Final Report - June 1997

FIELD SURVEYS FOR *BOTRYCHIUM GALLICOMONTANUM* AND PHENOLOGY OF *BOTRYCHIUM MORMO* IN MINNESOTA

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ABSTRACT

Ecological studies of *Botrychium* were conducted by the author during the 1996-1997 field season. This included conducting field searches for *B. gallicomontanum*, monitoring the phenology of *B. mormo* and annual monitoring of permanent *B. mormo* plots.

Field surveys for *B. gallicomontanum* were conducted in June of 1997 for the purpose of establishing additional sites for this rare species. Sites were selected in accordance with personnel from the Minnesota DNR. Three sites, two in Kittson County and one in Marshall County were searched. *Botrychium campestre* was found in both counties and represents new county records and sizable range extensions in Minnesota. Previously the northernmost location was Lincoln County. *Botrychium lunaria*, also a new county record was discovered at Lake Bronson State Park and also represents a range extension for this species. *B. matricariifolium*, also a county record, was found in Kittson County.

Four species of moonworts were found at Lake Bronson State Park including: *B. simplex, B. campestre, B. lunaria* and a fourth undetermined species. The fourth species may be *B. gallicomontanum* or related species, *B. spatulatum* or *B. pallidum*. Any of these species would represent significant finds for Minnesota and identification will be confirmed through isozyme analysis. All plants were yellowed and deformed due to the application of a herbicide earlier in the year.

The phenology of *Botrychium mormo* was monitored at two sites in the Chippewa National Forest every two weeks from June through September 1996. A total of 284 plants were monitored. Plants emerged earlier (June 2) than previously suggested and the population size likewise peaked earlier than previously hypothesized (July 14). The largest plants occurred late in August with an average size of 2.6 cm. Plants emerging early in the season had a prolonged "seasonspan", or period of emergence, whereas plants emerging later had a relatively short "seasonspan".

Annual monitoring of five permanent *B. mormo* plots occurred July 14-20, 1996. Population sizes increased significantly in 1996.

INTRODUCTION

Ecological studies of *Botrychium* were begun by the author in 1986 with the monitoring of prairie moonworts (*B. campestre*, *B. gallicomontanum* and *B. simplex*). These studies have resulted in reports and publications on the ecology and morphology of the species (Farrar and Johnson-Groh, 1986; Johnson-Groh, 1988; Farrar and Johnson-Groh, 1990; Farrar and Johnson-Groh, 1993), a new species description (Farrar and Johnson-Groh, 1991) and several papers currently in preparation. The 1996 field season represents the tenth year of monitoring these and other populations.

Previous studies have revealed the unusual biology of the moonworts. *Botrychium* produces one leaf annually consisting of two portions, a trophophore, or the photosynthetic lamina and a sporophore or the spore producing lamina. Moonworts have underground gametophytes which are not photosynthetic, but are mycorrhizal. Some species in the subgenus have gemmae, vegetative propagule which detach and initiate new plants. New plants resulting from gemmae apparently are maintained by the mycorrhizal relationship for a number of years before the first leaf emerges. Our studies have shown that individual plants typically do not emerge annually and may "skip" years. Removal of the above ground leaf does not negatively effect the emergence in subsequent years and damage to the leaf through collection, fire, herbivory appears inconsequential.

B. gallicomontanum, discovered in 1986, is the rarest moonwort species in Minnesota. It is endemic to Minnesota, restricted in its known occurrences to two nearby prairies in Norman County. The primary site, Frenchman's Bluff Preserve, is in the ownership of the Minnesota Chapter of The Nature Conservancy. The second site is privately owned. Additional populations have not been found despite extensive searches conducted by the author over several years.

Monitoring of *B. mormo* permanent plots has been conducted since 1992 and 1994. Because *B. mormo* was considered a late summer species the annual monitoring was conducted generally in late August or early September, a date chosen in hope of maximizing the number of plants visible during a single visit. It was unknown what proportion of plants in the total population may have emerged previously and reached senescence by the monitoring date or how many may emerge subsequent to this date.

METHODS AND MATERIALS

1. Field searches for B. gallicomontanum. Sites for field search were selected in collaboration with DNR and TNC staff. These sites represented dry gravely prairies in northwestern Minnesota which are most similar to the only known sites for *B. gallicomontanum*, Frenchman's Bluff in Lincoln County. The sites visited included a site near Middle River in Marshall County (T158N R43W, S1/2 Sec 35), Skull Lake Wildlife Manangement Area in Kittson County (T163N R47W, NW1/4 Sec 15) and Lake Bronson State Park in Kittson County (T161N R46W NW1/4 Sec 34).

The searches were conducted June 4-5, 1997. Approximately 3-4 hours were spent surveying each site. Releves were conducted at each site and each *Botrychium* species was vouchered from each site. Samples were collected for isozyme analysis by D. Farrar.

2. *Phenology monitoring of Botrychium mormo*. Two permanent plots previously established by the author were monitored every two weeks during the 1996 growing season to determine the phenology of

B. mormo. Both sites selected were located in the Chippewa National Forest and were selected to represent typical *B. mormo* plots, maximize the data collected and minimize the travel time (close proximity of plots). Plot 1, located on the Ottertail Peninsula of the Chippewa National Forest (T143N R29W SENE 6) includes a mature maple-basswood stand which the author has monitored since 1992. A second plot (plot 3), located in the Meadow Lake District of the Chippewa National Forest (T147N R31W NENW 8) is a maple-basswood stand with scattered aspen monitored since 1994.

Each plot measures 5.7m² in which each individual plant is marked by a numbered tag attached to an aluminum wire inserted into the ground two centimeters north of the plant. (Negative effects of the tags have been ruled out through comparative studies on "tagless" plots.) Each of the plots were monitored every two weeks using the following procedure. The entire plot was extensively searched for reoccurring tagged plants and new un-tagged plants. Each tag was checked for presence or absence of plants. Plants were measured and notes were recorded on the degree of development (just emerging, releasing spores, etc.) as well as disturbances such as herbivory or fire damage. New plants were tagged. Extreme caution was taken to avoid damaging plants and thereby affecting subsequent measurements.

The same procedure was followed every two weeks during the 1996 field season. Monitoring was conducted on: June 1, 15, 29, July 13, 27, August 10, 24, September 7, 21, and October 4. This time period represented the entire duration of emergence for the population.

3. Annual monitoring of B. mormo plots. Five permanent plots throughout northern Minnesota were monitored for population dynamics. The method for this survey is the same as described above for phenology monitoring except that only one visit was made in the 1996 field season. Plots were visited July 14-20, 1996. The locations and methods for establishing these plots is described in a report to the DNR, "Monitoring and Life History of *Botrychium mormo* in Minnesota" submitted in 1995. Locations for plots are listed in Appendix A.

RESULTS

1. Field searches for B. gallicomontanum.

Field surveys for *B. gallicomontanum* were conducted for the purpose of establishing additional sites of occurrence for this rare species. Following are descriptions of what was found at each of the three sites visited. Releves for each of the sites are in Appendix B.

Skull Lake Wildlife Manangement Area, Kittson County

The northwestern portion of this prairie was surveyed for *Botrychium*. *B. simplex* was found in abundance (>200) throughout the prairie. All plants were healthy and some were quite large. *B. simplex* could also be found in areas adjacent to the prairie which had recently become overgrown with *Populus tremuloides* along with *B. matricariifolium*. *B. matricariifolium* represents a new county record.

One full day was spent searching this site, however because of difficulty of access only a small portion of the site was viewed. It seems probable that this site would have *B. campestre* and possibly others. This site is worth revisiting especially following a fall burn. (The thick thatch impeded the search for moonworts considerably.)

Middle River, Marshall County

Botrychium campestre was found at this site and represents a new county record and a sizable range extension in Minnesota. (Previously the northernmost location for *B. campestre* was in Lincoln County.) Three hours were spent searching at this site primarily in prairie adjacent to the boundary road on the south side of the preserve. Reconnaissance of areas north of these sites revealed more degraded prairie with less potential for moonworts.

B. simplex was also found at this site growing with *B. campestre*. Both species were found in dense thatch and were distributed in patches. Over 100 individuals of *B. simplex* and 20 *B. campestre* were found within one hour of searching. It seems probable that there are many more scattered throughout this site. The moonworts were primarily found in hummocks resulting from gravel extraction.

Lake Bronson State Park, Kittson County

Four species of moonworts were found at Lake Bronson State Park including: *B. simplex, B. campestre, B. lunaria* and a fourth undetermined species. The fourth species may be *B. gallicomontanum*, though *B. spatulatum* or *B. pallidum* are also possibilities. Any of these species would represent significant finds in Minnesota. All species will be confirmed through isozyme analysis conducted by D. Farrar. *Botrychium lunaria*, also a new county record was discovered at Lake Bronson State Park and represents a range extension for this species. Likewise the occurrence of *B. lunaria* in prairie is unusual in that most *B. lunaria* collections in Minnesota represent forest communities.

Moonworts were found on the prairie adjacent to the tent campground. Many plants were found in the burned eastern section (100's of plants) and also in the section adjacent to the gravel pit. Unlike the burned area where it was very easy to locate plants, the area adjacent to the gravel pit contained a great deal of thatch which inhibited the search. Though not in great abundance *B. simplex* and *B. campestre* were still found in the unburned area. In addition to this site, a 30 minute search in a prairie adjacent to the group campground on the south side of the park revealed *B. simplex* and *B. campestre*.

The eastern portion of the prairie north of the tent campground had been burned in the fall of 1996 according to the park superintendent, Garry Barvels. In an effort to control leafy spurge the prairie had been seeded on May 10, 1997 and then sprayed with herbicide (Round-up) on May 23, 1997. Most plants were yellowed and deformed due to the application of the herbicide. This herbicide deformation made identification of some moonworts difficult and may possibly interfere with the isozyme analysis. Many moonworts could be positively identified and a few, which were bright green and healthy had apparently emerged after the application of the herbicide.

An additional day was spent at this site establishing two permanent plots marking over 100 plants for the purpose of studying the effect of herbicide on moonworts. Monitoring these plots in 1998 will provide significant data on the effects of herbicide with important management implications.

2. Phenology monitoring of Botrychium mormo.

Botrychium mormo was monitored from June 1 to September 22, 1996. An October visit was not needed due to low numbers of remaining plants and a killing frost.

There was a steady rise in the number of plants in each of the plots monitored every 2 weeks. Plot 1 peaked with a population of 201 on July 14 and plot 3 peaked with a population of 83 on June 26. The overall rise in population was very steady with an average two week increase of 11%. Senescence comes

less rapidly to these plants as compared with *B. gallicomontanum* resulting in a more gradual decline in numbers of plants. The average population decline for the last five visits was 16%.

Size of individuals increased steadily each two week period and peaked on September 22 for both plots (Figure 1). The maximum size is attained at the end of the growing season for these plants with an average maximum size of 2.6 cm. *B. mormo* continue to grow until they senesce with very little leveling off of growth. The largest moonworts are found at the very end of the growing season. Because they are so small and difficult to find it is not surprising that most of them have been found in the late summer and early fall leading most people to assume they are most abundant in the fall. However the results presented here indicate that the population peaks in mid summer and that a few individuals continue to increase in size and thereby become more visible.

Events during growth were divided into four stages. These included juvenile, leaf separation, spore release and senescence. The juvenile stage lasted an average of four weeks (Figure 2). Newly emerging plants could be found from June 2 until June 27. Leaf separation, or the period after the sporophore begins to extend beyond the trophophore and before the spores release, lasts 6.5 weeks. Plants in the leaf separation stage could be found from June 26 until August 24. This is considerably longer than *B. gallicomontanum* which has a separation stage lasting 1.5 weeks. Both the spore release and senescence stages last 1.5 weeks each in *B. mormo*. The first plants releasing spores and senescing occurred on August 10.

The population size peaked much earlier than previously thought. The presence of significant numbers of plants during the earliest visit (June 1) was surprising. In general *B. mormo* has a very long season first emerging in June and senescing in August. Previously annual monitoring had been done in late August based on the assumption that this was when the populations peaked.

3. Annual monitoring of B. mormo plots.

The annual monitoring of *B. mormo* was conducted July 14-20, 1996. Five permanent plots were surveyed for the reoccurrence of existing tagged individuals and the appearance of new individuals. 1996 Appeared to be a "good year" for moonworts with significant increases in all five plots monitored (Table 1).

Plot	1994 Return	1994 New	1995 Return	1995 New	1996 Return	1996 New
1	50	35	82	33	134	95
2	-	-	new plot	27	34	30
3	new plot	24	4	10	21	83
4	new plot	74	16	30	31	42
5	new plot	12	4	10	16	12

Table 1. *Botrychium mormo* **permanent plots.** Number of returning and new individuals 1994-96. Occasionally plots have greater returns than existed there the previous year such as is the case in plot 2. This is caused by the appearance of multiple plants at a tag where previously there had been only one plant.

Plot 1 still continues to support high numbers of *B. mormo*, but plot 3 also displayed a significant increase in new individuals in 1996. Conditions in 1996 were favorable to moonworts which may partially account for the notable increase in numbers. However, a second more likely cause of this substantial increase is that the monitoring more closely coincides with the population peak as determined by the phenology study. Monitoring in 1994-95 was done in August which may have been after the population had declined.

DISCUSSION / MANAGEMENT

1. Field searches for B. gallicomontanum.

A significant portion of the Lake Bronson prairie has not been burned (or treated with herbicide) for several years. *B. simplex* and *B. campestre* were found here, however it was extremely difficult to find them given the amount of dense thatch. The author recommends that a fall burn be conducted on this site. Fall burns greatly facilitate the search for moonworts. Given that this may be a potential *B. gallicomontanum* site we would recommend burning in the fall to enhance searching for moonworts the following spring. Equally significant is the avoidance of damage to the populations resulting from a late spring burn.

It is recommended that the two new plots established for effect of herbicide be monitored for a minimum of three years or until a measurable impact is noted. This site presents an ideal setting to conduct a thorough search for moonworts to determine the distribution of plants relative to parameters such as soil chemistry, moisture, topography, mycorrhizae, etc. The author will be requesting funding (National Science Foundation) to continue work at this site, as well as support and permission to work at this site from the Minnesota DNR.

2 - 3. Phenology monitoring of Botrychium mormo. Annual monitoring of B. mormo plots.

This study has shown that *B. mormo* emerges earlier than previously suggested and likewise that the population number peaks earlier in the season. These phenology results are based on one field season. The results obtained appear to be typical among years when compared with preliminary results from 1995 and early 1997 results. However it is important to recognize that one year of phenology is insufficient upon which to base projections for all years. It is recommended that one more year of data be collected to provide a more substantial basis for understanding the phenology of *B. mormo*.

The permanent plots used to study moonworts represent a sample of the population from which we make size estimates. If we are sampling at a time when the population is senescing, we are getting a false estimate of the population size. These phenology results will allow us to estimate population sizes more accurately and in turn better population size estimates will help us to more accurately assess the "rareness" of these species. Based on these results it is recommended that future monitoring of permanent plots should be conducted to coincide with the maximum population size in mid July. This will allow a more accurate assessment of the population size.

Similarly these phenology results can help us determine "windows" of time during which human impact on these species should be minimized. From these results we know that management activities conducted in June may have a more significant impact than previously thought. Management such as timber harvest or soil compacting will impact fewer plants in August following the population peak when plants are senescing than in June when the plants are just emerging. However, we do not know how various management practices impact the specific life stages. Plants releasing spores may be more or less susceptible to damage than earlier stages such as emergence. Additional studies need to be conducted to access the impact of management relative to the life stages.

LITERATURE CITED

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

Farrar, D. R., and C. Johnson-Groh. 1993. Permanent plot monitoring of *Botrychium campestre* in Iowa. Report of research submitted to the Iowa Chapter of the Nature Conservancy. 22 pages.

<u>Appendix A</u>: *Botrychium mormo* Plot Locations

PLOT 1: Ottertail Peninsula, Chippewa National Forest, Cass County T143N R29W SENE 6

Approximately 7 miles west of Bena, MN, turn south on Sucker Bay Road. Proceed south approximately 9 miles beyond where the road turns to gravel. At Y intersection (F.S. road 2914) go west (right). The plot is located 0.2 miles beyond F.S. road 2914. The plot is on the south side of the road 59 meters and 160° from road.

PLOT 2: Hagen Wildlife Protection Area, Polk County T148N R39W SWSESE 34

Approximately 7 miles west of Bagley turn north on Clearwater County Road 7 (church in NE corner of intersection). Proceed north on paved road for 3 miles. Turn left (west) on county road 22. Proceed 1 mile to where the road changes to Polk County road 1. Continue 2.5 miles (total 3.5 miles since turning west). Turn right (north) on gravel road. Go 1 mile to Hagen WPA. Turn left (east) on south side of refuge and go 0.35 miles. From the road proceed north approximately 175 meter following plot instructions.

PLOT 3: Meadowlakes, Chippewa National Forest, Beltrami County T147N R31W NENW 8

Going north of Bemidji on highway 71 turn right (east) on Beltrami county road 20. Proceed approximately 10 miles to F.S. road 2393. Turn north (left). Proceed 2.2 miles north on gravel road until you find F.S. road 2393A which is part of the Meadowlakes trail system. Park and hike east on 2393A. Follow the trail until it "T"s approximately 1/4 mile from the road. Turn left (north) and go approximately 225 paces until you find a large white birch tree on east side of road. Follow plot instructions from birch.

PLOT 4: Ottertail Peninsula, Chippewa National Forest, Cass County T144N R29W SENE 30

Approximately 7 miles west of Bena, turn south on Sucker Bay Road. Proceed south 7.8 miles on paved road. On the west side of the road is a green mailbox marked "Senic". On the east side of the road is a small road leading to a Potlatch clear cut. Follow plot instructions from this small side road.

PLOT 5: Pike Mountain, Superior National Forest, St. Louis County T59N R17W ENW 36

From Virginia go north on highway 53. Turn east on highway 169. Approximately 4.5 miles from highway 53 turn right (south) on gravel road. (Approximately 0.5 miles east of Florenton Cemetery.) Proceed south for one mile until you come to Inland Steel Mining Gate. Permission for access is required. From end of Inland Steel Property, follow trail along clear cut due south. Main trail will eventually turn west, another (4-wheeler) trail continues south up Pike Mountain. Follow this trail up the mountain and continue beyond lookout tower site. Follow plot instructions from lookout site.

Appendix B: 1997 Kittson and Marshall County Botrychium Site Releves

Skull Lake Wildlife Manangement Area, Kittson County:

4 June 1997 Kittson County T163N R47W, NW1/4 Sec 15 Size: 100m² Slope and position: flat Remarks: Prairie interspersed with areas overgrown with a young *Populus tremuloides* canopy with prairie elements underneath; Two *B. matricariifolium* was found in the wooded area.

Litter:	5
Bare Soil:	+
Bryophytes:	+
Woody Cover:	2
Total Cover:	5
Botrychium simplex	1
Botrychium matricariifolium	а
Poa pratensis	5
Andropogon gerardii	5
Populus tremuloides	2
Prunus virginiana	1
Amelanchier sp.	1
Lithospermum canescens	1
Lathyrus venosus	1
Aster sericeus	1
Solidago rigida	1
Galium boreale	+
Antennaria neglecta	+

Middle River, Marshall County - Releve 1:

5 June 1997
Marshall County.
T158N R43W, S1/2 Sec 35

easternmost site approximately 50 meters from the road

Size: 100m²
Slope and position: flat, slightly undulating, hummocks
Soil: gravely, porous
Remarks: moonworts in swales between hummocks; hummocks often with woody species; 5 plants (probably *B. campestre*) in 2m² area; plants are just beginning to spread sporophore and trophophore;

Litter:	5
Bare Soil:	1
Bryophytes:	2

Woody Cover:	2 (patchy)
Total Cover:	5
Botrychium campestre	а
Andropogon gerardii	5
Poa pratensis	4
Commandra umbellata	2
Lithospermum canescens	2
Solidago rigida	1
Populus balsamifera	1
Petalostemon purpureum	+
Lathyrus venosus	+
Astragalus crassicarpus	+
Fragaria virginianum	+
Monarda fistulosa	+
Artemisia frigida	+
Antennaria neglecta	+
Prunus virginiana	+
Arctostaphylos uva-ursi	+
Populus tremuloides	а
Galium boreale	а
Potentilla arguta	а
Quercus macrocarpa	r
Corylus cornuta	r

Middle River, Marshall County - Releve 2:

5 June 1997 Marshall County.
T158N R43W, S1/2 Sec 35 western site approximately 25 meters from the road in swale (old road bed?) between 2 raised areas of *Prunus americana*Size: 100m²
Slope and position: flat, slightly undulating, hummocks
Soil: gravely, porous
Remarks: moonworts in swales between hummocks; hummocks often with woody species; area in swale has many *B. simplex* (>50), 2 plants of what is probably *B. campestre* were found; most are young just beginning to expand.

Litter: Bare Soil: Bryophytes: Woody Cover: Total Cover:	5 1 2 2 (patchy)
Botrychium campestre	a
Boirychium simplex Andropogon gerardii Poa pratensis	5 3

Prunus americana	3
Stipa comata	2
Amelanchier sp.	1
Lithospermum canescens	1
Lathyrus venosus	1
Aster sericeus	1
Solidago rigida	1
Geum triflorum	+
Galium boreale	+
Antennaria neglecta	+
Quercus macrocarpa	а

Middle River, Marshall County - Releve 3:

5 June 1997
Marshall County.
T158N R43W, S1/2 Sec 35

western site approximately 50 meters from the road in swales

Size: 100m²
Slope and position: flat, slightly undulating, hummocks
Soil: gravely, porous
Remarks: moonworts in swales between hummocks; hummocks often with woody species; over 100 *B. simplex* flagged in 10m² area

Litter:	5
Bare Soil:	+
Bryophytes:	+
Woody Cover:	2 (patchy)
Total Cover:	5
Botrychium simplex	1
Andropogon gerardii	5
Poa pratensis	2
Aster sericeus	2
Lithospermum canescens	2
Lathyrus venosus	2
Solidago rigida	2
Cypripedium calceolus var. parviflorum	1
Potential arguta	1
<i>Rosa</i> sp.	а
Equisetum hymale	a
Artemisia frigida	+
Antennaria neglecta	+
Geum triflorum	+
Geum triflorum	+

Lake Bronson State Park in Kittson County - Releve 1:

5 June 1997 Kittson County T161N R46W NW1/4 Sec 34 on northwest edge of old gravel pit
Size: 100m²
Slope and position: flat
Soil: gravely, porous
Remarks: 3 *B. campestre* and 6 *B. simplex* found in 20 minutes search; Many *B. simplex* (>50)
within 50 meters of releve; plants difficult to find because of dense thatch.

Litter:	5
Bare Soil:	-
Bryophytes:	-
Woody Cover:	-
Total Cover:	5
Botrychium simplex	1
Botrychium campestre	+
Poa pratensis	5
Stipa comata	2
Schizachyrium scoparium	2
Artemisia frigida	1
Anemone patens	+
Solidago sp.	+
<i>Rosa</i> sp.	+
Petalostemon purpureum	+
Sporobolus heterolepis	+
Bouteloua curtipendula	а

Lake Bronson State Park in Kittson County - Releve 2:

5 June 1997
Kittson County
T161N R46W NW1/4 Sec 34

in eastern portion of prairie hit by herbicide

Size: 100m²
Slope and position: flat
Soil: gravely, porous
Remarks: Many *B. simplex* (>300) throughout site; scattered *B. campestre*; 1 *B. lunaria* and several deformed unidentified *Botrychium*. Only a partial releve was conducted as it was impossible to identify most plants due to the application of herbicide and fall burn.

Litter:	-
Bare Soil:	3
Bryophytes:	-
Woody Cover:	-
Total Cover:	3
Botrychium simplex	1
Botrychium campestre	+
Botrychium lunaria	r
Botrychium sp.	+

Poa pratensis Stipa comata Schizachyrium scoparium Artemisia frigida Solidago sp. Rosa sp. Euphorbia corollata unable to determine unable to determine