

Sword Fern Microbiome Project:

Preliminary Findings



Haven
Ecology & Research

Prepared for:

Friends of Seward Park

Prepared by:

Dylan Mendenhall

Andres Barrera



Figure 1: Example of a small patch of sword ferns that recently died in Seward Park. This mortality event occurred in 2020 in an area that was previously unaffected by the phenomenon.

Abstract

Sword ferns (*Polystichum munitum*) are rapidly declining in Seward Park, one of the few remnant old growth forests in the Puget Sound lowlands. Based on recent transmission experiments, we hypothesize that a pathogen may be responsible for the phenomenon. We also consider drought stress as an alternative explanation. We compared areas of Seward Park containing symptomatic sword ferns with areas of Seward Park and Schmitz Preserve Park containing healthy ferns. In each experimental group, we surveyed the soils, site conditions and plant communities. We also collected tissue samples from sword ferns to measure their foliar moisture content and to evaluate the microbial endophyte community using the Illumina MiSeq platform. Foliar moisture content was significantly lower in symptomatic sword ferns compared with healthy ferns. However, foliar moisture content was not correlated with soil moisture content, which was similar between all three experimental groups. Soil bulk density was also similar between the symptomatic and healthy areas of Seward Park. Widespread mortality was not observed among sword ferns in Schmitz Preserve Park, where the soils are more coarsely textured and better draining than in Seward Park. Although the microbiome component of our study is ongoing, these preliminary results do not support the drought hypothesis. The sword fern decline is likely driven by a process that is independent from the site conditions that influence soil moisture content.



Introduction

Over the past decade, sword ferns (*Polystichum munitum*) have died in sudden mortality events at dozens of locations throughout the Puget Sound region (Shannon, 2020a). In 2013, a particularly large mortality event was observed in Seward Park (Doughton, 2019). Although a range of possible explanations have been explored, including plant pathogens, herbivory by mountain beaver (*Aplodontia rufa*), and nutrient deficiencies (Kildisheva and Aghai, 2018), the phenomenon remains unexplained. Perhaps most perplexing, the symptoms of the decline are inconsistent and often resemble drought stress. However, an ecophysiology study that examined the water relations of *Polystichum munitum* concluded that moisture stress is not the primary driver of the phenomenon (Goldstein-Miller et al., 2019). Given that sword ferns are well-adapted to extended periods of drought (Baer et al., 2016; Brodersen et al., 2016), it seems unlikely that the phenomenon would be caused by a moisture deficit.

Curiously, the phenomenon does not appear to affect co-occurring plant species. For example, as the symptoms and mortality have spread through the sword fern population at Seward Park, other native plants have continued to persist, including *Mahonia nervosa*, *Sambucus racemosa* and *Oemleria cerasiformis*. These observations suggest that a host-specific pathogen may be responsible for the phenomenon. Although previous efforts using culturing techniques and qPCR have been unable to identify a likely candidate (Kildisheva and Aghai, 2018), two recent transmission studies demonstrated that symptoms can be experimentally induced in healthy ferns (Mendenhall et al., 2019; Shannon, 2020b). Shannon (2020b) demonstrated that healthy sword ferns, planted in areas where the phenomenon is actively spreading, develop symptoms of the phenomenon after two years. Based on these findings, we hypothesize that a microorganism is responsible for the die-off phenomenon.

However, there are reasons to suspect that site conditions may contribute to the sword fern decline. Previous research has shown that the proximity of sword ferns to large diameter trees and coarse woody debris is correlated with higher survival rates in an area affected by the phenomenon (Shannon, 2019). Furthermore, soil bulk density was found to be higher in an area of Seward Park where a large mortality event occurred (Masaoka, 2017). Because soil compaction can limit the water-holding capacity of the soil (Archer and Smith, 1972), we also consider a competing hypothesis: sword ferns are declining due to seasonal drought stress, aggravated by local site conditions that influence soil moisture content.

Our study compares areas of Seward Park containing symptomatic ferns with areas predominantly composed of healthy ferns. We included Schmitz Preserve Park as an additional control due to it being another remnant old growth forest with a similar structure and composition as Seward Park. As these two parks are located approximately 11 km (7 mi) from each other, both occurring at low elevations and both surrounded by large bodies of water, we assume them to have a similar climate.

To test the pathogen hypothesis, our study uses a high-throughput sequencing approach to screen the endophytic microbial communities of sword ferns, with a focus on bacteria, fungi and oomycetes. This component of the study is ongoing. To explore the drought hypothesis, we compared the foliar moisture content of sword ferns with the moisture content of the sub-soil. We also analyzed soil bulk density.

The primary purpose of this report is to provide a detailed record of our methods so they may be adapted for future research and monitoring. We also present some preliminary results. We found that areas composed of healthy or symptomatic ferns had similar edaphic properties. Although symptomatic ferns do show signs of water stress, the phenomenon cannot be explained by a deficit in soil moisture.



Methods

Experimental Design

Our study compared forested areas of Seward Park containing sword ferns (*Polystichum munitum*) that were predominantly healthy or predominantly symptomatic. We included forested areas of Schmitz Preserve Park containing healthy sword ferns as an additional control. Together, these sites represent three experimental groups: i) Schmitz–Healthy, ii) Seward–Healthy, and iii) Seward–Symptomatic (Figure 1). We considered using a blocked design, however ferns with symptoms matching the phenomenon were generally not observed in Schmitz Preserve Park.

In each experimental group, we established 12 plots, each with an area of 100 m² (0.025 acres). At each plot, we surveyed the plant community, site conditions and noted personal observations. We collected tissue samples from sword ferns to measure foliar moisture content, and for amplicon sequencing on the Illumina MiSeq platform. We also collected soil samples for analyzing bulk density and texture.

		Park	
		Schmitz Preserve	Seward
Ferns	Healthy	1 Schmitz Preserve Healthy	2 Seward Healthy
	Symptomatic	NA	3 Seward Symptomatic

Figure 2: Experimental design.

Initially, we had intended on delineating a large area containing healthy sword ferns in Seward Park and comparing it with another area containing predominately symptomatic and/or dead ferns. However, during our preliminary reconnaissance, we observed dead and symptomatic ferns in nearly all areas of the park. The phenomenon has become so extensive in Seward Park that there is no longer a contiguous area with healthy sword ferns large enough for a survey like ours. Therefore, we adapted our protocol by developing a set of criteria for defining healthy and symptomatic areas.



Symptomatic plots were randomly placed in forested areas of Seward Park where: i) the understory plant community is dominated by native plants; ii) living sword ferns are present; and iii) more than 50% of the sword ferns have symptoms or have recently died.

We defined symptomatic ferns as those with any combination of the following criteria: i) crispate and/or contorted pinnae; ii) pinnae with atypical hues; iii) fronds with aborted or undeveloped pinnae at the distal end; iv) fronds with partial die-back at the distal end; v) fronds with a contorted rachis; and/or vi) a ratio of young fronds to old fronds less than 0.5 (Figure 9). We defined healthy ferns as lacking the above symptoms.

Healthy plots were randomly placed in forested areas of Seward Park and Schmitz Preserve park where: i) the understory plant community is dominated by native plants; ii) living sword ferns are present; iii) more than 50% of the sword ferns are healthy; and iv) less than 25% of the sword ferns have recently died. As an exception, plot 21 was mistakenly placed in an area where 28% of the sword ferns had recently died.

Thus, our plots in Seward Park are best interpreted as representing fragmented patches of healthy sword ferns, surrounded by a matrix of dead and symptomatic ferns.

Our survey should not be construed as being proportionally representative of the plant communities in these parks. As a result of our strict criteria for comparing healthy and symptomatic plots, our survey omitted non-forested areas of the parks, areas dominated by non-native species, areas where living sword ferns are no longer present due to the die-off phenomenon (Figure 11), and areas where sword ferns were not a component of the understory in recent history. Our criteria also resulted in the omission of areas with ambiguous or intermediate effects of the phenomenon such as isolated patches of healthy ferns in areas with high mortality or areas with low mortality and ambiguous symptoms.

As additional constraints, we avoided 6 long-term monitoring plots established by EarthCorps and 20 long-term monitoring plots established by researchers at the University of Washington. For safety reasons, we avoided overhead hazards and areas with slopes over 100%. We also avoided sampling within 10 m of the trails due to the ongoing pandemic.

Field Survey

Plots were surveyed by Dylan Mendenhall and Andres Barrera between August 27, 2020 and September 10, 2020. At every plot, we recorded site conditions and location data, including: survey date, latitude, longitude, horizontal accuracy, slope, slope position, aspect and canopy cover.

We used a modified Braun-Blanquet cover-abundance scale to visually estimate the abundance of each vascular plant species observed within 10 m of the ground (Braun-Blanquet et al., 1932; Mueller-Dombois and Ellenberg, 1974). Trees and non-vascular plants were not included in the cover-abundance survey. The mid-point values of the cover classes were used in subsequent analysis. We measured the diameter at breast height (i.e. bole diameter at 1.4 m above the ground) on the uphill side of every tree. Trees below 1.4 m in height (i.e. “seedlings”) were rarely observed and were not included in the survey.



Cover Class	Cover Range (%)	Cover Midpoint (%)
A	0 - 1	0.5
B	1 - 5	3
C	5 - 15	10
D	15 - 25	20
E	25 - 50	37.5
F	50 - 75	62.5
G	75 - 95	85
H	95 - 100	97.5

Table 1: Modified Braun-Blanquet cover-abundance scale.

Sample Collection and Preparation

Sample Collection

In each plot, a single fern was selected for tissue sampling using the criteria described above for distinguishing healthy and symptomatic ferns. Prior to sampling, we photographed the fern and noted general observations about its health.

We counted the total number of young and old fronds as a measure of the fern's recent productivity. We defined young fronds as those that were produced during the most recent spring and summer, and old fronds as those that were produced during any previous year. We used morphological, developmental, and physiological traits to determine their relative age. Sword ferns generally produce fronds in discrete age-cohorts, with a new set of fronds produced each year. Each set of fronds is generally retained for 2 to 3 years. As fronds only produce sori during their first year aboveground, we primarily used the presence of sori on the underside of the pinnae for identifying young fronds. We also interpreted the relative age of the fronds based on the abundance and orientation of the scales at the base of their stipes. During their first spring and summer aboveground, the stipes are covered by a dense layer of scales. Over time, the scales become more appressed to the stipe and gradually detach, presumably through senescence, abrasive action and rainfall. We interpreted fronds with more sparsely covered stipes and more appressed scales as having been produced during a previous year.

From each fern, we collected samples from the roots, rhizome, stipe and pinnae. We collected 20 random pinnae and placed them in non-sterile plastic bags. From a random frond, we collected a single section of the stipe, approximately 2 cm in length, and immediately placed it in a sterile microcentrifuge tube. We then excavated one of the rhizomes. Mature sword ferns are almost universally comprised of rhizomes with multiple branches. Thus, we were generally able to sample a single branch while leaving the rest of the plant undisturbed. We cut an approximately 10 cm section from the terminal end of the rhizome and placed it in a non-sterile plastic bag. We collected approximately 1 g of the root tissue, which was immediately placed in a sterile microcentrifuge tube. Tissue samples were stored at 4°C for approximately 24 hours before being transported the lab.



Soil samples were collected from the uppermost B horizon, approximately 5 to 10 cm below the mineral soil surface. For the primary soil sample, we aggregated five 200 ml sub-samples from random locations in the plot. From this aggregate sample, 200 ml was placed in a non-sterile plastic bag for subsequent analysis. We also collected a single bulk density sample using a core with a volume of 78.5 cm³, which was immediately placed in a sealed tin.

Sample Preparation

The stipe and root samples were stored in a freezer at -20°C without additional preparation. The pinnae and rhizome samples were stored at 4°C for another 2 weeks before further processing. Surface sterilization of the pinnae followed the methods of Younginger and Ballhorn (2017). Pinnae were then placed in sterile centrifuge tubes and stored in a freezer at -20°C.

Rhizomes were dissected in two stages. On an open bench, we used a razor to remove the remaining soil, stipes, and the outer layer of the rhizome. We removed a section approximately 5 cm long from the terminal end of the rhizome and transferred it to a laminar flow hood for further preparation. In the second stage, the rhizome was cut into smaller sections approximately 2 cm in diameter. The rhizome sections were surface sterilized in the same manner as the pinnae (Younginger and Ballhorn, 2017) with a final rinse in deionized water. The rhizome sections were then cut into small pieces approximately 5 mm in diameter and then aggregated together. Approximately 4 g of random pieces from the rhizome were placed in a sterile centrifuge tube and stored in a freezer at -20°C until further use.

All 36 rhizome samples and 4 pinnae samples (#25, #28, #33, #34) were shipped on dry ice to MPG Ranch for further laboratory analysis.

The primary soil samples were sieved to 2 mm and air-dried. Bulk density samples were oven-dried at 105°C to a constant weight. To measure foliar moisture content, 10 random pinnae were oven-dried at 95°C to a constant weight. Gravimetric moisture content was measured using the bulk density samples.

Statistical analysis

In our preliminary review of the data, we used analysis of variance (ANOVA) followed by a Tukey HSD test to compare the foliar moisture content, soil moisture content and soil bulk density of the three experimental groups. We used Pearson's correlation coefficient to compare soil moisture content with foliar moisture content. For all statistical tests, we used an alpha level of 0.05. All analyses were conducted using the R programming language (R Core Team, 2019).



Results

Anecdotal Observations

In Seward Park, we observed a wide range of morphological, developmental, and physiological traits associated with the die-off phenomenon, including: ferns with a low ratio of young to old fronds; fronds with a contorted rachis; fronds with crispate or contorted pinnae; fronds with an atypical hue; fronds that had not fully expanded at their distal end; and fronds that had partial die-back at their distal end after fully developing (Figure 9).

Some of the symptomatic ferns showed clear evidence of a gradual decline over multiple years. For example, we inferred that fern #33 produced an apparently healthy set of fronds in 2018, a set of discolored fronds in 2019 and no new fronds in 2020 (Figure 3).



Figure 3: Example of a symptomatic fern (#33) showing evidence of it declining over multiple years. Arrows indicate two distinct cohorts of fronds that presumably emerged in 2018 and 2019. No fronds were produced in 2020.



To the best of our knowledge, there is no evidence of widespread mortality among the sword fern population in Schmitz Preserve Park. We observed only one sword fern that had recently died (plot #4), which was readily explained by competition for soil moisture with a large diameter tree growing in close proximity. All other sword ferns in the plot appeared healthy.

On a small subset of the sampled ferns, we conducted an impromptu dissection in the field by cutting a longitudinal section through a rhizome. We found a set of fully developed leaf buds clustered around the apical meristem of the rhizome of all healthy ferns. In contrast, the symptomatic ferns generally had no leaf buds present, or in some cases, leaf buds that appeared to have withered after their initial development. We confirmed this observation in the lab, where we made longitudinal sections of individual leaf buds. Leaf buds from healthy ferns had a fully developed spiral shape. In contrast, leaf buds from symptomatic ferns failed to develop past their primordial stage, or appeared to have withered away after initially developing into a spiral.

As another pertinent observation, we found an Anderson's fern (*Polystichum andersonii*) in an area of Seward Park with over 50% mortality among sword ferns (*Polystichum munitum*). Despite being adjacent to several dead and symptomatic sword ferns, the Anderson's fern appeared relatively healthy. Its leaves did not have any symptoms associated with the die-off phenomenon and there was a set of fully developed leaf buds at the base of its fronds.

Preliminary Analysis

Mean foliar moisture content (FMC) of the pinnae was significantly different between the three experimental groups based on ANOVA ($F_{2,33} = 27.52$, $p < 0.001$). A post-hoc Tukey HSD test revealed the mean FMC of the pinnae was significantly lower in the symptomatic ferns at Seward Park ($0.98 \text{ g}\cdot\text{g}^{-1} \pm 0.11 \text{ SE}$) compared with the healthy ferns at both Seward Park ($1.67 \text{ g}\cdot\text{g}^{-1} \pm 0.07 \text{ SE}$, $p < 0.001$) and Schmitz Preserve Park ($2.11 \text{ g}\cdot\text{g}^{-1} \pm 0.14 \text{ SE}$, $p < 0.001$). The FMC of the healthy ferns in Seward Park was also significantly lower than the healthy ferns in Schmitz Preserve Park ($p = 0.039$).

The soil bulk density was significantly different between the experimental groups ($F_{2,33} = 5.122$, $p = 0.012$). The mean bulk density in the Schmitz–Healthy group was $0.96 \text{ g}\cdot\text{g}^{-1} \pm 0.04 \text{ SE}$, significantly lower than the bulk density of the Seward–Healthy group ($1.11 \text{ g}\cdot\text{g}^{-1} \pm 0.04 \text{ SE}$, $p = 0.081$) and the Seward–Symptomatic group ($1.17 \text{ g}\cdot\text{g}^{-1} \pm 0.03 \text{ SE}$, $p = 0.011$). However, the bulk density of the Seward–Healthy and Seward–Symptomatic groups were similar to each other ($p = 0.658$).

The gravimetric soil moisture content was similar between all three experimental groups ($F_{2,33} = 0.663$, $p = 0.522$), with means ranging from $0.10 \text{ g}\cdot\text{g}^{-1}$ to $0.12 \text{ g}\cdot\text{g}^{-1}$. Furthermore, the foliar moisture content of the ferns was not correlated with soil moisture content ($r = 0.07$, $p = 0.675$).



Figure 4: Longitudinal sections through the rhizomes of a healthy fern (left) and a symptomatic fern (right). Arrow indicates leaf buds that failed to develop on the symptomatic fern. Bars represent 5 cm.



Figure 5: Longitudinal sections through the dormant leaf buds of a healthy fern (left) and a symptomatic fern (right). Bars represent 2 cm.



Figure 6: Polystichum andersonii. This fern appeared relatively healthy despite being in close proximity to several dead and symptomatic sword ferns (Polystichum munitum). Arrow indicates a dead sword fern.



Foliar Moisture Content *Polystichum munitum*

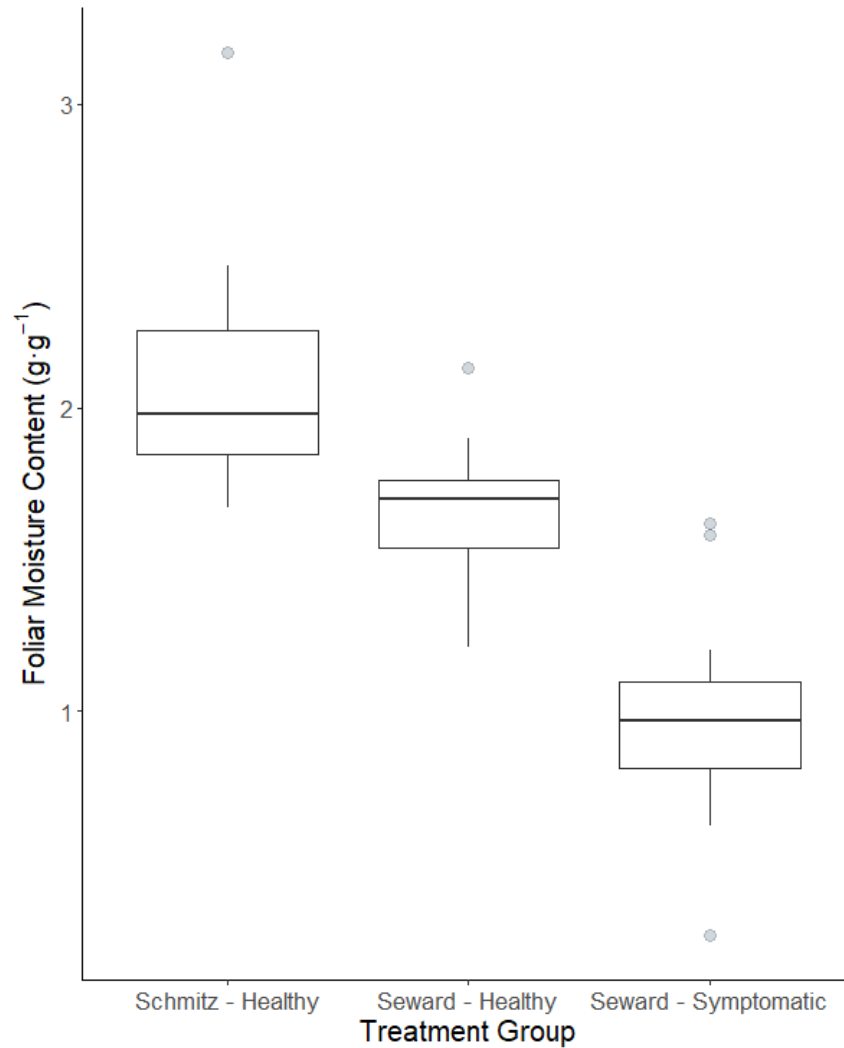


Figure 7: Foliar moisture content ($\text{g}\cdot\text{g}^{-1}$) of sampled *Polystichum munitum* ferns. Data are displayed as Tukey-style boxplots; horizontal bar represents the median value; box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to $1.5 \times \text{IQR}$; points represent outliers.



Soil Bulk Density

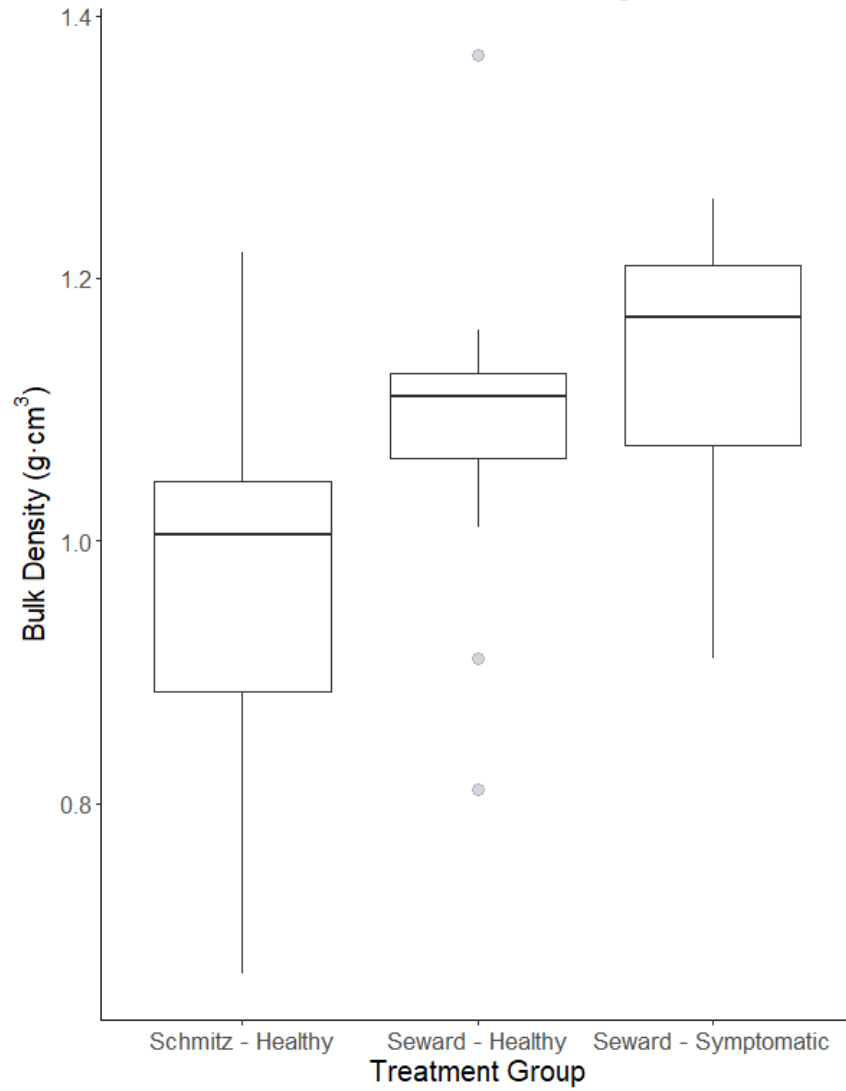


Figure 8: Soil bulk density ($\text{g}\cdot\text{cm}^{-3}$). Data are displayed as Tukey-style boxplots; horizontal bar represents the median value; box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to $1.5 \times \text{IQR}$; points represent outliers.



Discussion

Under the drought hypothesis, we predicted that differences in foliar moisture content (FMC) would be correlated with differences in soil moisture content. We expected these patterns to be particularly evident during the dry season when we collected our samples. During this season, the chlorophyll fluorescence values (F_v/F_m) of sword ferns decline in parallel with lower soil moisture content (Kildisheva et al., 2018). These changes in chlorophyll fluorescence are likely an indication of water stress (Maxwell and Johnson, 2000). In our study, FMC was indeed lower in symptomatic ferns compared with healthy ferns. However, this pattern was not correlated with differences in soil moisture content, which was similar between the three experimental groups. These results do not support the drought hypothesis.

Our results are consistent with the findings of two previous studies. In a greenhouse experiment, cut fronds that were exposed to symptomatic tissue developed significantly lower FMC compared to controls exposed to healthy sword fern tissue (Mendenhall et al., 2019), which suggests that a transmissible agent obstructed the vascular tissue. Likewise, in a field study based in Seward Park, symptomatic ferns had significantly lower midday xylem water potential compared with healthy ferns found elsewhere in the park (Goldstein-Miller et al., 2019). These patterns could potentially be explained by xylem cavitation or tylose formation induced by a vascular pathogen, which can result in symptoms that are similar in appearance to drought stress (Yadeta and Thomma, 2013).

The effect of this phenomenon on the FMC of *Polystichum munitum* has potential implications for wildfire management in the Western Cascades. The length of the active burning season and the behavior of wildfires are partially influenced by the foliar moisture content of understory plants (Agee et al., 2002). For example, the back rate of fire spread is controlled by the moisture content of surface fuels (Fernandes et al., 2009; Rossa, 2017). In the current study, many of the symptomatic ferns had FMC values well below $1.00 \text{ g}\cdot\text{g}^{-1}$ (100%), a critical threshold for wildfire behavior in Mediterranean shrublands and forests (Nolan et al., 2020, 2016). For areas affected by the sword fern die-off phenomenon, predictive models of fuel loading may need to be adjusted to account for unusually low moisture content in the surface fuels.

In our study, the soils associated with symptomatic ferns and healthy ferns in Seward Park had a similar bulk density, which conflicts with the results of a previous study in which soil bulk density was found to be significantly higher in an area of Seward Park affected by the phenomenon (Masaoka, 2017). This discrepancy is likely due to differences in our sampling approach. Masaoka (2017) focused on sampling soils from a relatively confined area of Seward Park where a mortality event occurred in 2013 (i.e. “ground zero”). In contrast, our current study used plots that were dispersed over a wider area, which are therefore more representative of the phenomenon. Nonetheless, we agree with Masaoka’s overall interpretation; soils with bulk densities in the range observed are unlikely to restrict plant growth.

We did find significant differences in bulk density between Schmitz Preserve Park and Seward Park, which is unsurprising given that these soils likely developed from different parent materials. Based on our personal observations of the soil texture in the A and B horizons, the areas that we surveyed in Schmitz Preserve Park are consistent with the Indianola series, which is characterized by deep loamy sands that developed from glacial outwash (Soil Survey Staff, 2009). In contrast, the soils of Seward Park are predominantly silty loams and gravelly loams, which likely developed from glacial till (Hanson and Davidson, 2005). As the surrounding lake was formed by a receding glacier (Sherwood, 1973), lacustrine



deposits may have also influenced the parent materials of Seward Park, which explains the relatively silty texture of soils that we encountered. We speculate that the soils of Seward Park may represent an intergrade between the Alderwood and Kitsap series (Soil Survey Staff, 2018a, 2018b), which are strongly associated with each other in King County (Snyder et al., 1973).

Because the soils of Schmitz Preserve Park are more coarsely textured, and because the bulk density is significantly lower, we would expect Schmitz Preserve Park to have better drainage than in Seward Park. As a result, the effects of seasonal drought stress should be more pronounced in the upland areas of Schmitz Preserve Park compared with Seward Park. However, the FMC of the Schmitz–Healthy group was significantly higher than the Seward–Healthy group, despite the soil moisture content being similar. This observation leads us to believe that the die-off phenomenon may be so widespread in Seward Park that visual assessments may not be entirely reliable for identifying “healthy” ferns, except in terms of their relative differences. Future studies would be cautioned against using any areas of Seward Park as a reference site for healthy sword ferns.

Our most consequential finding may be the accidental discovery of a lone *Polystichum andersonii* fern in an area of Seward Park where the die-off phenomenon was especially severe. *Polystichum andersonii* is thought to be a tetraploid hybrid between *P. munitum* and *P. kwakiutlii* (Hitchcock and Cronquist, 2018). As these species are closely related to each other, this observation may suggest that the purported pathogen is host-specific to *Polystichum munitum*. Future research is needed to evaluate the susceptibility of other *Polystichum* species that are native to the Pacific Northwest, such as *P. imbricans* and *P. californicum*. Identifying a resistant *Polystichum* species would provide an option for partially restoring the understory structure in areas affected by the phenomenon.

Lastly, it was clear from our personal observations in Schmitz Preserve Park that mature stands of sword fern have a low mortality rate under normal circumstances. The rapid decline of sword ferns in Seward Park cannot be attributed to the typical succession of old growth forests.

Conclusions

The sword fern decline at Seward Park is far from an isolated phenomenon; new die-offs continue to be observed at other locations throughout Western Washington. Although our study is ongoing, our preliminary analysis explored the hypothesis that the phenomenon is primarily caused by drought stress. More specifically, we predicted that foliar moisture content (FMC) would be lower in symptomatic ferns and that these differences would correlate with soil moisture content. We also expected differences in soil bulk density to be associated with the phenomenon by moderating the soil water holding capacity.

Although we did find significantly lower FMC in the symptomatic ferns, these differences could not be explained by differences in soil moisture content or soil bulk density. Our results suggest that the phenomenon is driven by a factor that is independent from soil water availability. Our preliminary results are consistent with the pathogen hypothesis.

Our study raises concerns about the potential effects of the phenomenon on wildfire behavior, particularly in areas of the Olympic Peninsula and the Western Cascades where the understory is



dominated by *Polystichum munitum*. As another implication of our research, we identified *Polystichum andersonii* as a candidate species for restoring the understory structure of forests affected by the sword fern die-off phenomenon. However, further research is needed to confirm its resistance.

In the next stage of our study, we will use a metabarcoding approach to identify potential bacteria, fungi or oomycetes that may be responsible for the phenomenon, building on previous research that identified fungal endophytes in the pinnae of sword ferns (Younginger and Ballhorn, 2017). Further research would be needed to understand how the pathogen is transmitted and its mechanism for inducing water stress, among other symptoms. As a potential limitation, our amplicon sequencing approach will not be able to identify RNA viruses, which are known to affect other fern species (Valverde and Sabanadzovic, 2009). It also remains possible that the phenomenon is caused by changes in the life cycle or behavior of an insect vector, rather than through the introduction of a non-native pathogen.

In the absence of a discrete die-off event, the phenomenon is difficult to detect, even while it continues to spread through the understory. Many of the symptoms are identical to the temporary effects of seasonal drought. However, repeated observations at the same location can reveal the rapid decline of sword ferns over time. We recommend that land managers establish monitoring programs to evaluate potential changes in their local sword fern populations and to prioritize areas for management.

Acknowledgements

We gratefully acknowledge the support of our collaborators, Ylva Lekberg, Mary Ellyn DuPre and all staff at MPG Ranch. We thank Friends of Seward Park for commissioning this study and for their ongoing work to preserve, restore and celebrate Seward Park. We received valuable information and support from Tim Billo at the University of Washington, Kim Frappier at Mercer Island Parks and Recreation, and Lisa Ciecko at Seattle Parks and Recreation. We also thank David Perasso for educating us about the biology and life history of sword ferns. This project was partially funded through a grant from 100 Women Who Care. Lastly, we acknowledge that our study sites are located in the historical, traditional, and ancestral territory of the Duwamish Tribe.



References

- Agee, J.K., Wright, C.S., Williamson, N., Huff, M.H., 2002. Foliar moisture content of Pacific Northwest vegetation and its relation to wildland fire behavior. *Forest Ecology and Management* 10.
- Archer, J.R., Smith, P.D., 1972. The Relation Between Bulk Density, Available Water Capacity, and Air Capacity of Soils. *Journal of Soil Science* 23, 475–480. <https://doi.org/10.1111/j.1365-2389.1972.tb01678.x>
- Baer, A., Wheeler, J.K., Pittermann, J., 2016. Not dead yet: the seasonal water relations of two perennial ferns during California’s exceptional drought. *New Phytologist* 210, 122–132. <https://doi.org/10.1111/nph.13770>
- Braun-Blanquet, J., Fuller, G.D., Conard, H.S., 1932. *Plant Sociology: The Study of Plant Communities*. McGraw-Hill.
- Brodersen, C.R., Rico, C., Guenni, O., Pittermann, J., 2016. Embolism spread in the primary xylem of *Polystichum munitum* : implications for water transport during seasonal drought: Embolism spread in *Polystichum munitum*. *Plant, Cell & Environment* 39, 338–346. <https://doi.org/10.1111/pce.12618>
- Doughton, S., 2019. The Case of the Vanishing Ferns: Citizen sleuths can’t figure out what’s killing Seattle’s sword ferns [WWW Document]. *The Seattle Times*. URL <https://www.seattletimes.com/pacific-nw-magazine/whats-killing-our-sword-ferns/> (accessed 9.9.19).
- Fernandes, P.M., Botelho, H.S., Rego, F.C., Loureiro, C., 2009. Empirical modelling of surface fire behaviour in maritime pine stands. *Int. J. Wildland Fire* 18, 698–710. <https://doi.org/10.1071/WF08023>
- Goldstein-Miller, C., Ramirez, A.R., Kildisheva, O., Cieko, L., 2019. *Polystichum munitum* (Western Sword Fern) Decline in the Pacific Northwest. Report.
- Hanson, T., Davidson, E., 2005. *Seward Park Vegetation Management Plan*. Seattle Parks and Recreation.
- Hitchcock, C.L., Cronquist, A., 2018. *Flora of the Pacific Northwest*, 2nd ed. University of Washington Press.
- Kildisheva, O., Aghai, M., 2018. *Seward Park Sword Fern Decline Strategic Plan*. Seattle Parks and Recreation.
- Kildisheva, O., Canup, K., Schwartz, N., Billo, T., Aghai, M., 2018. *Sword Fern Ecophysiology Report 2018 - Seward Park, WA* 17.
- Masaoka, G., 2017. *Investigation of the Western Sword Fern Disappearance in Seward Park*. University of Washington, Seattle, WA.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany* 51, 659–668. <https://doi.org/10.1093/jexbot/51.345.659>
- Mendenhall, D., Elliott, M., Shannon, P., 2019. *Sword ferns from the Seward Park die-off site can induce wilting and obstruct water uptake in healthy ferns*. Report.
- Mueller-Dombois, D., Ellenberg, H., 1974. *Aims and Methods of Vegetation Ecology*. Wiley, New York.
- Nolan, R.H., Blackman, C.J., de Dios, V.R., Choat, B., Medlyn, B.E., Li, X., Bradstock, R.A., Boer, M.M., 2020. Linking Forest Flammability and Plant Vulnerability to Drought. *Forests* 11, 779. <https://doi.org/10.3390/f11070779>



- Nolan, R.H., Boer, M.M., Dios, V.R. de, Caccamo, G., Bradstock, R.A., 2016. Large-scale, dynamic transformations in fuel moisture drive wildfire activity across southeastern Australia. *Geophysical Research Letters* 43, 4229–4238. <https://doi.org/10.1002/2016GL068614>
- R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rossa, C.G., 2017. The effect of fuel moisture content on the spread rate of forest fires in the absence of wind or slope. *Int. J. Wildland Fire* 26, 24–31. <https://doi.org/10.1071/WF16049>
- Shannon, P., 2020a. Regional Map [WWW Document]. URL http://pshannon.net/swordFerns/regionalMap/2020_alSmith.html (accessed 12.2.20).
- Shannon, P., 2020b. 5-year sword fern survival experiment: 26 months along, strong results (Email).
- Shannon, P., 2019. Seward Park Sword Fern Die-off: Is there a pattern to the 10% sword fern survival at Ground Zero? Seward Park Sword Fern Die-off. URL <https://sewardparkswordferndieoff.blogspot.com/2019/09/is-there-pattern-to-10-survival-at.html> (accessed 1.27.20).
- Sherwood, D., 1973. Seward Park, Sherwood History Files. Seattle Parks and Recreation.
- Snyder, D.E., Gale, P.S., Pringle, R.F., 1973. Soil Survey of King County Area, Washington. Soil Conservation Service, USDA.
- Soil Survey Staff, 2018a. Kitsap Series, Official Soil Series Descriptions. Natural Resources Conservation Service, USDA.
- Soil Survey Staff, 2018b. Alderwood Series, Official Soil Series Descriptions. Natural Resources Conservation Service, USDA.
- Soil Survey Staff, 2009. Indianola Series, Official Soil Series Descriptions. Natural Resources Conservation Service, USDA.
- Valverde, R.A., Sabanadzovic, S., 2009. A novel plant virus with unique properties infecting Japanese holly fern. *J. Gen. Virol.* 90, 2542–2549. <https://doi.org/10.1099/vir.0.012674-0>
- Yadeta, K., Thomma, B., 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. *Front. Plant Sci.* 4. <https://doi.org/10.3389/fpls.2013.00097>
- Younginger, B.S., Ballhorn, D.J., 2017. Fungal endophyte communities in the temperate fern *Polystichum munitum* show early colonization and extensive temporal turnover. *Am. J. Bot.* 104, 1188–1194. <https://doi.org/10.3732/ajb.1700149>



Appendix



Figure 9: Signs and symptoms of the sword fern die-off phenomenon: A) reduced production of new fronds; B) contorted rachis; C) crispate and contorted pinnae; D) discolored pinnae; E) underdeveloped and aborted fronds; F) die-back at distal end of a frond. In areas affected by the phenomenon, these signs and symptoms can occur in any combination and vary in severity.



Figure 10: Example of an individual sword fern that died in Upper Luther Burbank Park on Mercer Island. Some of the nearby sword ferns did not have symptoms, despite their close proximity.



Figure 11: Example of an area in Seward Park previously dominated by sword ferns. Areas with 100% mortality could not be included in our survey. Arrows indicate the remnants of dead sword ferns.



Figure 12: Frond from an apparently healthy fern (left) compared with a frond from a symptomatic fern (right) in Upper Luther Burbank Park on Mercer Island.