Examination of soils for underground propagules (gametophytes, juvenile sporophytes, and gemmae) of *Botrychium pumicola* (pumice moonwort) in the Deschutes and Winema National Forests, Oregon.

Susan Roe-Andersen
Botrychulator-at-Large
PO Box 36, Mesa Verde, CO 81330

August 5, 2015

Purpose and Need:

Botrychium pumicola (Ophioglossaceae) the pumice moonwort is currently listed as Threatened by the State of Oregon (ODA, 2013). It is also on the U.S. Forest Service Region 6 Sensitive Species List, the Bureau of Land Management Special Status Plant List and is a California Special Status Plant (CDFG, 2015; ISSSSP, 2015). The decline in several *B. pumicola* populations in montane habitats in the Deschutes National Forest from over 1000 individuals 10-20 years ago, to populations of 3 to zero is of great conservation and land management concern. Montane populations of *B. pumicola*, discovered in the early 1990's during surveys for rare plants in proposed timber sale areas, were sizable ($n \sim 1000+$) in frost-pocket openings in forests of lodgepole pine (*Pinus contorta*) on pumice soils. Recent surveys of these areas noted the absence of *B. pumicola* and the encroachment of *P. contorta* into previously open pumice areas (Figs. 1-2). Aerial photographs from 1943, 1987, and 2011 confirm this recent *P. contorta* invasion (Charmane Powers, personal communication). Factors driving the decline in *B. pumicola* are unknown but could be the result of climate change which has reduced snow pack and water availability and has changed frost heaving action which may have previously prevented establishment of *P. contorta*. This research examined soils in areas of current and former *B. pumicola* populations to determine if an underground reservoir of propagules (gametophytes, juvenile sporophytes, and gemmae) exists.

Figure 1. Frost-pocket opening at Katati basin, Deschutes National Forest. Photo courtesy of Charmane Powers.

Figure 2. *Pinus contorta* (lodgepole pine) encroachment into former *Botrychium pumicola* frost-pocket habitat at Katati basin, Deschutes National Forest. Photo courtesy of Charmane Powers.

Management implications:

If *B. pumicola* plants are dormant with a reservoir of underground propagules waiting for conditions to improve, concerns about possible extinction of montane populations may be reduced. Management

actions such as removing *P. contorta* seedlings and/or mulching to increase moisture availability could then be tested to see if *B. pumicola* emergence increases at these sites.

Methods:

Soils were collected at sites where *B. pumicola* are abundant in the Deschutes National Forest at Mahogany Butte and Water Trough 22 (BoPu Present Sites) and from areas in the Deschutes and Winema National Forests at Katati (BoPu plots 3003 and 3009), Rowdy (BoPu plots 3010 and 3011), Woof (BoPu plots 3013 and 3015), and Chemult (study site 1335, BoPu2 plot 4) where *B. pumicola* has presumably been extirpated or where population levels are extremely reduced (BoPu Absent Sites). Sampling occurred from June 23-25, 2015, after *B. pumicola* plants had emerged, or would have been expected to be emerged. At BoPu Present Sites, ten 200 cm^3 samples were taken from each site within 1-cm of emerged plants using a 5-cm diameter bulb planter. At each sampling hole the top10-cm of soil was pushed away, five cores were taken from the 10-20 cm depth and five cores from the 20-30 cm depth, kept on ice or refrigerated and processed in the laboratory at Southern Oregon University from June 29-July 15, 2015. At BoPu Absent sites six 200 cm^3 samples (three at 10-20 cm depth and three from 20-30 cm depth) were taken from each site next to old tags that formerly marked *B. pumicola* individuals using the same methods as above.

Soil from BoPu Present sites was processed first to vet the procedure and to determine the depth of propagule occurrence (Figs. 3-6). Using a modified method of Mason and Farrar (1989), soils (500 mL) were mixed with 500 mL water; soaked, stirred and decanted through Tyler Standard Screen soil sieves with 2 mm openings (#10 sieve) to remove rocks and roots and capture large propagules, and 0.5 mm openings (#35 sieve) to capture smaller *B. pumicola* propagules. Sievings from the #10 sieve were placed into a pan and checked for juvenile sporophytes. Sievings from the #35 screen were centrifuged in 50-ml centrifuge tubes for 5 min at 2000 RPM in a Beckman-Coulter GS-6R centrifuge. The pellet was then re-suspended in 30% sucrose, stirred, and centrifuged for 4 min at 2000 RPM. Organic material from the top of each tube was transferred to microscope slides and examined using a compound microscope to search for gametophytes, juvenile sporophytes and gemmae (vegetative propagules that detach from the stem of a parent plant to form a new sporophyte). Propagules were photographed and identity of gemmae were confirmed by Dr. Donald Farrar, Iowa State University, Ames, Iowa and Dr. Cindy Johnson-Groh, Gustavus Adolphus University, St. Peter, Minnesota.

Figure 3. Sieving of soils through #10 and #35 sieves. Bottom sieve (#18) used to improve water flow.

Figure 4. Sievings from #10 sieve await checking for juvenile sporophytes.

Figure 5. Sievings from #35 sieve ready for processing.

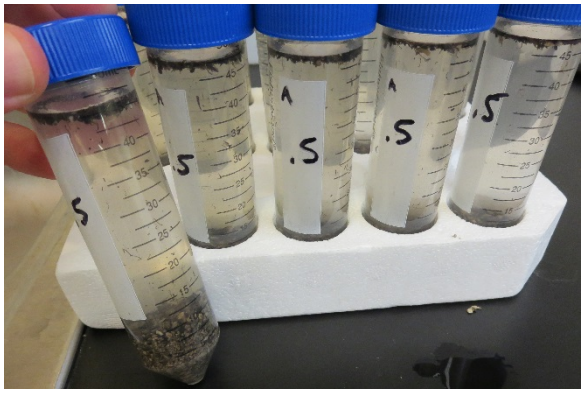

Figure 6. Sievings from #35 sieve ready for centrifuge.

Results:

<u>BoPu Present Sites</u>

At the 20-30 cm depth, 22 gemmae were recovered from Water Trough 22 soils and 21 gemmae were recovered from Mahogany Butte soils (Figs. 7-8). No other propagules (gametophytes or juvenile sporophytes) were recovered. Arbuscular mycorrhizal (AM) spores were found in Water Trough 22 and Mahogany Butte soils at the 10-20 and 20-30 cm levels.

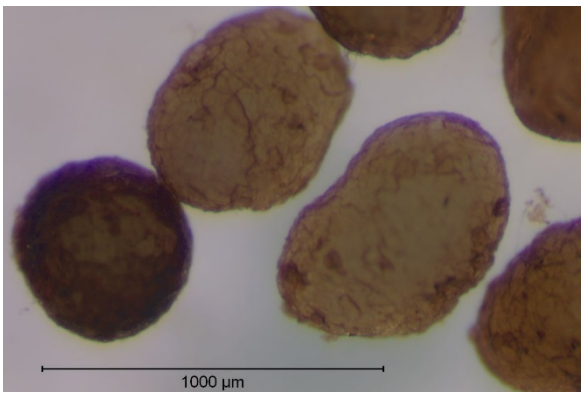

Figure 7. Gemmae recovered from Water Trough 22 site soils. 1000 µm=1mm.

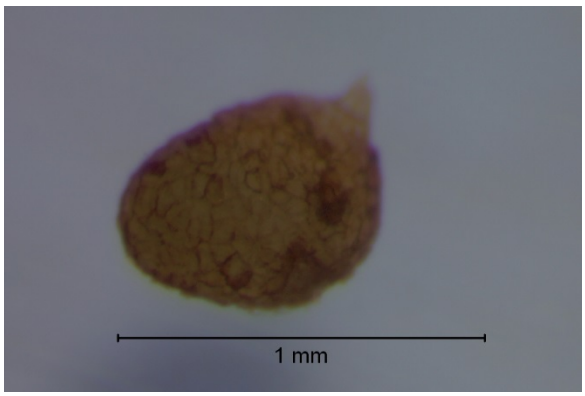

Figure 8. Gemmae recovered from Mahogany Butte site soils showing detachment point (upper right) from *B. pumicola* stem.

BoPu Absent Sites

One gemmae was recovered from 20-30 cm depth soils from the Rowdy site (Fig. 9). No other propagules were recovered in these soils. Arbuscular mycorrhizal (AM) spores were found in 20-30 cm depth soils from Katati, Woof, Rowdy and Chemult.

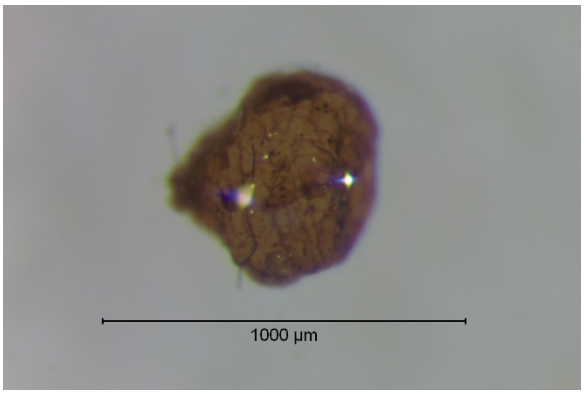

Figure 9. Gemmae recovered from Rowdy site soils. 1000 µm=1mm.

Discussion and Recommendations:

The lack of propagules recovered from three of the four BoPu Absent Sites indicates that either those sites no longer have an underground reservoir of propagules or that this study did not process enough soils to recover them. The recovery of a single gemmae from the Rowdy site indicates there are propagules remaining in the soil of that site despite the decline in above-ground *B. pumicola* individuals. The presence of arbuscular mycorrhizal (AM) spores indicates the availability at all sites of fungal inoculum for *B. pumicola*, an obligately mycorrhizal fern.

Since propagules still exist at one site and may be present at the other sites I recommend management action be taken to reduce *Pinus contorta* in former frost pocket habitat at these sites. To test the hypothesis that *P. contorta* encroachment may be negatively impacting *B. pumicola* habitat, the former footprint of the *B. pumicola* populations at Katati, Rowdy and Woof sites should be thoroughly surveyed. If the former footprint of the populations at these sites is large enough (approximately 20x20 meters) the sites should then be split into treatment and control areas with all *P. contorta* removed from treatment areas. The number of years for a juvenile sporophyte to reach the soil surface is unknown, however some studies estimate 5-10 years for emergence (Farrar and Johnson-Groh, 1990; Muller, 1993). Treatment and control areas at each site should therefore be re-surveyed yearly for ten years to determine if the treatment had the desired effect of increasing *B. pumicola* populations. If there are dormant *B. pumicola* individuals at these sites, the removal of *P. contorta* may also improve conditions so that emergence rates increase.

Acknowledgements:

I wish to thank Dr. Darlene Southworth, Southern Oregon University for use of her lab; Charmane Powers, Deschutes National Forest, for assistance with locating and sampling sites on the Bend/Fort Rock District; Lily Pifferini-Carter, Student Intern at the Deschutes National Forest, for assistance with soil collection; Faith Wilkins and Missy Voit-Anderson, Fremont-Winema National Forest, for their assistance with locating sites on the Chemult District.

Literature Cited:

California Department of Fish and Game (CDFG). 2015. Natural Diversity Database. Special Vascular Plants, Bryophytes, and Lichens List. http://www.dfg.ca.gov/biogeodata/cnddb/pdfs/SPPlants.pdf. Accessed 5 August 2015.

Farrar, D. R., and Johnson-Groh, C. L. 1990. Subterranean sporophytic gemmae in moonwort ferns, *Botrychium* subgenus *Botrychium*. American Journal of Botany 77(9):1168-1175.

Interagency Special Status Sensitive Species Program (ISSSSP). 2015. U.S. Department of Agriculture, U.S. Forest Service and U.S. Department of the Interior, Bureau of Land Management. Available at: http://www.fs.fed.us/r6/sfpnw/issssp/species-index/flora-vascular-plants.shtml. Accessed 5 August 2015.

Mason, N. A. and Farrar, D. R. 1989. Recovery of *Botrychium* gametophytes, gemmae, and immature sporophytes by centrifugation. American Fern Journal 79(4):143-145.

Muller, S. 1993. Population dynamics in *Botrychium matricariifolium* in Bitcherland (Northern Vosges Mountains, France). Belg. J. Bot. 126:13-19.

Oregon Department of Agriculture (ODA). 2015. List of threatened, endangered and candidate plant species. Oregon Department of Agriculture, Plant Conservation Division. Available at: http://www.oregon.gov/ODA/programs/PlantConservation/Pages/ListedPlants.aspx. Accessed: 5 August 2015.