Competition may be for access to the mycorrhizae, if it is occurring at all. No research has been done on *Botrychium* species with respect to these issues. There are no reports of parasitism or disease in the literature for any *Botrychium* species.

CONSERVATION

Threats

Observations suggest that there are several tangible threats to the persistence of *Botrychium hesperium* in Region 2. In rough order of decreasing priority these are habitat loss, recreation, succession, overgrazing, effects of small population size, sedimentation, timber harvest, exotic species invasion, global climate change, and pollution.

Threats to *Botrychium hesperium* are not well understood (NatureServe 2003). As a species that requires some level of disturbance to create and maintain suitable habitat, it is difficult to define threats to it. Summarizing information from NatureServe (2003), Chadde and Kudray (2001, pg. 11) remarked thus: "Because this species occurs in both naturally and artificially (human-caused) disturbed sites, threats include natural plant succession as well as the same human activities (recreation, road and trail maintenance activities, grazing) that also apparently resulted in creating the initial suitable habitat." Obviously, a better understanding of the role of disturbance in the autecology of *B. hesperium* is of great importance from a management perspective. Threats to the belowground life stages of *B. hesperium* may be more serious than threats to the aboveground (sporophyte) stages, given the greater importance of the belowground portion of the lifecycle (Chadde and Kudray 2001). Threats to populations of *B. hesperium* in the Wallowa Mountains of Oregon include fire suppression, pack animal grazing, wood-cutting, and recreation-associated activities (Zika et al. 2002).

Global climate change is likely to have wide-ranging effects in the near future. Projections based on current atmospheric CO_2 trends suggest that average temperatures will increase while precipitation will decrease in Colorado (Manabe and Wetherald 1986). This will have significant effects on nutrient cycling, vapor pressure gradients, and a suite of other environmental variables. Decreased precipitation could have dire consequences for many populations of *Botrychium hesperium* in Region 2. Temperature increase could cause vegetation zones to climb 350 feet in elevation for every degree F of warming (U.S. Environmental Protection Agency 1997). This could have large impacts on low-elevation populations. That *B. hesperium* is not typically found on south aspects suggests that it would be sensitive to climate changes that cause conditions to become warmer and more xeric.

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

Due to its rarity in Region 2 and the small number of individuals in known occurrences, any land use activity within an occurrence of *Botrychium hesperium* may potentially threaten it. Although this species is often found in moderately disturbed areas and may depend on some level of natural disturbance, these same disturbance regimes could serve to extirpate a very small population, particularly in a small habitat unit. The small populations documented in Region 2 are at risk from stochastic events beyond the control of managers.

Influence of management activities or natural disturbances on habitat quality

Large numbers of *Botrychium hesperium* individuals have been found in areas in which a human-induced disturbance regime is imposed. Logging, ski trail development, building of roads, and other human disturbances have created a great deal of habitat for this species. On some level, these activities appear to benefit *Botrychium* species including *B. hesperium*, as cited in numerous biological evaluations and surveys (e.g., Kolb and Spribille 2000, Thompson 2000, Buell 2001, Thompson 2001, Wilfahrt 2001). However, it has not been shown that human disturbance can be counted on to ensure the long-term viability of this species. It is possible that human-created habitats, such as ski runs, that are currently inhabited by large, healthy *Botrychium* populations may become inhospitable later due to processes we do not currently understand, such as microbial or fungal succession. Thus it is not known if maintaining habitats in a state of arrested succession through the continuance of an imposed disturbance regime provides persistent habitat for *B. hesperium*. While human disturbance has created habitats for *Botrychium*, management practices such as fire suppression and commercial grazing over the last century may have reduced available habitat.

Although *Botrychium* species rely on light to moderate disturbance, it may be important to minimize soil disturbance for several reasons. Soil disturbance can increase the proportion of inorganic nutrients (Vitousek and Reiners 1975 as cited in Allen and Allen 1990). An increase in this proportion may allow non-mycotrophic species to out-compete mycorrhizal dependent species such as *Botrychium* (Allen and Allen 1990). The presence of *B. hesperium* on road and trail margins, where it is less trampled than it would be in the roadbed or trailbed, is also suggestive that this species is sensitive to heavy disturbance. On the other hand, removal of light disturbance events could endanger populations by allowing succession to proceed (Lesica and Ahlenslager 1996, Johnson-Groh and Farrar 2003).

Influence of management activities or natural disturbances on individuals

Because *Botrychium hesperium* is inconspicuous and populations may remain undocumented, surveys should take place before management actions within potential habitat. Please see Johnson-Groh and Farrar (2003) and the Tools and Practices section of this document for discussions of species inventory methods.

Recreational use of *Botrychium hesperium* habitat presents a threat to individuals that may be killed or damaged directly by these activities. Off-road vehicle use (both motorized and non-motorized) represents a significant threat to *B. hesperium* from recreation. Use of mountain bikes and "mountainboards" (similar to snowboards but equipped with wheels for use on ski slopes in the summer) on ski slopes during the growing season has the potential to impact individual plant.

Construction of facilities to support recreational skiing presents threats to specific moonwort populations. Because of a lack of baseline data, it is not known to what extent the creation of ski runs and ski areas has impacted populations of *Botrychium* species including *B. hesperium*. Construction of a ski hut near the population on Vail Pass presents a potential threat due to disturbance associated with construction and increased use of the hut. Because the presence of *B. hesperium* was known before construction, the hut was located in a site where impacts to the population would be reduced. Nonetheless, summer use of the hut could result in trampling of individuals.

Numerous management practices used to create and maintain ski runs pose potential threats to populations of *Botrychium hesperium*. Summer maintenance practices have a greater potential than winter maintenance to impact populations since they are more likely to disturb soil and damage or kill individuals. These activities include pulling stumps, creating and maintaining roads, using summer snowcats, controlling weeds mechanically or chemically, grooming the earth on ski runs, installing and maintaining waterlines and electrical lines, and maintaining lift corridors (Johnston personal communication 2003, Popovich personal communication 2003).

Fire is not detrimental to *Botrychium*, and secondary effects have a greater impact than the fire itself (Johnson-Groh and Farrar 2003). Fire impacts individuals directly by burning their aerial portions, but *Botrychium* species including *B. hesperium* appear to suffer no ill consequences of this (Johnson-Groh and Farrar 2003). Particularly hot fires or fires that desiccate the soil could result in mortality, but due to this species' strong dependence on mycorrhizae, removal of leaf tissue via burning or other means is probably inconsequential to the plant's survival (Montgomery 1990, Wagner and Wagner 1993, Johnson-Groh and Farrar 1996a, Johnson-Groh and Farrar 1996b, Johnson-Groh 1999). Fires that occur during phenologically sensitive times (July and August, when forest fires are most frequent) would preclude any reproductive output for that year and might kill spores lying near the surface (Root personal communication 2003).

Sedimentation resulting from fire or timber harvest is a threat to individuals. Burial by sediment has resulted in the apparent mortality of individuals of other *Botrychium* species (Johnson-Groh and Farrar 2003). Gopher excavation has resulted in the temporary loss of *B. gallicomontanum* individuals in permanent plots at Frenchman's Bluff, Minnesota. Part of a plot was buried by soil excavated by gophers, but after 11 years of monitoring at this site the *B. gallicomontanum* population had largely rebounded (Johnson-Groh 1999, Johnson-Groh and Farrar 2003).

While there have been no direct impacts documented from livestock and wild ungulate grazing on populations of *Botrychium hesperium* in Region 2, grazing is known to occur and impacts to habitat have been documented. In Region 2, sheep and horses both graze in subalpine meadows and other areas of the mountains to some extent. Elk and other ungulates frequent the meadow habitats of *B. hesperium*, where they may occasionally graze it. Disturbance of loose soil by sheep that had moved through the site was observed on Molas Pass (element occurrence 15). Sheep grazing in Norway has been observed to eliminate *B. lunaria* individuals from an area (Anonymous reviewer personal communication 2003). Disturbance of the surface by livestock may injure some individuals (potentially above and below ground). Grazing can eliminate a season's contribution to the sporebank (Johnson-Groh and Farrar 2003). Since it is likely to cause some level of erosion, trampling, alteration of plant community composition, damage to the soil structure (particularly when wet), and introduction of invasive plants, the use of livestock grazing as a management tool for the enhancement of habitat is risky for a plant as rare as *B. hesperium*.

Interaction of the species with exotic species

No research has investigated the effects of weeds on *Botrychium*. However, their mutual affinity for disturbance may cause *Botrychium* species and their habitat to be vulnerable to negative impacts from weeds. Marler et al. (1999) observed indirect enhancement of the competitive ability of *Centaurea maculosa* with a native bunchgrass in the presence of arbuscular mycorrhizal fungi. *Centaurea maculosa* is extensively mycorrhizal. Thus mycorrhizae, possibly the species on which *Botrychium* species depend, augment the ability of *C. maculosa* and perhaps other noxious weeds to invade native grasslands. Several exotic species have become significant problems in mountainous areas of Region 2, including *Linaria vulgaris*, *Chrysanthemum leucanthemum*, and *Matricaria perforata*. Although these species have not been documented with *B. hesperium*, they present a significant threat. Because new exotic species are arriving all the time, vigilance in monitoring for their impacts is crucial. *Bromus inermis* has been documented with *B. hesperium* at Copper Mountain in Summit County, Colorado (Root personal communication 2003). *Trifolium repens* and *Taraxacum officinale* are also common in *Botrychium* habitats (Root personal communication 2003).

Earthworms are a diverse group of over 3,500 species worldwide, and the expansion of global commerce may be increasing the likelihood of exotic earthworm invasions with potential adverse affects on soil processes and plant species (Hendrix and Bohlen 2002). In the deciduous hardwood forest habitats of *Botrychium mormo*, invasion of non-native earthworms has resulted in dramatic decreases in mycorrhizal fungi, which could threaten the obligate mycorrhizal symbiont, *B. mormo* (Nielsen and Hole 1963, Cothrel et al. 1997, Berlin et al. 1998, Gundale 2002). The activity of earthworms has also resulted in the elimination of the duff layer and a shift in species composition in *B. mormo* habitat (Berlin et al. 1998). Although earthworms present a possible threat to *B. hesperium*, no research has shown that species of *Botrychium* other than *B. mormo* are being impacted by them. Region 2 moonwort habitats are extremely cold in the winter and have little litter accumulation and a poorly developed O horizon. Therefore, moonwort habitats in Region 2 probably have very few earthworms, and it is unlikely that earthworms have a significant effect on moonworts or their mycorrhizae (Root personal communication 2003). While there are few reports of earthworms in the subalpine zone, Steinmann reports having found small annelids in high elevation caves that probably came from outside the caves (Root personal communication 2003).

Threats from over-utilization

Collection has been noted as a potential threat to *Botrychium hesperium* populations (Johnson-Groh and Farrar 2003). Although evidence suggests that leaf removal does not have a significant long-term effect on *Botrychium* species (Johnson-Groh and Farrar 1996a, 1996b), collection of the species in Region 2 is only advisable in larger populations. Johnson-Groh and Farrar (2003) state that no collections should be made in populations of less than 20 plants; instead they recommend that photos should be taken. Even if the plants would probably survive, collection of material from populations already of questionable viability is a risky endeavor. For example, in a population of three sporophytes, there is almost no margin of error, and accidentally removing the apical bud could potentially result in the extirpation of the species at this site. This is a difficult issue for some *Botrychium* species, including *B. hesperium*, since collection is important for verification by experts. Vouchers are valuable and assist greatly with taxonomic research on the species. Weber and Wittmann (2001a, 2001b) recommend not collecting plants with the roots, because there are no diagnostic characteristics associated with the roots and collecting them kills the plant. To minimize the risk of infection and of removing the apical bud, Johnson-Groh and Farrar (2003) recommend cutting the leaf with a knife near ground level rather than pinching or pulling with the fingers. They also recommend that no more than 10 percent of a population be collected.

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

Conservation Status of the Species in Region 2

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

No rigorous quantitative research has been conducted on population trends for this species in Region 2. The observations of Kolb and Spribille (2000), Thompson (2002), Buell (2001), and Thompson (2001) suggest that the abundance of *Botrychium hesperium* has increased in the vicinity of ski resorts. It is difficult to infer the effects of other forest management practices such as fire suppression on the abundance of *B. hesperium*. There is no evidence that the range of *B. hesperium* has expanded or contracted recently. The recognized range of *B. hesperium* will change in Region 2 as some of the occurrences are identified as *B. 'michiganense'*.

Do habitats vary in their capacity to support this species?

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

Vulnerability due to life history and ecology

The vulnerability of *Botrychium hesperium* due to its life history and ecology remains uncertain, as our knowledge of its life history and ecology is limited. The species does depend on disturbance to maintain suitable habitat, and thus it is vulnerable to habitat change, succession, and absence of disturbance. It is not known how vulnerable *B. hesperium* populations are in this regard.

While this species appears to have the ability to reproduce asexually via gemmae, it has not been observed to produce copious quantities of them and thus probably remains largely reliant on reproduction involving the gametophyte portion of its lifecycle. The gametophyte stage is probably more susceptible to drought than reproduction via gemmae (Camacho 1996). However, its ability to associate with fungi to remain dormant for one or more years enables *Botrychium* species to better withstand drought conditions (Lesica and Ahlenslager 1996, Johnson-Groh 1999). The apparent tendency of *Botrychium* species, including *B. hesperium*, to reproduce asexually may leave them vulnerable to ecosystem change. While reproduction by cloning is good in static environments, sexual reproduction and long-distance dispersal are better suited to facile environments where recombination of alleles and higher genetic diversity leave some individuals better suited to new conditions.

The tendency of *Botrychium* species to grow in small, somewhat isolated populations with highly variable numbers of individuals makes them susceptible to local extirpation due to stochastic processes, succession, and environmental variation (Johnson-Groh 1998). However, the findings of Johnson-Groh et al. (2002) suggest that with observable populations of emergent sporophytes, there typically resides an underground "structurebank" in varying stages of maturation that can buffer a population.

Because much of their life history occurs underground, and because they are generally small and cryptic plants, *Botrychium* species are easily overlooked and are thus poorly understood. This leaves them vulnerable where populations have not been found.

Evidence of populations in Region 2 at risk

Because so little is known about the distribution and ecology of *Botrychium hesperium*, it is difficult to make inferences about the degree of imperilment of this species in Region 2. Some data suggest that *B. hesperium* is highly imperiled, while other data suggest it is not. Below we present a summary of both types of evidence.

Numerous facts about *Botrychium hesperium* suggest that it is an imperiled species. Even if it is not imperiled because it is more common than we think, there are several "red flags" worth summarizing here. Currently there are 33 populations known in Region 2 (29 on USDA Forest Service lands). Populations of *B. hesperium* are small (ranging in size from 1 to "less than 100") and fluctuate greatly, resulting in a high probability of local extinction as a result of stochastic processes and even normal environmental variation. The total population in Region 2 is not known, but available information suggests that it is somewhere between 300 and 400 individuals, which is very small and of questionable viability. *Botrychium hesperium* also has very specific habitat requirements. Many of the old, arrested successional sites that it occupies are inherently facile and destined to become unsuitable to *B. hesperium* as a result of natural succession. It appears to have a metapopulation structure that obligates it to long distance dispersal to new sites as succession renders current sites unsuitable for it, which is both risky and costly. Some studies have shown that it may not be particularly good at long distance dispersal. It is wholly dependent on mycorrhizae in both the gametophytic and sporophytic life history stages, and its spores will cease to develop into a gametophyte without the presence of a mycorrhizal symbiont. The reliance of *B. hesperium* on disturbed sites predisposes it to negative impacts from exotic species, which also may thrive in these habitats. It is frequently found next to roads and trails, which are perfect sites for weeds and leave plants vulnerable to trampling. New exotic species are arriving constantly, and it may be only a matter of luck that the habitat for *B. hesperium* has not already been substantially invaded by exotics. Because the ecology of this species is poorly understood, current management may be placing demands on the species despite good intentions. Element occurrence records for Colorado (Colorado Natural Heritage Program 2002) document impacts or the potential for impacts from hikers, sediment washing off a road, roadwork, ski hut construction and trampling by hut visitors, and elk trampling. Although it is adapted to light to moderate levels of disturbance, the severe and chronic disturbance imposed by many human activities will extirpate populations where such disturbance occurs. Many occurrences have not been revisited in many years, and their current status is unknown. The number of *B. 'michiganense'* populations in Region 2 that are currently misidentified as *B. hesperium* is not known, but when the relative distributions of these taxa are better understood it is possible that *B. hesperium* will be appear rarer than is currently believed. This may even result in changes to its subnational or global imperilment ranks assigned by NatureServe and its affiliates (i.e., the Colorado Natural Heritage Program).

Although there is much evidence suggesting that *Botrychium hesperium* is highly imperiled, other qualities suggest otherwise. That *B. hesperium* is successful in some sites altered by human disturbance suggests that it may benefit from human activities such as maintenance of road edges and ski slopes. Recent observations where hundreds of plants were found on old ski slopes suggest that this sort of human disturbance is not incompatible with, and perhaps beneficial to *B. hesperium*. There is an abundance of naturally disturbed habitat (steep slopes, openings between krummholz near treeline, and avalanche chutes to name some examples) that is known to support some individuals. Many of these sites are difficult to access and thus have not been thoroughly searched for *B. hesperium*. Although *B. hesperium* is often found along heavily used thoroughfares, there is very little off-trail trampling in many sites because these areas tend to be unattractive to people (Root personal communication 2003). Because *B. hesperium* is not listed as a sensitive species in Region 2 by the USDA Forest Service, there has been little survey work to identify more populations (Root personal communication 2003). More populations are being found all the time, and as survey work continues in both natural and human disturbed sites, it is likely that more populations of *B. hesperium* will be found in Region 2.

Management of the Species in Region 2

Implications and potential conservation elements

Historically, insect attacks and fire probably created large areas of suitable habitat for *Botrychium hesperium* throughout Region 2. Although it may take hundreds of years for these habitats to return to forested conditions, the habitat created by these ecological processes may be ephemeral for *B. hesperium*, and we cannot know what the pre-settlement distribution and population size of *B. hesperium* was. However, those habitats may have been as important as other habitats, such as avalanche chutes and treeline sites.

Management actions in the forests of Region 2 over the last 100 years have probably had mixed effects on *Botrychium hesperium*. While the clearing of forests for the ski industry has inadvertently created large amounts of suitable habitat, fire suppression policies may have resulted in a net loss of habitat. However, formerly logged sites currently support at least two populations of *B. hesperium* in Region 2, so it is possible that timber harvest may be ecologically analogous to fire for *B. hesperium* in certain (probably very limited) circumstances. However, this is highly speculative and requires research before management decisions based on this supposition can be made. *Botrychium hesperium* is unlikely to be negatively impacted by any foreseeable natural catastrophe in Region 2, although global climate change has the potential to drastically alter the habitats where *B. hesperium* is found.

Desired environmental conditions for *Botrychium hesperium* include sufficiently large areas where the natural ecosystem processes on which it depends can occur, permitting it to persist unimpeded by human activities and their secondary effects, such as weeds. Given the current paucity of information on this species, it is unknown how far this ideal is from being achieved. It is possible that most or all of the ecosystem processes on which *B. hesperium* depends are functioning properly at many or most of the populations of this species. Further research on the ecology and distribution of *B. hesperium* will help to develop effective approaches to management and conservation. Until a more complete picture of the distribution and ecology of this species is obtained, priorities lie with conserving the known occurrences in Region 2, particularly those in persistent habitat in natural settings.

Tools and practices

Botrychium hesperium, like other species of *Botrychium*, is small, inconspicuous, and difficult to find. Although the probability that other occurrences remain to be found in Region 2 is high, it is nonetheless very rare in the West. Year-to-year fluctuation in aboveground sporophytes (with years of no individuals aboveground in a population possible) also makes inventory work difficult with this species. Additionally, this species was only recently described (Wagner and Wagner 1983b) and is difficult to identify. For these reasons inventory work remains the highest priority for this species in Region 2.

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

Buell (2001) described a simple method for conducting inventories for *Botrychium* species along pipeline alignments. Elements of this method are widely applicable for searching for *B. hesperium*. A zigzag-shaped survey path was followed by one or more surveyors through appropriate habitat, deviating as necessary to highly suitable habitat when it was observed adjacent to the surveyors' path. Upon detection of moonworts, surveyors searched for more plants in a radial expanding pattern emanating from the first plant found until no more individuals were detected. This approach could be useful in large areas of suitable habitat, while in small areas or when sufficient human resources are available, searching the entire area of suitable habitat at a site may be appropriate. In large potential habitat units, having more than one surveyor is useful.

Botrychium species are notoriously difficult to investigate. Their small size, inconspicuous appearance, sporadic distribution, prolonged dormancy, and cryptic nature make them challenging subjects for research, yet these are also the attributes that make them fascinating to us. In the past, experts (Drs. Warren and Florence Wagner, Don Farrar, Cindy Johnson-Groh, Peter Root, Dean Erhard, Leslie Stewart, Nancy Redner, Annette Kolb and Toby Spribille, Dave Steinmann, and others) have had great success in finding populations of *B. hesperium* in Region 2 using a search image for habitat that they have developed from years of study and survey work. For identifying habitat and finding more populations of *B. hesperium*, engaging experts on *Botrychium* to the maximum extent possible will help greatly in expanding our knowledge of this species.

Identifying suitable habitat in which to focus searches for *Botrychium hesperium* could be aided by modeling habitat based on the physiognomy of known occurrences. Intersecting topography, substrate, and vegetation could be used to generate a map of potential sites for *B. hesperium*. This would be a valuable tool for guiding future searches. Aerial photography and satellite imagery may also assist with the identification of areas worth searching.

Annual monitoring of selected populations of *Botrychium hesperium* in Region 2 could help to understand its ecology and population trends. Establishing permanent plots following the methods of Lesica and Ahlenslager (1996) would address questions regarding population stability and trends. However, these methods are labor-intensive and expensive, and they yield data for only part of one or a few populations (Anonymous reviewer personal communication 2003). A simple annual census of representative colonies (perhaps 10) that are accessible and in well-defined habitats could provide valuable data inexpensively. Randomized permanent plots in which individuals are tracked by marking or mapping them within each sampling unit could help elucidate issues such as life span, dormancy, recruitment success, and population trends. Adding a photoplot component to this work following recommendations offered in Elzinga et al. (1998) could facilitate the tracking of individuals and add valuable qualitative information. Monitoring sites should be selected carefully, and a sufficient number of sites should be selected if the data is intended to detect regional population trends.

To address the hypothetical metapopulation structure of *Botrychium hesperium*, one approach might be to select highly suitable but unoccupied sites and to attempt to observe colonization events. In a stable population, colonization rates should roughly equal extinction rates, so this approach might permit additional inference into population trends.

A detailed and scientifically rigorous monitoring effort employing the methods of Johnson-Groh and Farrar (2003) will not be possible with smaller populations. Thus, larger populations must be selected for monitoring. At present the priorities for Region 2 lie in basic survey work, since we still do not know the full distribution of *B. hesperium* and its conservation status is uncertain. Gathering population size data can be done rapidly and requires only a small amount of additional time and effort (Elzinga et al. 1998). Thus, presence/absence monitoring is not recommended for *B. hesperium*.

Populations of *Botrychium* are inherently variable (Johnson-Groh 1999). The number of emergent sporophytes in a given year is highly variable and is an incomplete, and potentially misleading, indicator of total population numbers in *B. hesperium* and other moonwort species. *Botrychium hesperium* may be prone to local extinction because it tends to occur in early successional sites following disturbance, and apparently it does not persist in later seres. Thus, the long-term viability of the species may depend on the availability of a shifting mosaic of suitable habitats in appropriate early successional stages that *B. hesperium* can colonize (Lesica and Ahlenslager 1996, Chadde and Kudray 2001). If this is the case, then the metapopulation dynamics of this species become crucial to its management and conservation, and underscore the need to conserve areas of suitable habitat that are not currently inhabited by *B. hesperium*. It also underscores the need for forest management practices that allow the natural disturbance regime to create and maintain suitable habitats, since reliance on human disturbance may or may not assure the long term viability of the species. Future metapopulation studies will need to investigate migration, extinction, and colonization rates (Elzinga et al. 1998) and will be extremely difficult (though technically feasible) to assess for any *Botrychium* species.

Due to anatomical and environmental constraints that limit its ability to outcross, *Botrychium hesperium* appears predisposed to low outcrossing rates. As with other species of *Botrychium*, gene flow has probably always been limited.

Estimating cover and/or abundance of associated species within the plots described above could permit the investigation of interspecific relationships through ordination or other statistical techniques. This may be of limited practical value to predict relationships, but it might show optimal vegetation density and litter depth and other habitat variables favoring *Botrychium hesperium*. Understanding environmental constraints on *B. hesperium* would facilitate the management of this species. Gathering data on edaphic characteristics (primarily moisture and texture) and associated weather data from the permanent plots described above would permit the analysis of species-environment relationships. Such data gathered carefully at the known populations in Region 2, and then compared with census data, would provide some basic insight into the causes of the fluctuation in aboveground sporophytes in Region 2, and would help with hypothesis generation for further studies of the ecology of this species. The use of photopoints for habitat monitoring is described in Elzinga et al. (1998). This is a powerful technique that can be done quickly in the field, and could be easily done during annual census visits to selected populations. Though it does not provide detailed cover or abundance data, it can help to elucidate patterns observed in quantitative data.

The ecology of *Botrychium hesperium* remains poorly understood, and the species has not been studied long enough to develop management strategies that could guarantee success. However, some generalities regarding management can be made based on current knowledge. Because *B. hesperium* shows a general preference for open sites, it may benefit from management activities that maintain reduced canopy cover. Maintaining the health of mycorrhizae is certainly crucial to the species as well. Spring burning may be an effective management tool for *B. hesperium* if the soil is moist and may have been important for creating habitat for the species historically. However, there have been no studies on the effects of fire on *B. hesperium*. Burning appears to have positive effects on *B. campestre* populations in Iowa, but fire combined with erosion and drought, both natural results of fire, may be deleterious (Johnson-Groh and Farrar 1996b, Johnson-Groh 1999). Creation of ski runs and roads has apparently created and maintained large areas of suitable habitat for *Botrychium* species, including *B. hesperium*. However, it would be rash to suggest that these human disturbances assure the long-term viability of *B. hesperium*, since ski runs and roads are managed for skiing and transportation, not *Botrychium*. We know nothing about the long-term suitability of these habitats for *Botrychium* species, or whether they act as population sources or sinks. For plants growing in persistent natural sites, such as those in the upper subalpine zone between tree islands of krummholz, the most beneficial management actions are probably those that dissuade excessive visitation (i.e., trampling) and development of these sites.

It is extremely difficult to grow *Botrychium* species in the greenhouse or lab (Whittier 1972). No spores are currently in storage for *Botrychium hesperium* at the National Center for Genetic Resource Preservation (Miller personal communication 2002). Collection of spores for long-term storage may be useful for future restoration work.

Buell (2001, page 11) describes a method for transplanting *Botrychium* species that has been employed by Nancy Redner of the USDA Forest Service. This method has been used to mitigate impacts on *Botrychium* populations at the Copper Mountain Ski Resort from pipeline and road projects. No data on survivorship of the transplanted populations is available, but Buell (2001) describes transplantings that followed this methodology as "reasonably successful." Several species of moonwort (but not *B. hesperium*) were transplanted in 2003 to mitigate road-widening impacts along Guanella Pass Road (ERO Resources Corporation 2003), but it is not yet known if any plants survived. Cody and Britton (1989) note that transplanting of *Botrychium* species is usually fatal. Because there has not been any long-term assessment of the success of the Summit County and Guanella Pass transplantings, the value of this practice for conservation is extremely dubious and cannot be relied upon to maintain populations in project areas.

Information Needs

Distribution

Given the probability that more populations await discovery on USDA Forest Service lands and elsewhere in Region 2, further survey work is an important research need for *Botrychium hesperium*. Recent work in Colorado at Pikes Peak, Indian Peaks Wilderness, and Summit County suggests that there are occurrences of *B. hesperium* in Region 2 not yet discovered. Further targeted inventory work would allow land managers and NatureServe to accurately assess the rarity and conservation priority of this species. Until specimens and occurrences are verified as either *B. hesperium* or *B. 'michiganense'*, it will not be possible to formulate conservation strategies that address the specific needs of either taxa.

Lifecycle, habitat, and population trend

Very little is known about the population ecology of *Botrychium hesperium*. In particular, the below-ground portion of this species' lifecycle remains poorly understood, although much of its lifespan occurs underground. The way in which subterranean life stages influence population dynamics needs to be understood before we can accurately model population dynamics. The longevity and dispersal ability of spores, and the persistence, size, and longevity of spore banks will also need to be understood. Although all *Botrychium* species depend on a relationship with mycorrhizae, the nature of this relationship remains largely unknown. Investigations of this symbiosis promise to yield valuable information for the management and conservation of *Botrychium* species. For all *Botrychium* species, the reasons that they cannot persist in the climax community when they so heavily depend on mycorrhizae are yet unknown. To manage for *Botrychium* is to manage for mycorrhizae, and to truly understand this species we must understand this interaction.

Revisits are needed for selected populations in Region 2 annually to obtain population size data. Marking and tracking individuals at these sites following methods of Lesica and Ahlenslager (1996) or Johnson-Groh and Farrar (2003) would provide population trend data. Until we have more confidence in our knowledge of the distribution of this species in Region 2, any inferences drawn from the known populations with regard to population trend will by highly speculative. Annual population counts of sporophytes at 10 accessible, large populations of *Botrychium hesperium* in Region 2 would provide valuable data that could help to better assess its population size, trend, and conservation status.

Response to change

The specific responses of *Botrychium hesperium* to disturbance and succession are not clear and warrant further investigation. There has been no specific research on *B. hesperium* addressing these issues. There are numerous and some fairly detailed observations of *B. hesperium* in sites that have resulted from and been maintained by human disturbance. From these data we can draw some inferences regarding the effects these activities will have on populations of *B. hesperium*. A better understanding of the amount of time elapsed since the disturbance occurred is an important research need for *B. hesperium*. However, there is no baseline population data or survey work to reference prior to the disturbances, since in most cases the disturbances occurred before *B. hesperium* was even formally described. The effects of exotic species on the viability of *B. hesperium* populations have not been investigated.

Metapopulation dynamics

The metapopulation dynamics of *Botrychium hesperium* and other *Botrychium* species are not understood. Migration, extinction, and colonization rates are unknown for all *Botrychium* species and will be difficult to determine, given the difficulties in finding and observing this species. Johnson-Groh and Farrar (2003) note four factors that complicate the characterization of the metapopulation structure of *Botrychium* species: 1) the difficulty in finding plants, which results in low confidence that all plants are accounted for and poor understanding of their distribution on the landscape; 2) the predominance of underground life history stages, which precludes the determination of true population size and population dynamics; 3) the underground population, which makes it impossible to determine if a new population arose from spores or dormant gametophytes; and 4) the need for very long term studies to determine population dynamics and the vulnerability of populations to extinction.

Demography

Our current knowledge of demographic processes is not advanced for any member of the *Botrychium* genus, as demographic studies are lacking. *Botrychium mormo* is the best-studied member of the genus (see Berlin et al. 1998), but many assumptions were made in estimating crucial life history parameters even for this species. Thus, any analyses made using current data for *B. hesperium* would be largely conjectural and based on populations in Waterton Lakes National Park, as no demographic data are available for populations in Region 2.

Population trend monitoring methods

Standard population monitoring trend protocols have been developed by Johnson-Groh and Farrar (2003) and have been used successfully in studies of other *Botrychium* species. Other sampling designs have also been used in the study of *Botrychium* species (e.g., Lesica and Ahlenslager 1996, Berlin et al. 1998, Johnson-Groh 1999). Because there are probably many unknown populations (NatureServe 2003), observations at known sites may or may not reflect real population trends (Johnson-Groh 1999). The problem with any methodology is that it is very difficult to determine the true population size of any *Botrychium* species due to the high proportion of the population that remains underground as gametophytes and juvenile sporophytes, and due to the high annual variation in the aboveground sporophyte population (Lesica and Steele 1994). Thus the available methods are not effective for understanding region-wide trends, unless most of the populations of the species are known and incorporated into the monitoring program. Multiple seasons and sampling large populations will be required to detect trends. Due to the inherent difficulties in monitoring *Botrychium* populations, determination of population trend requires tens of years (Johnson-Groh and Farrar 2003).

Restoration methods

There are many barriers to habitat restoration for *Botrychium hesperium* and other *Botrychium* species. *Botrychium* species are extremely difficult to propagate (Whittier 1972, Gifford and Brandon 1978, Wagner and Wagner 1983a), and propagating them for reintroduction to the wild is probably not feasible given the difficulties they present. The below-ground ecology of these species is crucial to understanding their autecology, yet it is also very poorly understood. As obligate mycorrhizal symbionts, they cannot survive without suitable fungal partners, but very little is known about the specifics of this relationship. The difficulties in growing *Botrychium* species are presumably the result of their delicately attuned mycorrhizal relationships (Wagner and Wagner 1983a). The mycobionts of *B. hesperium* have not been identified. Buell (2001) recommends using a fungal inoculum in areas that have had historic soil disturbance to accelerate the recolonization of the site.

Restoration or maintenance of native vegetation will certainly be a crucial part of any restoration effort on behalf of *Botrychium hesperium*. Restoration of native vegetation in the vicinity of known *B. hesperium* occurrences is likely to benefit these populations as possible colonization sites and buffers and in reducing the influx of exotic species. Incorporating natural burning and disturbance regimes into the management of restored habitat will probably be needed to maintain the suitability of habitat for *B. hesperium*.

Research priorities for Region 2

The most obvious research priorities in Region 2 are a better understanding of the range and distribution of *Botrychium hesperium*, and an assessment of population trends in known colonies in wild and human-created habitats. It is very likely that more populations await discovery for this and other species of moonworts (Farrar and Johnson-Groh 1986, Wagner and Wagner 1986), and many of these may fall on USDA Forest Service lands in Region 2. Inventories for both *B. hesperium* and *B. 'michiganense'* are needed to document the range and revise the status of both taxa. Genetic analyses of samples from populations of *B. hesperium* are also needed for verification (Farrar personal communication Farrar 2004).

Kolb and Spribille (2000) offer numerous excellent suggestions for further research on *Botrychium hesperium*. Vanderhorst (1997) also offers numerous suggestions for research and management for *Botrychium* populations on the Kootenai National Forest (in Region 1). Numerous research needs are cited by Farrar and Johnson-Groh (1986), Berlin et al. (1998), and Johnson-Groh (1999), and because of similarities in the life history and ecological needs of *Botrychium* species, many of these apply to *B. hesperium* as well. With respect to *Botrychium* species in general, these include further research on the life history and demography, focusing on underground life history stages. Sporebank longevity could be investigated using the methods of Dyer and Lindsay (1992) and Whittier (1972), which included the germination of spores from herbarium specimens.

In general, there is a great need to understand the ecological requirements of *Botrychium hesperium*. Research on the autecology of *B. hesperium* in needed, particularly with regard to its responses to burning, grazing, disturbance, and succession. More research is needed to determine the types and periodicity of disturbance that create and maintain suitable habitat for *Botrychium* species including *B. hesperium*. The development of potential habitat maps for *Botrychium* species would provide a valuable tool for the identification of new populations (Kolb and Spribille 2000). Habitat manipulation studies (i.e., addition of artificial snow to plots, soil scarification) in robust moonwort populations, in conjunction with monitoring, could begin to identify critical ecosystem properties controlling the distribution and persistence of *B. hesperium* (Kolb and Spribille 2000). Research is needed to determine the differences in soil character and mycorrhizae, exotics, community associates, etc. at recent disturbances versus maintained early successional seres such as road verges and ski slopes, railroad right-of-ways, and powerlines. These questions would be ideal for pursuit as a graduate research project.

Monitoring of *Botrychium hesperium* populations is needed to better understand their life history characteristics including age, dormancy, growth rates, and reproductive rates in Region 2. Monitoring of exotic species is also needed. Monitoring transplant experiments at Copper Mountain and Breckenridge ski areas will determine whether these techniques are effective management practices (Kolb and Spribille 2000). Responses to human disturbance and natural disturbance (including fire, frost action, flooding, and landslides) could be assessed through population monitoring (Zika et al. 2002). Monitoring populations on ski slopes and in timber harvest areas under different management regimes will also be important for identifying appropriate management practices.

A clearer understanding of the relationship between *Botrychium hesperium* and its mycorrhizal symbionts will also have considerable practical value. An assessment of the effects of disturbance quality and periodicity, fire, and grazing on its mycorrhizae will assist managers in ascribing appropriate management protocols. Research is also needed to assess the effect on spore output of different mycorrhizal species and infection levels. Studies on the role of mammals and other potential vectors in the dispersal of spores will assist with the management of *B. hesperium*.

Metapopulation studies are very difficult to conduct for *Botrychium* species, but it is likely that the metapopulation structure is important for them (Johnson-Groh and Farrar 2003). Investigation of migration, extinction, and colonization rates could yield valuable data for the conservation of *B. hesperium* and other *Botrychium* species.

Identifying habitat characteristics through further plot sampling would add some analytical power to the assessment of *Botrychium hesperium* habitat. Kolb and Spribille (2000) used releves but other quantitative methods would be applicable as well. Estimating cover and/or abundance of associated species could permit the investigation of interspecific relationships through ordination or other statistical techniques. Understanding environmental constraints on *B. hesperium* could facilitate the conservation of this species.

Additional research and data resources

Extensive data and resources are available regarding *Botrychium hesperium*. These resources were summarized for assimilation into this report, but some of these resources will be particularly useful for management and conservation planning for *B. hesperium*. Element occurrence data and Potential Conservation Areas developed by the Colorado Natural Heritage Program (2002) will be useful for identifying areas for management actions and conservation initiatives for *B. hesperium* in Region 2. Other reports that are particularly rich in useful data include Lesica and Ahlenslager (1996), Kolb and Spribille (2000), Buell (2001), Chadde and Kudray (2001), and Steinmann (2001a).

An assessment of this species for the Pacific Northwest is forthcoming. Authored by Drs. Don Farrar, Kathleen Ahlenslager, and possibly others, it will complement this report and probably offer much clarification on the issue of *B. 'michiganense'*. The formal description of *B. 'michiganense'*, to be authored by Farrar, Gilman, Zika, and F. Wagner, will also soon be published (Farrar personal communication 2004).

DEFINITIONS

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

Allopolyploidy: The union of genetically distinct chromosome sets, usually two different species, to form a polyploid (Allaby 1998).

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

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

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

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

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

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

Gemmae: Minute vegetative propagules, abscised at maturity from the parent plant (Farrar and Johnson-Groh 1990).

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

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

Mycobiont: The fungal partner in a mycorrhizal symbiosis.

Rank: System used by Natural Heritage Programs, Natural Heritage Inventories, Natural Diversity Databases, and NatureServe.

Global imperilment (G) ranks are based on the range-wide status of a species. State-province imperilment (S) ranks are based on the status of a species in an individual state or province. State-province and Global ranks are denoted, respectively, with an "S" or a "G" followed by a character. **These ranks should not be interpreted as legal designations.**

G/S1	Critically imperiled globally/state-province because of rarity (5 or fewer occurrences in the world/state; or very few remaining individuals), or because of some factor of its biology making it especially vulnerable to extinction.
G/S2	Imperiled globally/state-province because of rarity (6 to 20 occurrences), or because of other factors demonstrably making it very vulnerable to extinction throughout its range.
G/S3	Vulnerable through its range or found locally in a restricted range (21 to 100 occurrences).
G/S4	Apparently secure globally/state-province, though it might be quite rare in parts of its range, especially at the periphery.

G/S5	Demonstrably secure globally, though it may be quite rare in parts of its range, especially at the periphery.
GX	Presumed extinct.
G#?	Indicates uncertainty about an assigned global rank.
G/SU	Unable to assign rank due to lack of available information.
GQ	Indicates uncertainty about taxonomic status.
G/SH	Historically known, but not verified for an extended period, usually.
G#T#	Trinomial rank (T) is used for subspecies or varieties. These taxa are ranked on the same criteria as G1-G5.
S#B	Refers to the breeding season imperilment of elements that are not permanent residents.
S#N	Refers to the non-breeding season imperilment of elements that are not permanent residents. Where no consistent location can be discerned for migrants or non-breeding populations, a rank of SZN is used.
SZ	Migrant whose occurrences are too irregular, transitory, and/or dispersed to be reliable identified, mapped, and protected.
SA	Accidental in the state or province.
SR	Reported to occur in the state or province, but unverified.
S?	Unranked. Some evidence that the species may be imperiled, but awaiting formal rarity ranking.

Note: Where two numbers appear in a G or S rank (e.g., S2S3), the actual rank of the element falls between the two numbers.

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

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

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

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

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