

***Botrychium* (Moonwort) Rare Plant Surveys for Polymet Project July 2004**

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Overview

This summarizes the results of *Botrychium* surveys conducted for Barr Engineering for the Polymet Mining Project. This work was conducted in July 2004 on lands within the Superior National Forest in St. Louis County, Minnesota that are managed by either the U.S Department of Agriculture - Forest Service or the Minnesota Department of Natural Resources (MDNR).

INTRODUCTION

The purpose of the Polymet *Botrychium* survey was to search specified areas for the presence of rare *Botrychium*. All localities were documented including species and population size. The survey was conducted in St. Louis County in the area east of Hoyt Lakes. Forest cover includes jack pine (*Pinus banksiana*), aspen (*Populus tremuloides*), balsam fir (*Abies balsamea*), paper birch (*Betula papyrifera*) and black spruce (*Picea mariana*). These stands are bisected by forest logging roads (FS Road 108 and FS Road 108), Dunka Road, a railroad and a power-line-right of way.

The genus *Botrychium* (moonworts) is among the rarest of fern genera. These small diminutive plants are found in a variety of habitats ranging from native prairies to rich northern hardwoods to calcareous fens to sandy beaches. Several of the species in this genus are listed as endangered and have very limited distributions. Three species are officially listed as endangered in Minnesota (*B. gallicomontanum*, *B. oneidense*, *B. pallidum*), three species are listed as threatened (*B. lanceolatum*, *B. lunaria* and *B. rugulosum*) and four species are listed as special concern (*B. campestre*, *B. mormo*, *B. simplex*, *B. manganense*). Species found in this survey include *B. dissectum*, *B. matricariifolium*, *B. michiganense*, *B. multifidum*, *B. pallidum*, *B. simplex*, *B. virginianum* and possibly *B. rugulosum*.

Botrychium Life History / Life Cycle

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

Botrychium are unusual among ferns in many respects with regard to their life history and reproduction (figure 1). *Botrychium* produce a small, single leaf (frond, 2-10 cm) that is divided into a once- or twice-pinnate sterile segment, known as the trophophore, and a once to trice-pinnate fertile segment, known as the sporophore. These frond segments fuse in a common stipe. The entire structure is usually green and photosynthetic. The trophophore is leaf-like and its primary function is photosynthesis. Because it encloses the sporophore during emergence, it also protects the sporophore. The sporophore produces spores in globose sporangia (ranging from ten sporangia per sporophore on small plants to over 100 on large plants).

Botrychium produce one leaf (including two segments, a trophophore and a sporophore) annually that is attached to a belowground rhizome that is upright and short (4-15 cm). Just below the soil surface (1-5 cm) is a single leaf-producing bud at the rhizome apex. The bud may contain up to six preformed leaves (Imaichi 1989). The rhizome and attached roots are off-white, stout and succulent. The roots typically branch off at right angles to the rhizome and vary in length (up to 20 cm).

The sporophore produces spores that filter into the soil and germinate in darkness (Whittier 1973). Following germination, a belowground achlorophyllous, fleshy gametophyte is produced. These gametophytes are small (usually less than 0.5 cm), irregularly shaped (often knobby and tuberous), off-white with numerous rhizoids. The gametophyte produces gametangia (archegonia and

antheridia) and sexual reproduction occurs resulting in a belowground juvenile sporophyte. (*Botrychium* are primarily self-fertilized.)

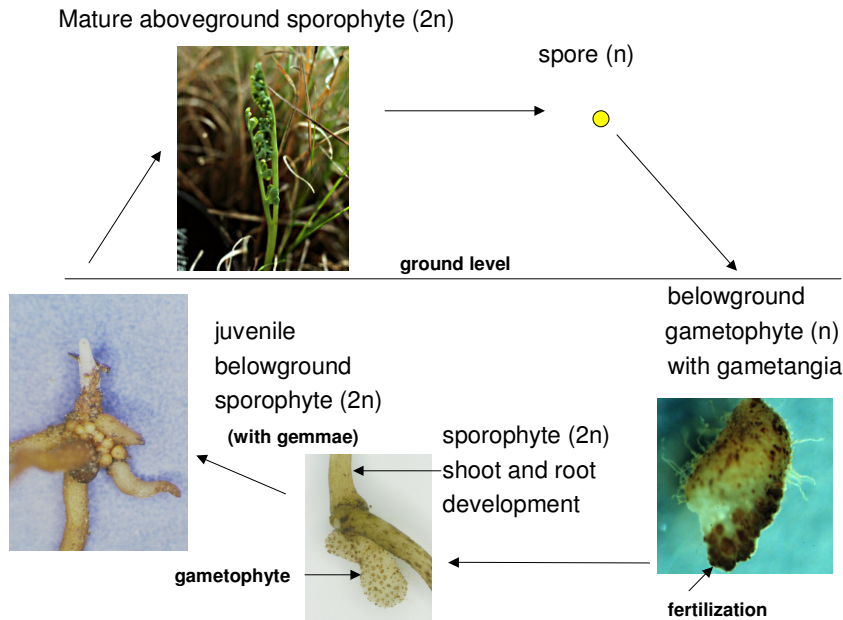


Figure 1. Generalized *Botrychium* life cycle (Johnson-Groh et al. 2002).

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

In addition to the belowground stages described above, some species such as *B. pallidum* reproduce asexually via belowground gemmae, small (0.5-1mm) propagules that can independently give rise to a new plant once detached from the parent plant (Farrar and Johnson-Groh 1990). The presence of vegetative reproduction greatly influences the population dynamics of these gemmiferous species. It is common in the field to see two or more leaves of gemmiferous *Botrychium* emerging in close proximity. Excavation of these clusters usually reveals a large number of belowground sporophytes in various stages of development.

Unlike most other ferns, the juvenile sporophyte stages of *Botrychium* remain belowground for a number of years. The belowground recruitment of gametophytes and juvenile sporophytes therefore can be compared to seedling or sporeling recruitment aboveground for flowering plants and other ferns. As with other plants, juvenile mortality is probably very high for *Botrychium*. Johnson-Groh, et al. (2002) found that for most species the belowground density exceeds the density of aboveground sporophytes. In other words the population of plants belowground is generally higher than the visible population aboveground.

Moonworts require endophytic mycorrhizae for gametophyte and sporophyte development (Berch and Kendrick 1982, Schmid and Oberwinkler 1994). Germinating gametophytes are infected by vesicular arbuscular mycorrhizae (VAM, Schmid and Oberwinkler 1994). The mycorrhizae facilitate nutrient and water uptake and transfer carbon resources to *Botrychium*. Little is known about how or when the gametophyte is infected or the identity of the fungal partners.

Moonworts depend on mycorrhizae as a significant source of carbohydrate, minerals, and water. This observation is based on several ecological behaviors. First, similar to orchids, moonworts do not emerge every year (Johnson-Groh and Farrar 1993). They frequently fail to emerge for one to three consecutive years, with no subsequent decrease in size or other observable negative effects (Lesica and Ahlenslager 1996, Johnson-Groh 1997, Johnson-Groh and Farrar 1993). Second, “albino” *Botrychium*s have been observed that are equally vigorous as photosynthetic individuals (Johnson-Groh, et al. 2002). Another indication that *Botrychium* depends relatively little on its own leaves for photosynthesis is the observation that these leaves frequently do not emerge above the litter. In fact only a small proportion of the total population of *B. mormo* emerged from the litter (Johnson-Groh and Lee 2002, Johnson-Groh 1998). Herbivory and loss of leaves through fire do not affect the size and vigor of plants in the subsequent year (Hoefflerle 1999, Johnson-Groh 1998, Johnson-Groh and Farrar 1996a, 1996b). Finally, if leaves of juvenile plants are produced one per year, as in adults, 3-8 years may be required for development from gametophyte to a mature sporophyte with an emergent photosynthetic leaf (Johnson-Groh et al. 2002). Juvenile plants must rely totally on mycorrhizae for carbohydrates while underground. Whittier (1984) noted that gametophytes may remain dormant (not actively growing) for up to four months without a exogenous carbon source, but resume growth in the presence of sucrose. Thus, although there has been no physiological studies to confirm this, it seems certain that moonworts (*Botrychium* subg. *Botrychium*) may depend largely on mycorrhizae for carbon transferred from other green plants, to supplement that produced by their own photosynthesis. This dependence on mycorrhizae emphasizes the importance of soil habitat for these species.

Disturbance

The impact of disturbance on *Botrychium* is poorly understood. Some species of *Botrychium* clearly prefer old disturbance sites, whereas others do not. Sites, which were disturbed approximately ten years ago, seem ideal habitat for many species of *Botrychium*. Roadside ditches, campgrounds, ski hill runs, avalanche shoots and railroad right-of-ways are common habitat for moonworts. Seemingly suitable sites that have been disturbed more recently or longer ago often do not support moonworts or support smaller populations. *Botrychium* are frequently associated with old road construction. Logging roads in particular support healthy populations of moonworts. Farrar (1996) reported a distinct association of *B. matricariifolium* with old logging roads in the Ottawa National Forest in Michigan.

This disturbance relationship has not been quantified and does not apply to all moonworts. Some species such as *B. mormo* and *B. montanum* require old-growth forests. Disturbance may redistribute spores exposing them to nutrients or mycorrhizae favorable to germination. The relationship with disturbance is likely mediated by the development of the mycorrhizal community post disturbance.

Historic disturbance appears to be an important factor for moonwort establishment. Nothing is known about the mechanisms of this behavior and little is known about the impact of recent disturbances on extant populations. Colonization of *Botrychium* following disturbance is difficult to observe and careful consideration of the time scale of colonization and the microhabitat is warranted. *Botrychium* spores percolate underground and may lie dormant for many years before

they germinate. Once they do germinate it takes 3-5 years of development before they actually emerge aboveground (Johnson-Groh unpublished data). The colonization of new *Botrychium* populations likely is on a time scale of 10's of years rather than years. Colonization following disturbance is not immediate, but delayed. The type of disturbance is also important. It is not the case that *Botrychium* inhabit all disturbed sites or that disturbing a site will promote the establishment of *Botrychium* populations.

METHODS

The author and assistants (Samantha Engh and Gina Quiram) searched the Polymet area for *Botrychium* (moonworts and grapeferns) from July 2-July 8, 2004. A total of seven days were spent in the field. Because of limited time the search was conducted in habitats that had the highest probability of having *Botrychium*.

Search Methodology

Successfully searching for moonworts requires targeting high potential habitats and careful search on hands and knees for plants. The following protocol has been incorporated into a national Forest Service protocol for inventorying and monitoring for *Botrychium* (Johnson-Groh 2004, in progress).

Habitats within the entire survey area were evaluated based on similarity in disturbance, cover, canopy closure, soil type and moisture to sites with known populations of *Botrychium*. There were two known sites for *Botrychium* in the survey area including populations 1 and 2 discovered and described by Gary Walton in 1998 (Walton 1998). Walton reported the discovery of *B. simplex* and *B. matricariifolium* as well as unconfirmed specimens of *B. ascendens*, *B. michiganense* and *B. acuminatum*.

Both of these sites were resurveyed and additional sites similar to these sites were searched. This included roads, ditches, the railroad right-of-way and the power line right-of-way. Areas that were different (aspen and jack pine stands) were also surveyed though considerably less time was spent on these habitats.

Searches were conducted for *Botrychium* using the timed meander search approach (Goff et al. 1982). Up to 1-2 hours per site was spent searching for *Botrychium*. Because *Botrychium* are extremely small plants and very difficult to see it was important to develop a good search image and periodically search on hands and knees, carefully sorting through vegetation and litter. Once discovered plants were flagged with survey pin flags and the area immediately surrounding the plants (5m² radius) was carefully searched on hands and knees for additional plants. The herbaceous litter was carefully parted and sorted to look for plants that are below the litter. Given the limited time to conduct this search, it was only possible to conduct careful searches in target areas rather than broadly searching the entire site.

The number of plants discovered per site was recorded by species. Voucher specimens were collected and have been deposited at the University of Minnesota – Duluth herbarium. Collections include only the above ground portion of the plant. The underground portions of the plant are unnecessary (and unhelpful) in species identification. Removing only the top does not harm the plant (Hoefflerle 1999, Johnson-Groh and Farrar 1996b) and allows it to produce more leaves in subsequent years.

The following are limitations of the survey protocol.

- Localized population distributions and abundances are only estimates, not known values. The aboveground population size of *Botrychium* plants varies seasonally and annually. Erroneous results may be obtained from surveys conducted at only a single point in time. Determining abundance / distribution may require multiple inventories over several years.
- Finding these small cryptic plants is difficult. Most *Botrychium* are quite small and often overlooked. Crawling on hands and knees and parting the standing vegetation and litter is the best means of locating plants, but this is time consuming and doesn't allow comprehensive surveys of large areas.
- A significant problem relates to the peculiar life cycle of *Botrychium*, in which, relatively little of the population is visible aboveground. Following emergence aboveground, the plants generally produce one leaf annually, but it is common for *Botrychium* plants to remain dormant belowground in a given year and produce no aboveground leaf (Johnson-Groh et al. 2002). In addition to these belowground stages, some species reproduce asexually via belowground gemmae (*B. pallidum*), resulting in abundant underground plants in various stages of development. The net result is that the number of plants underground generally exceeds the number with aboveground leaves, and in some years (especially those following drought) few plants may be visible above ground although the belowground population remains healthy.
- Mixed species assemblages of *Botrychium* are common in a diversity of habitats and are well documented (Wagner and Wagner 1983). 'What's good for one' is likely good for many *Botrychium* species, namely adequate soil, moisture and mycorrhizal resources.

In two areas (railroad and forest service road 108) a different sampling technique was used after determining that populations were present in each of those habitats. In these sites a point sample method was used as described below:

- Railroad: Starting at the intersection of Dunka Road and where the railroad branches southward, ten points were searched at intervals of 50 paces (~50 meters) along the railroad. Every 50 meters the slopes of the railroad were searched for plants on alternating sides of the tracks. At each point the number and identity of species found in 10 minutes were recorded along with the GPS coordinates. An additional ten points were sampled at intervals of 100 meters bringing the total number of points sampled to twenty. All searches were conducted between the railroad tracks and forest edge.
- Forest Service Road 108: Starting at the intersection of Dunka Road and forest service road 108 a point sample technique was used to search for plants along this old logging road. A total of 15 points were sampled the first eleven points at intervals of 100 meters and the remaining 4 points at intervals of 200 m. At each point the number and identity of species found in 10 minutes were recorded along with GPS coordinates. The woods adjacent to the trail were also search at most of the points. Search was conducted from the road into the woods approximately 20 meters.

RESULTS

Distribution – Abundance

The areas surveyed and species found are listed in Table 1-3. A total of 19 populations containing *Botrychium* were documented and 26 points in the point surveys had *Botrychium* present. Species of *Botrychium* found include: *B. dissectum*, *B. matricariifolium*, *B. michiganense*, *B. multifidum*, *B.*

pallidum, *B. rugulosum*, *B. simplex*, *B. virginianum*. Populations ranged in size from one individual to over 500 individuals. All populations were found in old disturbance sites such as old logging roads or railroad right-of-way. A few plants were found scattered in wooded habitats adjacent to these disturbed sites. Because of time limitations wooded sites were not targeted for this survey. Disturbed sites have the highest probability of supporting *Botrychium* populations so these sites were disproportionately targeted in this survey. Woods adjacent to *Botrychium* populations in disturbed sites were searched briefly but not exhaustively and often a few plants could be found a few meters into the woods. Though it was impossible to search the entire area, searches were selected in areas that had good habitat potential and were geographically spread.

In all cases the population sizes represent a conservative estimate of the true population size. There are two reasons for this. First, limited time was spent searching each site. Searching was only conducted long enough to ascertain the general population size and diversity of species. Additional search could reveal larger populations and additional species. Second, moonworts have a large buffer of individuals underground as noted in the introduction. Total populations of moonworts are higher than just the plants that have emerged in any given year (Johnson-Groh et al. 2002).

The railroad point survey technique illustrates how widespread *Botrychium* is in this area. *Botrychium* were found at 16 out of 20 points in this survey within a 10-minute sampling period at each point (Table 2). Likewise the FS Road 109 point survey had 10 out of 15 points with *Botrychium* (Table 3). This point survey is a quick method of determining whether there are moonworts in a given location, but it is not long enough to definitively determine the species diversity of moonworts. It is likely that additional species would be found with longer search at the points. It is also probable that *Botrychium* are found the entire distance of the railroad and logging road. Plants are likely scattered throughout this habitat. This point survey represents a conservative approximation of species diversity, abundance and distribution.

Table 1. Site data for *Botrychium* populations.

Site Number	Site Description	Longitude Latitude	Habitat	Species with listed or special concern status	Species with common status	Voucher No.
Polymet 1	FS Road 108c	N47°36.976', W091°58.553	Shaded logging road	<i>B. simplex</i> (179)		2830
Polymet 2	FS Road 108c	N47°7.025', W091°58.507'	Shaded logging road		<i>B. matricariifolium</i> (2)	
Polymet 3	FS Road 108c	N47°37.042', W091°58.389'	Shaded logging road	<i>B simplex</i> (32) <i>B. michiganense</i> (1)		2831b 2832
Polymet 4	FS Road 108c	No satellites, 10 m east of Polymet 3	Shaded logging road	<i>B simplex</i> (1)		
Polymet 5	FS Road 108c	N47°37.061', W091°58.379'	Shaded logging road	<i>B simplex</i> (3)	<i>B. multifidum</i>	
Polymet 6	FS Road 108c	N47°37.079', W091°58.326'	Shaded logging road	<i>B. simplex</i> (12)		

Polymet 7	Dunka Road – railroad right-of-way	N47°36.378' W091°58.538' to N47°36.467' W091°58.309'	Full exposure, road and rail right-of-way	<i>B. simplex</i> (69) <i>B. pallidum</i> (4) <i>B. michiganense</i> (4)	<i>B. matricariifolium</i> (29) <i>B. multifidum</i> (3)	2833b 2833a 2835
Polymet 8	Dunka Road – railroad right-of-way	N47°36.392', W091°58.485' to N47°36.375', W091°58.533'	Full exposure, road and rail right-of-way	<i>B. simplex</i> (450)	<i>B. multifidum</i> (22)	2829
Polymet 9	North side of Dunka Road in ditch – across from Polymet 8	N47°36.375', W091°58.533'	Ditch, edge of woods	<i>B. simplex</i> (76)		
Polymet 10	Walton pop 2 (A) North side of Dunka Road	N47°36.006', W092°00.182'	Ditch, edge of alder habitat	<i>B. simplex</i> (33)	<i>B. matricariifolium</i> (20)	
Polymet 11	FS Road 109	N47°37.301', W091°57.129'	Shady logging road	<i>B. simplex</i> (5)	<i>B. multifidum</i> (3)	
Polymet 12	FS Road 109	N47°37.328', W091°57.132'	Shady logging road	<i>B. simplex</i> (1) <i>B. michiganense</i> ?(2)	<i>B. multifidum</i> (6)	2859
Polymet 13	FS Road 109	N47°37.564', W091°56.964'	Shady logging road	<i>B. simplex</i> (16)		
Polymet 14	FS Road 109	N47°34.345', W091°57.091'	Shady logging road		<i>B. multifidum</i> (1) <i>B. matricariifolium</i> (5)	
Polymet 15	Across from Walton's pop 1 (B); intersection of FS Road 109 and Dunka, south ditch	N47°36.888', W091°57.112'	Ditch of Dunka Road, full exposure	<i>B. simplex</i> (216)	<i>B. matricariifolium</i> (4) <i>B. multifidum</i> (2)	2862
Polymet	Power	N47°35.988',	Full	<i>B. simplex</i> (181)	<i>B. matricariifolium</i> (238)	2844

16	line right-of-way	W092°00.179'	exposure, high cover mosses	<i>B. michiganense</i> (209) <i>B. michiganense</i> / <i>matricarifolium</i> ? (79) <i>B. pallidum</i> (27)	<i>B. dissectum</i> (1) <i>B. multifidum</i> (4)	2845 2849
Polymet 17	Ditch north of Dunka Road, adjacent to wet area	N47°36.293', W091°58.923'	Shady, edge of Alder thicket	<i>B. simplex</i> (49) <i>B. pallidum</i> (2) <i>B. michiganense</i> / <i>B. matricarifolium</i> (3) <i>B. michiganense</i> (8)	<i>B. multifidum</i> (356) <i>B. dissectum</i> (4)	2852 2853
Polymet 18 (point 1 of railroad pt survey)	Railroad right-of-way	N47°36.745', W091°57.491'	Steep embankment adjacent to railroad	<i>B. simplex</i> (7) <i>B. michiganense</i> (18) <i>B. matricarifolium</i> / <i>michiganense</i> ? (15) <i>B. pallidum</i> (3) <i>B. pallidum</i> ? (22)	<i>B. matricariifolium</i> (38) <i>B. multifidum</i> (1)	2864
Polymet 19 (point A of FS Road 108 survey)	FS Road 108	N47°36.519', W091°58.276'	Shaded logging road	<i>B. simplex</i> (4)	<i>B. multifidum</i> (3)	

Table 2. Site data for railroad point surveys.

Point #	Location	Species with listed or special concern status	Species with common status	Comments
A (Polymet 18)	N47°36.745', W091°57.491'	<i>B. simplex</i> (7) <i>B. michiganense</i> (18) <i>B. matricarifolium</i> / <i>michiganense</i> ? (15) <i>B. pallidum</i> (3) <i>B. pallidum</i> ? (22)	<i>B. matricariifolium</i> (38) <i>B. multifidum</i> (1)	Distance between points = ~ 50 m (100 railroad ties) for first 10 points (A-J); rocky dry shoulder
B	N47°36.764', W091°57.420'	<i>B. simplex</i> (24)		Rocky, dry shoulder
C	N47°36.788', W091°57.367'	<i>B. simplex</i> (1) <i>B. michiganense</i> / <i>matricariifolium</i> ? (1)	<i>B. matricariifolium</i> (7)	Rocky, dry shoulder
D	N47°36.780', W091°57.420'		<i>B. matricariifolium</i> (4) <i>B. spp.</i> ? (2)	In hummock in dense patch of <i>Rubus idaeus</i>
E	N47°36.787', W091°57.291'	<i>B. simplex</i> (1)	<i>B. matricariifolium</i> (3) <i>B. spp.</i> ? (1)	In hummock in dense patch of <i>Rubus idaeus</i>
F	N47°36.789', W091°57.227'	<i>B. simplex</i> (8)		Very rocky, many lichens
G	N47°36.800', W091°57.164'	<i>B. michiganense</i> (1) <i>B. michiganense</i> / <i>matricariifolium</i> (1)		Rocky, grassy shoulder
H	N47°36.791', W091°57.090'		<i>B. multifidum</i> (9) <i>B. dissectum</i> (1)	Edge of clear cut in <i>Rubus idaeus</i>
I	N47°36.806', W091°57.033'	<i>B. simplex</i> (25) <i>B. michiganense</i> (1)	<i>B. matricariifolium</i> (1) <i>B. multifidum</i> (1)	Grassy slope, bench dominated by

			<i>B. spp.?</i> (1)	<i>Equisetum sylvaticum</i>
J	N47°36.784', W091°57.976'	<i>B. simplex</i> (2)	<i>B. matricariifolium</i> (2) <i>B. multifidum</i> (1)	Edge of <i>Populus balsamifera</i> , lots of <i>Cladonia</i> (lichen)
K	N47°36.745', W091°57.491'	<i>B. simplex</i> (8) <i>B. pallidum</i> (1)		Distance between points K-T = ~100 m (200 railroad ties) apart; grassy mesic bench
L	N47°36.745', W091°57.491'		<i>B. virginianum</i> (2) <i>B. multifidum</i> (6)	Mound with <i>Rubus</i> adjacent to drainage culvert
M	N47°36.745', W091°57.491'	No <i>Botrychium</i>		<i>Carex</i> swamp
N	N47°36.745', W091°57.491'	No <i>Botrychium</i>		Dry, rocky edge of <i>Picea</i> / <i>Pinus</i> woods, <i>Pleurozium</i> (moss)
O	N47°36.745', W091°57.491'	No <i>Botrychium</i>		pond
P	N47°36.745', W091°57.491'	<i>B. simplex</i> (9)	<i>B. multifidum</i> (1)	Steep slope in <i>Agrostis</i> and <i>Rubus idaeus</i>
Q	N47°36.745', W091°57.491'	No <i>Botrychium</i>		Very steep embankment of slate, bottom with dense <i>Equisetum</i>
R	N47°36.745', W091°57.491'	<i>B. simplex</i> (10)	<i>B. multifidum</i> (4)	Gravelly slope with <i>Equisetum</i> and <i>Picea</i>
S	N47°36.745', W091°57.491'	<i>B. simplex</i> (4)	<i>B. multifidum</i> (5)	Steep embankment, bottom with <i>Pteridium</i> and <i>Lycopodium</i>
T	N47°36.745', W091°57.491'	<i>B. rugulosum</i> ? (2)	<i>B. multifidum</i> (14)	Grassy, not steep

Table 3. Site data for FS Road 109 point survey.

Point #	Location	Species with listed or special concern status	Species with common status	Comments
AA (Polymet 19)	N47°36.519', W091°58.276'	<i>B. simplex</i> (4)	<i>B. matricariifolium</i> (3) <i>B. multifidum</i> (3)	Distance between points = ~ 100 m (100 paces) for first 11 points (AA-KK); Center of road
BB	N47°36.597', W091°58.271'		<i>B. multifidum</i> (3)	Edge of <i>Pinus banksiana</i> woods
CC	N47°36.652', W091°58.233'	<i>B. simplex</i> (26)		Edge of road in <i>Pteridium</i> below <i>Populus tremuloides</i>
DD	N47°36.519', W091°58.276'	<i>B. simplex</i> (1)		East side of road
EE	N47°36.809', W091°58.206'	No <i>Botrychium</i>		Looks good, but found nothing
FF	N47°36.889',	No <i>Botrychium</i>		Looks good, but found

	W091°58.175'			nothing
GG	N47°36.947', W091°58.089'	No <i>Botrychium</i>		Looks good, but found nothing
HH	N47°37.013', W091°58.076'	<i>B. simplex</i> (8)		In larger opening, log landing adjacent to road
II	N47°37.104', W091°58.095'	No <i>Botrychium</i>		Looks good, but found nothing
JJ	N47°37.154', W091°58.084'	<i>B. simplex</i> (18)	<i>B. multifidum</i> (14)	FS Road 108B (east west road); road less used
KK	N47°37.213', W091°57.994'		<i>B. multifidum</i> (4)	
LL	N47°37.259', W091°57.959'		<i>B. matricariifolium</i> (1) <i>B. multifidum</i> (174)	Distance between points LL-00 = 200 m (200 paces) apart; very dense population, all young
MM	N47°37.273', W091°57.848'	<i>B. multifidum</i> / <i>rugulosum</i> ? (19)		Center of road
NN	N47°37.346', W091°57.763'	No <i>Botrychium</i>		Looks good, but found nothing
OO	N47°37.405', W091°57.728'		<i>B. multifidum</i> (2)	Intersection of unmarked logging road

Species – Status

Moonworts are unusual in that only one leaf of the plant is manifest above ground annually. As with all plants there is a high degree of plasticity or variability among leaves with regard to morphology. Because only one leaf is present with its inherent annual variation in morphology, species determinations may be difficult or impossible on the basis of one leaf. This is comparable to identifying a tree using only one leaf without the advantage of viewing the range of morphological variation in all the leaves. In some cases it may be impossible to identify plants without genetic tools.

For species that are especially problematic, enzyme electrophoresis may be used to confirm identification. Genetic analysis of moonworts through enzyme electrophoresis is effective in distinguishing species. In this process a standard set of common metabolic enzymes extracted from the plants are subjected to an electric field within a starch gel medium. These enzymes migrate differentially to produce characteristic “fingerprint” patterns for each species. Donald Farrar, Iowa State University, confirmed several species found in this survey using isozymes in August 2004. Dr. Farrar has conducted genetic analyses of >500 plants from Minnesota (Farrar, 2001).

All of the species reported here have been confirmed using isozymes, though not all individual plants. The conservation statuses of species found in this survey are outlined in Table 4. Following is a brief discussion of each of the species found in this survey

Table 4. Conservation status of *Botrychium* species.

Moonwort Species	DNR Status	Superior Status	Comments
<i>Botrychium matricariifolium</i>			
<i>Botrychium michiganense</i> (<i>hesperium</i>)	Species tracked by DNR	R =Regional Forester Sensitive	
<i>Botrychium pallidum</i>	Endangered	R =Regional Forester Sensitive	
<i>Botrychium simplex</i> var <i>simplex</i>	Special Concern		

Grapefern Species

<i>Botrychium dissectum</i> var <i>dissectum</i>			
<i>Botrychium multifidum</i>			Difficult to distinguish from <i>B. rugulosum</i> ,
<i>Botrychium rugulosum</i>	Threatened		Difficult to distinguish from <i>B. multifidum</i> ,
<i>Botrychium virginianum</i>			

Moonworts

Botrychium acuminatum

Walton (1998) reported this species for a site he designated population 1. This is not a true species, but is a variation of *B. matricariifolium*.

Botrychium ascendens

Botrychium ascendens was also reported by Walton (1998) for population 1. This was not found in this survey at that location or any other. Though it is possible that *B. ascendens* could be found in these habitats, *B. ascendens* has currently only been found in a few sites in Minnesota (mine-tailings impoundments). It is possible that the *B. ascendens* reported by Walton were *B. pallidum*.

Botrychium matricariifolium

B. matricariifolium is a very common species throughout Northern Minnesota. It is an extremely variable species and is often difficult to distinguish from *B. michiganense*.

Botrychium michiganense

This species is not listed by the Minnesota DNR primarily because of the newness of the discovery of this species in Minnesota. However it is one of the species the DNR tracks. The Forest Service lists it as sensitive. This species was initially described by W. H. Wagner as *B. hesperium*. Upon further study of this taxon in the western US, Wagner concluded that it was distinct from the western *B. hesperium* and proposed the new name, *B. michiganense*, recognizing its first discovery in Michigan. Publication of the new name is forthcoming (Farrar pers.comm.). *Botrychium michiganense* has been documented in Minnesota from approximately 20 sites. It is difficult to distinguish from *B. matricariifolium* and it is not always possible to make the distinction between these two species, especially when plants are small.

Botrychium pallidum

Botrychium pallidum is listed by the state of Minnesota as endangered. *B. pallidum* is largely known in Minnesota from disturbed sites (railroads, mine-tailings impoundments, logging roads). It is one of the earliest species (phenology) and was releasing spores and senescing in many sites.

Botrychium simplex

Botrychium simplex is listed by the state of Minnesota as special concern. It has no listed Forest Service status. *B. simplex* occurs in grassy native habitats in western and northern Minnesota.

Grapeferns

Botrychium dissectum

The state of Minnesota or the Superior National Forest does not list *Botrychium dissectum*. It has a limited distribution and is one of the less common of the grapeferns. It is an easy species to identify so it is not likely overlooked.

Botrychium multifidum

B. multifidum is a very common species throughout Northern Minnesota. It is difficult to distinguish from *B. rugulosum* (see discussion under *B. rugulosum*).

Botrychium rugulosum

B. rugulosum is listed as threatened by the state of Minnesota. It is very difficult to distinguish from the common *B. multifidum* especially early in the season when this survey was conducted. Several plants suspected to be *B. rugulosum* were collected for this survey and sent to D. Farrar for isozyme analysis. These plants were run in August and appear to be *B. multifidum* (Farrar, pers. comm. 2004).

B. rugulosum is poorly understood. Wagner and Wagner (1982) described it as a distinct species based on differences in leaf morphology and phenology. The leaves of *B. rugulosum* are rugose or wrinkled. The leaves are convex with long stalked pinnae and a large space between the basal pair of pinnae and the upper pinnae (Lellingner 1985). Wagner and Wagner (1982) noted a phenology difference between *B. multifidum* and *B. rugulosum*, the former developing earlier. Neither the morphology nor phenology are easy to recognize making *B. rugulosum* very difficult to distinguish from *B. multifidum*.

There are several problems with the identification of *B. rugulosum* / *multifidum*. First, it is unclear whether or not isozyme data will eventually be helpful for identifying grapeferns. Isozyme data has been used extensively for moonworts and is a very reliable method of species identification. Only recently has D. Farrar attempted to use isozymes on grapeferns. Thus far there appears to be much less genetic differentiation among grapeferns. This lack of difference could represent a true genetic conformity among grapeferns or it may mean that this technique is not useful for grapeferns.

Until our knowledge of the genetic and morphological traits of *B. rugulosum* are more complete, we must treat the taxon conservatively. Morphological differences can be used to distinguish species, but these do not work on juvenile leaves (July). Based on a July survey I cannot say that *B. rugulosum* does not exist in the area surveyed. It is likely that most of the grapeferns are *B. multifidum* (confirmed with isozymes), but that a few might be *B.*

rugulosum. A survey later in the growing season would allow a more definitive conclusion based on morphology.

Botrychium virginianum

B. virginianum is the most common grapefern species throughout Minnesota.

CONCLUSIONS

Botrychium are widespread in the area surveyed. Rare moonworts, *B. pallidum* (endangered), *B. simplex* (special concern) and *B. michiganense* (monitored), were found in several sites. *B. rugulosum* (threatened) may occur in this site, but because surveys were conducted in July (too early for mature leaf morphology) it is impossible to conclude that it does or doesn't occur in the Polymet project area.

LITERATURE CITED

- Berch, S. M. and B. Kendrick. 1982. Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern allies. *Mycologia* 74:769-776.
- Farrar, D. R. 2001. Identification of Minnesota *Botrychium* specimens using electrophoresis: Report of enzyme electrophoretic analysis of plant specimens collected in Minnesota from May 1999 through June 2001. Report to MN DNR, St. Paul, MN.
- Farrar, D. R. 1996. Genetic analysis of *Botrychium matricariifolium*. Report to the Ottawa National Forest.
- Farrar, D. R., and C. L. Johnson-Groh. 1990. Subterranean sporophytic gemmae in moonwort ferns, *Botrychium* subgenus *Botrychium*. *American Journal Botany* 77: 1168-1175.
- Goff, F. G. and G. A. Dawson, and J. J. Rochow. 1982. Site examination for threatened and endangered plant species. *Environmental Management* 6:307-316.
- Hoefflerle, A. 1999. Impacts of aerial leaf removal on leaf size of the daisy leaf moonwort (*Botrychium matricariifolium*) and the triangle moonwort (*Botrychium lanceolatum* var. *angustisegmentum*) in the subsequent year. MS thesis, Michigan Technological University, Houghton, Michigan.
- Imaichi, R. 1989. Early leaf development and leaf sheath formation in *Botrychium strictum* and *B. virginianum* (Ophioglossaceae). *Annals of Botany* 63:249-256.
- Johnson-Groh, C. L. 1997. Field surveys for *Botrychium gallicomontanum* and phenology of *Botrychium mormo* in Minnesota. Report to the Minnesota Dept. Nat. Resources, St. Paul, MN.
- Johnson-Groh, C. L. 1998. Population demographics, underground ecology and phenology of *Botrychium mormo*. Pp. 103-108 in N. Berlin, P. Miller, J. Borovansky, U. S. Seal, and O. Byers, eds. *Population and habitat viability assessment (PHVA) for the goblin fern (Botrychium mormo) Final Report*. Conservation Biology Specialist Group, Apple Valley, MN.
- Johnson-Groh, C. L. 2004 (in progress). Biology and Ecology of *Botrychium*, subgenus *Botrychium*. Inventory and Monitoring Protocol for Moonworts (*Botrychium*). US Forest Service Report.
- Johnson-Groh, C. L., and D. R. Farrar. 1993. Population dynamics of prairie moonworts (*Botrychium* subgenus *Botrychium*) in Iowa and Minnesota (abstract). *American Journal of Botany* 80: 109 (Abstract).

- Johnson-Groh, C. L., and D. R. Farrar. 1996a. The effects of fire on prairie moonworts (*Botrychium* subgenus *Botrychium*). *American Journal of Botany* 83:134 (abstract).
- Johnson-Groh, C. L., and D. R. Farrar. 1996b. Effects of leaf loss on moonwort ferns (*Botrychium* subgenus *Botrychium*). *American Journal of Botany* 83: 127 (abstract).
- Johnson-Groh, C. L. and Jennifer M. Lee. 2002. Phenology and demography of two species of *Botrychium* (Ophioglossaceae). *American Journal of Botany* 89: in press.
- Johnson-Groh, C. L., C. Riedel, L. Schoessler and K. Skogen. 2002. Belowground distribution and abundance of *Botrychium* gametophytes and juvenile sporophytes. *American Fern Journal* 92:80-92.
- Kelly, D. 1994. Demography and conservation of *Botrychium australe*, a peculiar, sparse mycorrhizal fern. *New Zealand Journal of Botany* 32: 393-400.
- Lellinger, D.B. 1985. A field manual of the ferns and fern-allies of the United States and Canada. Smithsonian Institution Press, Washington, DC.
- Lesica, P., and K. Ahlenslager. 1996. Demography and life history of three sympatric species of *Botrychium* subg. *Botrychium* in Waterton Lakes National Park, Alberta. *Canadian Journal of Botany* 74: 538-543.
- Montgomery, J.D. 1990. Survivorship and predation changes in five populations of *Botrychium dissectum* in Eastern Pennsylvania. *American Fern Journal* 80: 173-182.
- Schmid, E. and F. Oberwinkler. 1994. Light and electron microscopy of the host-fungus interaction in the achlorophyllous gametophyte of *Botrychium lunaria*. *Canadian Journal Botany* 72:182-188.
- Wagner, W. H. and F. S. Wagner. 1982. *Botrychium rugulosum* (Ophioglossaceae), a newly recognized species of evergreen Grapefern in the Great Lakes region of North America. *Contr. Michigan Herb.* 15:315-324.
- Wagner, W. H. and F.S. Wagner. 1983. Genus communities as a systematic tool in the study of new world *Botrychium* (Ophioglossaceae). *Taxon* 32:51-63.
- Walton, G. B. 1998. The 1998 Reservoir Project Rare Plant Survey in the Superior National Forest Laurentian District. Report submitted to the Superior National Forest, Aurora, MN.
- Whittier, D. P. 1973. The effect of light and other factors on spore germination in *Botrychium dissectum*. *Canadian Journal Botany* 51: 1791-1794.
- Whittier, D. P. 1984. The organic nutrition of *Botrychium* gametophytes. *American Fern Journal* 74:77-85.

Appendix A:
Site Data

Polymet 1

Latitude and longitude: N47°36.976', W91°58.553

General Location: FS Road 108B

Habitat: middle of old logging road, partial to full sun

Associated species: *Fragaria virginiana*, *Danthonia spicata*, *Diphasiastrum tristachyum*, *Vaccinium* sp., *Diervilla lonicera*, *Abies balsamea* (seedling), *Cornus*, *Rubus pubescens*, *Maianthemum canadense*, *Salix* sp., *Trientalis borealis*, *Aster macrophyllus*, *Lotus corniculatus*, *Betula papyrifera*, *Hieracium aurantiacum*, *Hieracium kalmii*, *Anaphalis margaritacea*

Population size: 179 *B. simplex*

Phenology: full height, close to dehiscing.

Date: 2 July 2004

Notes: there are likely many more plants in vicinity of populations 1- 6

Polymet 2

Latitude and longitude: N47° 37.025', W91° 58.507'

General Location: FS Road 108B

Habitat: side of old logging road road, full sun

Associated species: *Fragaria virginiana*, *Viola* sp., *Polytrichum commune*, *Anaphalis margaritacea*, *Salix* sp., *Sphagnum* sp., *Cornus canadensis*, *Luzula* sp., *Aster macrophyllus*, *Solidago* sp., *Hieracium aurantiacum*, *Epilobium angustifolium*, *Athyrium filix-femina*, *Calamagrostis canadensis*

Population size: 2 large *B. matricariifolium*

Phenology: full height, not yet dehisced

Date of Observation: 2 July 2004

Notes: there are likely many more plants in vicinity of populations 1- 6

Polymet 3

Latitude and longitude: N47° 37.042', W091° 58.389'

General Location: FS Road 108B

Habitat: side of road, full sun

Associated species: *Fragaria virginiana*, *Achillea millefolium*, *Toxicodendron radicans*, *Rubus idaeus*, *Hieracium aurantiacum*, *Hieracium kalmii*, *Cerastium* sp., *Viola* sp., *Salix* sp., *Cornus canadensis*, *Lathyrus ochroleucus*

Population size: 32 *B. simplex*, 1 *B. michiganense*

Phenology: full height, close to dehiscing

Date: 2 July 2004

Notes: there are likely many more plants in vicinity of populations 1- 6

Polymet 4

Latitude and longitude: no satellite reception, ~ 10 m east of Polymet 3

General Location: FS Road 108B

Habitat: middle of road, full sun

Associated species: *Fragaria virginiana*, *Salix* sp., *Diervilla lonicera*, *Trifolium pratense*, *Betula papyrifera*, *Hieracium aurantiacum*, *Anaphalis margaritacea*, *Cerastium* sp., *Viola* sp., *Poa pratensis*

Population size: 1 *B. simplex*

Phenology: full height, close to dehiscing

Date of Observation: 2 July 2004

Notes: there are likely many more plants in vicinity of populations 1- 6

Polymet 5

Latitude and longitude: N47° 37.061', W091° 58.379'

General Location: FS Road 108B

Habitat: *B. multifidum* south edge of road, full sun; *B. simplex*, center of road, full sun

Associated species: *Cornus canadensis*, *Viola* sp., *Fragaria virginiana*, *Diervilla lonicera*, *Anaphalis margaritacea*, *Betula papyrifera*, *Poa pratensis*, *Trifolium pratense*, *Lathyrus ochroleucus*

Population size: 1 *B. multifidum*, 3 *B. simplex*

Phenology: *B. multifidum* young leaf, last year's leaf still present

Date: 2 July 2004

Notes: there are likely many more plants in vicinity of populations 1- 6

Polymet 6

Latitude and longitude: N47° 37.079', W091° 58.326'

General Location: population at the end of drivable forest service road, intersection of FS Road 108 and 108c

Habitat: old logging road, population spread over three m along south side of road

Associated species: *Cornus stolonifera*, *Fragaria virginiana*, *Hieracium aurantiacum*, *Anaphalis margaritacea*, *Erigeron* sp, *Vaccinium* sp., *Trifolium*, *Equisetum sylvaticum*, *Diervilla lonicera*, *Rubus idaeus*, *Achillea millifolium*, *Aster macrophyllus*,

Population size: 11 *B. simplex*, one *B. simplex* across road ~7 m away

Phenology: full height, close to dehiscing

Date: 2 July 2004

Notes: there are likely many more plants in vicinity of populations 1- 6

Polymet 7

Latitude and longitude: N47° 36.378', W091° 58.538' to N47° 36.467', W091° 58.309'

General Location: Between Dunka Road and railroad west of forest road 108

Habitat: gravelly ridge between railroad and the south side of Dunka Road, sunny

Associated species: *Bromus inermis*, *Fragaria virginiana*, *Rubus idaeus*, *Hieracium aurantiacum*, *Equisetum sylvaticum*, *Tragapogon dubius*, *Solidago gigantea*, *Potentilla norvegica*, *Rubus pubescens*, *Chrysanthemum leucanthemum*

Population size: 69 *B. simplex*, 29 *B. matricarifolium*, 3 *B. multifidum*, 4 *B. pallidum*, 4 *B. michiganese*

Phenology: full height, most dehisced, few senescing

Date: 3 July 2004

Notes: plants are likely distributed in similar habitats throughout length of Dunka Road and railroad right-of-way

Polymet 8

Latitude and longitude: N47° 36.392', W091° 58.485' extending 66 m west to N47° 36.375', W091° 58.533'

General Location: Between Dunka Road and railroad west of forest road 108

Habitat: gravelly ridge between railroad and the south side of the road

Associated species: *Castilleja coccinea*, *Ophioglossum pusillum*, *Chrysanthemum leucanthemum*, *Poa pratensis*, *Bromus inermis*, *Fragaria virginiana*, *Rubus pubescens*, *Trifolium pratensis*, *Liparis loeselii*, *Platanthera hyperborea*, *Equisetum hymale*, *Hieracium aurantiacum*

Population size: 450 *B. simplex*, 22 *B. multifidum*

Phenology: *B. simplex* = full height, most dehisced, few senescing; *B. multifidum/rugulosum* = plants very young, last year's leaf still present

Date: 3 July 2004

Notes: large population of *Ophioglossum* (>350 plants); plants are likely distributed in similar habitats throughout length of Dunka Road and railroad right-of-way

Polymet 9

Latitude and longitude: N47° 36.375', W091° 58.533'

General Location: North of Dunka Road across from Polymet 7 and 8, west of Forest Service road 108

Habitat: gravelly ridge between the north side of Dunka Road and the forest, open ditch with thick *Bromus*, plants adjacent to *Alnus*.

Associated species: *Poa pratensis*, *Bromus inermis*, *Hieracium aurantiacum*, *Anaphalis margaritacea*, *Agrostis stolonifera*, *Taraxacum officinale*, *Equisetum pretense*, *Fragaria virginiana*, *Alnus* sp.

Population size: 76 *B. simplex*

Phenology: plants are full height, most dehiscing

Date of Observation: 3 July 2004

Notes: habitat appears patchy, but likely extends in patches along Dunka Road

Polymet 10

Latitude and longitude: N47° 36.006', W092° 00.182'

General Location: Walton's population A (pop 2 on maps); north side of Dunka Road in ditch

Habitat: ditch open grass gravelly, growing on edge and inside of *Alnus* thicket and in *Populus* forest; thick leaf litter

Associated species: *Aster macrophyllus*, *Fragaria virginiana*, *Corylus cornuta*, *Rubus pubescens*, *Rubus idaeus*, *Achillea millefolium*, *Diervilla lonicera*, *Aralia nudicaulis*, *Carex* sp., *Populus tremuloides*, *Alnus* sp.

Population size: 33 *B. simplex*, 20 *B. matricariifolium*

Phenology: *B. matricariifolium* sporangia yellow not releasing

Date: 4 July 2004

Notes: relocated population discovered by Walton; searched woods north of site for 1 hour; plants only found on edge of *Populus* forest south into ditch

Polymet 11

Latitude and longitude: N47° 37.301', W091° 57.129'

General Location: Logging road 109

Habitat: old logging road, open to partially shaded; forest = *Acer rubrum*, *Betula papyrifera*, *Pinus banksiana*, *Alnus* sp.

Associated species: *Fragaria virginiana*, *Aster macrophyllus*, *Plantago major*, *Galium boreale*, *Diervilla lonicera*, *Taraxacum officinale*, *Trientalis borealis*, *Pteridium aquilinum*, *Lathyrus ochroleucus*, *Epilobium angustifolium*, *Chrysanthemum leucanthemum*, *Salix* sp., *Achillea millefolium*,

Population size: 5 *B. simplex*, 3 *B. multifidum*

Phenology: plants are full height, beginning to dehisce

Date: 5 July 2004

Notes: there are likely many other populations along FS Road 109 as the habitat appears to be good

Polymet 12

Latitude and longitude: N47° 37.328', W091° 57.132'

General Location: Logging road 109

Habitat: old logging road, open to partially shaded; forest = *Acer saccharum*, *Betula papyrifera*, *Pinus banksiana*, *Populus balsamifera*

Associated species: *Fragaria virginiana*, *Hieracium aurantiacum*, *Castilleja coccinea*, *Taraxacum officinale*, *Rubus idaeus*, *Betula papyrifera*, *Anaphalis margaritacea*, *Solidago* sp., *Salix* sp.,

Population size: 1 *B. simplex*, 6 *B. multifidum*, 2 *B. michigense*?

Phenology: plants are full height, beginning to dehisce

Date: 5 July 2004

Notes: there are likely many other populations along FS Road 109 as the habitat appears to be good

Polymet 13

Latitude and longitude: N47° 37.564', W091° 56.964'

General Location: Logging road 109

Habitat: old logging road, open to partially shaded; forest = *Acer rubrum*, *Pinus banksiana*, *Salix* sp., *Populus tremuloides*, *Alnus* sp.

Associated species: *Chrysanthemum leucanthemum*, *Fragaria virginiana*, *Hieracium aurantiacum*, *Anaphalis margaritacea*, *Achillea millifolium*, *Rubus pubescens*, *Lactuca* sp., *Aster macrophyllus*

Population size: 16 *B. simplex*

Date: 5 July 2004

Notes: there are likely many other populations along FS Road 109 as the habitat appears to be good

Polymet 14

Latitude and longitude: N47° 34.345', W091° 57.091'

General Location: Logging road 109

Habitat: old logging road, open to partially shaded; forest = *Betula papyrifera*, *Pinus banksiana*,

Associated species: *Hieracium aurantiacum*, *Aster macrophyllus*, *Ranunculus* sp., *Pteridium aquilinum*, *Fragaria virginiana*, *Salix* sp., *Achillea millifolium*, *Potentilla* sp., *Rubus pubescens*, *Chrysanthemum leucanthemum*, *Solidago* sp.

Population size: 1 *B. multifidum*, 5 *B. matricarifolium*

Date: 5 July 2004

Notes: plot extends 26 m north, 1 *B. matricarifolium* 9 m north, 1 *B. matricarifolium* 26 m north;

Polymet 15

Latitude and longitude: N47° 36.888', W091° 57.112'

General Location: Walton's population 1 (map B) near FS Road 109

Population A located south of junction of FS Road 109 and Dunka Road

Population B located west of junction of FS Road 109 and Dunka Road

Population C located 50 m west of junction of FS Road 109 and Dunka Road

Population D located east of junction of FS Road 109 and Dunka Road

Habitat: roadside ditch, open, growing on gravelly shoulder of road

Associated species: *Fragaria virginiana*, *Equisetum arvense*, *Melilotus officinalis*, *Tragopogon dubius*, *Anaphalis margaritacea*, *Pteridium aquilinum*, *Hieracium aurantiacum*,

Poa pratensis, *Lathyrus ochroleucus*, *Salix* sp., *Solidago* sp. *Carex* sp., *Tanacetum vulgare*,
Rubus idaeus,

Population size: A: 20 *B. simplex*, 2 *B. matricariifolium*, 2 *B. multifidum*

B: 11 *B. simplex*, 1 *B. matricariifolium*

C: 177 *B. simplex*, 1 *B. matricariifolium*

D: 8 *B. simplex*

Date: 5 July 2004

Notes: same area as Walton pop 1 (map B); area documented by Walton was destroyed by logging activity; populations discovered in this survey were in areas nearby that were undisturbed

Polymet 16

Latitude and longitude: N47° 35.988', W092° 00.179'

General Location: South of Walton population 2 (map A) in power line right-of-way

Habitat: open, high moss coverage, scattered young trees, lots of large rocks

Associated species: *Fragaria virginiana*, *Cladonia* (lichen), *Pleurozium* (moss), *Chrysanthemum leucanthemum*, *Lactuca* sp., *Lathyrus ochroleucus*, *Trifolium pratensis*, *Rubus idaeus*, *Anaphalis margaritacea*, *Cirsium* sp., *Dantonina spicata*, *Bromus inermis*, *Vicia americana*, *Athyrium felix-femina*, *Epilobium angustifolium*, *Salix* sp., *Polytrichum commune* (moss), *Poa pratensis*, *Maianthemum canadense*, *Acer rubrum*, *Abies balsamea*, *Corylus cornuta*, *Larix laricina*, *Pteridium aquilinum*

Population size: 181 *B. simplex*, 238 *B. matricariifolium*, 209 *B. michiganense*, 79 *B. matricariifolium/michiganense*, 4 *B. multifidum*, 27 *B. pallidum*, 1 *B. dissectum*

Date: 6 July 2004

Notes: this area is extremely rich with *Botrychium* of all species; area searched (50m x 10 m) had >750 individuals discovered in 3 hours search

Polymet 17

Latitude and longitude: N47° 36.293', W091° 58.923'

General Location: north side of road in ditch between Dunka Road and swamp, west of FS Road 109

Habitat: road ditch, steep, most plants on lower portion adjacent to *Salix*

Associated species: *Rubus idaeus*, *Fragaria virginiana*, *Equisetum sylvaticum*, *Poa pratensis*, *Chrysanthemum leucanthemum*, *Salix* sp., *Carex* sp., *Taraxacum officinale*, *Cirsium* sp., *Solidago* sp.,

Population size: 356 *B. multifidum*, 49 *B. simplex*, 4 *B. dissectum*, 2 *B. pallidum*, 3 *B. matricariifolium/michiganense*, 8 *B. michiganense*

Date: 6 July 2004

Notes: *B. pallidum* and *B. matricariifolium* are found on ditch shoulder, *B. simplex*, *B. dissectum* and *B. multifidum* are at bottom on ditch near *Salix*; area searched = ~ 32m x 5m

Polymet 18 (point 1 of railroad point survey)

Latitude and longitude: N47° 36.745', W091° 57.491'

General Location: railroad right-of-way, on south side of tracks where railroad bends southeast away from Dunka Road

Habitat: dry slopes adjacent to railroad, open to partially shaded edge of *Populus balsamifera* stand just beyond clear cut

Associated species: *Rubus idaeus*, *Asarum canadense*, *Fragaria virginiana*, *Cirsium* sp., *Dryopteris cristata*, *Pyrola* sp., *Abies balsamea*,

Population size: 7 *B. simplex*, 38 *B. matricarifolium*, 18 *B. michiganense*, 15 *B. matricarifolium/michiganense*, 3 *B. pallidum*, 1 *B. multifidum*, 22 unknown (narrow linear pinnae, probably *B. pallidum*),

Date: 7 July, 2004

Notes: searched 10 minutes – first point on railroad point-survey

Polymet 19 (point A of Forest Service Road 108 point survey)

Latitude and longitude: N47°36.519', W091°58.276'

General Location: center of forest service road 108, south end near Dunka Road

Habitat: open to shaded forest road, grassy, plants on center of road into edge of woods

Associated species: *Populus tremuloides*, *Pinus banksiana*, *Fragaria virginiana*, *Cornus canadensis*, *Rubus pubescens*, *Aster macrophyllus*, *Lotus corniculatus*, *Hieracium aurantiacum*, *Anaphalis margaritacea*

Population size: 4 *B. simplex*, 3 *B. multifidum*

Date: 7 July, 2004

Notes: searched for 10 minutes, first point on forest road 108 point-survey

Appendix B:

Photos



Photo 1. Polymet 1, FS Road 108c.



Photo 2. Polymet 5, FS Road 108c.



Photo 3. Polymet 7, Dunka Road right-of-way (looking west).



Photo 4. Polymet 8, Dunka Road right-of-way (looking east).



Photo 5. Polymet 9, north side of Dunka Road (looking west).



Photo 6. Polymet 10, Walton pop 2 (looking east).



Photo 7. Polymet 13, FS Road 109.



Photo 8. Polymet 15, Walton pop 1, Dunka Road and FS Road 109 (looking west).



Photo 9. Polymet 16, power line right-of-way.



Photo 10. Polymet 16, power line right-of-way.



Photo 11. Railroad right-of-way.



Photo 12. Polymet 18, railroad right-of-way (looking east).



Photo 13. *B. dissectum* (Polymet 17).



Photo 14. *B. matricariifolium* (Polymet 7).



Photo15. *B. michiganense* (Polymet 3).



Photo 16. *B. multifidum / rugulosum* (Polymet 7).



Photo 17. *B. pallidum* (Polymet 16).



Photo 18. *B. pallidum* (Polymet 16).



Photo 19. *B. simplex* (Polymet 1).