Botrychium echo W.H. Wagner (reflected grapefern): A Technical Conservation Assessment

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

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COVER PHOTO CREDIT

Botrychium echo (reflected grapefern). © Loraine Yeatts.

Summary of Key Components for Conservation of *Botrychium echo*

Status

Botrychium echo (reflected grapfern) is known from 60 locations in Region 2 of the USDA Forest Service. Occurrences at seven of these locations have not been seen within the last 20 years. The total population size in Region 2 is not known, but the total population from locations where population size has been estimated is approximately 1,500 to 2,300 plants. Many other populations probably support significant numbers, but the population size of these sites is not known. *Botrychium echo* was formerly designated as a sensitive species in Region 2 but was not designated sensitive by the Regional Forester in Region 2 in 2003. It is ranked globally vulnerable (G3) by NatureServe. Within Region 2, it is known only from Colorado where it is ranked vulnerable (S3). *Botrychium echo* has no federal status.

Primary Threats

Observations and quantitative data have shown that there are several tangible threats to the persistence of *Botrychium echo*. The primary threats are habitat loss, recreation, succession, overgrazing, effects of 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 occurrences in Region 2 are small, they are also threatened by stochastic processes.

Primary Conservation Elements, Management Implications and Considerations

Of the 60 known occurrences of *Botrychium echo* in Region 2, 55 or 56 have been documented on land managed by the USDA Forest Service. Three occurrences are known from Rocky Mountain National Park. One occurrence is known from land owned by the City of Denver, and another is known from land owned by the City of Colorado Springs. One occurrence is also located at least in part on private land. Seven occurrences have not been seen in over 20 years. At present, conservation efforts to protect the known occurrences of *B. echo* in Region 2 are most likely to be effective. Restoration of populations of members of *Botrychium* subgenus *Botrychium* is probably precluded by the great difficulties in propagating them. Further species inventory efforts are needed to better understand the full range of *B. echo*. 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. echo* 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 USDA Forest Service (USFS), Rocky Mountain Region (Region 2). *Botrychium echo* is the focus of an assessment because of its high degree of rarity and imperilment in Region 2, and because of concern for its viability in Region 2. While *B. echo* was formerly listed as sensitive by the USDA Forest Service Region 2 (USDA Forest Service 1993), it is no longer a sensitive species in Region 2 (USDA Forest Service 2003). However, *Botrychium* species have been the focus of increasing interest by the USDA Forest Service and other federal and state agencies due to their rarity, difficulty in detection, and highly variable populations (Johnson-Groh and Farrar 2003). This assessment addresses the biology of *B. echo* throughout its range in Region 2. This introduction outlines the scope of the assessment and describes the process used in producing the assessments.

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 to 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 echo* with specific reference to the geographic and ecological characteristics of the USFS Rocky Mountain Region. Although some of the literature relevant to the species may originate from field 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. echo* 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. All known publications, reports, and element occurrence records on *Botrychium echo* are referenced in this assessment, and most of the experts on this species were consulted during its synthesis. All available specimens of *B. echo* were viewed to verify occurrences and incorporate specimen label data. Specimens were searched for at COLO (University of Colorado Herbarium), COCO (Carter Herbarium), CS (CSU Herbarium), GREE (University of Northern Colorado Herbarium), RM (Rocky Mountain Herbarium), KH (Kalmbach Herbarium), and SJNM (San Juan College Herbarium). The assessment emphasizes refereed literature because this is the accepted standard in science. However, some non-refereed literature was used in the assessment when information was unavailable elsewhere. Unpublished data (e.g. natural heritage program records) were important in estimating the geographic distribution, and contain the vast majority of the useful information known on *B. echo*. However, these data required special attention because of the diversity of persons and methods used in their collection. Non-refereed publications and reports were regarded with greater skepticism.

Treatment of Uncertainty

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.

Publication of Assessment on the World Wide Web

To facilitate their use in the Species Conservation Project, species assessments are being published on the USFS 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 facilitates 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

While *Botrychium echo* was formerly considered sensitive in USFS Region 2 (USDA Forest Service 1993), it is not currently listed as a sensitive species by that agency in Region 2 (USDA Forest Service Rocky Mountain Region 2003) or the Bureau of Land Management in Colorado (Bureau of Land Management 2000). *Botrychium echo* is not listed as Threatened or Endangered in accordance with the endangered species Act. However, all *Botrychium* species have attracted much attention over the last decade from federal and state agencies (Johnson-Groh and Farrar 2003).

The International Union for Conservation of Nature and Natural Resources does not list *Botrychium echo* as endangered or vulnerable (International Union for Conservation of Nature and Natural Resources 1978). NatureServe changed the global rank of *B. echo* from globally imperiled (G2) to globally vulnerable (G3) on February 11, 2003, which means that few verified occurrences are known and those occurrences are typically small (with less than 10 individuals) (Colorado Natural Heritage Program 2004). This change is not yet reflected on the NatureServe Web site (NatureServe 2003). *Botrychium echo* is considered vulnerable (S3) in Colorado (**Table 1**; Colorado Natural Heritage Program 2004). A change in state status (from S2 to S3) was made at the same time as the global status change and is also not updated yet on the NatureServe Web site (NatureServe 2003). *Botrychium echo* is not known from the other states of Region 2. It is considered critically imperiled (S1) in Utah and is reported (SR) from Arizona (**Table 1**; NatureServe 2003). It is newly recognized to occur in New Mexico (Spellenberg 2004) and has not yet been assigned a state rank there. Please see the Distribution and abundance section of this document for a summary of the rangewide status of *B. echo* and for summary data for the known occurrences. For explanations of NatureServe's ranking system, please see the Definitions section of this document.

Table 1. Summary of the known global distribution and status of *Botrychium echo* (from Kartesz 1999, NatureServe 2003, and Spellenberg 2004). Region 2 state is in bold.

Nation	State	S rank	State Designation	Notes
USA	Arizona	SR	None	
USA	**Colorado**	**S3**	**None**	
USA	New Mexico	No rank	None	Noted by Spellenberg 2004
USA	Utah	S1	None	

Existing Regulatory Mechanisms, Management Plans, and Conservation Strategies

Adequacy of current laws and regulations

Botrychium echo has no legal protection unto itself that would prevent the destruction of its habitat or individual plants. It is not listed as threatened or endangered in accordance with the Endangered Species Act, and therefore there are no federal laws concerned specifically with its conservation. Because *B. echo* is not a designated sensitive species in Region 2 or in other regions of the USFS, it is not considered in biological evaluations that are required by policy for all USFS projects to determine impacts to sensitive species.

Thus it is not offered any specific protective measures on USFS lands. Given the rarity (small population size and small number of known populations) of *B. echo* in Region 2 and the ongoing human impacts to individuals and habitat, current laws and regulations appear inadequate to conserve this species.

Adequacy of current enforcement of laws and regulations

At least one occurrence of *Botrychium echo* may be extirpated by road widening on Guanella Pass in Colorado (Popovich personal communication 2003). This is occurring on the Arapaho National Forest in a permanent easement held by the Federal Highways Administration. Although *B. echo* was listed as a sensitive species when this project was finalized, it was determined that the possible loss of this occurrence would probably not impact the viability of this species in Region 2. Existing regulations do not have any provisions for the protection of individual occurrences or habitat. Justifying the protection of occurrences of *B. echo* during project planning activities on USFS land will be more difficult without sensitive species status. Since this species was recognized, there has been no other known case in which an occurrence was extirpated due to human activities or the failure to enforce any existing regulations. Please 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. echo*.

Biology and Ecology

Classification and description

Botrychium echo 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 and moonworts) is the most diverse of these genera with 50 to 60 species (Wagner and Wagner 1993). Hassler and Swale (2001) list 61 species and five hybrids of *Botrychium* worldwide. The genus *Botrychium* contains three subgenera: *Osmundopteris*, *Sceptridium*, and *Botrychium* (Wagner and Wagner 1993). Subgenus *Botrychium* (moonworts), which contains *B. echo*, is the most diverse of the three subgenera with perhaps 25 to 30 species. Members within the subgenus share many morphological traits, and subtle interspecific differences make field identification difficult. In addition, members within 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 elucidation of interspecific differences has been confounded by the cryptic nature of *Botrychium* species (Paris et al. 1989, Wagner 1998, Hauk and Haufler 1999). Please see **Table 2** for a summary of the classification of *B. echo*.

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

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

[1]Wagner and Wagner 1993

[2]Clausen 1938

[3]Wagner and Wagner 1983b

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 in North America (Wagner and Wagner 1994) as compared to six in 1938 (Clausen 1938).

Botrychium echo was described in 1983 by Drs. Herb and Florence Wagner along with *B. hesperium* (Wagner and Wagner 1983b). Before this, specimens of this species were usually identified as *B. matricariifolium* ssp. *hesperium* or *B. lanceolatum*. However, the distinctness of *B. echo* had been recognized by early collectors, such as E. Bethel and Ira Clokey, who in 1914 separated their collections of what they thought were *B. matricariifolium* ssp. *hesperium* into two groups- "typical," and "segments narrow, more acute" (Wagner and Wagner 1983b). The type specimen was collected at Glacier Lake in Boulder County, Colorado by Bethel and Clokey (collection 3937a) and is housed at the Smithsonian Institution (Wagner and Wagner 1983b). This species was named for its tendency to reflect the characteristics of other species including *B. lanceolatum* and *B. pinnatum*, and for Echo Lake in Clear Creek County, Colorado where the Wagners clarified their concept of *B. hesperium* and *B. echo* (Wagner and Wagner 1983b, Root personal communication 2003).

Botrychium species can be extremely difficult to identify, due to their subtle diagnostic characters, frequent co-occurrence of multiple *Botrychium* species at a location, and high morphological variability. Positive identification requires comparison with silhouette outlines of verified specimens (such as those presented in Wagner and Wagner 1986) and the use of dichotomous keys (see Weber and Wittmann 2001). Small samples and immature individuals often cannot be identified with certainty.

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

Botrychium echo averages 9.5 cm (3 to 15 cm) tall (**Figure 1** and **Figure 2**; Wagner and Wagner 1983b). It has a short stalk (usually 0 to 4 mm long), while the fertile blade (sporophore) is relatively short, only half again as long as the sterile blade (trophophore) (Wagner and Wagner 1983b, Wagner and Wagner 1993). It often has a reddish-brown stripe along the common stalk from the base of the trophophore stalk (Wagner and Wagner 1993, Spackman et al. 1997). The features of the sterile portion of the trophophore are valuable diagnostic characteristics for this and other *Botrychium* species. The trophophore is shiny green on live plants (Wagner and Wagner 1983b), which is a useful characteristic for field identification (Root personal communication 2002). The trophophore is short stalked, broadly oblong to oblong-deltate, 4 cm by 3 cm, and firm (Wagner and Wagner 1983b, Wagner and Wagner 1993). There may be up to four pairs of pinnae, usually crowded or overlapping, and narrowly attached to a relatively narrow rachis (Wagner and Wagner 1983b, Wagner and Wagner 1993). The basal pair of pinnae is usually cleft into a smaller lower segment and a larger upper segment (Wagner and Wagner 1993, Spackman et al. 1997). Often there is slightly more distance between the 1^{st} and 2^{nd} pinna pairs than between the 2^{nd} and 3^{rd} pinna pairs (Wagner and Wagner 1993). There are also dissected forms of *B. echo* that have a few to many lobes on their pinnae. Colorado occurrences may have as many as 30 percent of the individuals with this dissected form. Some dissected *B. echo* leaves closely resemble those of *B. alaskense*, a species from Alaska described in 2002 (Root personal communication 2003). Lorain (1990) speculated that material found in the Idaho Panhandle may be an unusually dissected form of *B. echo*, but this apparently has not been verified. The lower pinnae of *B. pallidum*, *B. lunaria*, *B. minganense*, and *B. simplex* are fan or wedge-shaped, while those of *B. hesperium* and *B. pinnatum* are oblong to ovate with rounded tips and not clearly separated (Spackman et al. 1997).

Figure 1. *Botrychium echo*. Photograph provided by Loraine Yeatts.

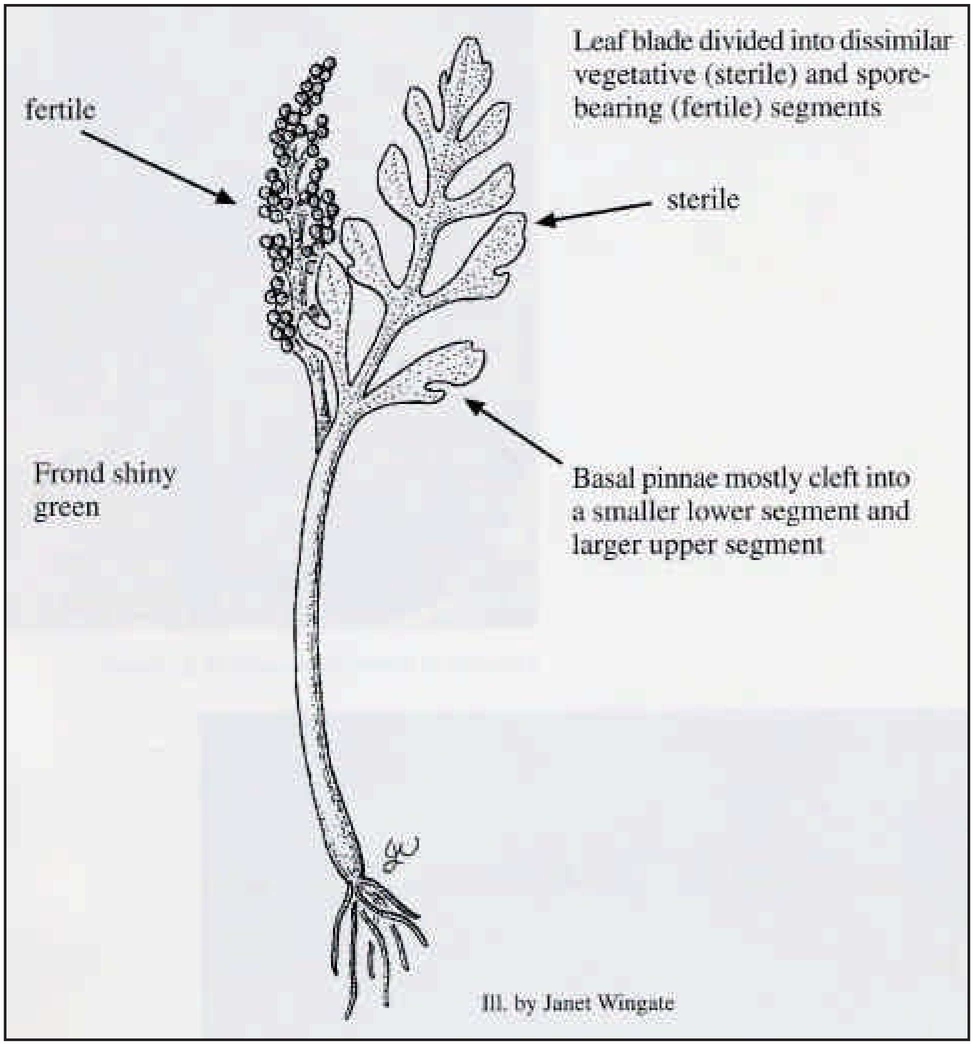

Figure 2. Illustration of *Botrychium echo*, showing its diagnostic characteristics. Illustration by Janet Wingate (used with permission).

Botrychium echo is one of five *Botrychium* species (others are *B. pumicola*, *B. gallicomontanum*, *B. campestre*, and *B. hesperium*) that produce gemmae on their root bases (Farrar and Johnson-Groh 1990, Camacho 1996, Camacho and Liston 2001, Johnson-Groh et al. 2002). Gemmae are minute, spheric, budlike structures that abscise at maturity from the parent plant and are a means of asexual reproduction (Farrar and Johnson-Groh 1990). They permit sporophytes to give rise to other sporophytes directly without passing through a gametophyte generation, which may be an adaptation to drought. No other fern genus produces such structures, but analogous aboveground structures are common in *Huperzia* species (Lycopodiaceae) (Anonymous reviewer personal communication 2003).

Botrychium echo is believed to be the result of an allopolyploid cross between *B. lanceolatum* and *B. campestre*, between which it is intermediate in many characters (Kempema and Smart 2001). *Botrychium campestre* produces copious amounts of gemmae, while *B. echo* produces fewer. The gemmae in *B. echo* also are more broadly attached to the root base and more frequently develop into sporophytes while still attached to the parent plant (Farrar and Johnson-Groh 1990). *Botrychium echo* is tetraploid (n=90), while its purported parent species are both diploid (n=45) (Wagner 1993).

In Region 2, *Botrychium echo* is likely to be found with *B. hesperium*, from which it can be distinguished in the field by features of the trophophore as described in Wagner and Wagner (1983b). Distinguishing between 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 echo* is shiny green in life, while *B. hesperium* is dull green (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* also differs from *B. hesperium* in that it reproduces asexually with gemmae. Although highly diagnostic, this is a poor characteristic for field identification since using it requires excavation of the plant. See Wagner and Wagner (1983b) for further details and technical illustrations showing the differences between *B. hesperium* and *B. echo*. **Figure 3** is reproduced from this paper and well illustrates the diagnostic differences in the trophophores of *B. hesperium* and *B. echo*.

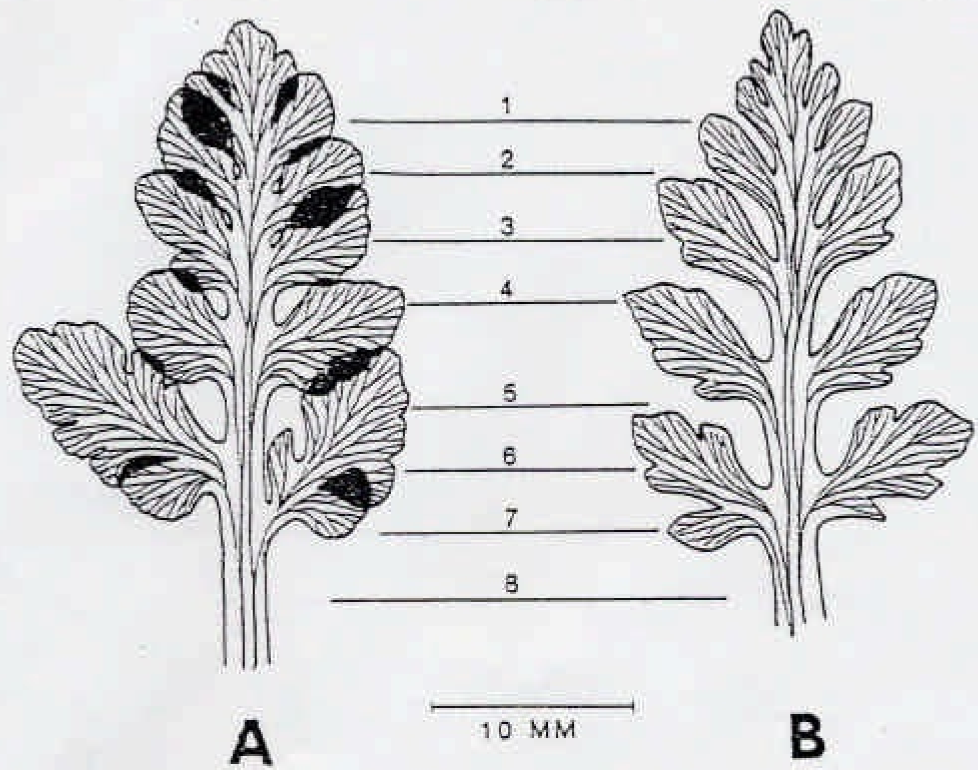

FIG. 4. Comparison of *B. hesperium* (A) and *B. echo* (B) from a mixed population at San Francisco Peaks, AZ. Tracings of cleared sterile blades. The characters are: 1 = ovate, broadly attached vs. linear-lanceolate, narrowly attached pinnae. 2 = Overlapping vs. separated pinnae. 3 = Repand vs. subentire 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 3. 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 echo*. See Colorado Native Plant Society (1997) and Spackman et al. (1997) for photographs, drawings, and descriptions. The original description can be found in Wagner and Wagner (1983b) with silhouettes of plants. The Flora of North America (Wagner and Wagner 1993) also offers a full description and a small range map of the species.

Distribution and abundance

Botrychium echo is endemic to the Southern Rocky Mountain cordillera, known only from Colorado, northern Utah, northern Arizona, and northern New Mexico (**Figure 4**; Lellinger 1985, Zika et al. 1995, NatureServe 2003, USDA Natural Resources Conservation Service 2001, Colorado Natural Heritage Program 2004, Spellenberg 2004). In Region 2, it is currently known from 60 occurrences in 27 counties throughout the mountains of Colorado (Colorado Natural Heritage Program 2004). Please see **Table 3** for summary information on the known occurrences of *B. echo* in Region 2, and **Figure 5** for a detailed map showing its distribution in Region 2.

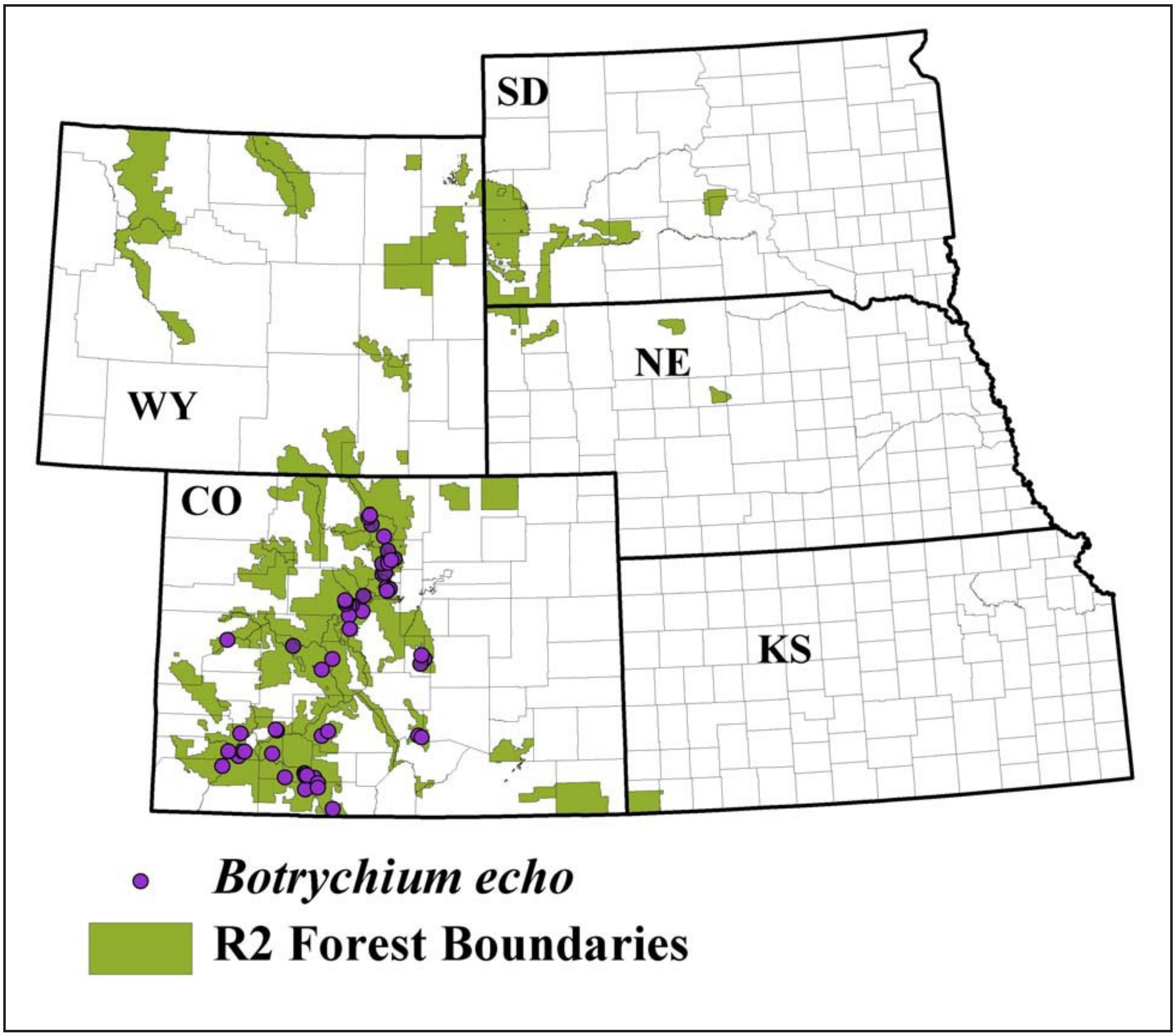

Figure 4. Distribution of the known occurrences of *Botrychium echo* in the states of USDA Forest Service Region 2 (R2).

Table 3. Summary information for the known occurrences of *Botrychium echo* in Colorado. CNHP=Colorado Natural Heritage Program.

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation (feet)	Habitat	Occurrence Size	Source I.D.
1	Archuleta	Nipple Mountain	8/1/2001	USFS: San Juan National Forest	10,360	Old clear-cut area, with young spruce.	100+ of four *Botrychium* species	CNHP EO 49
2	Boulder	Brainard Lake	8/4/2001	USFS: Arapaho-Roosevelt National Forest	10,340	Subalpine meadow. Plants in two subpopulations concentrated on 1/5 of an acre.	50 or more	D. Steinmann (2001b)
3	Boulder	Caribou Townsite	8/19/1992	USFS: Arapaho-Roosevelt National Forest	10,000	Not reported.	not reported	CNHP EO 4
4	Boulder	Coney Flats	8/11/1999	USFS: Arapaho-Roosevelt National Forest	9,900	Throughout the open moist meadow. *Botrychium hesperium*, *B. lanceolatum*, *B. pallidum*, and *B. minganense* are also found at this location.	not reported	D. Steinmann
5	Boulder	Devil's Thumb Trail	7/22/2000	USFS: Arapaho-Roosevelt National Forest	~10,900	In open subalpine meadow along trail.	20 to 50	D. Steinmann (2001b)
6	Boulder	Glacier Lake	9/8/1914	USFS: Arapaho-Roosevelt National Forest/ private	8,500	Not reported.	not reported	CNHP EO 14
7	Boulder	Arapaho Moraine	8/15/1947	USFS: Arapaho-Roosevelt National Forest	9,500	On east slope in gravelly, poorly drained soil.	not reported	CNHP EO 15
8	Boulder	Near Rainbow Lakes	7/31/1995	USFS: Arapaho-Roosevelt National Forest	9,685	Open, disturbed gravelly area in forest.	"few"	Jennings (95-26) and Lamand (COLO)
9	Clear Creek	Echo Lake	7/10/2003	City of Denver: Echo Lake Park	10,650	West side of road and disturbed area along trail under lodgepole pines; on gravelly slope between Echo Lake lodge and lake.	~20	CNHP EO 5
10	Clear Creek	Warrior Mountain	7/10/2003	USFS: Arapaho National Forest	11,020 to 11,080	Growing adjacent to a willow in a small roadside meadow.	~37	CNHP EO 6
11	Clear Creek	Warrior Mountain	7/10/2003	USFS: Arapaho National Forest	10,940 to 11,020	Along road cut (both sides), mostly the south side of the road. Roadside bank at a picnic ground.	~53	CNHP EO 7
12	Clear Creek	Kingston	8/11/1984	USFS: Arapaho-Roosevelt National Forest	10,710	Between boarding house and mine.	not reported	CNHP EO 18
13	Clear Creek	Devil's Canyon	8/15/1995	USFS: Arapaho National Forest	10,040	Roadside areas of lodgepole regeneration stand. Light exposure: open. Topographic position: lower slope. Moisture: dry to moist.	not reported	CNHP EO 24

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation	Habitat	Occurrence Size	Source I.D.
14	Clear Creek	Guanella Pass Road	9/19/2003	USFS: Arapaho National Forest	10,200	Appears to have been disturbed in the past, fine sandy loam soils with a sparse covering of *Fragaria* spp., *Achillea lanulosa*, *Taraxacum officinale*, *Solidago spathulata* ssp. *nana*, *Trisetum spicatum*, *Festuca ovina*, and other species. All moonworts were transplanted from this site in 2003, and this site will be heavily disturbed during road construction.	4	ERO Resources 2003
15	Clear Creek	Guanella Pass Road	9/20/2003	USFS: Arapaho National Forest	11,200	Gravelly, sparsely vegetated areas on west side of road and on disturbed banks of an old spur road. Twenty-seven *Botrychium echo* individuals were transplanted from this site in 2003. Most of this location will be heavily disturbed during road construction; 121 *Botrychium* individuals (species not specified) fall outside the project area but are at risk from disturbance during construction.	~425 of 5 *Botrychium* spp.	ERO Resources 2003
16	Conejos	Iron Creek	8/30/1994	USFS: Rio Grande National Forest	10,500	Total tree cover 5 percent. Total shrub cover 5 percent. Total forb cover 45 percent. Total graminoid cover 25 percent. Total moss/ lichen cover 10 percent. Total bare ground cover 10 percent. Site logged pre -1967 and probably much earlier based on the condition of rotting stumps. Area is regenerating very slowly. Aspect: south. Percent slope: 20. Slope shape: straight. Light exposure: open. Topographic position: lower slope. Moisture: dry. Parent material: qf- alluvial fan deposits (holocene) poorly sorted material ranging from silt to boulders, locally deposited largely by mud flows. Geomorphic land form: moderate mountain slope. Soil texture: very strong silt loam. Soil surface gravelly. Soil map unit 125. (cryoboralf-rock outcrop complex.)	est. 70 or more	CNHP EO 19
17	Conejos	Platoro Reservoir	9/10/1998	USFS: San Juan-Rio Grande National Forest	10,400	Habitat type: *Picea engelmannii/vaccinium myrtillis*. Tree cover: trace. Shrub cover: 0 percent. Forb cover: 10 percent. Graminoid cover: 2 percent. Moss/lichen cover: trace. Bare ground cover: 88 percent. Aspect: southeast. Slope: 60 percent. 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.	9	CNHP EO 33

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation	Habitat	Occurrence Size	Source I.D.
18	Conejos	Pinorealosa Mountain	8/24/2000	USFS: Rio Grande National Forest	10,840	Total tree cover: trace of *Picea engelmanni*. Total shrub cover: 0 percent. Total forb cover: 20 percent. Total graminoid cover: 15 percent. Total moss/lichen cover: trace. Total bare ground cover: 65 percent. Habitat type: sparse Engelmann spruce/ *Festuca thurberi* (this area burned historically, perhaps from the huge 1879 Osier fire, and spruce did not regenerate well in this area; spruce cover was probably very sparse historically). Aspect: plants are most abundant on the west aspect. Slope: 0 to 10 percent. Slope shape: convex. Light exposure: open. Moisture: dry. Parent material: tto-ojito creek member - nonwelded gray to densely welded brown quartz laitic ashflow sheet containing 10 to 20 percent phenocrysts. Lithic fragments characteristically are sparse in comparison with other major units of treasure mountain tuffs. Geomorphic land form: glaciated mountain slopes and ridges. Soil texture: fine sandy loam. Tally of *Botrychium* species found: *B. echo*: 61, *B. lanceolatum*: 25, *B. minganese*: 2, *B. lunaria*: 2, unknown: 47 (too cold damaged to identify species).	61	CNHP EO 38
19	Custer	GreenHorn Mountain Road	7/20/1999	USFS: San Isabel National Forest	10,900	Habitat type: roadside bank in spruce forest.	"few"	CNHP EO 48
20	Custer, Huerfano	GreenHorn Mountain Road	7/20/1999	USFS: San Isabel National Forest	9,850	Habitat type: roadside gravel pit/bank on west side of road.	6	CNHP EO 47
21	Delta	Island Lake Campground	8/29/1996	USFS: Grande Mesa National Forest	10,839	Roadcut and disturbed roadside, both sides of road and south of a parking area.	not reported	CNHP EO 46
22	Dolores	Barlow Road	7/26/2002	USFS: San Juan National Forest	10,800 to 11,000	Total tree cover: 0 percent. Total shrub cover: 0 percent. Total forb cover: 5 to 20 percent. Total graminoid cover: 1-5 percent. Total moss/lichen cover: 0 percent. Total bare ground cover: 80 to 95 percent.habitat type: road margin. Aspect: southwest. Slope: 30 to 40 percent. Slope shape: flat margin of road on steep talus. Light exposure: open. Moisture: dry. Parent material: igneous. Geomorphic land form: talus slope on canyon slope.	6	CNHP EO 50
23	Eagle, Summit	Shrine Pass	8/14/2000	USFS: White River National Forest	10,564	Associated plant community: open sparsely vegetated slopes with *Festuca* spp., *Heterotheca pumila*, and *Cirsium eatonii*. Aspect: east. Slope: 15 to 25 percent. Light exposure: open. Moisture: dry/mesic. Parent material: sandstone.	200 to 500	CNHP EO 41
24	El Paso	Pikes Peak Trail	7/9/1887	USFS: Pike National Forest	10,600	Pike's Peak trail.	not reported	CNHP EO 12

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation	Habitat	Occurrence Size	Source I.D.
25	El Paso	Pikes Peak Highway	7/8/2000	USFS: Pike National Forest, City of Colorado Springs	11,070 to 11,275	Roadside areas that appear to have recovered from disturbance, in open meadows and near wetlands in the subalpine zone.	50 to 100	Steinmann (2001a)
26	Gilpin	Kingston	8/12/1984	USFS: Arapaho National Forest	10,720	Not reported.	not reported	CNHP EO 8
27	Grand	Crater Creek	7/27/1961	NPS: Rocky Mountain National Park	~10,200	Mud slope below talus on bare surface exposed to sun.	not reported	J. Douglass and M. Douglass (61394) (COLO)
28	Gunnison	Rustlers Gulch Trail	7/19/1954	USFS: Gunnison National Forest	~9,700	In aspens.	not reported	CNHP EO 22
29	Gunnison	Cottonwood Pass	8/26/1999	USFS: Grande Mesa-Uncompahgre, Gunnison National Forest	12,080	Total tree cover: 0 percent. Total shrub cover: 10 percent. Total forb cover: 15 percent. Total graminoid cover: 20 percent. Total moss/lichen cover: 15 percent. Total bare ground cover: 20 percent. Habitat type: alpine meadow. Aspect: south to southwest. Slope: 15 percent. Slope shape: concave. Light exposure: open-full sun. Topographic position: midslope. Moisture: seasonally wet (snowmelt). Parent material: gravelly granite. Geomorphic land form: glaciated mountain slopes. Also on barren roadcut. With *Oxytropis deflexa*, *Fragaria virginiana*, *Achillea lanulosa*, *Botrychium lunaria*, *B. lanceolatum*, and *B. minganense*.	~10	CNHP EO 37
30	Gunnison	Cameron Gulch	7/9/2002	USFS: Gunnison National Forest	10,760 to 11,000	Total tree cover: 1 percent. Total shrub cover: 30 percent. Total forb cover: 10 percent. Total graminoid cover: 3 percent. Total moss/lichen cover: 25 percent. Total bare ground: 1 percent. Percent rock cover of surface: 25 percent. Aspect: west. Slope: 45 to 50 percent. Slope shape: convex. Light exposure: partial shade. Topographic position: midslope. Moisture: moist. Parent material: granitic. Soil texture: sandy loam.	50 to 150	CNHP EO 51
31	Hinsdale	Weminuche Creek	8/27/1995	USFS: Rio Grande National Forest	9,800	Tree cover: 0 percent. Shrub cover: 10 percent. Forb cover: 10 percent. Graminoid cover: 10 percent. Moss/lichen cover: 40 percent. Bare ground cover: 20 percent. Rest is boulders. Habitat type: scree slope with some vegetation (upper montane). Aspect: northwest. Slope: 20 percent. Slope shape: straight. Light exposure: open. Topographic position: midslope. Moisture: dry. Parent material: tuff (maybe rhyolitc). Geomorphic land form: rockslides. Soil texture: clay loam.	12	CNHP EO 25

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation	Habitat	Occurrence Size	Source I.D.
32	Hinsdale	Slumgullion Slide	9/6/1997	USFS: Grande Mesa-Uncompahgre, Gunnison National Forest	11,240	Habitat type: adjacent to spruce-fir habitat (150 ft to 175 ft away). Total tree cover: <1 percent *picea engelmannii* seedlings. Total forb cover: <1 percent. Total shrub cover: no shrubs. Total graminoid cover: maybe 1 percent. Total moss/lichen cover: trace. Total bare ground cover: 30 percent. Rocks/large boulders: 65 percent+. Slope: 1 percent. Slope shape: convex (slightly). Light exposure: open with full sun. Topographic position: drainage. Moisture: dry with seasonal moisture. Parent material: granitic. Geomorphic land form: massive slide. Soil texture: not sure-high percent course fragments, gravelly granitic.	~75	CNHP EO 29
33	Hinsdale	Slumgullion Gravel Pit	8/18/1999	USFS: Grande Mesa-Uncompahgre, Gunnison National Forest	11,320	Habitat type: old gravel pit on east facing hillside. Aspect: east. Light exposure: open. Moisture: dry. Parent material: volcanic tuff. Total tree cover: 5 percent. Total shrub cover: 0 percent. Total forb cover: 40 to 50 percent. Total graminoid cover: 10 percent. Total moss/lichen cover: 30 percent. Total bare ground cover: 10 percent. Aspect: nw. Slope: 0 to 3 percent. Slope shape: straight. Light exposure: open. Topographic position: midslope. Moisture: dry. Parent material: dark dacitic flows and minor breccias. Geomorphic landform: glaciated mountain slope.	4	CNHP EO 31
34	Jackson	Cameron Pass	8/1/1986	USFS: Arapaho National Forest	10,250	Not reported.	not reported	CNHP EO 9
35	Lake	Arkansas River Headwaters	9/12/2000	USFS: San Isabel National Forest	11,250	Light exposure: open. Moisture: dry.	50 to 100	CNHP EO 44
36	Lake	May Queen Campground	7/9/1998	USFS: San Isabel National Forest	9,900	On a boulder-strewn slope (~30 degrees) with grasses and forbs, underneath a powerline.	not reported	W. Hauk
37	Larimer	Glacier Knobs	8/12/2000	NPS: Rocky Mountain National Park	9,850 to 10,360	Shoulder of highway, bare soil and rock cover about 60 percent of area. In gravel along trail. Geol: gneiss. Aspect: south-southeast. Soil: scree. Slope: 45 [degrees]. Spruce forest opening. Geol: gneiss outcrops and cliffs. Soil: decomposed gneiss and organic soil. Slope: level.	~7	CNHP EO 1
38	Larimer	Loch Vale	7/1/1987	NPS: Rocky Mountain National Park	10,200	Geology: gneiss. Soil: scree. Aspect: south-southeast. Slope: 50 percent. Along 20 feet of trail.	not reported	CNHP EO 2
39	Larimer	Zimmerman Lake	7/18/1985	USFS: Arapaho-Roosevelt National Forest	10,180	Aspect: north, with young spruce.	~3	CNHP EO 3
40	Mineral	Black Mountain	7/30/1996	USFS: San Juan National Forest	10,040	Aspect: southwest. Light exposure: shaded under a small spruce tree. Topographic position: midslope. Moisture: dry. Parent material: volcanic ash flow. Geomorphic land form: mountain slope.	7	CNHP EO 26

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation	Habitat	Occurrence Size	Source I.D.
41	Mineral	Lobo Overlook	7/25/1998	USFS: Rio Grande National Forest	11,600	Aspect: south. Slope: <5 percent. Light exposure: open. Topographic position: upperslope, ridge. Moisture: dry. Parent material: volcanic extrusive. Geomorphic land form: glaciated mountain slopes/ridge. Tree cover: <5 percent; engelmann spruce. Forb cover: 50 percent. Moss/lichen cover: 10 percent. Shrub cover: <5 percent. Graminoid cover: 20 percent. Bare ground cover: 20 percent.	~100	CNHP EO 27
42	Mineral	Wolf Creek Pass	7/25/1998	USFS: Rio Grande National Forest	10,880 to 11,280	Aspect: west. Slope: 5 percent. Light exposure: partial shade from tall spruce canopy. Topographic position: midslope. Moisture: dry. Parent material: volcanic extrusive. Geomorphic land form: glaciated mountain slopes. Soil texture: rocky. Tree cover: 5 percent; engelmann spruce. Forb cover: 5 percent. Moss/lichen cover: 5 percent. Shrub cover: 0 percent. Graminoid cover: <5 percent. Bare ground cover: 90 percent.	~20	CNHP EO 28
43	Mineral	Wolf Creek Pass	7/16/1998	USFS: Rio Grande National Forest	10,980	Total tree cover: trace. Total forb cover: 15 percent. Total moss/lichen cover: 0 percent. Total shrub cover: trace. Total graminoid cover: 20 percent. Bare ground: 65 percent. Aspect: nne. Slope: 15 percent. 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 species is quite common all over the ski area.	5	CNHP EO 30
44	Mineral	Alberta Park	9/4/1998	USFS: Rio Grande National Forest	10,280	Habitat type: *Picea engelmannii/Vaccinium myrtillis*. Tree cover: trace. Shrub cover: trace. Forb cover: 15 percent. Graminoid cover: 5 percent. Moss/lichen cover: 0 percent. Bare ground cover: 80 percent (bare ground, gravel cobble).aspect: southeast. Slope: 40 percent. 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.	60	CNHP EO 34
45	Montezuma	Orphan Butte Plantations	7/20/1995	USFS: San Juan National Forest	10,680 to 10,840	Tree cover: 10 percent. Shrub cover: 5 percent. Forb cover: 40 percent. Graminoid cover: 60 percent. Moss/lichen cover: 2 percent. Bare ground cover: 15 to 20 percent. Slope shape: straight-concave. Light exposure: open. Topographic position: crest, upperslope. Moisture: seasonally moist. Parent material: sandstone. Geomorphic land form: mountain slope. Aspect: west and northwest.. Slope: 2 to 8 percent.	6	CNHP EO 23

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation	Habitat	Occurrence Size	Source I.D.
46	Park	Mount Sheridan	8/10/1990	USFS: Pike - San Isabel National Forest	11,700	Gravelly roadside.	not reported	CNHP EO 17
47	Park, Summit	Boreas Pass	8/9/2000	USFS: White River National Forest	11,483	Associated plant community: dry, gravelly, eroded slopes. Aspect: west. Light exposure: open.	100 to 200	CNHP EO 40
48	Rio Grande	Fivemile Park	9/16/1998	USFS: Rio Grande National Forest	11,000	Habitat type: Engelmann spruce/*Vaccinium myrtillis*. Total tree cover: 1 percent. Total shrub cover: 0 percent. Total forb cover: 30 percent. Total graminoid cover: 10 percent. Total moss/lichen cover: 4 percent. Total bare ground cover: 55 percent. Aspect: southeast. Slope: 10 percent. 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.	14	CNHP EO 32
49	Saguache	Lookout Mountain	9/18/1998	USFS: Rio Grande National Forest	10,600	Habitat type: *Picea engelmannii/Juniperus communis*. Tree cover: 2 percent. Shrub cover: trace. Forb cover: 20 percent. Graminoid cover: 20 percent. Moss/lichen cover: 10 percent. Bare ground cover: 48 percent. Aspect: north. Slope: 10 percent. 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.	1	CNHP EO 35
50	Saguache	Miner's Creek	9/22/1999	USFS: Rio Grande National Forest	11,200	Total forb cover: 55 percent. Total graminoid cover: 15 percent. Total moss/lichen cover: 20 percent. Total bare ground cover: 20 percent. Habitat type: *Picea engelmanni/Vaccinium myrtillus* (site is disturbed and heavily forb dominated community). Slope: 15 percent. Slope shape: concave. Light exposure: most of site is open. Topographic position: lowerslope. Moisture: dry. Parent material: tev-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.	23	CNHP EO 39
51	San Juan	Coal Bank Hill	7/30/1987	USFS: San Juan National Forest	10,000	Not reported	not reported	CNHP EO 10

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation	Habitat	Occurrence Size	Source I.D.
52	San Juan	Molas Pass	8/17/1994	USFS: San Juan National Forest	10,880	Total tree cover: 1 to 55 percent. Total forb cover: 15 to 20 percent. Total moss lichen cover: 1 to 5 percent. Total shrub cover: 1 to 3 percent. Total graminoid cover: 2 to 10 percent. Total bare ground cover: 50 percent. Rock: trace. Litter: 45 percent. Aspect: south and southwest. Slope: 20 percent. Slope shape: convex-straight. Light exposure: partial shade- open. Topographic position: upper slope. Moisture: dry. Parent material: sandstone. Geomorphic land form: glaciated mountain slope. Soil texture: sandy silt. Total bare ground cover: 95 percent. Litter/? 4 percent. Associated plant community: Englemann spruce~3' tall, willow ~2.5' tall.	~8	CNHP EO 20
53	San Juan	Lime Creek	Aug. 1994	USFS: San Juan National Forest	10,360	Road cut. Aspect: southwest. Slope: 2 percent. Slope shape: straight. Light exposure: open. Topographic position: flat roadcut - pulloff. Total forb cover: 30 percent. Total moss/lichen cover: 2 percent. Total graminoid cover: 10 percent. Total bare ground cover: 60 percent.	1	CNHP EO 21
54	San Miguel	Savage Basin	7/29/1999	USFS: Grande Mesa-Uncompahgre, Gunnison National Forest	11,000	Steep eroded bank above road. Also *Botrychium lunaria* and *B. multifidum* in same area.	12	CNHP EO 36
55	Summit	Copper Mountain	7/10/1998	USFS: White River National Forest	10,000	Associated plant community: *Botrychium lanceolatum*, *B. hesperium*, *B. echo* at the top of a steep east-facing slope in grass and forbs.	8	CNHP EO 42
56	Summit	Copper Mountain Ski Area	9/1/1999	USFS: White River National Forest	11,400	Total tree cover: 0 percent. Total shrub cover: 20 to 80 percent. Total forb cover: 5 to 60 percent. Total graminoid cover: 20 percent. Total moss/lichen cover: 10 percent. Total bare ground cover: 10 to 90 percent. Habitat: subalpine meadow/ski run. Associated plant community: timbered hillside with *Vaccinium myrtillus*, *V. caespitosum*, *Abies lasiocarpa*, *Picea engelmannii*, moss. Habitat type: subalpine forest with willow seep, and a ski run on a steep slope, which used to be a lodgepole and spruce/fir stand. Aspect: northeast. Slope: 25 percent. Slope shape: convex. Light exposure: winter; none, snow covered. Summer; early morning to mid-afternoon. Moisture: soil was saturated. Parent material: organic soil. Geomorphic landform: subalpine slope. Soil texture: cobbly.	over 100	CNHP EO 43

Arbitrary Number	County	Location	Date of Last Observation	Land Ownership	Elevation	Habitat	Occurrence Size	Source I.D.
57	Summit	Vail Pass	7/22/1999	USFS: White River National Forest	11,400	Habitat: subalpine meadow. Total tree cover: 0 percent. Total shrub cover: 5 percent. Total forb cover: 55 percent. Total graminoid cover: 20 percent. Total moss/lichen cover: 10 percent. Total bare ground cover: 10 percent. Associated plant community: timbered hillside with *Vaccinium myrtillus*, *V. caespitosum*, *Abies lasiocarpa*, *Picea engelmannii*, moss. Habitat type: subalpine forest with rivulet, steep slope, below a lodgepole and spruce/fir stand. Aspect: southeast. Slope: 15 to 35 percent. Slope shape: concave. Light exposure: summer; early morning to mid-afternoon. Winter; snow covered, morning to early afternoon. Topographic position: subalpine slope with rivulet marked with willows and grasses. Moisture: seasonal snow runoff, with soil becoming dry. Parent material: organic and granite soils. Geomorphic land form: subalpine slope with rivulet. Soil texture: cobbly.	~50	CNHP EO 45
58	Summit	Breckenridge Ski Area	8/21/2000	USFS: White River National Forest	11,190	Herb cover: 40 percent. Shrub cover: 5 percent. Cryptogam cover: 5 percent. Open, clearcut subalpine forest.	20 to 30	Kolb and Spribille 2000
59	Summit	Keystone Ski Area	8/10/2000	USFS: White River National Forest	10,800	Herb cover: 60 percent. Shrub cover: 10 percent. Cryptogam cover: 20 percent. Open, clearcut subalpine forest.	more than 10	Kolb and Spribille 2000
60	Teller	Halfway Picnic Ground	7/7/1998	USFS: Pike National Forest	9,800 to 10,000	In a small opening in a lodgepole forest with scattered aspen. Northeast-facing slope. 30 to 45 percent slope. Anthill creating more gravelly microhabitat. Gravel/moss/litter cover: 80 percent. Forb cover: 10 percent. Hikers are abundant in the area because of the picnic area. Sediment from the road may travel into the drainage and degrade the road over time.	~3	CNHP EO 11

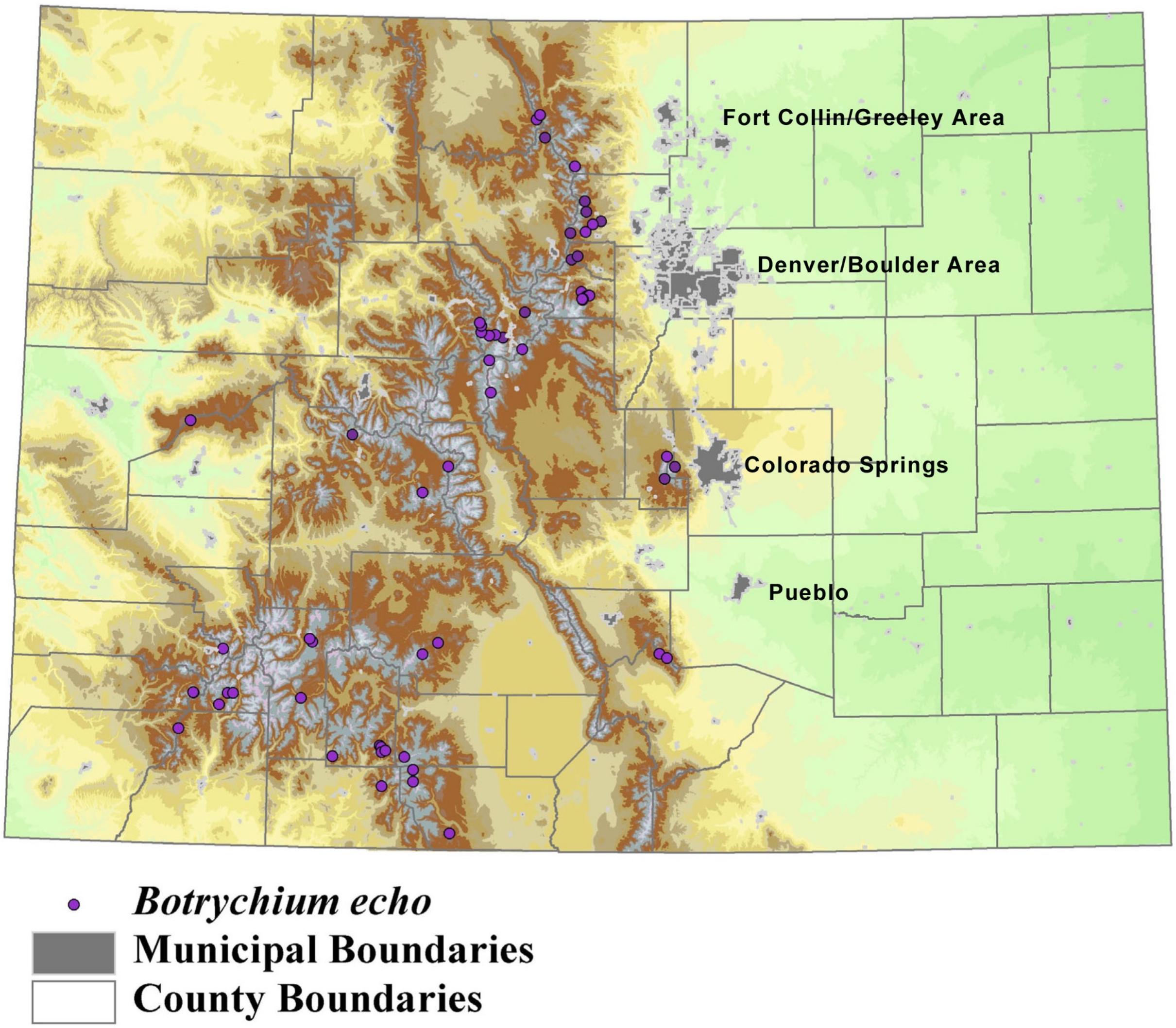

Figure 5. Detailed map of the distribution of *Botrychium echo* in USDA Forest Service Region 2 in relation to physiographic features and municipalities. Map extent is the state of Colorado.

In Utah, *Botrychium echo* is known from a single location in Summit County (1.5 miles south of Spirit Lake; Wagner and Wagner 1983b, Franklin personal communication 2002). In Arizona, it is known from two locations: one is on Mt. Baldy in Apache County, where Wagner and Wagner (1983b) noted 88 individuals, and the other is in the San Francisco Mountains in Coconino County. These occurrences collectively contain approximately 100 individuals (Wagner and Wagner 1983b, Franklin personal communication 2002).

Upon the return of the *Botrychium* specimens to the University of New Mexico Herbarium from the University of Michigan, Spellenberg (2004) found a specimen from Catron County, New Mexico that had been annotated as *B. echo* in 1984. This occurrence is found in the Gila Wilderness on land administrated by the USFS Region 3. This species has long been suspected to occur in New Mexico (Root personal communication), but this specimen had apparently been overlooked in the Flora of North America treatment (Wagner and Wagner 1993). All occurrences outside of Region 2 are poorly known and are in need of revisitation.

Recent surveys have identified many new occurrences of *Botrychium echo* in Colorado. Large numbers of *B. echo* 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 Colorado, with 19,000 occurring in primarily anthropogenic habitats. The 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. *Botrychium echo* was found at eight sites in Summit County during this work (some of which are combined in **Table 3** due to their close proximity to one another). Most of these data were derived from surveys done in and for ski areas on land leased from the USFS. Dave Steinmann, Deborah Edwards, and Peter Root have found two occurrences (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 occurrences in the Indian Peaks Wilderness (Steinmann 2001b).

The vast majority of occurrences of *Botrychium echo* in Region 2 fall on USFS land. Fifty-five or 56 occurrences are found on seven national forests in Colorado (**Table 3**). Three others are found on land managed by the National Park Service. One is found on land managed by the City of Denver, and one is on land managed by the City of Colorado Springs. The type locality for *B. echo* at Glacier Lakes in Boulder County, Colorado is apparently at least in part on private land and is inaccessible (Root personal communication 2003).

Available data suggest that the population size of *B. echo* in Region 2 falls somewhere between 1,500 and 2,300 individuals. An additional 100 individuals are documented in Arizona and Utah (Wagner and Wagner 1983b, Franklin personal communication 2002), and no population size data are available for New Mexico. The known occurrences vary greatly in quality and viability, and at least one may have been extirpated following road widening at Guanella Pass. No population size data are available for 16 locations, so these could not be included in the total population size estimate. The Colorado Natural Heritage Program changed the global rank of *B. echo* from G2 (imperiled) to G3 (vulnerable) as a result of newly discovered occurrences of this species in Colorado (Colorado Natural Heritage Program 2004). Although there have been numerous recent discoveries, the total estimated population size remains very low and is still based on limited data. It appears likely that future surveys in Region 2 will yield further discoveries of *B. echo* occurrences, but further survey work and monitoring are needed to more accurately assess the population size and degree of imperilment of this species.

Population trend

There are no data available from which Region 2 population trends for *Botrychium echo* can be determined. Very little work has been done following population trends in *Botrychium* species (but see Johnson-Groh 1998 and 1999, and Johnson-Groh et al. 2002). 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 (Lesica and Ahlenslager 1996, Johnson-Groh 1999). Lesica and Ahlenslager (1996) and Johnson-Groh (1999) observed that low rainfall appeared to reduce sporophyte emergence in the following year. However, Muller (1992) found that spring drought had no apparent relationship with emergence of sporophytes in *B. matricariifolium*.

Kolb and Spribille (2000) hypothesize that the abundance of *Botrychium* has increased in post-settlement times due to increased anthropogenic disturbances. 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 fire suppression on *B. echo*. Anthropogenic disturbances with which *B. echo* has been found include ski runs, roadsides, clear cuts, and mine sites.

In 2003 approximately 31 *Botrychium echo* individuals were transplanted from three sites along Colorado Forest Highway 80 Guanella Pass (ERO Resources Corporation 2003). The plants were transplanted in an attempt to mitigate impacts resulting from the widening of this road in 2004. Given the low probability that the transplanted individuals will survive (Cody and Britton 1989), it is likely that a downward population trend has occurred locally at these sites.

Habitat

A limited amount is known about the exact habitat associations and environmental tolerances of *Botrychium echo*. Its habitats tend to be early successional and subject to periodic disturbance (NatureServe 2003). Wagner and Wagner (1983b) note that *B. hesperium* and *B. echo* grow in very similar habitats where their ranges overlap, and these species are often found together (Kolb and Spribille 2000, Colorado Natural Heritage Program 2004). It is not known why they share so many habitat affinities in Region 2, yet *B. echo* is much more narrowly distributed. Lellinger (1985) and Wagner and Wagner (1993) describe the habitat of *B. echo* as grassy slopes, roadsides, and edges of lakes in rocky soil, often derived from granitic parent material. Similarly, Spackman et al. (1997) describe the habitats of *B. echo* as gravelly soils near roads and trails, rocky hillsides, grassy slopes, and meadows. Colorado Natural Heritage Program element occurrence records commonly cite the presence of coarse, gravelly soil and little or no tree cover. Element occurrence records from Colorado document occurrences in numerous settings including gravelly hillsides, disturbed trailsides through meadows, small openings in lodgepole or spruce forest, roadcuts, adjacent to roads, and near an old fire ring (Colorado Natural Heritage Program 2004).

Throughout most of Colorado *Botrychium echo* is found on soils derived from granitic parent material. In the San Juan Mountains this species occurs in soils derived from extrusive volcanics, such as tuff and andesite. It has also been found on sedimentary rocks in San Juan and Summit counties. Natural habitats identified by Kolb and Spribille (2000), Thompson (2000 and 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.

Botrychium echo occurs at high elevations. Wagner and Wagner (1993) report an elevation range of 8,200 to 12,140 feet, which concurs closely with Colorado Natural Heritage Program (2004) element occurrence records (8,500 to 12,080 feet). Please see **Table 3** for an overview of habitat descriptions for the occurrences in Region 2. **Figure 6** shows typical natural habitat for *B. echo*.

Figure 6. Habitat of *Botrychium echo* at Coney Flats, Boulder County, Arapaho-Roosevelt National Forest. *Botrychium hesperium*, *B. lanceolatum*, *B. pallidum*, and *B. minganense* are also found at this location. Photograph provided by Dave Steinmann.

There appear to be many species with which *Botrychium* species are often associated. These species probably share affinities for habitats as well as mycorrhizal symbionts with *Botrychium* species. Root (personal communication 2002) has observed a strong correlation between the presence of *Corydalis caseana*, *Solidago simplex*, and *Fragaria* species with the presence of *B. echo*. Kolb and Spribille (2000) and Colorado Natural Heritage Program (2004) also note frequent associations of *Botrychium* species, including *B. echo*, with *Fragaria* species. Lyon (personal communication 2002) often finds *B. echo* with *Senecio atratus*. To some extent, these species can be used as indicator species suggesting a high probability of the presence of *B. echo* and other moonwort *Botrychium* species. 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. *Botrychium echo* is often found in genus communities with any of several other *Botrychium* species (Wagner and Wagner 1983a, Wagner and Wagner 1983b). Other common *Botrychium* associates with *B. echo* include *B. lanceolatum*, *B. hesperium*, and *B. minganense* (Wagner and Wagner 1983b, Root personal communication 2002, Colorado Natural Heritage Program 2004). *Botrychium simplex*, *B. pallidum*, *B. pinnatum*, and *B. lunaria* are also noted with *B. echo* (Kolb and Spribille 2000, Colorado Natural Heritage Program 2004). Associated species commonly documented in Colorado element occurrence records include *Fragaria virginiana*, *Senecio atratus*, *Thermopsis divaricarpa*, *Achillea lanulosa*, *Rosa woodsii*, *Corydalis caseana*, *Potentilla* spp., and others. Please see **Table 4** for a complete list of associated taxa that have been documented with *B. echo*.

Table 4. Associated species reported with *Botrychium echo* in Region 2. Bolded species are commonly documented in Colorado element occurrence records.

Associated Species	Rare/ Exotic	Associated Species	Rare/ Exotic	Associated Species	Rare/ Exotic	Associated Species	Rare/ Exotic
Abies bicolor		*Campanula rotundifolia*		*Koeleria macrantha*		*Pseudocymopteris montana*	
Abies lasiocarpa		*Carex* spp.		*Ligusticum porteri*		*Pteridium aquilinum* var. *pubescens*	
Achillea lanulosa		*Carex foenea*		*Luzula parviflora*		*Pyrola* spp.	
Agoseris spp.		*Carex rossii*		*Mertensia ciliata*		*Pyrola chlorantha*	
Agrostis spp.		*Castilleja* spp.		*Moehringia lateriflora*		*Ribes* spp.	
Agrostis thurberiana		*Castilleja sulphurea*		*Moss* spp.		*Ribes montigenum*	
Anaphalis margaritacea		*Chamerion danielsii*		*Noccea montana*		***Rosa woodsii***	
Androsace septentrionalis		*Cirsium eatonii*		*Orophrysum parryi*		*Rubus idaeus*	
Anemone spp.		*Cirsium parryi*		*Orthilia secunda*		*Rumex acetosella*	E
Antennaria spp.		*Cirsium* spp.		*Oxytropis deflexa*		*Salix* spp.	
Antennaria corymbosa		***Corydalis caseana***		*Packera* spp.		*Salix brachycarpa*	
Antennaria dimorpha		*Cryptogramma* spp.		*Packera dimorphophylla*		*Sambucus racemosa*	
Antennaria parvifolia		*Dactylis glomerata*	E	*Penstemon* spp.		*Saxifraga micropetala*	
Antennaria rosea		*Delphinium* spp.		*Penstemon whippleanus*		*Selaginella densa*	
Antennaria umbrinella		*Deschampsia caespitosa*		*Phacelia* spp.		*Senecio* spp.	
Aquilegia spp.		*Elymus elymoides*		*Phacelia sericea*		***Senecio atratus***	
Arctostaphylos uva-ursi		*Elymus longifolia*		*Phleum alpinum*		*Shepherdia argentea*	

Associated Species	Rare/ Exotic	Associated Species	Rare/ Exotic	Associated Species	Rare/ Exotic	Associated Species	Rare/ Exotic
Arnica cordifolia		*Epilobium angustifolium*		*Phleum commutatum*		*Solidago missouriensis*	
Besseya ritteriana	R	*Erigeron* spp.		*Phleum pratense*	E	***Solidago simplex* var. *nana***	
Blepharoneuron tricholepis		*Erigeron vetensis*		***Picea engelmannii***		*Solidago spathulata* var. *neomexicana*	
Botrychium hesperium	**R**	*Festuca* spp.		*Pinus contorta*		*Spergulastrum lanuginosum*	
Botrychium lanceolatum		*Festuca saximontana*		*Pneumonanthe parryi*		*Taraxacum officinale*	E
Botrychium lunaria		*Festuca thurberi*		*Poa alpina*		*Tetraneuris grandiflora*	
Botrychium minganense	**R**	*Fragaria* spp.		*Poa nemoralis*		*Thalictrum alpinum*	
Botrychium multifidum	R	***Fragaria virginiana* ssp. *glauca***		*Poa pratensis*	E	***Thermopsis divaricarpa***	
Botrychium pallidum	R	*Gentian* spp.		*Polemonium pulcherrimum*		*Trautvetteria caroliniensis*	
Botrychium pinnatum	R	*Gentianella acuta*		*Polemonium viscosum*		*Trifolium repens*	E
Botrychium simplex	R	*Geranium richardsonii*		*Polytrichum piliferum*		*Trisetum spicatum*	
Bromopsis canadensis		*Heterotheca pumila*		*Populus tremuloides*		*Vaccinium caespitosum*	
Bromopsis porteri		*Heterotheca villosa*		*Potentilla* spp.		*Vaccinium myrtillus* ssp. *oreophilum*	
Bromus inermis	E	*Ipomopsis aggregata*		*Potentilla hippiana*		*Vaccinium scoparium*	
Bromus pumpelliana		*Juniperus communis*		*Potentilla pulcherima*		*Vaccinium* spp.	
Calamagrostis canadensis		*Juniperus communis* ssp. *alpina*		*Potentilla subjuga*		*Valeriana edulis*	

Buell (2001) notes that *Botrychium* species have a decidedly patchy within-site distribution. The causes for this pattern are unknown; 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 is an effective mode of dispersal for any species of *Botrychium*. Buell (2001) noted many occurrences in Summit County, Colorado where it appeared as though water flowing downslope had dispersed spores to other locations along drainage 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. echo*.

Several habitat attributes are commonly found in occurrences of *Botrychium echo*, as well as 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; 10 to 40 percent bare soil; rock cover frequently 5 to 15 percent; 20 to 30 percent slopes; not on south aspects; a history of disturbance; previously forested areas with a coniferous forest potential; often on calcareous substrates; usually at 3,210 to 3,510 meters elevation; sites well drained; and soils compact and eroded. Please see the Community Ecology section of this document for a discussion of this plant community.

The periodicity of disturbance that is required for *Botrychium echo* and other *Botrychium* species is not known. To a large extent this probably controls the suitability of habitats for *B. echo* as well as its metapopulation structure. Natural disturbance events that can create habitat for *B. echo* include frost, landslide, and fire; anthropogenic disturbances include bulldozer use, clearcutting, ski run maintenance, and road maintenance (Colorado Natural Heritage Program 2004). Clearly the tempo and intensity of these disturbances vary 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. echo*.

Johnson-Groh and Farrar (2003) suggest that sites that were disturbed approximately 10 years ago and then rested are most likely to support *Botrychium* occurrences. Buell (2001) found that *Botrychium* species, including *B. echo*, 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 the 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 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 percent). Slopes and roadcuts may provide an appropriate level of chronic disturbance due to periodic mass wasting events and erosion for maintaining suitable habitat. Constant small-scale frost heaving and needle ice formation on steep, exposed slopes probably maintain a chronic disturbance regime at high elevation sites. In general, *Botrychium* species are not often found on south-facing slopes in Colorado, suggesting that these sites are too xeric for *B. echo* (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. echo*, it may lack certain crucial attributes required for its establishment and persistence. 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. echo*.

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, epiphyte, and bryophyte species, moonworts are characterized by small statures, 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. echo* (Lellinger 1985, Wagner and Wagner 1993). The disturbance regime required by *B. echo* has not been studied and is not well understood. Habitat attributes for most moonwort species suggest that they depend on a natural disturbance regime imposed by wildfires, floods, landslides, or avalanches (Alverson and Zika 1996). Locations in which *B. echo* 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 tend to have a thick layer of duff, suggesting a low to moderate disturbance regime (Lesica and Ahlenslager 1996). Because *B. echo* depends on open sites, disturbances that create and maintain these openings are a key component of its autecology.

Botrychium echo 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 more than 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).

In addition to spore production, *Botrychium echo* is one of five *Botrychium* species that produce gemmae (Camacho 1996, Camacho and Liston 2001), which are minute vegetative propagules abscised at maturity from the parent plant (Farrar and Johnson-Groh 1990). This mode of reproduction has not been previously reported in any fern genus (Farrar and Johnson-Groh 1986). The tendency for *B. echo* to grow in clumps is probably the result of reproduction via gemmae. *Botrychium campestre*, one of the putative parents of *B. echo*, also produces gemmae, and was the first moonwort species in which they were documented (Farrar and Johnson-Groh 1986, Farrar and Johnson-Groh 1990, Johnson-Groh et al. 2002). However, subsequent research has found them on other diploid species including *B. pumicola*. *Botrychium gallicomontanum*, a rare allotetraploid species for which *B. campestre* and *B. simplex* are the putative parent species, is also known to reproduce with gemmae (Farrar and Johnson-Groh 1991). The production of gemmae as vegetative propagules by these three species, all of which are found in relatively xeric sites, suggests that they are an adaptation for reproduction in dry sites (Camacho 1996). Gametophytes may have a higher risk of desiccation than sporophytes, and the sex cells require a liquid environment in order to swim to the archegonia. Thus, by short-cutting the gametophyte stage of the life cycle altogether, gemmae, which produce sporophytes, may be a more reliable form of reproduction in the dry environments inhabited by *B. campestre* (Farrar and Johnson-Groh 1990). Gemmae production in *B. echo* has not been quantified, but it is less than that of *B. campestre*. A single *B. campestre* stem may contain between 20 and 30 and up to 100 of these 0.5 to 1 mm asexual propagules (Farrar and Johnson-Groh 1990). Older gemmae may also produce additional gemmae, and up to 500 gemmae can sometimes be found surrounding a single plant (Farrar and Johnson-Groh 1990).

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

Botrychium spores are small and light and are likely carried by winds in the air, and then by water percolation in the soil once they land. Researchers have hypothesized that the dispersal distance for some *Botrychium* species ranges from a few centimeters (Hoefferle 1999, Casson et al. 1998) to up to 3 m (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, many spores certainly travel great distances (Wagner and Smith 1993, Briggs and Walters 1997, Chadde and Kudray 2001). In addition to wind dispersal, animals 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). J.D. Montgomery recovered the spores of grape fern (*B. virginianum*) from the droppings of a vole after feeding them to it, after which they appeared to be intact (Root personal communication 2003). However, the viability of these spores was not assessed. Dependence on animal-mediated spore dispersal might also limit the dispersal ability of *Botrychium*. This is likely true for *B. mormo*, in which the sporangia do not dehisce, but it may be less important for all other *Botrychium* species in which the sporangia do dehisce (Anonymous reviewer 2003). The flow of rainwater downslope along drainage 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 (Campbell 1911, Campbell 1922, Bower 1926, Scagel et al. 1966, Whittier 1973, Wagner et al. 1985, Foster and Gifford 1989, Schmid and Oberwinkler 1994) stages. *Botrychium* spores need three to four weeks of darkness before they can germinate, with longer periods of darkness increasing the probability of germination (Whittier 1973). Germination can occur without mycorrhizal infection. However, the gametophyte will not mature without an arbuscular mycorrhizal symbiont (Campbell 1911, Whittier 1972, Whittier 1973). The subterranean, achlorophyllous gametophyte 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 gametophyte develops into a sporophyte. However, *Botrychium* sporophytes have reduced, non-proliferous roots that lack hairs (Wagner and Wagner 1993) and they depend upon mycorrhizae (Bower 1926, Foster and Gifford 1989). Winther (personal communication 2002) found that congeners had both endomycorrhizal and ectomycorrhizal associations.

Arbuscular (also referred to in the literature as vesicular-arbuscular) mycorrhizae are the known fungal symbiont with *Botrychium* species (Berch and Kendrick 1982, Schmid and Oberwinkler 1994). Johnson-Groh (1999) hypothesizes that the most important factor in the establishment and persistence of *Botrychium* populations is the presence of mycorrhizae. However, little is known about the specific nature of this interaction. Johnson-Groh (1999) found that water relations were extremely important for mycorrhizae and *Botrychium*. Farrar notes that mycorrhizal fungi are low in species diversity, ubiquitous in disturbed and undisturbed sites, and generalist in whom they infect (Farrar 1998, Smith and Read 1997). However, recent studies have measured surprisingly high species diversity of arbuscular mycorrhizal (AM) fungi in a single hectare (Bever et al. 2001). A single plant root has been observed to host up to 49 species of AM fungi 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 *Botrychium echo*.

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. With their tight association with mycorrhizae, similar work with *Botrychium* species is needed to understand the effects of mycorrhizae-mediated interspecific competition.

Hybrids between *Botrychium* species are rare, and when they are found they are typically sterile (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). *Botrychium campestre*, the prairie moonwort, is the purported ancestor of a complex of other species, including *B. echo*, which may have arisen from hybridization events. The descendants of *B. campestre* are tetraploids, which produce some gemmae but not in the same profusion as *B. campestre*. *Botrychium echo* is a probable descendant of *B. campestre* and *B. lanceolatum* (Colorado Native Plant Society 1997, Root personal communication 2002).

Demography

Members of the genus *Botrychium* appear to have naturally low rates of outcrossing (Farrar 1998). The anatomy of the gametophyte of *B. virginianum* (subgenus *Osmundopteris*) appears to be designed for self-fertilization, since the antheridia are positioned above the archegonia. Water moving through the soil is likely to bring the male sex cells to the archegonia on the same plant (Bower 1926). Soltis and Soltis (1986) used electrophoretic techniques to confirm that there are extremely high levels of inbreeding in this species. Allelic variability within each moonwort species consistently shows very low intraspecific variation when compared with other ferns and seed plants (Farrar 1998). McCauley et al. (1985) found the congener *Botrychium dissectum* (subgenus *Sceptridium*) to have an outcrossing rate of less than 5 percent. However, the 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. echo* 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 mycorrhizal fungi. As obligate mycorrhizal hosts who obtain their mineral nutrition and some carbohydrates from their fungal symbionts, the establishment and maintenance of this relationship is of paramount importance to *Botrychium* species. As such, genetic diversity would be more useful to *Botrychium* when present in their fungal symbionts, since they are the intermediaries between the roots and the rhizosphere and 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 life cycle (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). Johnson-Groh (1999) quantified gametophytes, sporophytes, and gemmae in a population of *B. campestre* in Iowa. In this study, densities of 21 gametophytes, 180 sporophytes, and 6,023 gemmae per m^2 were found in study plots. 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). Johnson-Groh (1998) recovered as many as 7,000 gametophytes and 250 sporophytes of *B. mormo* per square meter, although an unknown number of gametophytes may be B. virginianum. In an earlier study, Bierhorst (1958) found 20 to 50 gametophytes of *B. dissectum* per square foot, with relatively few mature gametophytes attached to juvenile sporophytes. 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). However, the destructive sampling techniques needed to infer the size of the subterranean populations of *B. echo* are inappropriate for use in most populations, since they are typically small. See **Table 3** and the Distribution and Abundance section of this document for details.

There has been no research on the demography and life history of *Botrychium echo*. In Waterton Lakes National Park, Alberta, Lesica and Ahlenslager (1996) have studied the demography and life history of *B. hesperium*, which is perhaps the most similar species to *B. echo* for which such data is available. Only aboveground portions of the sporophyte phase of the life cycle were studied. As also observed for *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* species that occur underground. Spores of *B. virginianum* (subgenus *Osmundopteris*) 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 importance of spore banks is unknown for *B. echo*, 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. echo* 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 grow very slowly to a sexually reproductive adult (Wagner 1998). It is not known how long it takes a spore to get underground or how it gets there (Root personal communication 2003). The longevity and the fate of the gametophyte after the production of a sporophyte have not been reported (Vanderhorst 1997). Sporophytes also may live heterotrophically underground for several years before producing aboveground structures (Kelly 1994). Upon emergence aboveground, the sporophytes begin spore production on their fertile lamina (sporophore). Please see **Figure 7** for a diagrammatic representation of the life cycle (after Lellinger 1985) of *B. echo*, and **Figure 8** for a life cycle graph (after Caswell 2001) for *B. echo*. Please see the Reproduction section of this document for further details on the life cycle of *B. echo*.

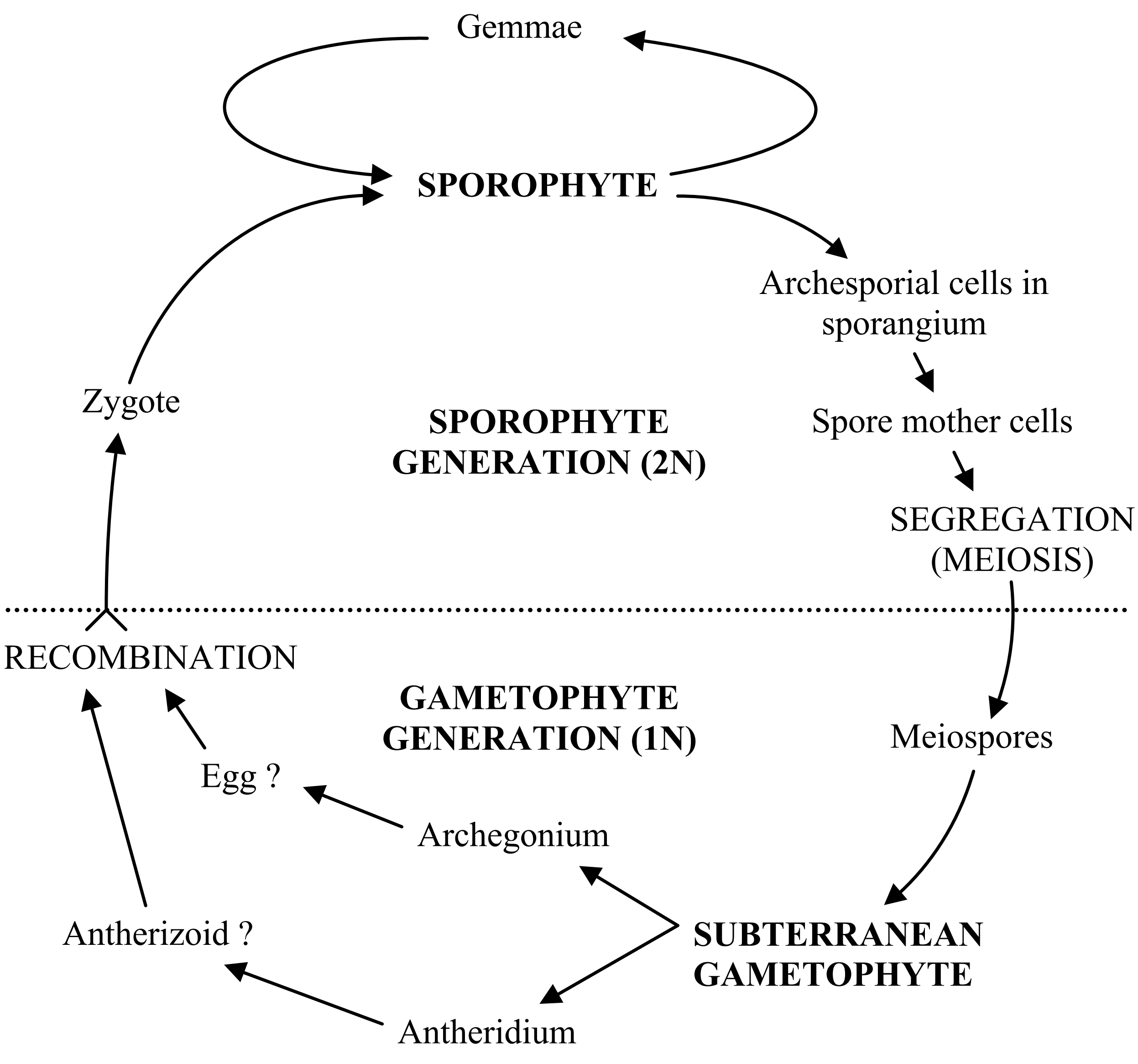

Figure 7. Life cycle diagram for *Botrychium echo* (after Lellinger 1985).

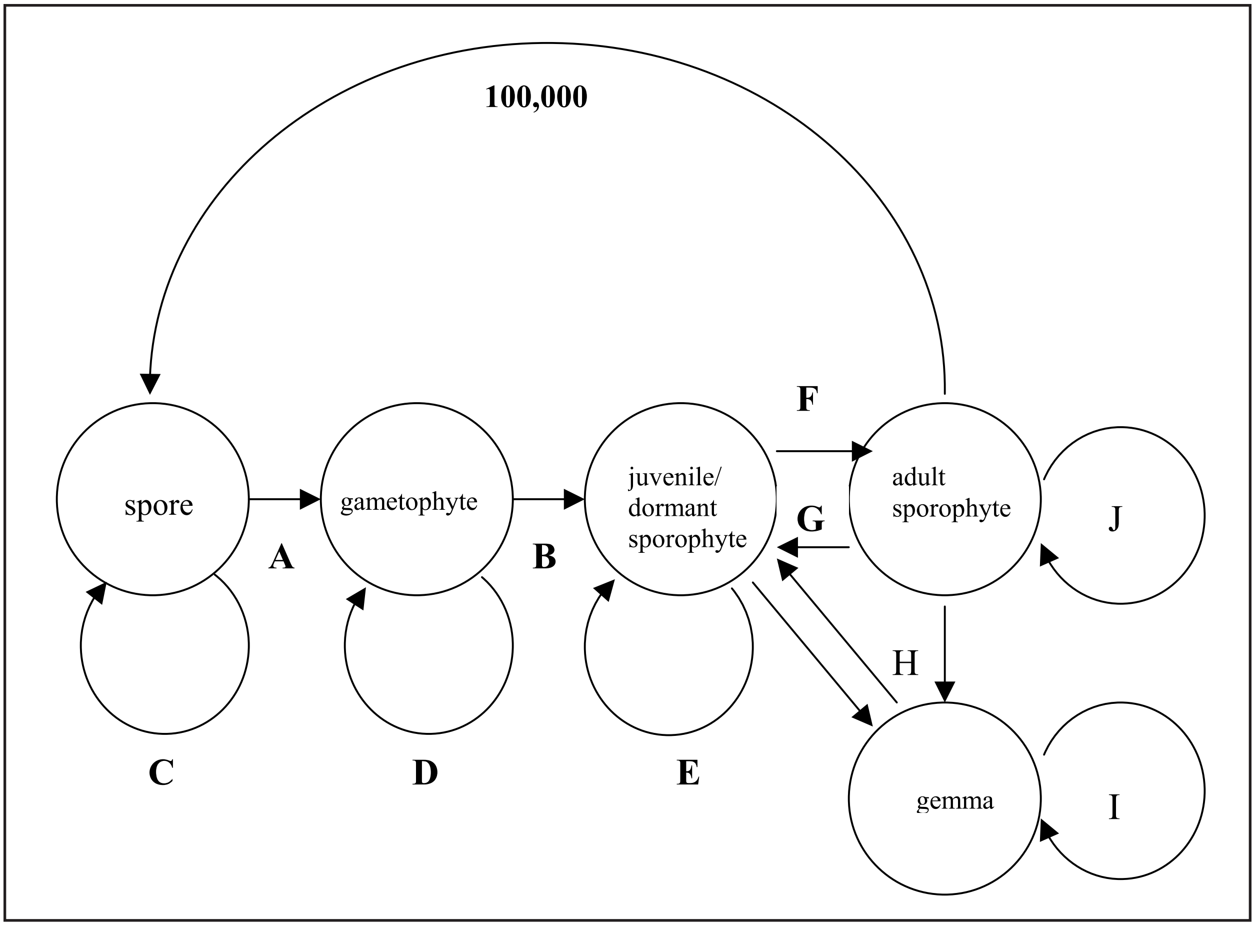

Figure 8. Hypothetical life cycle graph (after Caswell 2001) for *Botrychium echo*. Transition probabilities are not known and are difficult to quantify since important stages of the life cycle occur underground (A-G). Please see Johnson-Groh et al. (1998) and Johnson-Groh et al. (2002) for the best 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. Adults and juveniles bear gemmae (H), which permit *B. echo* to reproduce asexually. While gemmae production has been estimated for other *Botrychium* species, it has not been quantified for *B. echo*. The longevity of gemmae once abscised from the parent plant is not known (I). Spore production is estimated from Wagner (1998). No transition probabilities have been measured for *B. echo*. The lifespan of the sporophyte of *B. echo* has not been measured (J).

The lifespan of the *Botrychium echo* sporophyte has not been measured. Johnson-Groh and Farrar (2003) estimate that moonworts live approximately 10 years. The aboveground longevity of *B. campestre* is approximately four years, while *B. mormo* rarely live longer than two years (Johnson-Groh 1998). Lesica and Ahlenslager (1996) determined a half-life of approximately three years or less for *B. hesperium*. *Botrychium australe* is known to live 11.2 years (Kelly 1994), but *B. dissectum* (subgenus *Sceptridium*) individuals can live at least a few decades (Montgomery 1990, Kelly 1994), and *B. multifidum* (subgenus *Sceptridium*) may live for as long as 100 years (Stevenson 1975).

No population habitat viability analysis has been done for *Botrychium echo* as of this writing. The only *Botrychium* species for which such an analysis has been conducted is *B. mormo* (Berlin et al. 1998), which differs in many significant ways from *B. echo* and from most other moonworts as well. Nonetheless, some of the conclusions drawn from the model are relevant to the entire genus. Three factors were cited that have the most control in the model (because spores are so numerous), although these are also the factors about which the least is known. These factors are viable spore set per sporophyte, the nature and extent of a spore bank, and spore germination rate. Reproduction via gemmae, which may be a more common reproductive mode in *B. echo*, was not addressed in the model.

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). Because most *Botrychium* species are early to mid-seral species, they may be expected to drop out as succession proceeds to conditions unsuitable to them. The typically small, highly variable populations of *Botrychium* species are vulnerable to local extirpation (Johnson-Groh et al. 1998). Thus, *B. echo* 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. echo* migrated to new locations. The metapopulation dynamics of *B. echo* will be important to consider for conservation purposes (Pickett and Thompson 1978).

Occurrences of many *Botrychium* species tend to be small and localized (Colorado Natural Heritage Program 2004). 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. 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 echo* is lacking. Using phytosociological methods, Kolb and Spribille (2000) described the community in which *Botrychium* species (including *B. echo*) 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*. Detailed community description and plot data can be found in Kolb and Spribille (2000).

Elk grazing probably occurs in most *Botrychium echo* occurrences in Region 2. Moonworts clearly tolerate some degree of grazing, but no formal studies have been conducted on this topic (Johnson-Groh and Farrar 2003), and the sensitivity of *B. echo* to grazing is not known. Wagner and Wagner (1990) have reported browsing by deer and rabbits in other *Botrychium* species, and in some cases as many as 80 percent of the individuals in a population had been completely browsed (Wagner et al. 1985). Montgomery (1990) found that even repeated removal of the leaf of *B. dissectum* (subgenus *Sceptridium*) for three years did not kill the plants, and on this he commented (p. 178) "It is certainly remarkable that these plants persist." It is possible that elk may act as a dispersal vector for *B. echo*, so grazing in the fall during sporulation after the plant has had a summer to photosynthesize may serve an important role in the population biology of this species.

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 speculation that animals may disperse the spores of some *Botrychium* species (Wagner and Wagner 1990, Wagner and Wagner 1993, Wagner 1998, F. Wagner personal communication 2002).

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 are dependent on the same resources, coexisting plants are not engaged in direct intraspecific competition. Competition may be for access to the mycorrhizae, if it is occurring at all. No research has been done on *Botrychium* species with respect to these issues. There are no reports of parasitism or disease in the literature for any *Botrychium* species.

Please see **Figure 9** and **Figure 10** for envirograms outlining the resources and malentities for *Botrychium echo*. These envirograms summarize the relationships among different biotic and abiotic factors that weigh heavily in the autecology of this species.

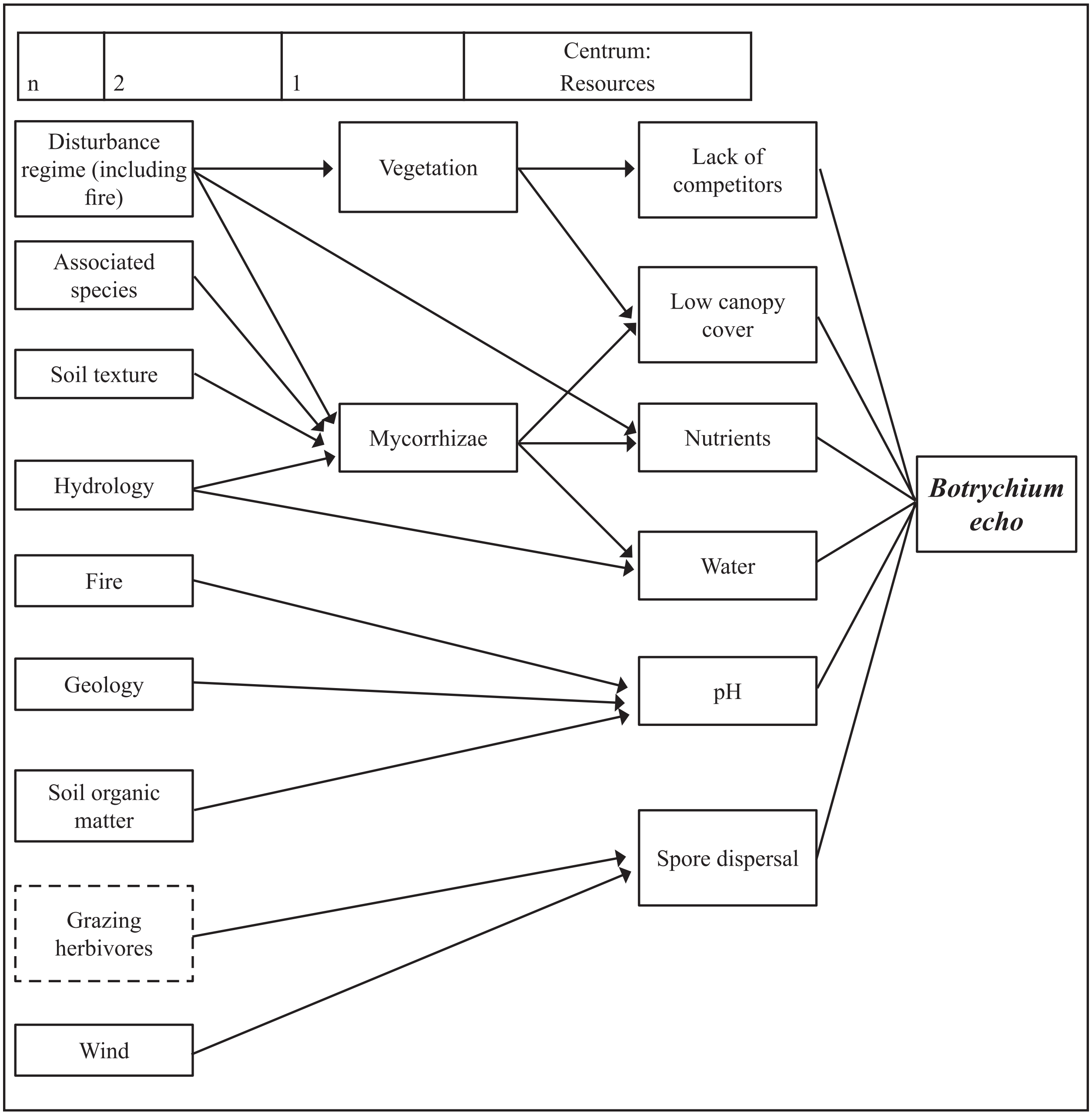

Figure 9. Envirogram outlining the resources of *Botrychium echo*. Cells with dotted borders are speculative.

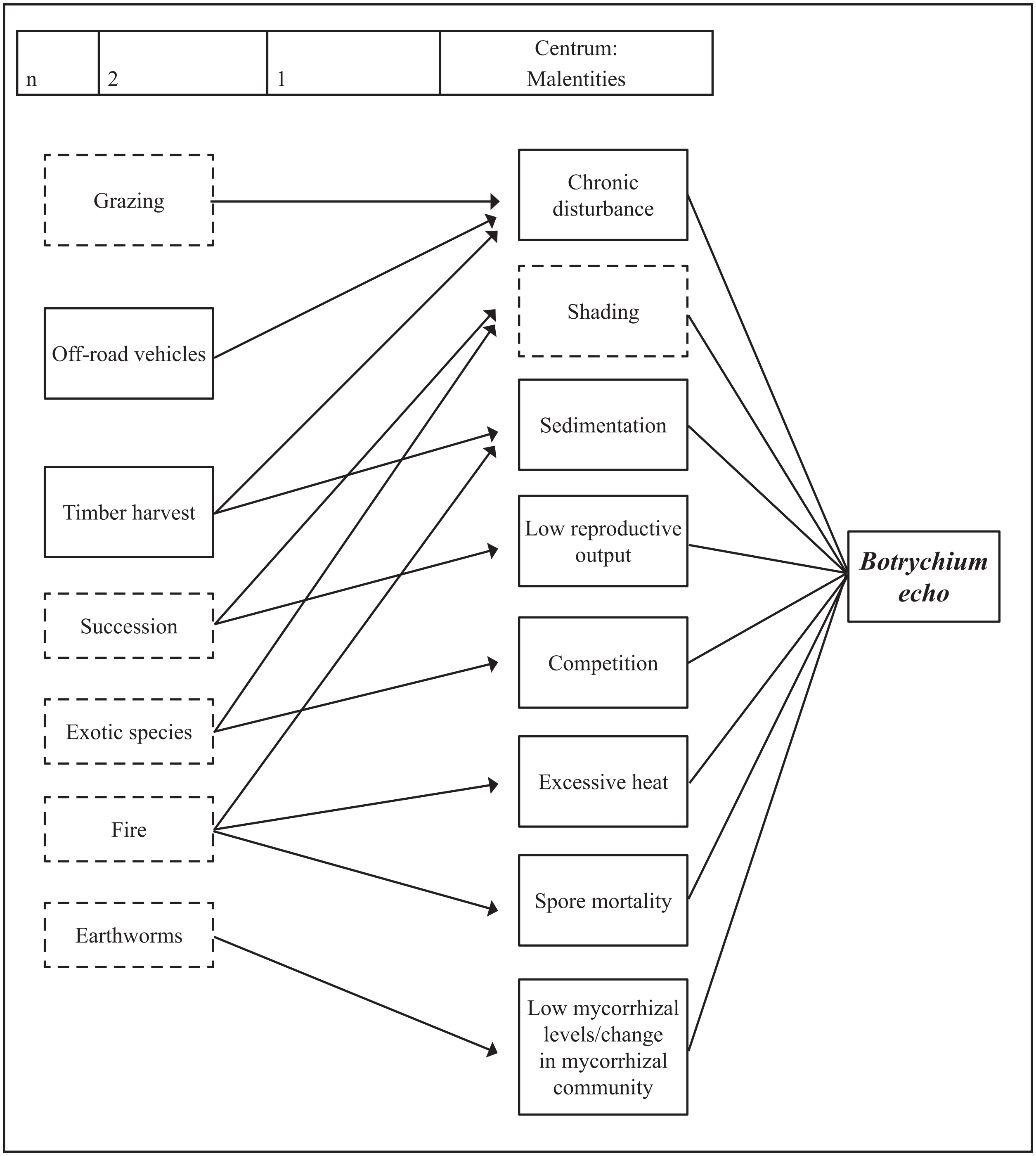

Figure 10. Envirogram outlining the malentities to *Botrychium echo*. Cells with dotted borders are speculative.

CONSERVATION

Threats

Observations suggest that there are several tangible threats to the persistence of *Botrychium echo* in Region 2 and elsewhere. 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.

The threats cited above for Region 2 and the hierarchy ascribed to them are somewhat speculative given the paucity of information, and more complete information on the biology and ecology of this species may elucidate other threats. Assessment of threats to this species will be an important component of future inventory and monitoring work. Please see the sections below for specific treatments of these threats to habitat, individuals, exotic species, and over-utilization.

Threats to *Botrychium echo* are not well understood (NatureServe 2003), but research from similar *Botrychium* species may apply to *B. echo*. As a species that requires some level of disturbance to create and to maintain suitable habitat, it is difficult to define threats to it. Summarizing information from NatureServe (2003) on *B. hesperium*, Chadde and Kudray (2001, pg. 11) remarked: "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." This statement is equally relevant for *B. echo*. Obviously, a better understanding of the role of disturbance in the autecology of *B. echo* is of great importance from a management perspective. As noted for *B. hesperium*, threats to the belowground life stages of *B. echo* are probably more serious than threats to the aboveground (sporophyte) stages, given the great importance of the belowground portion of the life cycle (Chadde and Kudray 2001).

Global climate change is likely to have wide-ranging effects in the near future. Projections based on current atmospheric CO2 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 echo* in Region 2. Temperature increase could cause vegetation zones to climb 350 feet in elevation for every degree Fahrenheit of warming (U.S. Environmental Protection Agency 1997). This could have large impacts on low-elevation populations. Occurrences of *B. echo* on south aspects may be more sensitive to climate changes that cause conditions to become warmer and more xeric. Root (personal communication 2003) has noted that moonworts are not typically found on south aspects below 9,000 feet in elevation. This threshold is currently below the elevation of most occurrences of *B. echo*, but this limit is likely to climb as temperatures rise, imperiling many occurrences of *B. echo*.

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 echo* may 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 echo* individuals have been found in areas in which a human induced disturbance regime is imposed. Logging, developing ski trails, building roads, and other human disturbances have created a great deal of habitat for this species. On some level, these activities may benefit *Botrychium* species including *B. echo*, 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* occurrences 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. echo*. While it is clear that some types of anthropogenic disturbance creates habitats that support *Botrychium* species, the widening of the Guanella Pass road clearly illustrates the fact that these sites cannot be relied upon to support the long-term viability of *B. echo*. 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 nonmycotrophic vascular species to out-compete mycorrhizal dependent species such as *Botrychium* (Allen and Allen 1990). The presence of *B. echo* on road and trail margins where it is less trampled than it would be in the roadbed or trailbed is also suggestive that it 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 echo* is inconspicuous and occurrences 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 echo* habitat presents a threat to individuals that may be killed or damaged directly by these activities. Off-road vehicle use (both motorized and not motorized) represents a significant threat to *B. echo* from recreation. Use of mountain bikes and “mountainboards” (similar to snowboards but equipped with wheels for use on ski slopes in the summer) has the potential to impact individuals on ski slopes when it occurs during the growing season.

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. echo*. Construction of a ski hut near the occurrence on Vail Pass presents a potential threat due to disturbance associated with construction and increased use of the hut. Because the presence of *B. echo* was known before construction, the hut was located in a site where impacts to the occurrence 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 echo*. Summer maintenance practices have a greater potential than winter maintenance to impact occurrences since they are more likely to disturb soil and damage or kill individuals. These include pulling stumps, creating and maintaining roads, using summer snowcats, mechanically or chemically controlling weeds, grooming the earth on ski runs, installing and maintaining waterlines or 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. echo* appear to suffer no ill consequences from this (Johnson-Groh and Farrar 2003). Particularly hot fires or fires when the soil is desiccated could result in mortality, but due to the strong dependence of the species on mycorrhizae, removal of leaf tissue via burning or other means is probably inconsequential to the plant’s survival (Montgomery 1990, Wagner and Wagner 1993, Johnson-Groh and Farrar 1996a, Johnson-Groh and Farrar 1996b, Johnson-Groh 1999). Fires that occur 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 buried 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). It is possible that fossorial mammals could play a role in the dispersal of gemmae, but there have been no observations of this.

While there have been no direct impacts documented from livestock grazing on occurrences of *Botrychium echo* in Region 2, it is known to occur and impacts to habitat have been documented. Disturbance of loose soil by sheep that had moved through the site was observed on Molas Pass (Element occurrence 20 in **Table 3**). In Region 2, sheep and horses both graze in subalpine meadows and other areas of the mountains to some extent. 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 belowground). Grazing can eliminate a season's contribution to the sporebank (Johnson-Groh and Farrar 2003). The use of livestock grazing as a management tool for the enhancement of habitat is risky for a plant as rare as *B. echo*, since it is likely to cause some level of erosion, trampling, alteration of plant community composition, damage to the soil structure (particularly when wet), and introduction of invasive plants.

Interaction of the species with exotic species

Seven exotic plant species have been documented with *Botrychium echo* (**Table 4**). Four of these species are exotic grasses (*Bromus inermis*, *Dactylis glomerata*, *Phleum pratense*, and *Poa pratensis*). These species are common along roadsides in Region 2. *Trifolium repens* and *Taraxacum officinale* are also common in *Botrychium* habitats (Root personal communication 2003).

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. echo*, they present a significant threat. Because new exotic species are arriving all the time, vigilance in monitoring for their impacts is crucial.

While direct impacts from weeds have not been measured, their management may be a cause for concern with respect to *Botrychium* species. Johnson-Groh (1999) observed the effects of herbicide and fire on prairie moonworts (*B. simplex*, *B. campestre*, and *B. gallicomontanum*). Plants that had been hit directly with the herbicide Roundup were apparently killed (Johnson-Groh 1999, Johnson-Groh and Farrar 2003). Thus efforts to manage populations of noxious weeds will probably impact moonwort populations in rights-of-way or other sites targeted for weed management.

In the deciduous hardwood forest habitats of *Botrychium mormo*, invasion of non-native earthworms has resulted in dramatic decreases in mycorrhizal fungi (Nielsen and Hole 1963, Cothrel et al. 1997, Berlin et al. 1998, Gundale 2002). As an obligate mycorrizal symbiont, this poses a significant threat to *B. mormo*. Most earthworm activity takes place in the O horizon (Langmaid 1964), while mycorrhizal activity is greatest at the interface of the O and A horizons (Smith and Read 1997). The activity of earthworms has resulted in the elimination of the duff layer and a shift in species composition in *B. mormo* habitat (Berlin et al. 1998). Although earthworms present a possible threat to *B. echo*, no research has shown that species of *Botrychium* other than *B. mormo* are being impacted by them. 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 in Region 2 (Root personal communication 2003). Region 2 moonwort habitats are extremely cold in the winter, have little litter accumulation, and a poorly developed O horizon. There are few reports of earthworms in the subalpine zone, but Steinmann reports having found small annelids in high elevation caves that probably came from outside the caves (Root personal communication 2003). Earthworms are a diverse group of over 3,500 species worldwide, and the expansion of global commerce may increase the likelihood of exotic earthworm invasions with potential adverse affects on soil processes and plant species (Hendrix and Bohlen 2002).

Threats from over-utilization

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

There are no known commercial uses for *Botrychium echo*. 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 *Botrychium echo* in Region 2 or elsewhere. The observations of Kolb and Spribille (2000), Thompson (2000), Buell (2001), and Thompson (2001) suggest that the abundance of *B. echo* has increased in the vicinity of ski resorts. It thus appears that the population on anthropogenically created and maintained sites will continue to increase in coming years as more ski runs and resorts are created. This might similarly be inferred for roadside occurrences. However, the threats to these occurrences are great, and as seen in the occurrences along the Guanella Pass road, activities related to the maintenance and expansion of these developments are likely to result in local population decline or extirpation. It is difficult to infer the effects of other forest management practices such as fire suppression on the abundance of *B. echo*. Reduction in the periodicity of fire increases the chance of catastrophic crown fires that destroy the soil, rather than create or maintain openings that are suitable habitat for *B. echo*. There is no evidence that the range of *B. echo* has expanded or contracted recently. However, the lack of historical collections from distant locales suggests that *B. echo* has always had a narrow range.

Do habitats vary in their capacity to support this species?

Succession may lead to unsuitable conditions for *Botrychium echo* at a site in the absence of a disturbance regime. Variables such as fire regime, amount of litter, soil moisture variation, and soil texture may affect habitat suitability. The suitability of a site to the appropriate mycorrhizae is equally important for *Botrychium* species as obligate mycorrhizal symbionts. The physical properties and moisture of the soil, as well as the associated species in the plant community, are perhaps the most relevant factors with respect to mycorrhizae (Allen and Allen 1990).

Vulnerability due to life history and ecology

There remains some uncertainty in the assessment of the vulnerability of *Botrychium echo* due to its life history and ecology since these remain poorly understood. *Botrychium echo* is vulnerable to habitat change due to its dependence on disturbance to maintain the suitability of its habitat. The dependence of most *Botrychium* species on disturbance for creating and maintaining appropriate habitat leaves them vulnerable to factors such as succession and the absence of disturbance. It is not known how vulnerable *B. echo* populations are in this regard.

While *Botrychium echo* 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 partially reliant on reproduction involving the gametophyte portion of its life cycle. The gametophyte stage is probably more susceptible to drought than reproduction via gemmae (Camacho 1996). However, the 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.*

echo, 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). Only 15 of the occurrences known from Region 2 in which population size was counted or estimated include 50 or more individuals. 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 cryptic plants, *Botrychium* species are easily overlooked and are thus poorly understood and easily missed. This leaves them vulnerable where occurrences have not been found.

Evidence of populations in Region 2 at risk

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

Numerous facts about *Botrychium echo* 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 few occurrences (60) known in Region 2. Populations of *B. echo* are small (most populations documented report less than 50 individual sporophytes) and fluctuate greatly, resulting in a high probability of local extirpation as a result of stochastic processes and even normal environmental variation. *Botrychium echo* also has very specific habitat requirements. Many of the old, yet early successional sites it occupies are inherently facile and destined to become unsuitable to *B. echo* as a result of natural succession. This species appears to have a metapopulation structure that obligates it to long distance dispersal to other suitable sites as succession renders other sites unsuitable for it. Such dispersal is both risky and costly. Some studies have shown that *B. echo* may not be particularly good at long distance dispersal. It is wholly dependent on mycorrhizae in both the gametic 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. echo* 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. echo* 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 2004) 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 in which they occur. Many occurrences have not been revisited in many years, and their current status is unknown.

Because *Botrychium echo* is no longer designated as a sensitive species in Region 2, there will be less impetus to attempt to mitigate impacts to occurrences on USFS land, where the vast majority of the known occurrences are found. Mitigation and survey efforts done in the past have resulted largely from its sensitive status. Thus it will probably no longer benefit from these efforts to the extent that it did as a sensitive species.

Although there is much evidence suggesting that *Botrychium echo* is highly imperiled, other evidence suggests otherwise. That *B. echo* is successful in some sites altered by human disturbance suggests that it might benefit from human activities such as the building of roads and the creation of ski resorts. Recent observations where tens to hundreds of plants were found on ski slopes suggest that this sort of human disturbance is not incompatible with, and perhaps beneficial, to *B. echo*. 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. echo*. Although *B. echo* 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). As survey work continues in both natural and human disturbed sites, it is likely that more occurrences of *B. echo* 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 echo* throughout Region 2. The habitat created by these ecological processes may be ephemeral for *B. echo* (although they may take hundreds of years to return to forested conditions), and we cannot know what the pre-settlement distribution and population size of *B. echo* were. 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 echo*. 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 occurrences of *B. echo* in Region 2, so it is possible that timber harvest may be ecologically analogous to fire for *B. echo* 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 echo* 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. echo* is found.

Desired environmental conditions for *Botrychium echo* include sufficiently large areas where the natural ecosystem processes on which *B. echo* 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. echo* depends are functioning properly at many or most of the occurrences of this species. Further research on the ecology and distribution of *B. echo* will help 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

Species and habitat inventory

Botrychium echo, 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, the species is nonetheless very rare. 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 a high priority for this species in Region 2.

Often, due to limitations in time and funding, attempts to search for rare plants involve looking for multiple species in large areas. While this approach has been effective in finding many rare plant occurrences, it may not be effective for *Botrychium echo* given the factors cited above. Because searching for *B. echo* 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.

At present the priorities for Region 2 lie in basic survey work and monitoring, since we still do not know the full distribution of *Botrychium echo* and its conservation status is uncertain. Gathering population size data (a census of sporophytes) 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. echo*.

Johnson-Groh and Farrar (2003) offer excellent suggestions for conducting surveys for *Botrychium* species. The protocols defined in this document will serve as standard protocols for all survey work on subgenus *Botrychium*. Suggestions for surveys include search methods, marking plants, collection protocols, documentation, and other concerns. This protocol is based on the assumption that one week can be spent searching approximately 25 high priority sites per year, for about one hour each. Four or five people are recommended to walk transects at each area selected so that better coverage is achieved in the time of the visit. When plants are discovered, they should be marked with pinflags and the surrounding area should be searched intensively but carefully on hands and knees. Limitations of these methods are also discussed and are important considerations. The four limitations cited include low confidence in distribution and abundance due to population variability, difficulty in finding the plants, predominance of the belowground life cycle stages, and the occurrence of many species in genus communities which complicates identification. Popovich (personal communication 2003) implemented this protocol and noted that the area searched appeared to have been heavily impacted by the intensiveness of the search efforts. Johnson-Groh and Farrar (2003) also recommend using the "timed meander search procedure" described by Goff et al. (1982) for *Botrychium* search efforts.

Botrychium species are notoriously difficult to investigate. Their small size, inconspicuous appearance, sporadic distribution, prolonged dormancy, and cryptic speciation make them challenging subjects for research, yet these are also attributes that make them fascinating to us. In the past, experts have had great success in finding occurrences of *Botrychium* using a search image for habitat that they have developed from years of study and survey work (please see the Habitat section of this report for details). For identifying habitat and finding more occurrences of *B. echo*, engaging experts on *Botrychium* to the maximum extent possible will help greatly in expanding our knowledge of these species.

Identifying suitable habitat in which to focus searches for *Botrychium echo* 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 a probabilistic surface showing the likelihood of the presence of *B. echo* in given locations. 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.

Population monitoring

Annual monitoring of selected populations of *Botrychium echo* in Region 2 could help to understand its ecology and population trends. An annual census of 10 to 20 percent of the known occurrences would provide basic information on population status, trend, and variability. Establishing permanent plots following the methods of Lesica and Ahlenslager (1996) would provide more robust data for addressing questions regarding population stability and trends. 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. These methods are effective but are labor intensive and require many years of repeated sampling to glean meaningful demographic data (Johnson-Groh and Farrar 2003). Adding a photoplot component to this work, following the 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 selected if the data is intended to detect regional population trends.

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

Johnson-Groh (1999) notes that it can be difficult to be assured that an individual that had been marked in a previous year is the same individual again in subsequent years. This problem is exacerbated in *Botrychium echo* because it reproduces with gemmae, which remain close to the parent plant (Johnson-Groh 2001). Thus many sporophytes may emerge in close proximity to each other.

The number of emergent sporophytes in a given year is highly variable and is an incomplete indicator of total population numbers in *Botrychium echo*. Mason and Farrar (1989), Johnson-Groh (1998), and Johnson-Groh (2002) describe methods for extracting gemmae, gametophytes, and non-emergent sporophytes from soil samples. These methods would provide valuable data on the belowground structurebank of *B. echo*, but these methods are destructive and are not suitable for use in the known populations due to their high impact.

Populations of *Botrychium* are inherently variable (Johnson-Groh 1999). *Botrychium echo* may be prone to local extinction because it tends to occur in early successional sites following disturbance, and apparently 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. echo* can colonize (Lesica and Ahlenslager 1996). 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. echo*. It also underscores the need for forest management practices that allow the natural disturbance regime to persist that creates and maintains 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 to assess for any *Botrychium* species.

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. Understanding environmental constraints on *Botrychium echo* would facilitate the management of this species. Gathering data on edaphic characteristics (primarily moisture and texture) from the permanent plots described above would permit the analysis of species-environment relationships. Such data gathered carefully at the known occurrences in Region 2 when 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 comparative technique can be done quickly in the field. Although it does not provide detailed cover or abundance data, it can help to elucidate patterns observed in quantitative data.

Beneficial management actions

The ecology of *Botrychium echo* remains poorly understood, and the species has not been recognized long enough to develop effective management strategies. However, some generalities regarding management can be made based on current knowledge. Because *B. echo* shows a general preference for open sites, it may benefit from management activities that reduce canopy cover. Maintaining the health of the mycorrhizae is certainly crucial to the species as well.

Because *Botrychium echo* is inconspicuous and many occurrences may remain undocumented, surveys prior to management actions within potential habitat would help alleviate threats to this species from human impacts to individuals. Complete and detailed surveys are needed wherever there is the potential for impact to *Botrychium* populations (Kolb and Spribille 2000). This will help to identify new occurrences and to avert impacts to occurrences from development activities. The value of surveys prior to construction is evinced by the discovery of *B. echo* on Vail Pass at a site where a ski hut was to be constructed. This survey work has probably resulted in reduced impacts to this occurrence.

Further inventory and monitoring efforts would greatly benefit *Botrychium echo*. Identifying high quality populations in which the population size and condition, and the landscape context are excellent will help managers prioritize conservation efforts. Developing a better understanding of its centers of distribution will assist with the development of regional management protocols that favor the persistence of *B. echo*.

Mitigating recreation impacts on *Botrychium echo* may be important for some occurrences. Occurrences in ski areas are most likely to incur the loss of individuals due to summer recreational use of ski slopes. Signage or temporary fences that could be removed in the winter are possible mitigations. Hiking may result in trampling where *B. echo* occurs near trails or ski huts. Similar mitigations to those used on ski runs may also be effective in these scenarios.

Maintaining habitat in an open condition is the most prudent management decision until more is known about the impact of succession to a closed canopy (Johnson-Groh and Farrar 2003). Removal of woody species when the ground is frozen would minimize the risk to *Botrychium echo* sporophytes and gametophytes.

Thompson (2001) notes that the creation of ski runs has apparently created a great deal of suitable habitat for moonworts, which are not found in survey areas in Summit County, Colorado under tree canopies. While this may be true, ski runs cannot be depended upon for the long-term conservation of moonworts, since they are managed for the benefit of skiers, not moonworts. For example, the installation of pipelines in ski runs for the production of artificial snow will negatively impact individual plants in ski runs. It is not known if the autecological parameters needed by moonworts (e.g., appropriate mycorrhizal hosts, suitable disturbance regime) can persist indefinitely in ski runs. The observations of Muller (1999) suggest that maintaining occasional perturbations by humans or animals recreates pioneer habitats that benefit *Botrychium matricariifolium*. More research is needed on the practical application of management protocols for the maintenance of moonwort populations. Kolb and

Spribille (2000) recommend that *B. echo* populations should be protected from ground-disturbing activities.

Other human disturbances, including the construction of roads, have also created sites that *Botrychium* species, including *B. echo*, have successfully colonized. Although this might suggest that road construction is beneficial to *Botrychium* species, it is important to note that these occurrences are highly insecure and are wholly subjected to the vagaries of right-of-way management and transportation needs.

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 and development of these sites.

The utility of fire as a habitat management tool for *Botrychium echo* is not known. Evidence suggests that the direct impacts of fire are not detrimental to *Botrychium* (Johnson-Groh and Farrrar 2003). Though burning appears to have positive effects on prairie moonwort populations in Iowa (*B. campestre*, *B. gallicomontanum*, and *B. simplex*), fire combined with erosion and desiccation, both natural results of fire, may be deleterious. There were no significant differences in plant size in burned and unburned plots at these locations, but fires occurring after drought have resulted in population decline (Johnson-Groh and Farrar 1996b, Johnson-Groh 1999). Fire may play a role in preventing succession to closed canopy, in creating open habitats for *B. echo*, and in altering soil characteristics in ways that may also favor *B. echo*.

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

Any management strategies that work to prevent the infestation by weeds of uninfested occurrences of *Botrychium echo* are likely to confer the greatest benefits. Given the known impacts to moonwort species from accidental spraying with herbicide, the use of herbicides within occurrences of *B. echo* should be limited to direct application to target species. If noxious weeds are found in *B. echo* occurrences, eradication of the former is worthy of consideration, given the potential for negative impacts. Aggressive management of weeds before they become widespread in occurrences could avert costly and risky future eradication efforts. Where possible, hand pulling should be the favored method of managing weed populations within occurrences of *B. echo*.

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

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

Restoration

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

Buell (2001, page 11) describes a method for transplanting *Botrychium* species that has been employed by Nancy Redner of the USFS. This method has been used to mitigate impacts on *Botrychium* occurrences at the Copper Mountain Ski Resort from pipeline and road projects. No data on survivorship of the transplanted occurrences are available, but Buell (2001) describes transplantings following this methodology as "reasonably successful." Several moonwort species, including *B. echo*, 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. The methods they employed are described in detail in ERO Resources Corporation (2003). 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

Further survey work is among the greatest research needs for *Botrychium echo* in Region 2. As concern and awareness of *Botrychium* species has increased recently, more inventory work has focused on finding them. Consequently, there are many new discoveries of *B. echo* and other *Botrychium* species in Region 2. Recent work in Colorado at Pikes Peak, Indian Peaks Wilderness, and in Summit County have been fruitful in expanding our knowledge of the distribution of *B. echo*. It is very likely that further investigation will lead to more discoveries of occurrences as previously unsearched habitat is visited by botanists who are seeking these species. Given the rate at which new data are becoming available and the incompleteness of our current knowledge of *B. echo* in Region 2, it is difficult to formulate conservation strategies at present. More complete knowledge of the distribution of *B. echo* will permit the identification of areas most suitable for the development of conservation strategies and conservation management of *B. echo* in Region 2.

Life cycle, habitat, and population trend

Very little is known about the population ecology of *Botrychium echo*. In particular, the belowground portion of the life cycle remains poorly understood, although much of their 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 gemmae and spores, and the persistence, size, and longevity of spore banks will also need to be understood. Although all *Botrychium* species depend on their 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. 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. Census methods in a subset of the known occurrences, and marking and tracking individuals at these sites following the methods of Lesica and Ahlenslager (1996) would provide population trend data, which is currently unknown. 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 be speculative.

Response to change

The specific responses of *Botrychium echo* to disturbance and succession are not clear and warrant further investigation. There has been no specific research on *B. echo* addressing these issues. There are numerous and some fairly detailed observations of *B. echo* and *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 occurrences of *B. echo*. However, there are no baseline population data or survey work to reference prior to the disturbances, since in most cases the disturbance occurred before *B. echo* was even formally described. The amount of disturbance that *B. echo* can tolerate is not known but has considerable importance from a management perspective. The nature of observations of its response to both natural and anthropogenic disturbances in Region 2 is informal, and better data are badly needed to understand the role of disturbance in the life history, establishment, and persistence of *B. echo*. The intensity and tempo of disturbance needed to create and maintain suitable habitat for *B. echo* need to be addressed in future research. Reproductive rates and the ability to colonize new sites are important in better understanding the response to change of *B. echo*, but these remain highly speculative. The effects of herbivores and exotic species on the viability of *B. echo* populations have not been investigated.

Metapopulation dynamics

The metapopulation dynamics of *Botrychium echo* 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, resulting 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, precluding the determination of true population size and population dynamics; 3) the underground population, making 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

There have been very few demographic studies of *Botrychium* species. Currently our knowledge of demographic processes is not advanced for any member of this genus. *Botrychium mormo* is the best-studied member of the genus (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. echo* would be largely conjectural. No rigorous demographic data are available for populations of *B. echo* in Region 2. The longevity of the spores of *B. echo* could be assessed using the methods of (Dyer and Lindsay 1992), using spores obtained carefully from herbarium specimens. This could address the possibility that *B. echo* maintains a persistent spore bank.

Population trend monitoring methods

Methods are available to monitor population trends (Lesica and Ahlenslager 1996, Berlin et al. 1998, Johnson-Groh 1999). Even very simple and inexpensive methods, such as an annual census at 10 to 20 percent of the known occurrences over 10 years, could provide valuable trend data. Because there are probably many unknown occurrences (NatureServe 2003), observations at known sites may or may not reflect real population trends (Johnson-Groh 1999). 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. Monitoring efforts are further complicated by the highly variable population sizes, the prolonged dormancy of sporophytes, and the unknown dormancy and longevity of spores, gemmae, and gametophytes. Multiple seasons and large sample sizes will be required to detect meaningful change.

Restoration methods

Restoration or maintenance of native vegetation will certainly be a crucial part of any restoration effort on behalf of *Botrychium echo*. Restoration of native vegetation in the vicinity of known *B. echo* occurrences is likely to benefit them by providing possible colonization sites or buffers, and in reducing the influx of exotic species. The value of fire, logging, mowing, and other practices for restoration and maintenance of *Botrychium* habitat are discussed in detail in Johnson-Groh and Farrar 2003, and in this document where relevant.

There are many barriers to habitat restoration for *Botrychium echo* and other *Botrychium* species. *Botrychium* species are extremely difficult to propagate (Whittier 1972), and propagating them for reintroduction to the wild is probably not feasible given the difficulties they present. The belowground ecology of these species is crucial to understanding their autecology, yet it is also very poorly understood. As obligate mycorrhizal symbionts they cannot survive without suitable fungal partners, but very little is known about the specifics of this relationship. The fungal symbionts of *B. echo* 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. Using the symbionts needed by *Botrychium* species in inoculums, when these are identified, may assist in the recovery of *Botrychium* species following human impacts to a site. Transplant protocols have been developed and implemented for *Botrychium* species and are described in Buell (2001) and ERO Resources Corporation (2003), but it is not known if *B. echo* individuals tolerate transplanting.

Research priorities for Region 2

The most obvious research priority in Region 2 is the need for a better understanding of the range and distribution of *Botrychium echo*. It is likely that populations remain to be discovered for this and other species of moonworts in Region 2 (Wagner and Wagner 1986, Farrar and Johnson-Groh 1986).

Numerous other research needs are cited by Farrar and Johnson-Groh (1986), Lesica and Ahlenslager (1996), Berlin et al. (1998), and Johnson-Groh (1999), many of which apply to all *Botrychium* species. With respect to *Botrychium* species in general, these include further research on the life history and demography, focusing on underground life history stages. This research is complicated by the difficulties in growing *Botrychium* gametophytes and sporophytes under laboratory conditions. The specific role of gemmae in the life history of *B. echo* also needs further investigation. Finally, research on the ecology of *B. echo*, particularly with regard to its responses to burning, human disturbance, and succession, is needed.

Additional research and data resources

Extensive data and resources are available regarding *Botrychium echo*. 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. echo*. Element occurrence data and Potential Conservation Areas developed by the Colorado Natural Heritage Program (2004) will be useful for identifying areas for management actions and conservation initiatives for *B. echo* in Region 2. Other reports that are particularly rich in useful data include those of Lesica and Ahlenslager (1996), Kolb and Spribille (2000), Buell (2001), Steinmann (2001a).

DEFINITIONS

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

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

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

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

Congener — A member of the same genus. *Botrychium hesperium* is a congener of *B. echo*.

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, and ruderal species allocate resources primarily to reproduction. A suite of other adaptive patterns also characterize species under this model (Barbour et al. 1987). Some species, including *Botrychium echo*, 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* the gametophyte is subterranean and is parasitic on mycorrhyzal fungi (Foster and Gifford 1989).

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

Genus community — Several *Botrychium* species are commonly found growing together in close proximity. This is unusual in the plant world. Generally, 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*, 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.

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

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

Sporophyte — The diploid portion of the life cycle of plants. Haploid spores are produced by meiosis and give rise to gametophytes (Allaby 1998).

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

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

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