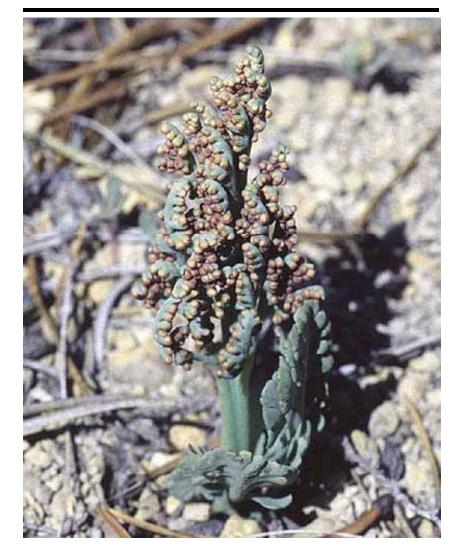
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NATIVE PLANT CONSERVATION PROGRAM

Responses of *Botrychium pumicola* to habitat manipulation in forested sites in South-central Oregon



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Introduction

Botrychium pumicola (Oregon moonwort) is an unusual vascular plant species in that it is rare, formally listed as threatened under Oregon law (Oregon Administrative Rules 603-73070), and inhabits areas that support marketable timber. The vast majority of globally rare plant species in Oregon occur in non-forested habitat, or if associated with forested ecosystems, the sites are generally unsuitable for timber harvest. Botrychium pumicola is similar to many rare species, however, in that its localized distribution and rarity are at least partially governed by unusual edaphic conditions. Another common name of the species (pumice grapefern) emphasizes its close relationship with loose volcanic soils, particularly (at the lower elevations) within frost pockets or comparable areas that retain soil moisture into late spring (Figure 1). The obligately mycorrhizal *Botrychium pumicola*, like other species in the genus, has an unusual relationship with its fungal associates, importing (rather than exporting) carbohydrates along with other nutrients from its fungal partners (Schmid and Oberwinkler 1993). Initially discovered at Crater Lake in 1897 (Coville 1901), and until recently considered to be the rarest moonwort (Wagner and Wagner 1983), this unique pteridophyte has been located in increasing numbers in the last several years within southcentral Oregon, bringing it into potential conflict with timber management programs mainly on the Deschutes and Fremont-Winema National Forests, and the Prineville District of the BLM. The study summarized in this report and evaluation of Botrychium pumicola in relation to logging and other management practices in xeric coniferous forests was begun in 1998 and completed in 2003.

Project Objectives

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To summarize the effects of a series of disturbance regimes developed to

- simulate logging and other forest practices on populations of *Botrychium pumicola*
- To make management recommendations regarding these disturbances and their effects on *Botrychium pumicola*
- To better understand the effects of disturbances potentially associated with logging and related forest management practices on the short-term survival and long-term demography of *Botrychium pumicola*



Figure 1. Emergent frond of *Botrychium pumicola*. Each frond is made up of a sterile and fertile portion - the fertile portion matures and begins to release copious numbers of spores in early summer. The coarse pumice substrate preferred by this species can be seen in the foreground.

Study Sites

Subsequent to field visits and consultation with agency personnel, seven sites were selected for inclusion in the project, based on proposed management activities, population size and viability, and ease of access (Figures 2 and 3). Although all sites are on pumice soils and all are vegetated with Pinus contorta and Purshia tridentata, within this general classification sites vary considerably in edaphic conditions and associated vegetation. Three sites on the Deschutes National Forest Bend-Fort Rock District (DNF 0100249, 0100080, 0100193 Figure 2) are in "frost pockets", with little associated vegetation. Populations at the 100080 and 100249 sites occur in lodgepole basins on pumiceous loamy sands and sands, with underlying materials of lavas or gravels. Due to limited air drainage, frost damage to invading seedlings is common in these sites, effectively eliminating recruitment of conifers, and keeping sites open. Site 10093 is also a barren flat, kept free of vegetation by frost, with soil materials that include coarse pumice cinders, volcanic ash, and aeloian rock fragments (Larsen 1976). Soils at the powerline site (DNF Crescent District - Figure 2) consist of loamy sands and would probably be vegetated with lodgepole pine (*Pinus contorta*) and bitterbrush (Purshia tridentata) if not manually kept clear of vegetation by Bonneville Power Administration's powerline management activities.

Sites 1462 and 1335 (Fremont-Winema NF - Figure 3) differ from the previously listed sites in that they are both shaded by a canopy of *Pinus contorta*, and they contain more organic matter than the DNF sites. Loamy to sandy loam "Wickiup-like" soils in site1462 support a dense understory of *Festuca idahoensis*, and a substrate congested with roots and duff, while site1335 has a groundcover consisting of fallen logs and branches interspersed with *P. tridentata*, and loamy sand to coarse loamy sand "Steiger-like" soils (Carlson 1979). Site 1360 (Fremont-Winema NF-Figure 3) occurs in a previously logged area, with *Botrychium* plants interspersed among young lodgepole pine. Substantial amounts of decomposing woody material produced during a thinning operation which occurred previous to the initiation of our study are dispersed throughout the site (Figure 4). Soils here are also "Wickiup-like" loam to sandy loams (Carlson 1979), and support a more extensive herbaceous layer than other sites, consisting of *Fragaria virginiana*, *Horkelia fusca*, and *Achillea millefolium*.

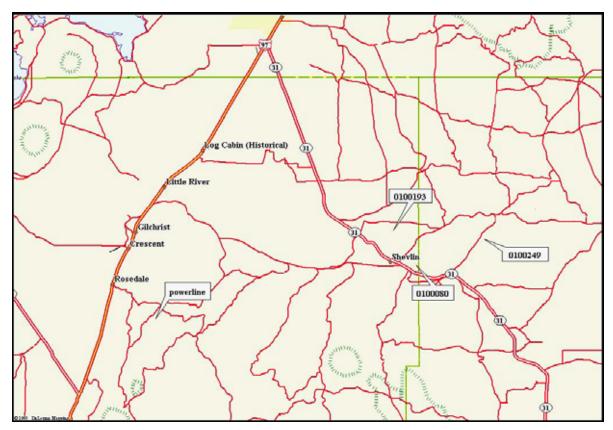


Figure 2. Location of study sites on the Deschutes National Forest. Figure 3. Location of study sites on the Fremont-Winema National Forest.

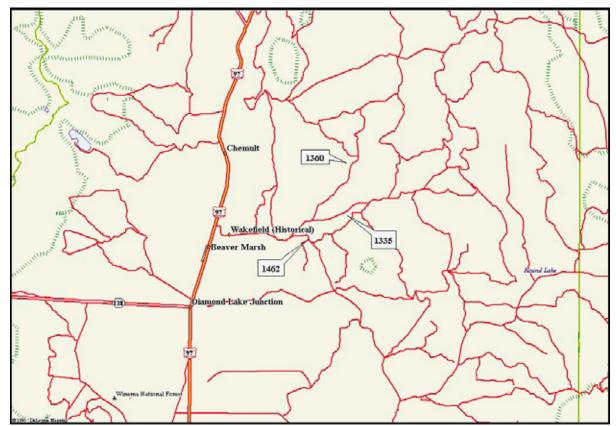


Figure 3. Location of study sites on the Fermont-Winema National Forest.



Figure 4. Soil in some sites was littered with organic debris. A plot marker from a previous study marks the site of a previously emergent plant that is now dormant or senescent. Plants can return after multiple years of dormancy.

Methods

Manipulative treatments. Once study sites were selected, and one hundred plants located and tagged at each site, the application of treatments was initiated. To maximize the ability to detect the effects of individual treatments, each plot consisted of either one individual plant, or of several stems initiating from the same point. The number of stems was recorded before treatment initiation, and throughout the study. To minimize confusion, plants chosen to be included in the study were isolated from other plants, preferably by at least one meter. This goal of a series of isolated study plots was easy to achieve, as *Botrychium pumicola* plants tend to be sparsely distributed over fairly large areas.

Three treatments (burial of plants, soil compaction, and subsurface scraping) were set up, using dormant plants that had been located and marked the previous spring (along with a control set which received no treatment), in October 1998. Shading and biomass removal treatments, using emergent plants, were subsequently completed in May and June of 1999.

Control. Sixteen of the tagged plants at each site were randomly assigned to serve as controls, and received no treatments. As with treated plants, these controls were monitored for mortality, size, and evidence of reproduction. Our annual monitoring of these control plants allows us to integrate this natural, site-specific variation into the evaluation of the effects of our disturbance treatments. Evaluation of controls throughout the study period may also help identify trends in the general health of individual populations. However, as we did not monitor the appearance of newly emergent plants (only the re-emergence of tagged plants) the value of our data in determining population viability is fairly limited.

Burial. Once sites were selected, and an adequate number of plants were located and tagged, application of treatments was initiated. To mimic the potential effect of burial by downed trees (currently a common phenomenon produced by the installation of activity-free buffer zones around *Botrychium pumicola* populations), as well as the effect of soil burial produced by use of mechanical equipment, 16 plants at each of the seven sites were randomly assigned to be buried under 15 to 20 cm of indigenous pumice soil, collected just outside each study population. This treatment, completed in late October, when plants were dormant, consisted of staking circular sheet metal strips approximately 25 cm tall around each tagged plant location and filling the resulting "container" with soil that was collected nearby, but outside of *B. pumicola* populations (Figure 5).



Figure 5. Dormant plants were buried by filling a sheet metal container with dirt from nearby, but outside of the *Botrychium* population.

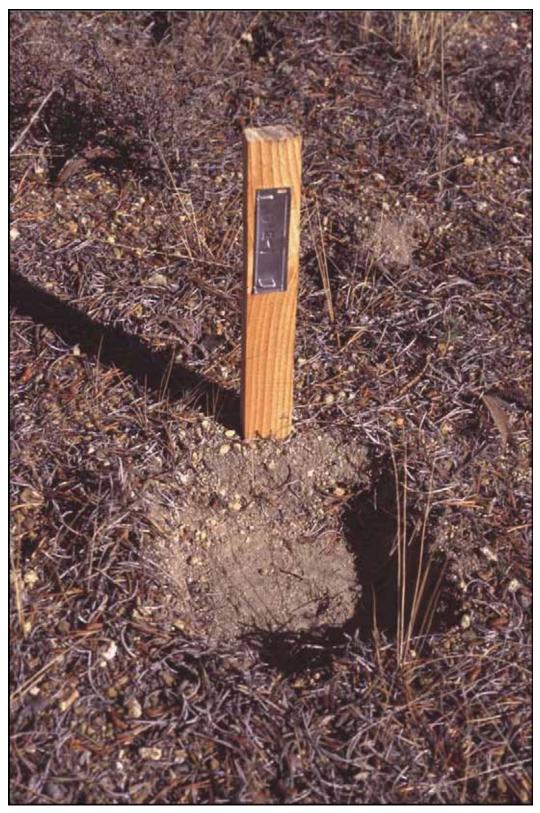


Figure 6. The soil above dormant plants was compacted with a sledge hammer. Soil in this site (DNF 100249) was covered with pine needles but did not contain much other organic matter. ₉

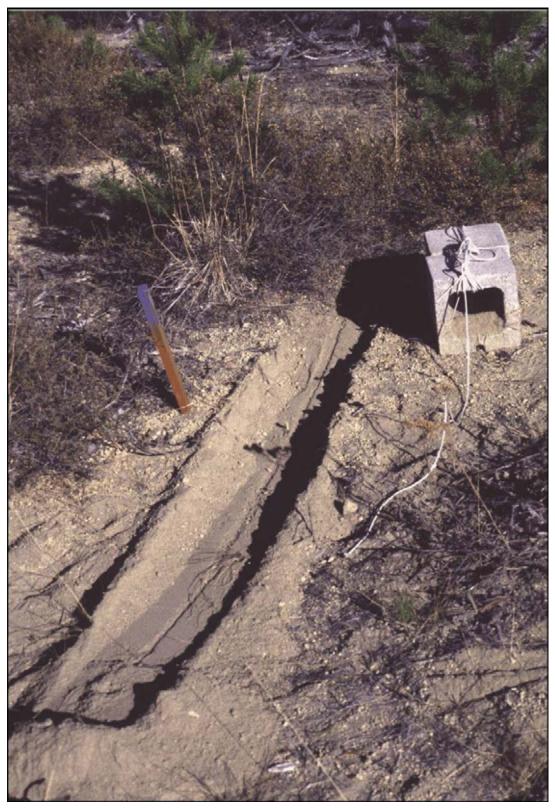


Figure 7. Scraping produced furrows of varying depths. A small *Botrychium* plant can be seen near the plot marker in the center of the photo.

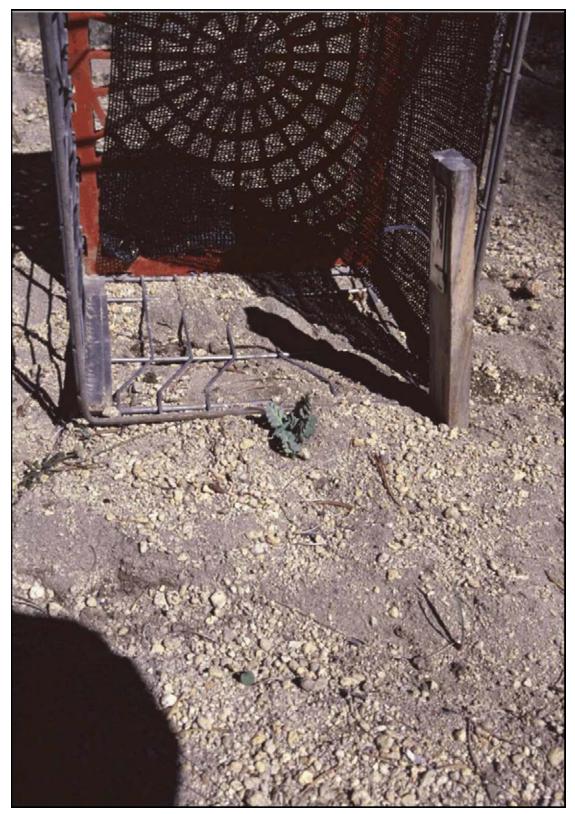


Figure 8. A shading box, tilted back to show the plant inside. Soil in this site (DNF 100193) is largely coarse-grained bare pumice.

Compaction. This treatment, also applied in October 1998, was produced by first measuring soil resistance (in kg/cm²) above tagged plants, using a pocket penetrometer. The soil over dormant tagged plants was then compacted with a sledge hammer, and another soil resistance measurement was completed. The difference between the two measurements estimates the ability of the soil to be compressed. Compaction was applied consistently, to the degree possible, by having the same individual wield the hammer and take the soil resistance measures throughout (Figure 6).

Subsurface scraping. This treatment, produced by dragging an apparatus made from cement bricks approximately 2 m across substrate that contained tagged plants, mimics the effect of skidding logs across the soil (Figure 7). The depth of the furrow produced by each scraping was measured immediately after treatment. The results of our post-treatment monitoring provides information on the effect of several cm deep skid trails, as well as similar activities potentially associated with road building or ORV use. This treatment was applied to 16 tagged plants in each of the seven sites in October, when plants were dormant.

Biomass removal. Once plants had emerged and developed sufficiently to exhibit their ultimate seasonal stature (by June 23, 1999), selected individuals were clipped off at ground level with pruning shears. Plants to be clipped had been designated during the previous field season. However, individuals of *B. pumicola* may not emerge in some years (Montgomery 1990, Kelly 1994), and as expected, not all of the plants that had been pre-selected in the summer of 1998 emerged in 1999 to participate in our study. To ensure statistical consistency, these non-emergent plants were replaced by adjacent emergent plants, which were then tagged and clipped in the same manner as pre-selected individuals.

Shading. This treatment was applied to barely emergent plants in the spring of 1999 (May 22-24). Plants were shaded by placing a sturdy metal frame, (31cm x 48cm) covered with 60% shade cloth over each individual that had been randomly chosen to receive this treatment at the initiation of the study (Figure 8). Shade cloth was securely affixed to frames with metal clips. Unfortunately, shading boxes were removed from the plots at the Crescent District's powerline site by unknown larcenists. Although the boxes removed during the first year were replaced on July 21, 1999, box removal continued to occur. Due to the variable nature of the treatment produced by irregular shading, these boxes were not replaced, and analysis of this treatment in this site was discontinued. As with the biomass removal treatment, pre-selected plants not evident at the time of treatment installation were replaced with nearby emergent plants. Monitoring. In each year after treatment, plants were monitored from the time of emergence (generally in early May) through senescence (August) for survival, number of stems, and presence or absence of reproductive fronds. Fronds were considered reproductive when sori or spores could be observed, but no attempt was made to quantify spore production or determine viability. If one or more plants were observed in the plot during any of the April August monitoring visits, this plot was counted as "emergent" for that year. As herbivory is prevalent in Botrychium species, and can affect plant size and survival (Montgomery 1990, Johnson-Groh and Farrar 1996), evidence of insect or animal herbivory was recorded during all monitoring visits. On July 22-23 in 1999, June 13-15, 2001, and June 3-5, 2002, the length and width of each frond was measured. Frond lengths were measured from the base of the frond to the tip, and did not include the frond stalk.

Frond widths were measured at the widest part (at the base of the frond just above the stalk). These measurements were used to estimate the area of each frond.

Analysis. Because emergence is a binary trait (plants emerge or they do not), emergence data were analyzed using logistic regression (Ramsey and Schafer 2002) in Statgraphics 5.1. (Manguistics, Inc. 2000). Simple regression and ANOVA were used to analyze the effects of the scraping and compaction treatments, and to evaluate differences among sites in treatment results. ANOVA was used to evaluate the effects of treatment on leaf area.

Plot Maintenance. Most plot markers survived the duration of the study in good condition rotting or splintered ones were replaced as soon as damage was noted, and identification numbers were remarked each year. Most treatment apparatus also remained in place throughout the study, although a few burial frames were dislodged, apparently by elk, in 2002. However, shading boxes at the powerline site were removed by unknown parties in 2000, and replacement boxes removed in 2001, with only three remaining at the end of the study. Continual removal of the boxes at unspecified times makes evaluation of the effect of the shading treatment difficult in this site. Therefore, we decided to discontinue replacement of the boxes in 2001, and to consider the effect of their disappearance to be an example of the "demonic intrusion" mentioned by Hurlbert (1984) as a source of confusion in experimental design and evaluation.

Plant excavations. In addition to the 96 plants used in manipulative treatments, four plants in each site were tagged and selected for ecological and biological observations. Emergent plants were excavated during the growing season to observe root development, determine the constitution of observed plant clumps, and search for sexual and asexual propagules. Further excavations were completed during the quiescent period to determine dormant plant depth and primordial structure.

Results

Controls. In 2002, as in all previous years of the study, not all plants tagged as controls emerged. Emergence of tagged control plants varied among sites, and ranged from 25% to 100% in 2002 (Table 1 and Figure 9). Numbers of control plants in the Fremont-Winema NF sites continued to decline, with site 1360 exhibiting particularly poor emergence in 2002.

An overall mean of 82% of control plants returned in 2002, as compared to 72% in 2001, 88% in 2000, and 86% in 1999. The extremely droughty winter and spring in central Oregon in 2001 may explain the reduced emergence of plants in that year - emergence seems to have returned to average levels in 2002. A summary of treatment effects (averaged over all sites for each year) is presented in Figure 10; see Figures 11 -17 to review site-specific results.

Plants generally began to emerge in mid-April, with peak emergence occurring in late May to mid-June for most sites (Figures 18 - 21), although the heavy snowpack and late spring in 1999 forced plants to emerge later. All plants were dormant by the middle of August. The mean leaf area of control plants also differed by site, and among years, although the

differences were not always significant (Figure 22). Leaf area in all sites was generally greater when measured later in the season, when plants had completely matured, and plants with large leaves at the initiation of the study produced large leaves throughout, while those with small leaves did not change greatly in size. The range of frond sizes was considerable; the smallest frond we measured had a leaf area of barely 1 cm², and the largest measured 37.5 cm². (Shaded plants at the Powerline site were not included in the analysis after 2001, due to repeated removal of shade boxes.)

Non-reproductive plants (both control and treated) were rare in all sites in all years, with the percentage of non-reproductive fronds per site ranging from 0.0 to 9.3% The ability to become reproductive did not vary significantly among sites, or among treatments, or among years (p > 0.05 from a logistic regression), with an overall mean of 7.4% non-reproductive plants recorded during our study.

Burial. Our 1999 monitoring did not discover any plants emerging from plots in which burial frames and soil were placed over dormant plants in the fall of 1998 (Amsberry and Meinke 1999*a*; 1999*b*). However, somewhat surprisingly, in 2000 one plant emerged from a burial plot in site FWNF 1360, indicating that it may be possible for buried plants to recuperate below ground after initially sustaining damage, and return in subsequent years. In 2001, this same plant returned, but no other plants emerged from burial plots. This single emergent plant was quite large, with a leaf area of 21 cm² in 2001. In 2002, this plant did not emerge, again reducing the number of plants that survived the burial treatment to zero.

Compaction. Soil compaction significantly reduced the emergence of *B. pumicola* (relative to the controls in each site) at all sites in the first year after treatment (Table 1). In all four Deschutes NF sites, emergence of compacted plants remained significantly lower than control plants throughout the study, but in the three Fremont-Winema NF sites, recovery of treated plants to the point that their emergence levels were similar to those of control plants had occurred by 2002 (Table 1; Figure 23). Recovery of populations is significantly related to mean soil "compactability" per site (p = 0.0335 from a linear regression). "Compactability" is a measure of the ability of the soil to be compacted and is defined as:

 $C = P_1 - P_2$; $P_1 = soil resistance (kg/cm^2)$ after compaction $P_2 = soil resistance (kg/cm^2)$ before compaction

As the mean difference between before- and after- treatment measurements of soil resistance per site increases, emergence (in 2002, at the end of the study) decreases (Figure 24).

Compaction also reduced the size of the fronds produced by surviving plants by 4.5 cm² (\pm 6.3 cm²), although this effect was not significant in 2001 (p > 0.05 from an ANOVA; Figure 25). In 2002, this effect became statistically significant, probably because more compacted plants emerged, giving us a larger sample size from which to calculate leaf area. Although fronds were smaller in the treated plots, compaction, like all other treatments, did not affect plant reproduction (p > 0.05 from an ANOVA).

Scraping. The mean depth of the furrows produced by the scraping treatment differed significantly among sites (p < 0.001 from an ANOVA; Figure 26). Although the treatment was administered in a similar manner in all sites, deeper furrows were produced in sites devoid of large amounts of organic debris and/or living plants of associated herbaceous species. As furrow depth also varied within sites, a logistic regression was needed to evaluate the effects of furrow depth on individual plants; depth significantly affected plant emergence in 1999 (p = 0.006; Figure 27). By the end of the study, although scraped plants continued to emerge more poorly than unscraped ones, the difference in emergence relative to the depth of the scraped furrow was no longer apparent.

Leaves produced by plants subjected to the scraping treatment were smaller than controls in both 2001 and 2002 (Figure 25), but the differences were not significant (p > 0.05 from an ANOVA).

Biomass removal (clipping). One-time removal of above-ground biomass did not have a significant affect on plant emergence in our study (Table 1). Although clipped plants emerged slightly better than their un-clipped counterparts in 2000 at site 100080, this one time result is probably not biologically meaningful. Clipping also did not affect leaf area clipped and control plants were of similar size in both monitoring years.

Shading. With two exceptions, shading did not generally have a significant affect on plant emergence (Table 1). However, shaded plants in site DNF 100193 did not emerge as well as controls, while shaded plants emerged better than controls in site FWNF 1360.

Shading, however did significantly (p <0.05 from an ANOVA) affect plant leaf area, with shaded plants growing 5.5 cm² (\pm 3.6 cm²) bigger in shaded plots than controls in 2001 (Figure 25). In 2002 shaded plants continued to produce larger fronds (1.8 cm² \pm 2.8 cm² larger) but the difference was no longer significant. As with all other treatments, shading did not affect plant reproduction in our study.

Plant excavations. During the emergent period, root growth was vigorous, especially in loose soils, and was characterized by the lack of root-hairs and thick rhizoids typical of mychorrizal ferns. Plant clumps consisted of groups of individual plants emerging together, with - invariably- one frond per plant. As well as emergent fronds, excavated clumps occasionally also revealed smaller, pre-emergent plants; presumably these plants will continue to become larger in subsequent years and eventually emerge from the soil. As reported by other workers (Camacho 1996, Farrar and Johnson-Groh 1990), asexual propagules (gemmae) were frequently observed on plant roots, especially on the larger plants, and are probably responsible for the clumped, multi-plant habit frequently observed in this species. This suggests that asexual propagation may figure prominently in the population recruitment in this species, although genetic studies indicate that sexual reproduction also occurs, at least occasionally (Camacho and Liston 1998). No gametophytes were ever seen. This lack of observed gametophytes was consistent with our expectations, however, as gametophytes are often small and unobtrusive in species of this genus (Mason and Farrar 1989), and have never been reported for *B. pumicola*.

Dormant plant buds (ranging from 3-10 cm) consisted of numerous fully-formed primordia, demonstrating that the potential growth for multiple years is commonly present within a single dormant plant (Figure 28 - 30). Preformed primordia (for up to five years of growth) have also been observed in Botrychium dissectum (Montgomery 1990), indicating that this is a widespread phenomenon in this genus. This information is useful for making decisions concerning management activities, as below ground disturbance during the dormant period may destroy multiple years's growth; the loss of a single individual may be more damaging than previously believed. One surprising discovery made during our dormant period excavations was the bright green, actively photosynthesizing nature of dormant plants. Although plants tips were far below the soil surface (1-3 cm in sites with dense, organic soils; 8-10 cm in looser soils), leaves and upper stems were green throughout. The ability to synthesize chlorophyll in the dark, solely through enzymatic activity, has occasionally reported for gymnosperms, algae, and a few ferns (Devlin and Barker 1971). This type of activity is evidently occurring here, although the phenomenon has not been previously reported for this species. This may be important in the early seasonal development of Botrychium pumicola plants, although the ecological significance of dark photosynthesis in ferns has not been established.

J.1 Values		0				
100249 (D	eschutes Na	tional Fores	t)			
year	burial	clipping	control	scraping	shading	compaction
1999	0.00	**	0.813	0.188	**	0.188
2000	0.00	1.00	1.0	0.50	1.00	0.438
2001	0.00	0.938	1.0	0.438	0.875	0.438
2002	0.00	1.00	1.0	0.563	1.00	0.50
100193 (D	eschutes Na	tional Fores	t)			
year	burial	clipping	control	scraping	shading	compaction
1999	0.00	**	0.875	0.313	**	0.063
2000	0.00	0.938	0.875	0.250	0.750	0.125
2001	0.00	0.688	0.688	0.188	0.688	0.563
2002	0.00	0.750	0.875	0.250	0.563	0.250
100080 (D	eschutes Na	tional Fores	t)			
year	burial	clipping	control	scraping	shading	compaction
1999	0.00	**	0.938	0.00	**	0.063
2000	0.00	1.00	0.750	0.313	0.750	0.125
2001	0.00	0.875	0.688	0.063	0.563	0.125
2002	0.00	0.875	0.750	0.250	0.688	0.063
Powerline	Site (Desch	utes Nationa	al Forest)			
year	burial	clipping	control	scraping	shading	compaction
1999	0.00	**	0.875	0.188	**	0.063
2000	0.00	0.875	0.813	0.125	0.688	0.00
2001	0.00	0.438	0.625	0.125	0.500	0.00
2002	0.00	0.500	0.563	0.063	0.688	0.063

Table 1. Probabilities for emergence of *B. pumicola* from logisitic regression in Statgraphics5.1 Values printed in red differ significantly from controls - those in black do not.

Table 1. - continued. Probabilities for emergence of *B. pumicola* from logisitic regression in Statgraphics 5.1 Values printed in red differ significantly from controls - those in black do not.

1462 (Fremont-Winema National Forest)									
year	burial	clipping	control	scraping	shading	compaction			
1999	0.00	**	0.813	0.313	**	0.063			
2000	0.00	0.938	0.938	0.375	1.00	0.438			
2001	0.00	0.938	0.813	0.375	0.688	0.500			
2002	0.00	0.375	0.438	0.375	0.375	0.313			
1360 (Fremont-Winema National Forest)									
year	burial	clipping	control	scraping	shading	compaction			
1999	0.00	**	0.875	0.563	**	0.375			
2000	0.063	0.875	0.938	0.750	1.00	0.438			
2001	0.063	0.438	0.750	0.375	0.938	0.375			
2002	0.00	0.500	0.250	0.188	0.625	0.250			
1335 (Frem	1335 (Fremont-Winema National Forest)								
year	burial	clipping	control	scraping	shading	compaction			
1999	0.00	**	0.688	0.125	**	0.188			
2000	0.00	0.875	0.813	0.188	0.938	0.500			
2001	0.00	0.563	0.500	0.125	0.688	0.375			
2002	0.00	0.313	0.438	0.063	0.500	0.313			

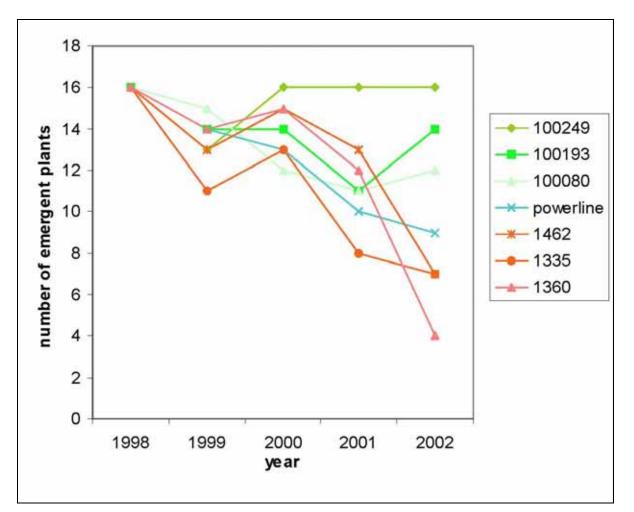


Figure 9. The emergence of control plants varied among sites, with a larger decline in the FWNF sites over the length of the study. Sixteen control plants were initially selected in each site.

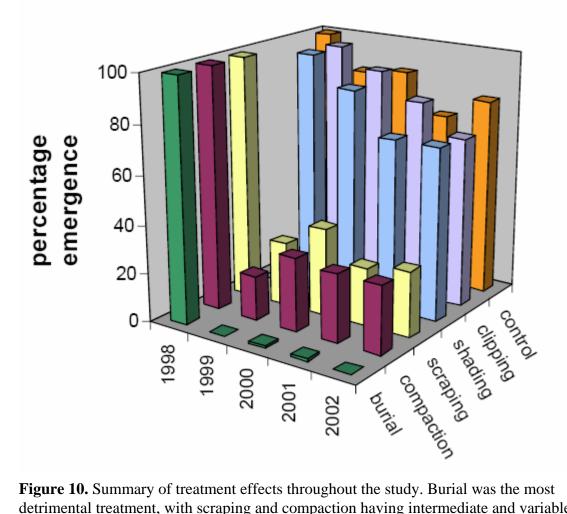


Figure 10. Summary of treatment effects throughout the study. Burial was the most detrimental treatment, with scraping and compaction having intermediate and variable effects, and shading and clipping having little impact.

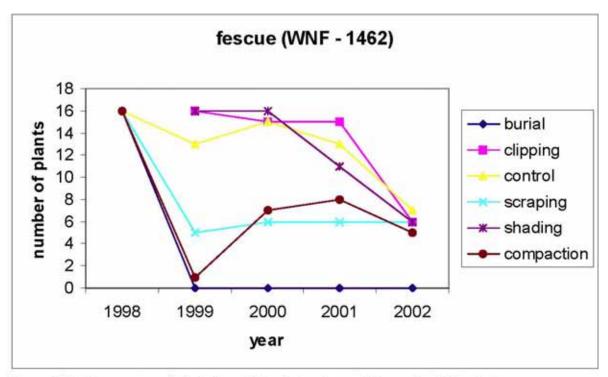


Figure 11. Emergence of plants in relation to treatments throughout the study.

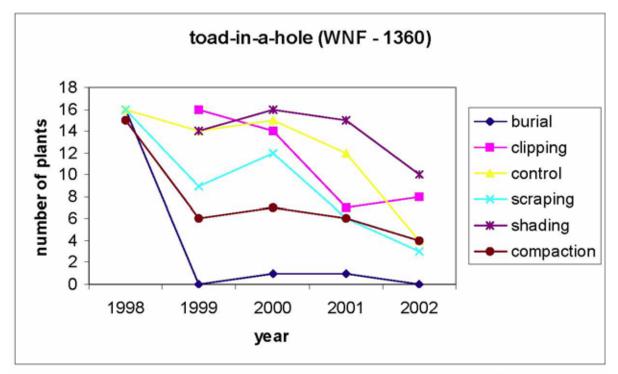


Figure 12. Emergence of plants in relation to treatments throughout the study.

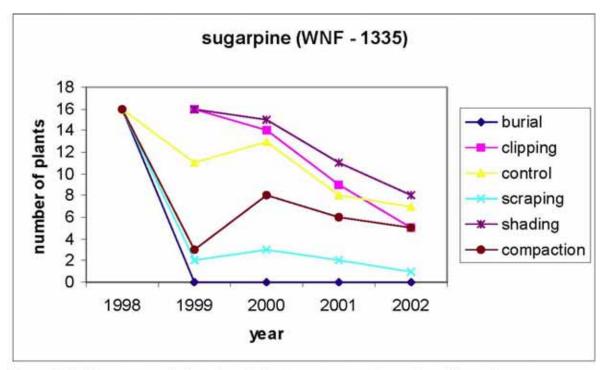


Figure 13. Emergence of plants in relation to treatments throughout the study.

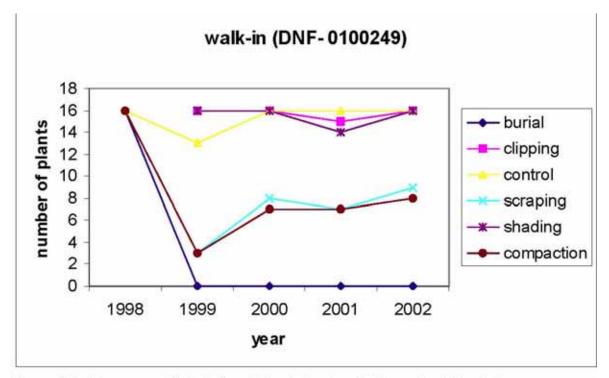


Figure 14. Emergence of plants in relation to treatments throughout the study.

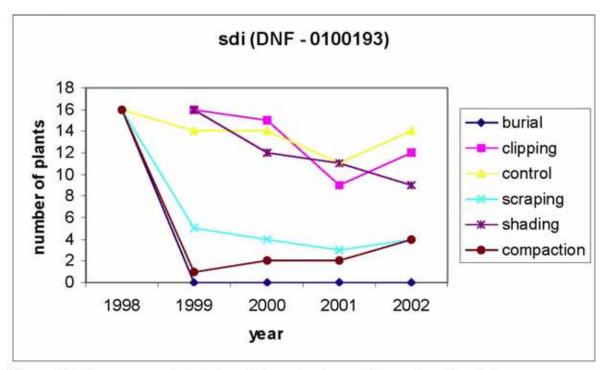


Figure 15. Emergence of plants in relation to treatments throughout the study.

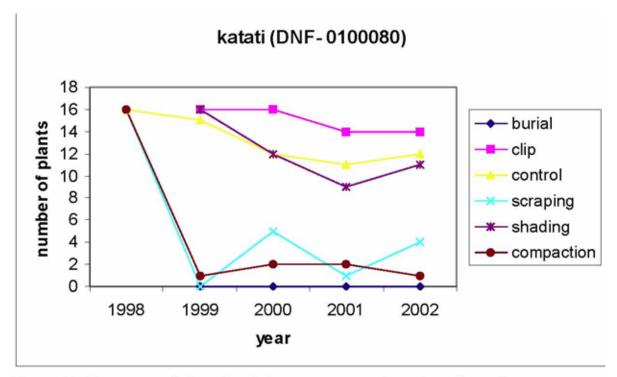


Figure 16. Emergence of plants in relation to treatments throughout the study.

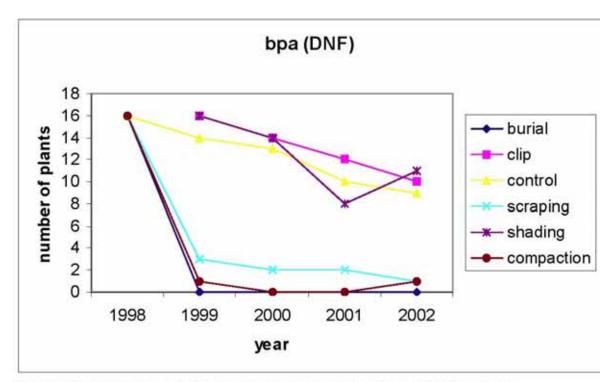


Figure 17. Emergence of plants in relation to treatments throughout the study.

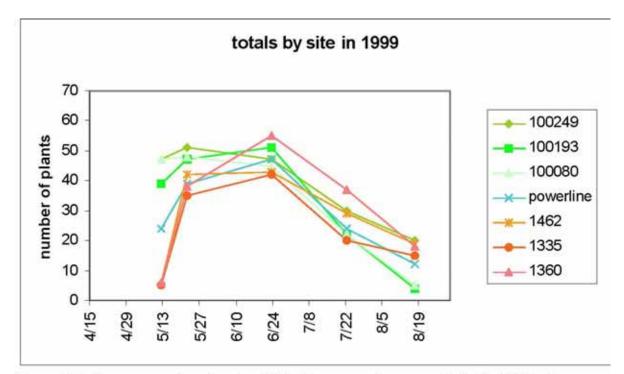


Figure 18. Emergence phenology in 1999. Emergence began earlier in the DNF sites.

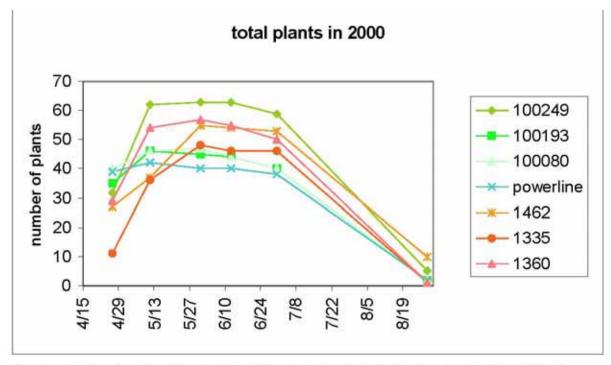


Figure 19. Plants emerged slightly earlier than in the previous year, and also senesced earlier.

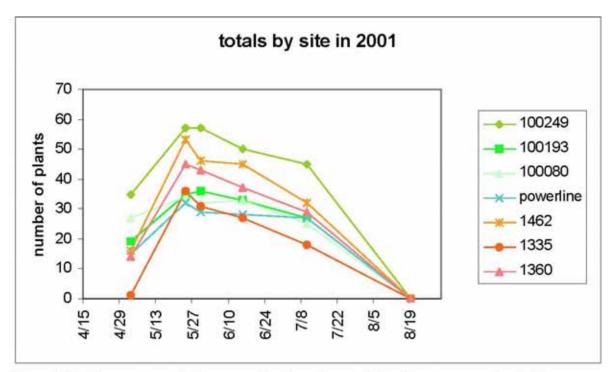


Figure 20. Emergence varied among sites to a degree, but patterns were quite similar.

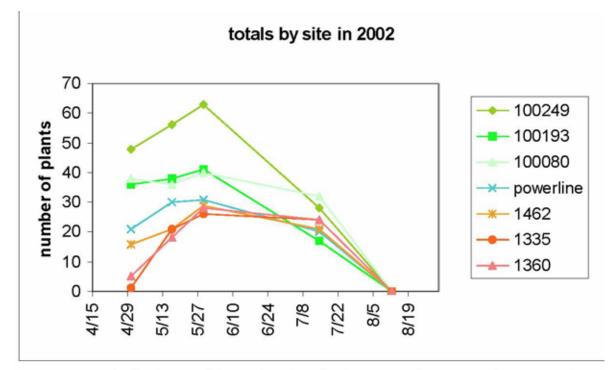


Figure 21. In the final year of the study, plants in the FWNF sites emerged more poorly than those in the DNF sites.

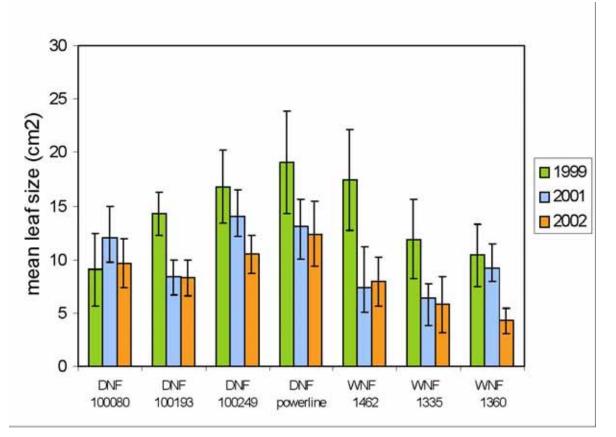


Figure 22. Mean leaf sizes of control plants varied among sites. Leaf sizes were measured on July 22nd in 1999, June 15th in 2001, and June 1st in 2002 - the difference in phenology probably contributes to the smaller leaf sizes in these years. Plants in the FWNF sites reached peak maturity at a later date than those in the DNF sites - this may also influence reported leaf sizes. "Leaf size" was calculated using the longest length and width measures for each frond - see Method section for details. (Error bars represent one standard error above and below the mean.)

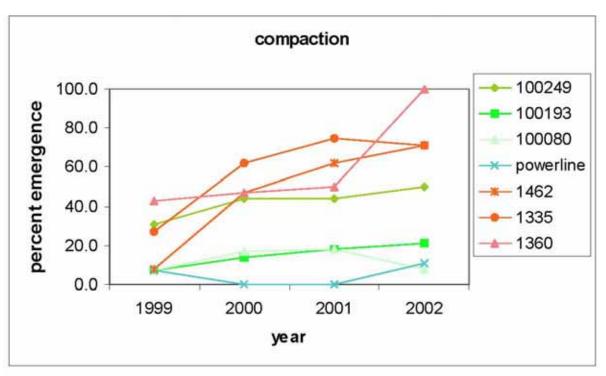


Figure 23. Compacted plants, when compared with controls, emerged in increasingly greater proportions subsequent to the decline in the first year after treatment, indicating that recovery of these plants from the initial ill effects of the compaction treatment occurred. "Percent emergence" is the emergence of treated plants relative to control emergence:

% emergence = emergence of treated plants/emergence of controls x 100

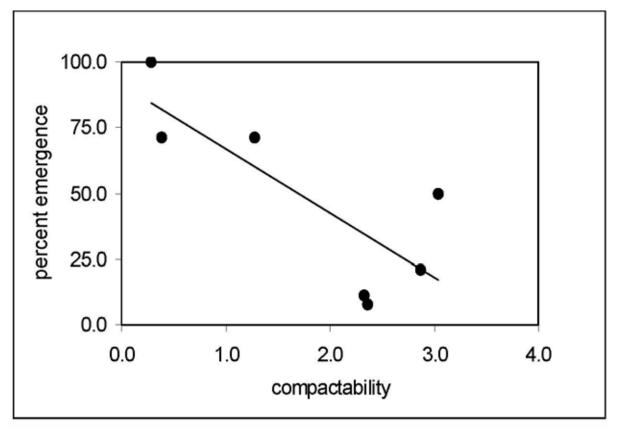


Figure 24. The percent emergence per site (relative to control emergence) of compacted plants in 2002 was related to the ability of the soil to be compacted. The four points in the lower right are the DNF sites, those in the upper left are the FWNF sites. (p = 0.034; R² = 62.8%; compactability = 3.0126 - 2.5747 x emergence; see text for details.)

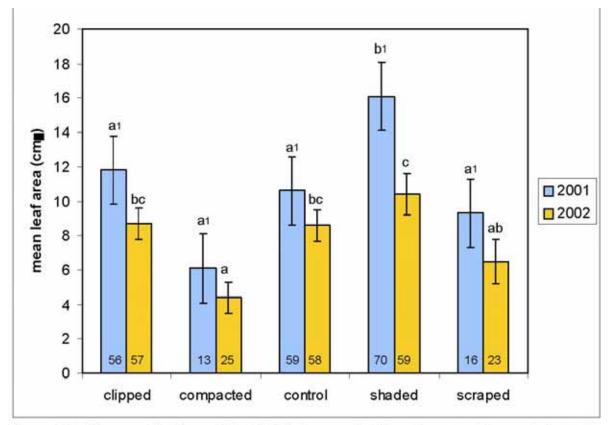


Figure 25. The mean leaf area of shaded plants was significantly greater than controls (and all other treated plants) in 2001, and compacted plants had smaller mean leaf areas than controls in 2002. (Error bars represent one standard error above and below the mean; treatments topped with the same letter are not significantly different from each other at p =

0.05 from an ANOVA; numbers at the bottom of each bar are sample sizes.)

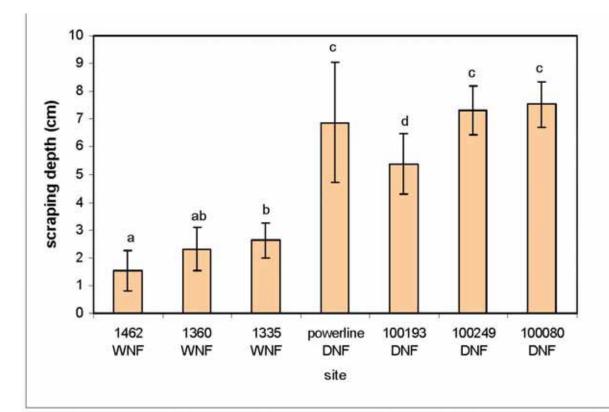


Figure 26. Depth of the furrows produced by the scraping treatment varied among sites. (Error bars represent one standard deviation above and below the mean; bars topped with the same letter are not significantly different from each other at p = 0.05 from an ANOVA; n = 16 for each site.)

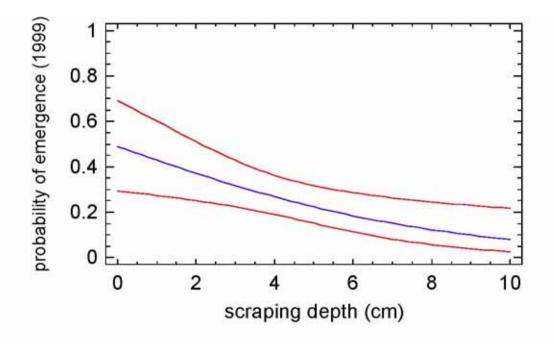


Figure 27. The depth of the scraped furrow significantly affected the probability of emergence in the year after treatment, with greater scraping depths reducing emergence. (p = 0.0057 from a logistic regression; n = 112; red lines represent 95% confidence intervals.



Figure 28. Excavated dormant plant. Large thick roots and few root hairs are characteristic of obligatory mycorrhizal plants. Dormant plants were enclosed in a protective sheath formed by the previous year's senescent frond – these sheaths were frequently damaged during the scraping treatment, exposing dormant primordia to the environment.



Figure 29. A dormant plant with the sheath removed. Fronds were green and turgid, although they had been dormant beneath the soil for at least two months.



Figure 30. Dissected dormant bud in the lab - sterile portion of largest primordium is shown on the left, fertile portion of the same primordium in the center, and a separate small primordium on the right. This small primordium would remain dormant throughout another growing season (when the larger one would emerge), and emerge the following spring, one and one-half years after this photo was taken. Dormant buds contained as many as four years of future fronds - damage to dormant buds impacts emergence for several years.

Discussion

Treatment effects on emergence

Throughout the study results have been site specific, with the extent of damage to plants, and their subsequent ability to recover, varying by location. As expected, emergence of controls also varied among sites. Previous studies on *B. pumicola* (Raven 1997), and other members of the genus (Montgomery 1990, Kelly 1994, Johnson-Groh et al. 2002) also documented irregular emergence of tagged plants, even in the absence of apparent disturbance. Emergence of controls in some sites remained constant throughout the study (i.e. site 100249), while others dropped off dramatically over time (i.e.1462). As treatment effects were determined by comparing the emergence of treated plants to controls, low overall emergence in some sites reduced our ability to evaluate treatment effects.

Emergence also varies with environmental conditions, with dry years negatively affecting *Botrychium* emergence and performance. In 2001, *Botrychium pumicola* plants in all study plots appeared to be suffering from the exceptionally low snow pack and dry winter weather (Oregon Climate Service 2003). Limp and discolored emergent vegetative fronds were frequently observed, and many fertile fronds turned brown before spores were dispersed. To evaluate the regional extent of these phenomena, we visited the alpine population of *Botrychium pumicola* at Lao Rock near Crater Lake. Plants in this site were healthy and vigorous, indicating that drought stress was probably confined to lower-elevation populations, and emphasizing the site-specific nature of *Botrychium*'s emergence and health.

Burial. Despite year-to-year variability, and site-specific variation, some trends are apparent. Burial is detrimental in all sites. Previous anecdotal reports also indicate that plants do not reemerge after frond burial (Stu Garrett, Native Plant Society of Oregon, personal communication), although the mechanism causing this apparent mortality is not known. Fronds may have insufficient resources to elongate an extended distance, excess moisture may accumulate in the increased depth of soil, or mycorrhizal associations may be disrupted by the burial treatment. Further studies, burying plants with various depths of soil, would quantify the effect of burial, and pinpoint the depth at which plant senescence occurs.

Compaction. Although previous management recommendations suggest that compaction due to logging practices does not impact pumice soils (Larsen 1976, Carlson 1979), skid trails produced an increase in soil resistence of 25-157% relative to nearby undisturbed soils in a study of pumice soil disturbance on the Ochoco National Forest (Allbrook 1986). Compaction due to skid trails reduced conifer growth (Wert and Thomas 1981), and, as expected, our compaction treatment negatively impacted the emergence of *Botrychium* plants in our study. However, the effect of compaction is complex, and varies by site. Our treatment produced an increase in soil resistence comparable to the previous study in the Fremont-Winema NF sites (101-181%), and a larger increase in the Deschutes NF sites (320630%). In sites with more woody debris or living vegetation (the FWNF sites), soils were less compactable, and plant damage seemed to be minimized. Compaction during the dormant period may not mechanically damage *Botrychium* primordia, but this process may condense soils to the point that primordia cannot penetrate the soil to emerge. Essential soil-water chemistry may

also be disrupted, or the suitability for mycorhizzal development reduced. These detrimental effects may be less pronounced in soils with more organic matter.

Recovery of compacted plants, at least to some degree, occurred in all sites (Figures 10-16), although treated plants in all DNF sites continued to emerge at levels that were significantly lower than emergence levels of control plants (Table 1). In the DNF 100080 and powerline sites (Figures 16 and17), only one or two plants re-emerged after compaction, although these same plants re-emerged periodically (but not each year). Populations in these sites appear to be declining, as control emergence generally decreased each year - continued monitoring will provide data on the long-term effect of compaction in these sites. Compacted plants in sites DNF 100249 and 0100193 (Figures 14 and 15), after their initial senescence, continued to reemerge at higher rates each year. As control plant emergence has remained fairly consistent each year, compacted plants may eventually reach emergence levels similar to those of controls, or they may stabilize at lower levels. Further monitoring data will help determine whether non-emergent plants are senescent or merely dormant.

Plants in the FWNF sites exhibited increasing or stable emerge levels, after the initial posttreatment year, and as control emergence declined in all sites throughout the study, treated and control plants eventually emerged at about the same rates. As pumice soils can recover from compaction, at least in the top 15 cm (Wert and Thomas 1981), and organic matter within the soil probably also contributes to the recovery process, this treatment does not appear to permanently damage plants in these sites. However, the declining overall emergence in these sites is puzzling, and obfuscates the ability to confidently assess the effects of our treatments.

Scraping. The negative effects of scraping, like those of compaction, were more pronounced in sites with less 'organic' soils. Greater scraping depths were produced by our treatment in soils devoid of organic matter, and these greater depths produced more senescence. Organic matter from fallen limbs and twigs, as well as living roots, prevented the scraping apparatus from digging deeply into the soil, and protected primordia from contact. In soils with less protection, scraping frequently damaged dormant buds, either by breaking frond stalks, or by injuring bud tips. Fronds that did appear in years subsequent to the scraping treatment were often deformed or broken, probably because they were mechanically damaged as dormant primordia, years before their scheduled emergence. Fronds that were not damaged to the point of breaking were often exposed by scraping, leaving them vulnerable to winter freezing, summer drought, and a greater potential for herbivory or mechanical injury.

Although damage caused by scraping was severe, especially in sites with loose soils, and affected plants for years after treatment, some recovery from this treatment did occur in some sites. The scraped portion of the population in site DNF 100249 initially emerged poorly in the year following treatment, but became increasingly vigorous each subsequent year. The population in this site is healthy overall, with consistent return of (untreated) marked plants each year. Dormant gemmae or immature subterranean sporophytes associated with plants that were exceptionally vigorous before they were scraped (or compacted - see above) may have the capability to mature quickly in response to damage of the parent plant, and emerge to replace senescent fronds. Up to ten gemmae per individual have been counted on plants of *Botrychium pumicola* (Camacho 1996), and healthy *Botrychium campestre* plants produce

copious numbers of gemmae (20-500/plant; Farrar and Johnson-Groh 1990). These gemmae persist underground for years, dependent on their fungal partner for nutrition (Mason and Farrar 1989, Kelly 1994), and progress through various stages of maturation, culminating in an emergent sporophyte. The time course of development from a gemma to a mature plant is not easily determined, and the mechanism that triggers emergence is not known (Farrar and Johnson-Groh 1990, Johnson Groh et al. 2002). However, although recovery seems to have begun in DNF 100249, emergence of treated plants remained significantly lower than the emergence of control plants throughout the length of the study - further monitoring will be needed to determine the potential for fully recovery.

Populations in other sites also exhibit some potential for recovery. In site FWNF 1462 (Figure 11), scraped plants exhibited a drop in emergence in the first year after treatment. Subsequently, they emerged at a constant level throughout the study, while the emergence of control plants (and other benignly treated plants., ie those that were shaded or clipped) dropped rapidly. By 2002, control and treated plants emerged at similar rates, indicating that scraping does not negatively impact plants in this site. Scraped plants in other sites exhibited erratic emergence rates, (possibly due to variable scraping depth), making the evaluation of the effect of this treatment difficult. Moderate disturbance has been associated with many species of *Botrychium* (Montgomery 1990, Wagner and Wagner 1983, Kelly 1994, Lesica and Ahlenslager 1996); the recovery of *B. pumicola* plants from low-level disturbance is consistent with the results of studies on related species.

Biomass removal. The lack of effect of one-time biomass removal on emergence in subsequent years corroborates the results of previous studies which reported no detrimental effects on *Botrychium* species in the year following leaf biomass removal, at least on an observational level (Montgomery 1990, Johnson-Groh and Farrar 1996). Although our treatment of removing fronds at ground level as they reached maturity allowed plants to accumulate little energy from photosynthesis, the endophytic fungus functioning as the mycorrhizal partner probably contributed substantially to their nutrition, allowing them to survive. The multiple preformed primordia we observed during our excavations of dormant plants also help explain the ability of treated plants to re-emerge in the years following biomass removal. When a currently emergent frond is removed, subterranean portions of a plant survive on nutrients derived from its fungal partner (and possibly 'dark photosynthesis' Devlin and Barker 1971). The next in the sequence of preformed fronds emerges the following year, with no loss in vigor.

However, although one-time removal of fronds produces no negative effects, the result of repeated loss of all photosynthetic biomass is variable. Plants of *Botrychium dissectum* that were eaten in three consecutive years continued to emerge (Montgomery 1990), although repeated frond loss through herbivory by slugs produced weakening of plants after four years in *Botrychium australe* (Sessions and Kelly 2002), and herbivory combined with disturbance caused by pigs also caused mortality (Kelly 1994). The effect of leaf loss (especially repeatedly) in conjunction with other environmental events such as fire or drought has not been evaluated; such combined effects may result in population decline and loss of vigor (Johnson-Groh and Farrar 1996).

Shading. In general, shading had little effect on emergence. However, in one site (DNF 100193; Figure 14), shade boxes appeared to function as havens for herbivores, as enclosed plants suffered from 40.3% more herbivory than control plants outside shade boxes. *Botrychium* plants remain green and succulent throughout the growing season, and are noticeably more juicy and edible-looking than surrounding xeric-adapted vegetation.

Reproductive fronds, with spores and sori, provide a high quality food source, especially as they ripen during a time when much of the associated vegetation no longer has fruit or flowers. Consequently, herbivory by rodents, caterpillars, and possibly deer was observed occasionally in all sites, although generally at fairly low levels (0-10.6%), with the exception of DNF 100193. The population in this site occurs on the edge of a pumice flat, in open areas among scattered stands of Purshia tridentata. Rodent herbivores that are present in the area used the shade boxes for protection from predators, resulting in an increase of Botrychium shoots in their diets; chewed leaf scraps and leafless Botrychium stalks were frequently observed. Previous studies have documented increased herbivory of Botrychium plants in shrubby fields when compared with those in woodland and open field habitat (Montgomery 1990); our shade boxes presumably served as substitute 'shrubs' in this site. These high levels of repeated herbivory may have reduced plant vigor, and eventually caused senescence (Sessions and Kelly 2002). Alternatively, lower reported emergence in this site may merely be due to frond removal by herbivores prior to our monitoring visits. Herbivory is quite common in various species of *Botrychium* (Kelly 1994, Montgomery 1990, Johnson-Groh and Farrar 1996, Sessions and Kelly 2002), including B. pumicola (Raven 1997), although the long-term effects of this type of frond removal have rarely been studied.

Treatment effects on frond size

The environmental and phenological variables affecting frond size in *Botrychium* have not been determined. The variation we observed in the frond size of control plants among populations, years, and individuals is most likely due to variation in microhabitat edaphic and climatic conditions, differences in the age of populations and individuals, and perhaps most importantly, the extent and nature of mycorrhizal colonization. Plants were larger when measured later in the season, when they had presumably reached full maturity. Frond sizes also varied widely in previous long term monitoring studies of *B. pumicola*, and the variation was related to the year, site, and habitat (Raven 1997). Despite our lack of knowledge regarding the mechanisms of *Botrychium* frond development, our study provides information on the effects of disturbance treatments. Only the compaction and shading treatments resulted in differences in frond size.

Compaction. The smaller fronds produced by compacted plants are probably caused by reduced plant vigor. Compaction due to skid trails reduced conifer growth (Wert and Thomas 1981); the decreased vigor of conifers, like that of *Botrychium*, could be caused by damage to the fungal partner, or by abiotic changes such as a change in water movement through the soil. Damage to the mycorrhizal connection may reduce the ability of plants to import nutrients, and force the preformed primordia within the dormant buds to develop at a reduced rate, resulting in a diminutive final stature. This effect may be reduced as soil recovers from compaction, with a consequent increase in frond size.

Shading. Plants in shade boxes are probably larger due to the effects of etiolation, a common phenomenon among photosynthetic plants, rather than increased vigor. Plants of *Botrychium australe* also produced larger fronds under shaded conditions, compared to those grown in higher light in the same site (Kelly 1994). These same plants later experienced an increase in light due to the removal of adjacent vegetation during a fire, and their mean leaf size decreased (Sessions and Kelly 2002). Shading also produced plants that were greener than their unshaded in counterparts in both the *B. pumicola* and *B. australe* studies.

Treatment effects on reproduction

No treatments affected the production of reproductive fronds in our study, and the percentages of reproductive plants we observed were similar to those reported previously for 24 populations of *Botrychium pumicola* over a five year period (71.4 - 100%, with a mean of 90.8%; Raven 1997). However, as other studies have shown *Botrychium* species to exhibit reproductive decline (measured by the number of plants producing reproductive fronds) in increasingly shaded conditions (Kelley 1994), several more years of data collection will be necessary to determine the ultimate effect of plant shading.

Due to the difficulties in quantifying reproductive output and sporophyte recruitment in *Botrychium* (Mason and Farrar 1989, Johnson-Groh et al. 2002), no attempt was made to measure spore or gemma production, or to determine levels of gametophyte/gemma germination and sporeling development in our study. As successful progression through these life history stages is critical to the long term persistence of *Botrychium* populations (Lesica and Ahlenslager 1996, Johnson-Groh et al. 2002), an understanding of the effects of disturbance on these propagules and immature plants is required in order to make useful predictions on the effect of disturbance on population viability. Because we are lacking information on life history stages, we cannot reliably determine the effects of any treatments, including shading, on reproduction and recruitment of *Botrychium pumicola*.

Summary

Burial of *Botrychium pumicola* plants is detrimental in all sites. Scraping and compaction also reduce plant vigor, with some recovery occurring over time, especially in sites with organic soils. One time biomass removal does not affect plants, although repeated removal may reduce vigor. Plants are not negatively impacted by shading, although this treatment produces morphological changes in plant size and color, and more data is needed to determine the long-term effects of shading on reproduction. Tagged control plants on the FWNF emerged in decreasing numbers each year, but as our study was not designed to evaluate the potential appearance of newly emergent plants, the overall health of these populations cannot be determined.

Recommendations

Activities that severely compact or disturb soils, or bury plants (such road or

• landing building, ORV use, or the sitting of recreational facilities) should not occur in *Botrychium pumicola* populations.

Activities that produce one-time biomass removal and one-time minimal ground disturbance, but do not bury plants or compact soils (such as low-impact, small-scale logging, or occasional recreational use) can occur in *Botrychium pumicola* sites with minimal impact on population viability.

More information is needed to understand the effects of shading on *Botrychium pumicola* populations. Further studies of canopy cover removal in sites with *B*.
pumicola will provide additional data on the effects of shading in this species.

Monitoring of study plots should continue. The life history of *Botrychium pumicola* makes it slow to react to environmental disturbance, and several years of data are needed to evaluate treatment effects.

Controls in some populations emerged in decreasing numbers throughout the study. In order to accurately evaluate trends in overall population health, monitoring of all plants in these populations (not just treated plants), as well as all plants in populations outside of the current study, should continue

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