This Conservation Assessment was prepared to compile the published and unpublished information on the subject species or community. It does not represent a management decision by the U.S. Forest Service. Though the best scientific information available was used and subject experts were consulted in preparation of this document, it is expected that new information will arise. In the spirit of continuous learning and adaptive management, if you have information that will assist in conserving the subject taxon, please contact the Eastern Region of the Forest Service Threatened and Endangered Species Program at 310 Wisconsin Avenue, Milwaukee, Wisconsin 53203.

CONSERVATION ASSESSMENT

Botrychium simplex
(Least Moonwort)

Prepared by Steve Chadde & Greg Kudray
For USDA Forest Service, Region 9
Requisition no. 43-54A7-0-0036 / Project no. Ottawa-00-06

I. EXECUTIVE SUMMARY

Botrychium simplex is a small moonwort found in many areas of the world including eastern North America, the Great Lakes region, and the Rocky Mountains. It is one of the more common moonworts, and a number of subspecies and varieties have been named. It is found in a variety of open and forested habitats. Some sites have been disturbed in the past, such as roadsides, tailings pond, and old fields, but a wide range of relatively undisturbed forests (including northern hardwood forests, jack pine barrens, and cedar swamps) are also common habitats. Most details about the biology of *B. simplex* are generalized from studies of other moonwort species. Much of the life-cycle occurs underground. Populations of aboveground sporophytes fluctuate and individual plants may not appear every year, complicating attempts to adequately inventory the population. A population study in a prairie environment reported aboveground plants appeared in less than half of the years monitored; drought combined with fire also caused a population decline. Like other moonworts, *B. simplex* is dependent on a mycorrhizal relationship; thus any concerns about species conservation must include consideration of this relationship. No information is available on managing habitat to maintain the species. Potential threats are not well understood; disturbance may stimulate plant establishment in some habitats. Natural plant succession may be a threat in open habitats, but no information is available on the response of *B. simplex* to site changes. Since the species is small and populations fluctuate, continued inventory efforts are

necessary to better refine population demographics, range, and habitat. Much basic research on *B. simplex* biology is lacking.

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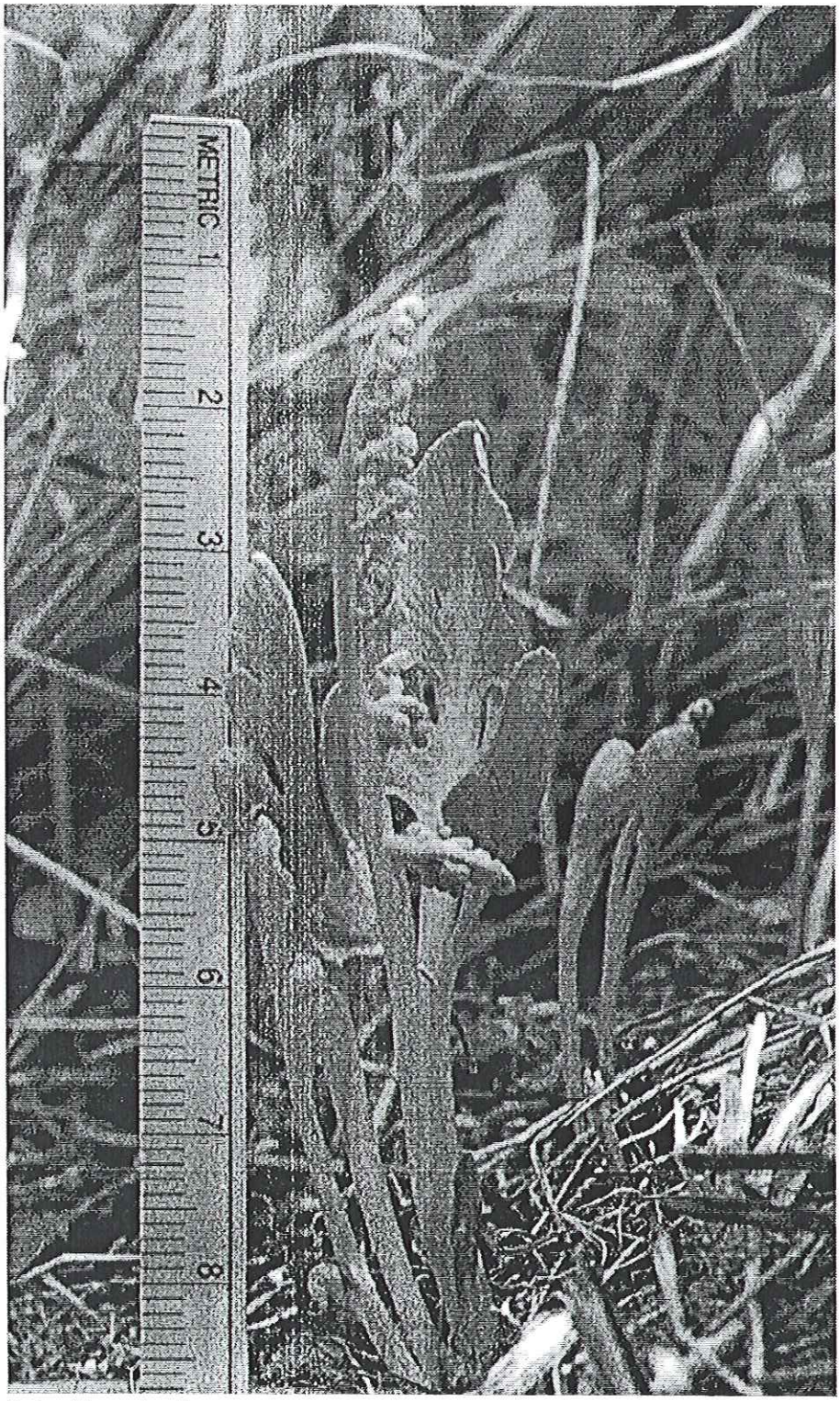
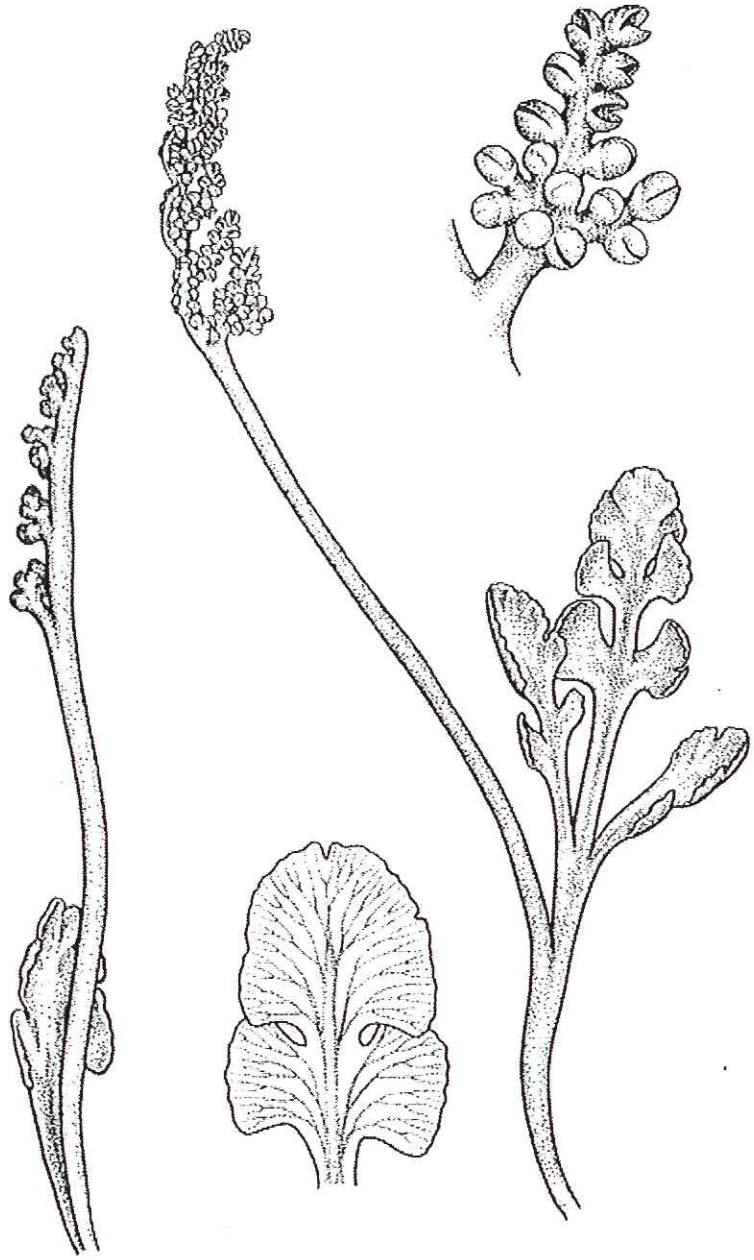


Botrychium simplex
Photo © Steve Mortensen.



Botrychium simplex
Illustration provided by USDA Forest Service.

II. INTRODUCTION/OBJECTIVES

One of the conservation practices of the USDA Forest Service is designation of Regional Forester's sensitive species. The Eastern Region (R9) of the Forest Service updated its Sensitive Species list on February 29, 2000. Part of that process included identification of priority species for Conservation Assessments and Strategies. A group of *Botrychium* species (Ophioglossaceae; Adder's-Tongue Family) was one of those priorities.

The objectives of this document are to:

1. Provide an overview of current scientific knowledge for *Botrychium simplex.*
2. Provide a summary of the distribution and status of *Botrychium simplex*, both rangewide and within the Eastern Region of the USDA Forest Service.
3. Provide the available background information needed to prepare a subsequent Conservation Strategy.

In North America, the genus *Botrychium*, family Ophioglossaceae, is comprised of three subgenera (Lellinger 1985, Wagner and Wagner 1993). One subgenus, *Osmundopteris*, is only represented in our area by *B. virginianum*, the rattlesnake fern, which is common around the world (Wagner 1998). The subgenus *Sceptridium* are the grapeferns, medium sized and decidedly evergreen plants (Lellinger 1985). Subgenus *Botrychium*, the moonworts, includes numerous species of often rare, local, and very small plants that are difficult to find and identify.

North America is a center of diversity for moonworts (Wagner and Wagner 1994) and the upper Great Lakes Region, along with the northwestern U.S. and nearby Canada, are two of the richest areas (Wagner and Wagner 1990a, Wagner 1998). Twenty-three species of North American moonworts are now recognized (Wagner and Wagner 1994) compared to the traditional interpretation of only six (Clausen 1938). The problems in distinguishing moonwort species are considerable (Wagner and Wagner 1990a), including the habit of different species of moonworts growing at one site, the natural variation in form due to microhabitat variability, their small size, and the difficulty of making good herbarium specimens. However, decades of work, primarily by the late Dr. Herb Wagner and associates, have clarified the taxonomy of the group, habitat preferences, and the ranges of individual species. Several rare species of subgenus *Botrychium* are now recognized in the Upper Great Lakes region.

Botrychium simplex is a small perennial fern, growing to only several inches tall. It is widely distributed around the world and also in disjunct distributions in eastern and western North America. *B. simplex* is considered one of the most common moonwort species (Wagner and Wagner 1986, Wagner 1991) and

grows in a wide variety of open and forested habitats. It is also one of the most variable of all moonworts in appearance, with different forms determined by site conditions (Hagenah 1966, Wagner and Wagner 1993) or perhaps by genetic differences. There may be further species or subspecies differentiation in the future (Wagner and Wagner 1993) for what is currently classified within a single *B. simplex* complex.

III. CONSERVATION ASSESSMENT

A. NOMENCLATURE AND TAXONOMY

- Scientific Name: *Botrychium simplex* E. Hitchc.
- Synonymy: *Botrychium simplex* E. Hitchc. ssp. *typicum* Clausen; *Botrychium simplex* E. Hitchc. var. *compositum* (Lasch) Milde; *Botrychium simplex* E. Hitchc. var. *laxifolium* (Clausen) Fern.; *Botrychium simplex* E. Hitchc. var. *tenebrosum* (A.A. Eat.) Clausen; *Botrychium tenebrosum* A.A. Eat.
- Family: Ophioglossaceae; Adder's-Tongue Family
- common names: Least Moonwort; Little Grapefern; botryche simple

B. DESCRIPTION OF SPECIES

B1. General description and identification notes

Botrychium simplex is a stout and rather fleshy perennial fern, growing 412 inches (10-30 cm) tall (Wagner and Wagner 1993, Rook 2001). There is a single erect frond divided into a sterile (trophophore) and a fertile (sporophore) segment. The single leaf is pale green, resembling a daisy leaf in shape; about 1 inch (2.5 cm) long, twice-cut, and erect. The stalk is pale green, slender, succulent, fragile, and about 4 inches (10 cm) long. The fertile frond rises above the leaf with erect, branched clusters of prominent yellow spore cases.

The many environmental and juvenile stages of *B. simplex* have resulted in the naming of numerous, mostly taxonomically insignificant, infraspecific taxa, although the western variety may merit separation as a distinct subspecies or species (Wagner and Wagner 1993). Eastern North American populations of *B. simplex* can be distinguished from western populations as follows: sporophore 1-4 times length of trophophore, arising from well-developed common stalk from below middle to near top, well above leaf sheath; trophophore non-ternate or if subternate, lateral pinnae smaller than central pinnae and simple to merely lobed (rarely pinnate); pinnae usually adnate to rachis, rounded and ovate to spatulate, segment sides at angles mostly less than 90°; trophophore tip undivided; texture papery to herbaceous.

The eastern, typical *B. simplex* has a common woodland and swamp shade form (sometimes termed *B. tenebrosum* A. A. Eaton) that appears to be a persistent

juvenile (Wagner and Wagner 1993). Plants are small and extremely slender, and the trophophore is simple, rudimentary, and attached near the top of an elongated common stalk (Wagner and Wagner 1993). Many intermediates between this and more typical forms exist. The variation appears to be the result of different growing conditions (Wagner and Wagner 1993).

A key to all *Botrychium* is provided in Wagner and Wagner (1993), but the difficulty of accurately identifying subtly different species of *Botrychium* often requires an expert. The treatment in Volume 2 of the Flora of North America (Wagner and Wagner 1993) is the most current published guide to all but the most recently described species (for example, since the release of Volume 2, a new species, *Botrychium lineare*, has been described by Wagner and Wagner [1994]). Lellinger (1985) includes descriptions and color photographs of many moonwort species. Cody and Britton (1989) provide descriptions and distribution maps of *Botrychium* species known to that time in Canada.

B2. Technical description

Trophophore stalk 0-3 cm long, 0-1.5 times length of trophophore rachis; blade dull to bright green to whitish green, linear to ovate-oblong to oblong to fully triangular with pinnae arranged ternately, simple to 2 (-3)-pinnate, to 7 x 0.2 cm, fleshy to thin, papery or herbaceous. Pinnae or well-developed lobes to 7 pairs, spreading to ascending, approximate to widely separated, distance between first and second pinnae frequently greater than between second and third pairs, basal pinna pair commonly much larger and more complex than adjacent pair, cuneate to fan-shaped, strongly asymmetric, undivided to divided to tip, basiscopic margins ± perpendicular to rachis, acroscopic margins strongly ascending, basal pinnae often divided into 2 unequal parts, margins usually entire or shallowly sinuate, apex rounded, undivided and boat-shaped to strongly divided and plane, venation pinnate or like ribs of fan, with midrib. Sporophores mainly 1-pinnate, 1-8 times length of trophophores. 2n = 90 (after Wagner and Wagner 1993).

C. LIFE HISTORY

B. simplex belongs to subgenus *Botrychium* (moonworts) within the genus *Botrychium*. In North America there are also subgenus *Osmundopteris* (rattlesnake fern) and subgenus *Sceptridium* (grapeferns) (Lellinger 1985, Wagner and Wagner 1993). The life-cycle of all three subgenera is similar (Lesica and Ahlenslager 1996). Moonworts are generally smaller than rattlesnake ferns and grapeferns. Plants have both a trophophore (vegetative segment) and a sporophore (fertile segment). Grapefern trophophores are present during the winter, while moonwort and rattlesnake fern leaves die back by winter.

Like all ferns, moonworts are characterized by alternation of generations between sporophytes and gametophytes. The sporophyte, the diploid (2N) generation of the plant, begins its life after fertilization of an egg by a sperm within the

archegonium of the gametophyte. Embryology of moonwort species has been little studied due to the difficulty of obtaining suitable material (Gifford and Foster 1989, Mason and Farrar 1989). Early morphological studies (e.g., Campbell 1922) described a diversity of patterns of embryo development among moonworts. For example, *Botrychium simplex* has a relatively large cotyledon and rapid development, perhaps capable of maturing a small aboveground fertile frond in its first year, while *B. lunaria* has a relatively small cotyledon, and may take as much as seven years to produce an emergent frond.

The following information is from research with a variety of *Botrychium* species. Reproduction in *B. simplex* has not been fully researched and there may be life history details specific to *B. simplex* that do not follow these general patterns for the genus. Lack of specific information on the life history of *B. simplex* is a significant management concern.

Vegetative reproduction was not thought to occur in *Botrychium* (Wagner et al. 1985), but Farrar and Johnson-Groh (1990) have documented underground gemmae in a few species of moonwort. They speculated that asexual reproduction may have evolved as an adaptation to the dry habitat that some of these moonwort species were found in. They also examined *B. simplex*, but found no evidence of gemma production, indicating that the primary mode of reproduction is sexually through spore production.

The spore cases of *Botrychium* are among the largest of all known ferns and appear like clusters of tiny grapes (hence the name *Botrychium*, from *botrus,* Greek for grapes) (Wagner 1998). The number of spores per case is probably the highest known for vascular plants, numbering in the thousands (Wagner 1998). Except for *B. mormo*, the sporangial opening to release the spores in most *Botrychium* is over 90° between the two sides of the gap (Wagner 1998). The spores have been measured to disperse by wind about one meter (Hoefferle 1999), but may typically travel much less, perhaps only a few centimeters (Casson et al. 1998). Peck et al. (1990) found that *B. virginianum* spores landed within 3 meters of the source if the plant was above the herbaceous layer, but much less when the sporophore was within the herbaceous layer. While most spores could be expected to land near the parent, some may travel considerable distances (Wagner and Smith 1993, Briggs and Walters 1997).

The succulent nature of the plant, the questionable spore dispersal mechanism, and the very thick spore walls that could help that spores to pass through an animal's gut, have suggested to some that herbivores such as small mammals may be involved in dispersal (Wagner 1998, Wagner et al. 1985, Wagner and Wagner 1993). The sporangia may also simply rot in the ground, thereby dispersing their spores (NatureServe 2001). It is uncertain how long *Botrychium* spores remain viable (Lesica and Ahlenslager 1996).

After the spores are released, they infiltrate into the soil and may germinate. Infiltration and subsequent germination may take up to 5 years, although some may germinate immediately (Casson et al. 1998). Spore germination requires darkness, (Whittier 1972, 1973; Wagner et al. 1985), a requirement that is not surprising in view of the subterranean habitat of the gametophyte and the need for the resultant gametophyte to be infected by an endophytic fungus in an obligate association (Whittier 1973). Details of this host/fungus interaction are provided in Schmid and Oberwinkler (1994). It has been suggested that *Botrychium* gametophytes may even delay growth until they are infected with the fungus (Campbell 1911; Whittier 1973, 1996). Essentially the *Botrychium* gametophyte becomes a parasite of the mycorrhizal fungus (Casson et al. 1998, Whittier 2000).

All *Botrychium* species are believed to be obligately dependent on mycorrhizal relationships in both the gametophyte (Bower 1926, Campbell 1922, Gifford and Foster 1989, Scagel et al. 1966, Schmid and Oberwinkler 1994) and sporophyte generations (Bower 1926, Gifford and Foster 1989, Wagner and Wagner 1981). The gametophyte is subterranean and achlorophyllous, depending on an endophytic fungus for carbohydrate nutrition, while the roots of the sporophyte lack root hairs and probably depend on the fungus for absorption of water and minerals (Gifford and Foster 1989). *Botrychium* gametophytes were formerly considered saprophytic (Bower 1926), but are now thought to obtain carbohydrates fixed by neighboring plants and transported by shared mycorrhizal fungi (Camacho 1996); they are thus better classified as myco-heterotrophic (Leake 1994).

A fungal associate is present within the plant at the earliest stages of development of the gametophyte and sporophyte (Bower 1926). There are no reports of successful completion of the lifecycle by *Botrychium* species without fungal infection, however, the degree of infection may vary between species and age of plants (Bower 1926, Campbell 1922). Little is known about the mycorrhizal fungi associated with *Botrychium* species other than their presence within the gametophyte and roots of the sporophyte (Camacho 1996). *Botrychium* mycorrhizae have been described as the vesicular-arbuscular (VAM) type by Berch and Kendrick (1982) and Schmid and Oberwinkler (1994).

The mycotrophic condition is important to the ecology of *Botrychium* species in several ways. Nutrition supplied through a fungal symbiont may allow the ferns to withstand repeated herbivory, prolonged dormancy, or growth in dense shade (Kelly 1994, Montgomery 1990). The fungal/fern relationship has implications for the occurrence of genus communities, the distribution of the species across the landscape, and associations with particular vascular plants. Mycorrhizal links may explain the often observed close associations between certain moonworts and strawberries (*Fragaria* spp.; Zika 1992, 1994) and between grapeferns (*Botrychium* subgenus *Sceptridium*) and Rosaceous fruit trees (Lellinger 1985). Due to the occurrence of heterotrophic life-stages, moonworts share many of the

morphological and habitat characteristics of myco-heterotrophic plants such as orchids (reviewed by Leake 1994) and in many respects behave much like mushrooms (Zika 1994).

Gametophytes and young sporophytes may exist underground for many years before an aboveground plant develops (Campbell 1911, Muller 1993). Mortality may be high during this period (Peck et al. 1990). The gametophyte produces male and female gametangia; fertilization of eggs occurs via free-swimming sperm under wet conditions (Lesica and Ahlenslager 1996). Most fertilizations are likely due to inbreeding, since the antheridia and archegonia are nearby and enzyme electrophoresis indicates a lack of genetic variability (McCauley et al. 1985, Soltis and Soltis 1986, Farrar and Wendel 1996, Farrar 1998). However, there is no reason that cross-fertilization should not occur (Wagner et al. 1985), especially in consideration of the existence of interspecific hybrids (Wagner et al. 1985, Wagner 1998). McCauley et al. (1985) calculated that *B. dissectum* outcrosses about 5% of the time. Extremely high levels of inbreeding were also found in *B. virginianum* although there was evidence for some outcrossing (Soltis and Soltis 1986).

Sporophytes develop on the gametophyte, forming roots and a single leaf each season from a short rhizome (Foster and Gifford 1974). Root development occurs before any leaf development (Casson et al. 1998), and the roots must also be colonized by the mycorrhizal fungi for a nutrient source (Farrar and Johnson-Groh 1990, Wagner 1998, Johnson-Groh 1998). The fungus involved is believed to be a vesicular arbuscular mycorrhizae (Berch and Kendrick 1982), which penetrates inside the plant cells of both the roots and the gametophytes in the case of *Botrychium* spp. The fungus may be transferring carbohydrates from other photosynthesizing plants in the vicinity, possibly species of herbaceous flowering plants (Farrar 1998). The species of mycorrhizae fungus involved with *Botrychium* is unknown (Casson et al. 2000). In a comparison of ferns and mycorrhizae colonization, the two *Botrychium* species surveyed had more extensively colonized roots than 37 other species of ferns (Berch and Kendrick 1982).

When the sporophyte eventually emerges, a sterile leafy blade (trophophore) and a fertile segment (sporophore) will develop. *Botrychium* plants may go dormant some years and not produce an aerial sporophyte (Wagner and Wagner 1981, Muller 1993). For example, *B. mormo* plants apparently do not produce aboveground sporophytes more than two consecutive years and there may be gaps as long as 6 years, although 1–3 years is more typical (Johnson-Groh 1998, Tans and Watermolen 1997). Johnson-Groh and Farrar (1996a) reported that *B. simplex* plants appeared aboveground in less than half of the years that were monitored. Species of *Botrychium*, with the exception of *B. mormo*, will not produce more than one sporophyte from a gametophyte within one growing season (Casson et al. 1998).

Several factors likely determine the size of the plant and how many spores it is capable of producing (Casson et al. 1998). These include the health of the plant and the associated fungi, climatic conditions, plant age, predators, and other factors. In discussing *B. mormo*, Casson et al. (1998) estimated that about 5–10 percent of aboveground plants would develop into larger plants with 20–50 sporangia (spore-bearing tissues) each.

B. simplex leaves appear in midspring to early fall (Wagner and Wagner 1993). In open droughty areas, plants appear and mature earlier, while woodland plants emerge and persist later into the season (Hagenah 1966). In Minnesota, least moonwort is reported to emerge in June and senesce by early August, making mid-June through July the best time to search for the species (Chippewa Rare Plants Field Guide 1996). The loss of plants to herbivory, fire, and collection did not affect the return of moonworts in later years (Johnson-Groh and Farrar 1996a, b). *Botrychium* may depend little on photosynthesis, and mycorrhizae alone may supply a significant amount of the plant's nutrients and energy (Johnson-Groh 1999, Casson 2000). However, it has been suggested that that photosynthesis may be important and that indiscriminate collecting could threaten *Botrychium* populations (USDA Forest Service, Eastern Region 1999).

Numerous hybrids between different species of moonworts have been found (Wagner et al. 1985; Wagner 1991, 1993; Wagner and Wagner 1988). The hybrids possess abortive spores and are intermediate in characteristics between the presumed parents (Wagner 1993). Wagner (1991) detailed a hybrid between *B. simplex* and *B. matricariifolium* from Quebec and also mentions two localities in Michigan. He provided a key to separate the hybrids. A suspected *B. simplex* hybrid with *B. lunaria* has also been reported (Wagner and Wagner 1988).

All 23 taxa of moonworts have chromosome numbers based on 45; half the members are tetraploids, and one is a hexaploid (Wagner 1993). Chromosome number has been useful in recognizing the distinctness of a new species; additionally, some species may have arisen through allopolyploids of interspecific hybrids (Wagner 1993). Farrar and Wendel (1996a, b) applied enzyme electrophoresis to the genetic relationships of eastern moonworts, including *B. simplex*, and suggested some relationships for moonwort species and hybrids. They subdivided *B. simplex* into three genetically divergent varieties with genetic differences between them approaching that of species (Farrar and Wendel 1996a). Wagner and Wagner (1993) also suggested that the western species may be distinct, meriting subspecies or even species status. However, Hauk and Haufler (1999) found genetic similarities between eastern and western *B. simplex* plants.

D. HABITAT

Lellinger (1985) described the North American habitat of *B. simplex* as meadows, barrens, and woods; plants were usually in subacid soil. In the eastern

United States, Gleason and Cronquist (1991) listed the habitat as open marshy places, meadows, and the edge of woodland ponds. Wagner and Wagner (1993) listed the habitat for eastern *B. simplex* as upland fields.

In Michigan, Hagenah (1966) listed typical habitats as jack pine barrens, highway shoulders, old fields, sandy borrow pits, and along the highwater mark of seasonal ponds in low, sandy woodlands.

In Minnesota, *B. simplex* was found in a wide variety of habitats including northern hardwood forests of sugar maple and basswood, rich black ash and cedar swamps, jack pine woods, prairies, and disturbed areas such as borrow pits, tailings ponds and road shoulders (Appendix A). Also in Minnesota, Rook (2001) listed habitats as dry or more often damp, partially shaded areas in coniferous forests, or rich deciduous woods on slopes. Lynden Gerdes has found this species at the base of black ash trees, usually growing in a carpet of mosses.

On the Chippewa National Forest in Minnesota, 4 locations of *Botrychium simplex* are reported (Ian Shackleford, Chippewa NF, pers. comm. September 2000). Habitats included a depression in an open area dominated by reed canary-grass, an open field with non-native grasses, and two northern hardwood stands.

In northern Wisconsin, the Chequamegon-Nicolet National Forest reported a wide range of habitat types associated with *B. simplex*, ranging from old fields and barrens to pine and hardwood forests (Appendix A). Common plant associates included jack pine and red pine, sugar maple, basswood and *Carex pensylvanica.* The species is found on glacial till and outwash on a wide variety of landforms, including pitted-outwash to drumlin ridges. Soils are sands, sandy loams, and loams, with low to moderate organic content. Plants are found in open to filtered light conditions and in dry to moist moisture regimes.

E. DISTRIBUTION, ABUNDANCE, AND STATUS

Botrychium simplex and *B. matricariifolium* are the most common moonworts in eastern North America (Wagner 1991). *B. simplex* is circumboreal and reported as common in Europe (Wagner and Wagner 1986 Gleason and Cronquist 1991). *B. simplex* is listed as "frequent" by Lellinger (1985). State and provincial conservation status rankings are listed below (see Appendix C for ranking definitions).

North American range of *Botrychium simpex* (Wagner and Wagner 1993).

The species is not listed as endangered, threatened, or special concern in Michigan or Wisconsin. It is listed as a species of special concern in Minnesota.

United States
Alaska (SR), California (SR), Colorado (S1), Connecticut (SH), Delaware (SR), District of Columbia (SR), Idaho (S1), Illinois (S1), Indiana (S1), Iowa (S2), Maine (SR), Maryland (SH), Massachusetts (SR), Michigan (S?), Minnesota (S3), Mississippi (SR), Montana (SU), Nevada (SR), New Hampshire (SR), New Jersey (S?), New Mexico (SR), New York (SR), North Carolina (S1), North Dakota (SU), Ohio (SH), Oregon (S4), Pennsylvania (S5), Rhode Island (S1), South Dakota (SU), Utah (S1), Vermont (SR), Virginia (S1), Washington (S3), West Virginia (S?), Wisconsin (S?), Wyoming (S2).

Canada
Alberta (S1S2), British Columbia (S1?), New Brunswick (SR), Newfoundland (S2), Newfoundland Island (Newfoundland) (SR), Northwest Territories (SR), Nova Scotia (S2S3), Ontario (S4?), Prince Edward Island (S1), Quebec (SR), Saskatchewan (S1).

Global Heritage Status Rank: G5 (20 May 1998)
Rounded Global Heritage Status Rank: G5

United States
National Conservation Status Rank: N5 (17 Dec. 1994)

Canada
National Conservation Status Rank: N? (08 Aug. 1993)

EO SUMMARY

GREAT LAKES STATES – NUMBER OF ELEMENT OCCURRENCES

State	No. of EOs	Status	Comments
Minnesota	70	S3	State special concern
Wisconsin	unknown	S?	Not tracked
Michigan	unknown	S?	Not tracked
Total	na		

GREAT LAKE STATES and NATIONAL FORESTS - SUMMARY OF ELEMENT OCCURRENCES

National Forest	No. of EOs
Minnesota	70
Chippewa National Forest	4
Superior National Forest	4
Michigan	na
Ottawa National Forest	na
Hiawatha National Forest	na
Huron-Manistee National Forest	na
Wisconsin	na
Chequamegon-Nicolet National Forest	na
Total State EOs	70 (MN only)
Total National Forest EOs	8 (MN only)
NF as % of EOs (MN only)	11%

F. POPULATION BIOLOGY AND VIABILITY

Little information is available about the population biology of *B. simplex*. Monitoring of a *B. simplex* population over several years in the presence of fire confirmed the variable nature of the plants' appearance aboveground (Johnson-Groh and Farrar 1996a). On average, less than half of the plants produced aboveground leaves in any given year. The total number of plants and plant size showed no difference between burned and unburned sites in most years. However, fires occurring during or after a drought resulted in population decline, suggesting that plants are killed or severely weakened by this combination of factors (Johnson-Groh and Farrar 1996a).

Population studies on other species of moonworts have also shown that there can be considerable annual variation in the number of aboveground plants at a given

site (Johnson-Groh 1999). Typically, populations fluctuated independently among plots at any given site, with some populations increasing while others decreased (Johnson-Groh 1999). These variations reflected microsite differences such as soil moisture, herbivory, or mycorrhizae (Johnson-Groh 1999), although populations of moonworts often fluctuate wildly from year-to-year without any apparent cause, and individual plants may not emerge every year (Muller 1993; Johnson-Groh and Farrar 1996a; Johnson-Groh 1998, 1999).

Botrychium probably appear or disappear, at least in part, due to the health of associated mycorrhizae fungi because of their obligate relationship with the fungi (Johnson-Groh 1998). Johnson-Groh (1999) concluded that mycorrhizae were the most important limiting factor for *Botrychium* establishment, distribution, and abundance. Environmental factors that may affect mycorrhizae, such as reductions in water availability, are also likely to have significant impacts on moonworts, whereas the repeated removal of leaf tissue may have little effect (Johnson-Groh 1999). Standard assumptions about the population biology of other, more 'typical' plants may be irrelevant to *Botrychium* because of this obligate relationship (Johnson-Groh 1999).

Since there is considerable variation in the numbers of aboveground sporophytes, a field measurement of only sporophytes does not completely indicate population numbers. Johnson-Groh (1998) developed a method to extract *Botrychium* gametophytes and belowground sporophytes from soil samples. Up to 7000 gametophytes and 250 non-emergent sporophytes per square meter of soil have been recovered, although an unknown number of these may be from the common *B. virginianum* (Johnson-Groh 1998). In another report Johnson-Groh et al. (2000) found gametophyte populations ranging up to 2000 gametophytes/m^2 for some moonwort species; other moonwort species had a much lower density. Bierhorst (1958) reported finding 20 to 50 gametophytes of *B. dissectum* beneath each surface square foot with a predominance of younger gametophytes versus older ones with attached sporophytes. These findings suggest that a finding even a single emergent sporophyte may indicate a self-sustaining population at that site (Casson et al. 1998).

A spore bank that consists of all ungerminated spores, including unopened sporangia, is present within the litter, duff, and soil (Casson et al. 1998). The spores persist in the soil for several years and, along with underground gametophytes and developing sporophytes, form a highly buffered population that can rebound from unfavorable years (Johnson-Groh et al. 1998, Johnson-Groh 1999). However, events that destroy the sporophytes may have an effect several years later (Johnson-Groh 1999). These underground stages have been compared to seed banks in angiosperms and could play an important role in population dynamics (Kalisz and McPeek 1992).

A population model for *Botrychium mormo* has been developed by a working group within the Population and Habitat Viability Assessment effort (Berlin et al.

1998) and Johnson-Groh et al. (1998). This model uses a variety of input variables such as number of spores in the soil, number of soil gametophytes, frequency of catastrophes, etc. They concluded that populations subjected to increased levels of annual environmental variation are at greater risk of population decline and extinction, although a single catastrophic year has relatively little effect on simulated populations. The population is likely more stable than would be predicted from monitoring only aboveground plants due to the large proportion of the population in underground stages. *B. simplex* may respond similarly.

Many species of *Botrychium* are associated with light to moderate disturbances (Lellinger 1985, Wagner and Wagner 1993a, Lesica and Ahlenslager 1996). As *B. simplex* is often found in open and disturbed areas, it may have a metapopulation structure whereby local populations are founded then go extinct as succession proceeds toward a closed climax community (Menges and Gawler 1986, Parsons and Browne 1982). The high variability in aboveground plant numbers found in some moonworts suggests a high probability of local extinction (Johnson-Groh et al. 1998). This kind of species may then depend on a regime of natural disturbances that creates a shifting mosaic of seral communities (Pickett and Thompson 1978).

Most moonworts, including *B. simplex*, are highly variable due to genetic differences and the effects of habitats (Wagner and Wagner 1990b). *B. simplex* is an especially variable moonwort with very different forms depending on environmental influences (Hagenah 1966, Wagner and Wagner 1993). The disjunct eastern and western populations may merit separate species or subspecies status (Wagner and Wagner 1993). However, one genetic study found similarities between the western and eastern populations (Hauk and Haufler 1999). An enzyme electrophoresis study of eastern *B. simplex* populations reported three genetically divergent varieties with the genetic distances between them approaching that of the species-level (Farrar and Wendel 1996a).

G. POTENTIAL THREATS AND MONITORING

Threats to *B. simplex* are not well understood but a serious underlying threat is the lack of information available on the species. The only well-documented threat resulting in a population decline was drought combined with fire (Johnson-Groh and Farrar 1996a). Because *B. simplex* often occurs in disturbed sites, threats may include natural plant succession and potentially the same human activities that have also apparently resulted in suitable habitat. Since *B. simplex* is also found in forested areas that have not been recently disturbed, forestry activities may affect existing populations negatively, although no research has been reported. Some threats will have their direct effect on the aboveground sporophyte and may be less serious, since the belowground part of the life-cycle is so important (see Sections C and F above).

Simple removal of leaf tissue may be inconsequential to the ability of moonworts to survive, although removing sporulating individuals may eventually have an effect (Johnson-Groh 1999). Wagner and Wagner (1993a) also stated that taking many samples will have little effect on the population as long as the underground shoots and roots are left intact. However, Hoefferle (1999) reported that if the aboveground plant was removed after spore release, the trophophore the following year was significantly smaller in size. Removal before sporulation had no effect. (It should be noted that this was a one-year study and weather conditions could have had a significant impact). Longer-term studies have indicated that the removal of leaves has no effect on subsequent leaf size or vigor (Johnson-Groh and Farrar 1996a, b). However, it has been suggested that that photosynthesis may be important and that indiscriminate collecting could threaten *Botrychium* populations (USDA Forest Service, Eastern Region 1999); thus leaf removal may have negative impacts on a population.

In a French study (Muller 1992), drought-like conditions resulted in wilting a sporophyte of a species of *Botrychium* prior to sporulation. The work of Johnson-Groh (1999) also emphasized the importance of water-relations to moonworts and their supporting mycorrhizae. Mycorrhizae are the most limiting factor for *Botrychium* establishment, distribution, and abundance (Johnson-Groh 1999); therefore adverse impacts to the mycorrhizae may be expected to also have deleterious effects on *Botrychium*.

Large decreases in mycorrhizal fungi have occurred following earthworm invasion in deciduous hardwood forests (Nielsen and Hole 1963, 1964; Cothrel et al. 1997; Nixon 1995). A similar effect may occur in the habitats favored by *B. simplex*, which include northern hardwood forests. Since most mycorrhizal activity occurs in the interface between the O and A horizons (Read 1994), the concurrent action of exotic earthworms in the same area may have significant effects. The exotic earthworms have their largest impact on the organic surface layer present in some soils (Langmaid 1964). However, the disturbed areas often favored by *B. simplex* likely would have less organic material; typical earthworm activity cycling organic material may not be a serious threat to *B. simplex* habitat there. Since *B. simplex* occurs with some regularity in forested environments that could be impacted by worms, habitat could be negatively affected.

G1. Stewardship overview and population viability concerns

Often it is difficult to determine what factor or combination of factors is impacting *Botrychium* populations (USDA Forest Service, Eastern Region 1999). Populations are inherently variable (Johnson-Groh 1999) but maintaining the health of the mycorrhizae seems to be an underlying necessity. Moisture relations are critical, as activities that dry the habitat may have deleterious effects on the population. *B. simplex* occurs in a wide variety of habitats, therefore a variety of techniques may be necessary to encourage the establishment and maintenance of the species. A variety of disturbed and relatively undisturbed habitats have been

reported; identifying a specific disturbance regime favorable to the species may be difficult.

Since *B. simplex* often exists in a habitat that is early successional due to disturbance (such as fields, tailings piles, roadsides, etc.), it may be prone to local extinctions. Maintaining viable populations may rely on a shifting mosaic of suitable habitats opening up for colonization (see Section F). Species protection efforts must account for the immediate area surrounding *B. simplex* populations to ensure that an adequate buffer is present to protect the population from potential threats. A buffer also allows for expansion of the population.

G2. Research and monitoring requirements

Like all *Botrychium*, *B. simplex* is small, inconspicuous, and fairly difficult to find. The fluctuating population also creates difficulties; plants may go dormant some years and not appear aboveground. There are probably undiscovered sites for *B. simplex*, inventories for the plant should continue. While some research data have been developed about population fluctuations for certain species of *Botrychium* (Johnson-Groh 1999), and one study of a *B. simplex* population in a prairie environment has occurred (Johnson-Groh and Farrar 1996a), much information about *B. simplex* population biology is lacking.

Almost no information is available on *B. simplex* life history in relation to disturbance and colonization of new sites. While its habitat is generally considered to be open areas, it also occurs in forested habitats. The specific habitat requirements of *B. simplex* and the sensitivity of this species to disturbance need to be determined. Succession toward closed-canopy conditions may be a threat, but it is unclear how *B. simplex* reacts to site changes over time. Long-term monitoring is necessary to determine life history characteristics, population stability, and dynamics over time.

Life history information for moonworts is mostly generalized from studies on various species within the group. Specific information on *B. simplex* life history is needed including its important relationship with mycorrhizal fungi and its belowground ecology in general. Data on spore dispersal are also lacking.

Exotic earthworms are a serious threat to some moonwort species, particularly *B. mormo* (Sather et al. 1998). It is unknown if exotic earthworms threaten *B. simplex* populations or habitats.

Berlin et al. (1998) make a number of specific research and monitoring recommendations for the moonwort, *B. mormo*. Many of their suggestions apply to other *Botrychium* species also, and that source should be consulted for detailed recommendations about *Botrychium* monitoring and research. There are also a number of specific suggestions about habitat and population monitoring for *B.*

rugulosum that generally apply to most rare *Botrychium* spp. at www.natureserve.org (NatureServe 2001).

In small populations, individual counts of the entire group should be made. In large populations, a representative sample of the population should be monitored through a randomized, permanent plot methodology. Individuals within each plot should be mapped as an aid to tracking, possibly providing detailed information pertaining to life span, dormancy, recruitment, etc.

Habitat monitoring is also a need for the species. Correlations between changes in habitat and reproductive success can give strong recommendations toward future management activities. Such monitoring will also indicate the appropriate time to initiate management activities. Perhaps the easiest and most effective way of monitoring habitat would be through permanent photo-points. Although photo-points may not provide the detailed information pertaining to species composition within a given site, rough changes in habitat should be observable. Photo-point analysis of canopy cover, and shrub and ground layer competition with respect to population trends would provide useful information for possible management procedures. Other more time-intensive procedures designed to statistically track changes in composition of the ground-layer associates at each site may be installed and monitored along with the methodology designed to track population trends, as discussed above.

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