

Seward Park Sword Fern Decline Strategic Plan



Sword Fern Decline Working Group
Seattle Parks and Recreation | Green Seattle Partnership
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Acknowledgements

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SECTION 1. EXECUTIVE SUMMARY

The decline or complete mortality of previously healthy stands of western sword fern (*Polystichum munitum*) have been reported throughout the Puget Sound Region of the western U.S. in the course of the past decade. One of the most iconic events occurred in 2013 at Seward Park, which led to the complete mortality of large stands of sword fern within the park, with no subsequent regeneration. By 2016, the affected area had grown to fifteen acres, heavily impacting the oldest portions of the forest. Symptoms of the decline begin with foliar browning, rotting, and discoloration, which eventually lead to mortality of the entire crown, leaving fern stumps that do not regenerate. These symptoms differ from the characteristic post-drought browning or flattening following winter snowpack.

This rapid decline of an iconic understory plant prompted a formation of the Sword Fern Decline Working Group (SFDWG) to address two key goals: (1) to determine the cause and drivers of the decline and (2) to identify effective strategies needed to restore and maintain the health of the native plant community. To date, members of the SFDWG (in partnership with local universities, municipalities, researchers, students, and park volunteers) have carried out 17 projects in the areas of mapping and monitoring, pathology and edaphic factors, vertebrate drivers, ecophysiology, morphology, and experimental restoration, with some projects currently ongoing.

The Strategic Plan provides a roadmap that communicates the current status and known needs for understanding and addressing the decline of western sword fern at Seward Park and the greater Puget Sound Region. This document provides background on western sword fern ecology and natural history, discusses population threats, provides a summary of all projects and actions of the SFDWG, synthesizes relevant findings, and proposes strategic objectives as a means of project prioritization in 2019.

The proposed objectives were largely synthesized from meetings and discussion among SFDWG members, municipal program managers, and consultants during 2018 and include:

1. Monitoring and Mapping

Quantifying the spread and distribution of sword fern decline is critical to understanding and managing the effects of this phenomenon. Strategies to achieve this include vegetation monitoring (fixed plots, Vegetation Management Plan re-survey) and regional mapping (iNaturalist, photo monitoring, and integrated methodology development).

2. Multi-disciplinary Research

Understanding the potential causes of the decline will require continued multi-disciplinary scientific collaboration. Strategies to accomplish this goal include continued work on pathogen identification (investigation of soil and waterborne transmission potential, eDNA metabarcoding), ecophysiological assessment of sword fern health (comparative analysis of healthy and affected populations, evaluation of survival potential of re-planted sword fern), multivariate analysis of biotic and abiotic factors (across healthy and affected populations), qualitative characterization of the decline (microscopy and photography), and the development of evidence-based restoration strategies.

3. Active Management and Restoration

The decline of sword fern in Seward Park is one of many pressing issues requiring ongoing management in Seattle City Parks. Management strategies should facilitate the protection of intact, non-impacted sword fern stands and restore affected sites with native understory vegetation using

evidence-based rationale for the proposed management actions.

4. Prevention and Mitigation

While a pathogen has not been identified as the definitive cause of the decline, pathogen-driven plant declines, are having a profound negative impact on many ecosystems in the Pacific Northwest, especially under increased environmental stress. In addition to native pathogens, novel or exotic pathogens introduced through restoration activities and visitor traffic can pose an additional risk to native vegetation. To mitigate the potential effects of pathogens, a precautionary approach can be beneficial in the context of park use and restoration work conducted on park property. These strategies include nursery stock tracking, decline severity risk map, and the development of risk mitigation protocols.

To support the proposed and future work of the SFDWG, the Strategic Plan outlines future priority efforts with regard to outreach and public engagement, which include building guidelines for communication planning, individual outreach, soliciting municipal and external support, as well as establishing institutional partnerships. To provide streamlined and efficient progress in 2019 and beyond, the Strategic Plan also includes a Working Group Charter, which outlines the group's structure, function, data management, communication expectations, and volunteer tracking.

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SECTION 2. INTRODUCTION

2.1. Strategic Plan Purpose

The document aims to inform the reader on the status of an ongoing phenomenon first observed in Seattle's Seward Park (and now more broadly), which has resulted in mortality of large sword fern populations. The following presents a comprehensive overview of past work and current endeavors of the Sword Fern Decline Working Group (SFDWG). Additionally, the document provides updated information drawn from peer-reviewed scientific literature on the known ecology, physiology, and pathology of sword fern (*Polystichum munitum*). The Strategic Plan describes. This document provides background on western sword fern ecology and natural history, discusses population threats, provides a summary of all projects and actions of the SFDWG, and synthesizes relevant findings. To address two key SFDWG goals of determining the source of the decline and identifying effective restoration and mitigation strategies, the plan lays out four strategic objectives as a means of project prioritization in 2019.

2.2. Sword Fern Decline Phenomenon

In the course of this decade, observations of declines (or die-off) of western sword fern have been reported throughout the Puget Sound Region of the western U.S. The majority of early and/or regional reporting was based on observations of land managers and concerned citizens. One of the most iconic events occurred in 2013 at Seward Park, with a rapid onset of the decline, which resulted in the complete mortality of large stands of sword fern plants with no subsequent regeneration (Figure 2.1). After an assessment in 2016, the affected area had grown to fifteen acres, heavily impacting the oldest portions of the interior forest that includes a stand of 150+ year-old trees (Figure 2.2). Symptoms of the decline begin with foliar browning, rotting, and discoloration, which differ from browning following drought or flattening following snowpack. As symptom severity increases, it eventually leads to mortality of the entire crown, leaving fern stumps that do not regenerate (Appendix 2.1). It is important to note that this decline is occurring in tandem with the decline of other iconic northwest forest species such as bigleaf maple (*Acer macrophyllum*), Pacific madrone (*Arbutus menziesii*), and Western redcedar (*Thuja plicata*) (Littell et al., 2010; McGregor et al., 2016; Rippey, 2018; Tobin, 2016).



Figure 2.1. Before (2011) and after (2017) photos showing the same location at Seward Park, Seattle, WA. Following the near-complete loss of sword fern populations in the understory. Photo Credit: (left) Jordan Jackson, (right) Paul Shannon



Figure 2.2. Distribution of the sword fern decline throughout Seward Park, Seattle, WA as of October 2017. The severity of the affected area varies from extreme (complete die-off) to mild (some effected ferns among a still extant understory).

2.3. Seward Park Background

Located in southeast Seattle, Seward Park is one of the city’s oldest and most-loved parks. Since its establishment in 1911, Seward Park has continued to serve as an urban refuge of native flora and as a center for recreation activities for Seattle’s residents and visitors. Spanning ca. 300 acres, Seward Park provides urban habitat for numerous species of plants and animals, serves as a critical component of south Seattle’s ecological history, and boasts some of the last remaining “old growth” trees within city limits (Hanson and Davidson, 2005).

Since the park’s establishment, Seattle’s population has grown 3-fold (Chisholm, 1910; Seattle Population, 2018), which has increased the parks’ use. Although in-line with park management goals (Hanson and Davidson, 2005), increased visitor use is associated with higher risk of disturbance and

pathogens introductions, which can severely impact the park's ecological health, especially in sensitive areas already experiencing plant decline. Maintaining the ecological health of Seward Park is essential to its role as an aesthetic, recreational, social, and environmental asset to the Seattle community and the increasingly populated metropolitan area.

2.4. Green Seattle Partnership Background

The Green Seattle Partnership (GSP) is a public-private venture to restore 2,500 acres of parklands across Seattle by 2025. The City and community commitment to improving the health of Seward Park is reflected in the stewardship activities. Seattle Parks and Recreation, individuals, organizations, and donors have dedicated significant funding and time to the restoration of Seward Park over the last 15 years. GSP work records show 40,426 volunteer hours, 23,709 professional crew hours, and 48,153 plants installed since 2005 (CEDAR, 2018). While the decline of sword fern at Seward and other city parks threatens the success of the restoration, it also provides an opportunity to examine forest disturbance and restoration strategies in the context of a well-loved urban park.

2.5. Working Group Background

To address the growing concerns surrounding the decline of sword fern populations within Seward Park and other regional natural areas, a number of park volunteers, concerned citizens, scientists, and land managers have come together to form the Sword Fern Decline Working Group (SFDWG). The group's mission and involvement has grown since its formation in 2015 and is outlined in detail in Section 5 of this document.

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SECTION 3. RESEARCH

3.1. Literature Review

3.3.1. Natural History of Western Sword Fern (*Polystichum munitum*)

Distribution and Habitat

Polystichum munitum (Kaufl.) Presl (Dryopteridaceae) or Western sword fern is a dominant understory species native to the coniferous and moist-mixed forests of the western United States, with distribution also extending into Canada and Mexico (Halverson, 1986; Hitchcock and Cronquist, 1973; Scoggan, 1978). In the Pacific Northwest, it occurs in association with Douglas fir – hemlock – cedar forest communities (Figure 3.1).

Although tolerant of a range of conditions, from the coastal fog belt to subalpine systems, it is generally an indicator of mesic, nutrient-rich, and productive sites (Agee and Huff, 1987; Halverson, 1986; Hemstrom and Logan, 1986; Klinka, 1977; Leshner and Henderson, 1989). The species is tolerant of an array of light conditions (2 to 40%) (Waring and Major, 1964), but low light facilitates optimal growth of this species. Consistent moisture (Halverson, 1986; Klinka, 1977; Leshner and Henderson, 1989; Pojar et al., 1991) and limited disturbance (Fonda and Bernardi, 1976) conditions can support robust sword fern stands. However, this species is also able to tolerate disturbances (e.g. logging) and occasionally persists on moderately disturbed sites (Cody and Britton, 1989; Shatford et al., 2003). The ability of this species to tolerate disturbance is likely linked to the interplay between light and moisture availability on a site.



Figure 3.1. Western sword fern (*Polystichum munitum*) distribution in the United States and Canada. Green designates states in which the species is considered native or naturalized.

Life History

Western sword fern reproduction occurs primarily through sexual means. Ferns reach reproductive age after 1-5 years, with little inter-annual variation in spore production (Haeussler et al., 1990). The sporangia dehisce, releasing wind-dispersed spores in early to mid-summer. Some spores may be retained over winter and released in late spring (Haeussler et al., 1990), although spore viability under these conditions has not been studied extensively. While vegetative regeneration after crown decline (e.g. following fire) occurs readily from rhizomes in healthy sword fern populations (Soltis and Soltis, 1987), it does not facilitate population spread (Coates and Haeussler, 1986).

Generally, populations consist mostly of mature plants, with a small proportion of juveniles, and the rare occurrence of gametophytes. Evidence from populations in the Olympic Peninsula suggests that 95% of individual ferns originate from an individual rhizome (Lezberg et al., 1999). Even in monoculture stands of sword fern, the genetic heterogeneity is likely high (Haeussler et al., 1990). While descriptions of the genetic population structure of sword fern stands across the Pacific Northwest is limited, some evidence suggests that inter-population gene flow is high (comparable to wind-pollinated gymnosperms) and populations contain a large degree of genetic variation (Soltis and Soltis, 1987).

The evergreen fronds of sword fern plants exhibit determinant growth and persist for several years, with older growth often appearing flattened following snowpack and frost (Baer et al., 2016; Coates and Haeussler, 1986). At the start of the growing season, in healthy Western sword fern populations, new fronds emerge from rhizomes (Coates and Haeussler, 1986). Similarly, sword fern crowns can regenerate following disturbance (e.g. fire, herbivory) (Cromack et al., 1979). Recent evidence suggests that despite a preference for mesic conditions, sword fern plants are well adapted to seasonal drought, through their ability to control stomatal aperture and to recover from cavitation following soil water recharge. However, sword fern roots do not extend below 15 cm, thus limiting the plant's ability to access water deeper in the soil profile during the prolonged drought periods, which may ultimately result in losses of cavitation-damaged fronds (Baer et al., 2016).

Ecological Value

Western sword fern is a critical food source for mountain beaver (*Aplodontia rufa*) during their entire life cycle (Fellers et al., 2004; Halverson, 1986; Hemstrom and Logan, 1986). This species also provides habitat for more than 15 species of birds (Wiens and Nussbaum, 1975), including the northern spotted owl (North et al., 1999), and Pacific ruffed grouse (Salo, 1978). Additionally, ferns, like other understory species can shape the composition of plant communities by serving as ecological filters (George and Bazzaz, 1999) and influencing nutrient cycling patterns within the soil profile (Allison & Vitousek, 2004).

3.1.2. General Population Threats

Environment

Historically, increased environmental stress has been a robust pre-cursor to increased susceptibility and population-wide decline among plant species (Anderson et al., 2004). Climate change and urbanization over the past several decades have made Pacific Northwest forests more susceptible to pathogens and disturbance due to an increase in the pronounced summer drought period (Dalton, 2013), higher air and soil temperatures, as well as greater disturbance due to increased human use. Operating under a continuous and unprecedented level of environmental stress makes native plants more susceptible to pathogen and insect risk (see below). This is evidenced by recent observations of native vegetation declines, such as that of the bigleaf maple (*Acer macrophyllum*), Pacific madrone (*Arbutus menziesii*), and Western redcedar (*Thuja plicata*) (Littell et al., 2010; McGregor et al., 2016; Rippey, 2018; Tobin, 2016).

Pathogens

Ferns are susceptible to most diseases that affect flowering plants (angiosperms), which may be caused by: bacteria (e.g. *Pseudomonas cichorii* and *P. gladioli*), fungi (e.g. *Alternaria*, *Calonectria*, *Colletotrichum*, *Completoaria*, *Cylindrocladium*, *Fusarium*, *Phyllosticta*, *Rhizoctonia*, *Milesia*, and *Taphrina* species), oomycetes (e.g. *Pythium* species), nematodes (e.g. *Aphelenchoides fragariae*, *Pratylenchus*, *Fumago* and *Teichospora* species), as well as numerous invertebrates (Chase et al., 1984; McCuiston et al., 2007; Sandeno, 1962; Strandberg, 2001). Specific fern pathogens have not been extensively examined, with most investigations limited to the

context of the horticultural industry. However, one study assessed key sword fern pathogens across natural stands throughout the coastal range of western Oregon (Sandeno, 1962). The most critical disease that affected natural stands of western sword fern was dry rot, which led to the appearance of dark discoloration of fern foliage and the desiccation of entire fronds. The disease was thought to develop following the impact of a successive compendium of organisms, which included fungi (*Taphrina faulliana* and *Fusarium* sp.) and a bacterium (*Pseudomonas* spp.). The foliar discoloration induced by dry rot was most prevalent through the summer months and initially appeared around the yellow spherical spots caused by *Taphrina faulliana* damage; however, the spread of foliar symptoms through sword fern stands (and in floricultural storage) was likely associated with *Fusarium* and *Pseudomonas* species. This work highlights the synergistic interplay that exists between pathogens in the natural environment.

Given the likelihood of introduction of novel pathogens to natural forest stands through restoration work (Jung et al., 2016; Rooney-Latham et al., 2015; Swiecki and Bernhardt, 2016), it is possible for novel or other complex pathogen interactions to develop into new plant diseases, and to first become evident in highly managed areas like urban parks. For example, in another report, an invasive soilborne water mold (*Phytophthora cinnamomi*) was isolated from roots, crowns, and fronds of declining sword fern plants and surrounding soil. *P. cinnamomi*, thought to have been introduced via rhododendron nursery stock, can cause root rot, which in sword fern results in foliar wilting and the eventual collapse of the plant (Tidwell et al., 1984).

Herbivores

Although sword fern is a valuable resource for a suite of wildlife species, it may also be susceptible to increased predation by herbivores if their populations rely on the species as a primary food source or consume it during critical portions of the plant's growing season, especially if sword fern populations are experiencing increased stress (Maser, 1998; Verts and Carraway, 1998). Herbivores may serve as direct pathogen vectors (Bashan, 1986) or contribute indirectly by altering the composition of entire ecological communities (Borer et al., 2009). For example, mountain beaver (*Aplodontia rufa*) and other resident mammals may cause substantial soil disturbance and come in direct contact with fern roots and aboveground tissue. Additionally, the ability to travel relatively long distances may promote the rapid spread of pathogens.

3.2. Overview of SFDWG Efforts

3.2.1. Summary of Research

Western sword fern (*Polystichum munitum*) once composed the dominant part of the understory and accounted for 23% of the herbaceous vegetative cover in Seward Park (Hanson and Davidson, 2005). By 2017, many well-established populations had been lost completely, while others declined by nearly a third. The sudden decline of this species serves as a reminder of the fragility of plant communities and the need to allocated resources to their continued monitoring and management.

With the growing pressure to understand the cause and drivers of the decline, SFDWG has initiated a number of research and investigative efforts, with many still ongoing. To date, the investigative efforts have not identified the cause of the mortality events, but point to the presence of a complex array of drivers that likely play a role in the decline. However, one theme that has become evident is that the sword fern decline is severe and likely extends across the state of Washington (Appendix 3.12). The following are an overview of all past and current projects associated with the SFDWG. A map (Figure 3.2) of all physical sword fern experiments is included along with [a direct link to the updated progress tracker](#). Relevant associated reports, data sets, and project materials have been systematically categorized and presented in the appendices.

Seward Park - Monitoring Overview

Sword Fern Decline Monitoring Plots and Transects



Figure 3.2. Map of physical location of experimental efforts within Seward Park at February 2018. The map does not include restoration planting installed at Ground Zero in fall of 2018.

i. Mapping and Monitoring the Western Sword Fern Decline

Project Title:	Assessment and monitoring of western sword fern (<i>Polystichum munitum</i>) and a description of the phenomenon decline with fixed plots
Date:	September 2015 - Current
Principal Investigator(s):	Tim Billo, University of Washington; timbillo@uw.edu
Project Contributor(s):	Kramer Canup, EarthCorps; kramer@earthcorps.org Paul Shannon, Friends of Seward Park; pshannon@systemsbiology.org Nelson Salisbury, EarthCorps; nelson@earthcorps.org Natalie Schwartz, University of Washington; nms35@uw.edu Tristan O'Mara, University of Washington; na
Research Questions & Objectives:	Is the area of decline spreading or slowly recovering? What percent of ferns are affected or dead, and is this changing? How do individual ferns change over time?
Methods:	<p>Twenty 3 × 3 m plots were randomly selected from within and around the boundary of the 2015 mapped die-off polygon (Appendix 3.1, Figure 1). Within each plot, ferns were identified and tagged at the individual level to enable repeated monitoring over time. All plots were surveyed in November 2015, May 2016, October 2016, and Fall 2017. In 2018, the network of plots was expanded and folded into a larger research endeavor that incorporated morphological and physiological assessments of ferns (Figure 3.2). Expansion of the plot network was designed to incorporate a wider scope spatially and across “healthy” and “impacted” populations of sword fern.</p> <p>During each survey, a team counted the number of live fronds on each individual fern. Qualitative estimations of desiccation [classified as % browning] were determined for the foliage of each fern (Appendix 3.1, Figure 2). Individual ferns were considered “alive” if they had any green aboveground tissue.</p> <p>To estimate boundary of the decline, Paul Shannon and Nelson Salisbury surveyed and mapped (coarse resolution) the perimeter of the zone experiencing the decline (Appendix 3.1, Figure 3).</p>
Results:	In 2015, 3% of ferns were completely dead, while by 2016 the number of dead ferns increased to 30%. (Appendix 3.1, Figures 4-7). Additionally, there was a 47% decrease in number of live fronds per fern over the same 1-year period. The 2017 results show a continued net loss of ferns, but with increased recovery rates (new fronds) observed in some plots (28 of 120 ferns showing new fronds in 2016-17, vs. 9/120 showing new fronds in 2015-2016).
Project Status:	Ongoing through 2017, with summary data available in the drive or via correspondence with PI. Because current plots are not fully representative of the sword fern population within the park, additional plots should be established to continually capture the distribution of the decline throughout the park.
Resources & External Links:	https://timbillo.wordpress.com/2017/05/26/seward-park-fern-die-off-update/

Project Title:	Mapping sword fern decline at Seward Park
Date:	2015-2017
Principal Investigator(s):	Nelson Salisbury, EarthCorps; nelson@earthcorps.org
Project Contributor(s):	Paul Shannon, Friends of Seward Park; pshannon@systemsbiology.org
Research Questions & Objectives:	Is the area affected by the decline spreading and to what extent?
Methods:	To estimate the initial boundary of the decline, Paul Shannon and Nelson Salisbury surveyed and mapped (coarse resolution) the perimeter of the zone experiencing the decline (area adjacent to the fish hatchery referred to as “Ground Zero”). A visual determination of a boundary around the affected area and the extent of the decline were made using the following guidelines: “greater than 30% of fronds brown on greater than 50% of the individuals in the area” (Appendix 3.1, Figure 3).
Results:	Mapping results from 2016 and 2017 suggested that the affected area had increased from initial mapping efforts in 2015. Outside the vicinity of the initially mapped at Ground Zero, the die-off boundaries became less discernable. This may have resulted from an overlap of possible pre-existing instances of decline and a mosaic of ferns in various stages of decline. In addition, other areas within the park (not adjacent to Ground Zero) also appeared to exhibit discrete instances of decline. Given the extensive nature of the spread of the decline through the park, attempts to map the affected area in this way were not feasible and the effort was ceased in fall of 2017.
Project Status:	Complete. A new mapping/tracking system will be developed to quantify the extent of the decline in the absence of a clear decline boundary.
Resources & External Links:	NA

Project Title:	Regional photo monitoring sword fern decline sites
Date:	2017
Principal Investigator(s):	Nelson Salisbury, EarthCorps; nelson@earthcorps.org
Project Contributor(s):	Al Smith, GSP Volunteer; alsticta@yahoo.com
Research Questions & Objectives:	To photographically document changes to the vegetative conditions at suspected sword fern decline sites
Methods:	<p>A limited number of semi-permanent photo points were established in three parks in Seattle: Seward, Lakeridge, and Cheasty Greenspace at Mountain View. Initial photographs were taken from June to August of 2017. Semi-permanent marks (i.e. stakes with flagging) at precise photo monitoring locations were installed. A photo location ID number was identified at each marked location. The following information was recorded at each photo location: (locality [city, park/location, address], unique point ID [e.g. SEWARD_PP1, SEWARD_PP2], date, cardinal direction in which photo is taken [e.g. N, NNE, NE, ENE, E], number of photos taken at each location). A map of collection points within each site was created to visually indicate the location of each photo point in relation to known objects or features (such as trails or roads; Appendix 3.2).</p> <p>In addition to the three designated parks, Al Smith has also begun documenting signs of the decline regionally. To date, more than 90 points in more than 30 parks or natural areas have been recorded (locations on an online map; Appendix 3.2).</p>
Results:	Initial points have been identified and will continue to be monitored.
Project status:	Ongoing, a revisit schedule should be formulated.
Resources & External Links:	http://www.earthcorps.org/ftp/ECScience/Projects/GSP/Sword_Ferns/Photo_Monitoring/ http://arcg.is/11X9Lu

Project Title:	Vegetation Management Plan (VMP) transect resurvey pilot study
Date:	October 2018 - Current
Principal Investigator(s):	Nelson Salisbury, EarthCorps; nelson@earthcorps.org
Project Contributor(s):	Kramer Canup, EarthCorps; kramer@earthcorps.org
Research Questions & Objectives:	How has the structure and composition of sword fern changed throughout Seward Park compared to data collected in 2004.
Methods:	Original 50-m vegetation transects established in 2004 during the vegetation inventory of Seward Park were relocated. Sword fern cover was surveyed using the line point intercept methodology at 0.5-m increments (Appendix 3.3). All sword fern plants that occur within the 1 × 50 m belt transects were identified, numbered, and subject to a visual health assessment (i.e. healthy, stressed, dead).
Results:	A total of 7 transects (out of 47 where sword fern was recorded in 2004) were revisited and monitored. Transects with the highest cover of sword fern recorded in 2004 were prioritized for this pilot. On average, sword fern cover decreased by 59% across all plots (from 60% in 2004 to 24% in 2018). Additionally, substantial numbers of dead or stressed ferns were counted in the density belt transects: healthy – 12.1, stressed – 6.6, dead – 12.7 (averages from all 7 plots).
Project Status:	Ongoing, to be determined if and to what extent additional transects should be revisited in 2019.
Resources & External Links:	NA

Project Title:	Mapping sword fern decline on the Kitsap Peninsula
Date:	June 2018 - Current
Principal Investigator(s):	John O'Leary, Department of Natural Resources, Suquamish Tribe; joleary@suquamish.nsn.us
Project Contributor(s):	NA
Research Question(s) & Objectives:	To test proposed mapping and monitoring methodology. To identify the area with the most intense/obvious die-off.
Methods:	<p>Conduct a walk-through visual assessment of areas affected by the decline. Quantitative assessments will be conducted using the following criteria:</p> <ol style="list-style-type: none"> 1) Dead crowns (no fiddleheads in spring/no green in any fronds in summer) - nearly all, more than half, or less than half 2) Dying/sick/wilting/stressed (any or all of these descriptions) - more than half, less than half but more than 1 in 10, or less than 1 in 10 but more than zero <p>The affected areas will be demarcated (GPS perimeter) with a polygon (10 ft × 10 ft boundary around “the hotspot” point). At each site, one or more photo points that allow a clear view of worst effects will be marked. Additional GPS points will be collected at the perimeter, ideally near features such as trails or large logs, place markers with flagging at GPS points. At these locations photos will be taken and labeled.</p> <p>In less affected areas, determinations will be made whether it is reasonable to create a GPS perimeter/polygon. If smaller polygons are created, additional GPS points at the perimeter (ideally near features such as trails or large logs) will be made and markers with flagging will be place at points.</p>
Results:	<p>The proposed methodology was not fully implemented, but was instead field-tested for feasibility and value. Specific notes are listed below:</p> <p>7/3/18 (Indianola Perimeter Trail) Presence of more examples of an apparent association between animal trails and die-off hotspots were reported. GPS mapping didn't capture this well in part because the distinct animal trails were often relatively short, quickly branching into more of a network pattern. Locating the “worst” 3-m square spot within each major hotspot provided the quickest way to document. The affected area appears to have expanded, but quantification may be unreliable. Die-off was clearly present beyond the flagging placed in 2017 near the outside edge of obvious die-off in area “1” – especially to the south and southwest (Appendix 3.4).</p> <p>Identifying a 50% stressed line was not practical because the tradeoff between effort and value of data given how large an area that would need to be included.</p> <p>7/11/18 (Indianola Perimeter Trail): Potential correlation between the presence of dead western cedar and sword fern decline in the same areas.</p>
Project Status:	Ongoing
Resources & External Links:	NA

Project Title:	Mapping sword fern decline in Mercer Island Parks
Date:	June 2018 – Current
Principal Investigator(s):	Kim Frappier, City of Mercer Island Parks and Recreation; kim.frappier@mercergov.org
Project Contributor(s):	Alaine Sommargren, Hannah Van Pelt (MIPR)
Research Questions & Objectives:	To identify and map potential sites of sword fern decline within Mercer Island open spaces.
Methods:	Mercer Island (MI) staff conducted a qualitative assessment of the site at Upper Luther Burbank Park in June 2018, which was first identified by volunteers Al Smith and Paul Shannon. Using Arc Collector, MI staff recorded each occurrence of dead or severely declining sword ferns (defined as ferns with two or less live fronds), flagged the perimeter of the die-off site, and then measured the area of the die-off in GIS. A second potential site was found at North Mercerdale Hillside, which was mapped in August of 2018. Staff plan to resurvey North Mercerdale in spring of 2019.
Results:	In Upper Luther Burbank Park, 116 ferns were affected, with 110 dead ferns and 6 with severe decline. The estimated area of decline is approximately 24,493 square feet (or 0.5 ac). The sword fern decline site is located on a west-facing slope within a forested ravine. In areas with the densest decline, staff observed other species including <i>Galium aparine</i> , <i>Mycelis muralis</i> , and <i>Lapsana communis</i> . There was also a significant amount of bare soil. <i>Mahonia nervosa</i> and healthy sword fern were observed along the north, south, and east edges of the die-off perimeter. The west perimeter within the ravine contains <i>Sambucus racemosa</i> . The canopy in the affected area is dominated by <i>Acer macrophyllum</i> . This site has had some removal of English ivy by volunteers, but no large scale contracted restoration work. In North Mercerdale Hillside, 34 ferns were affected, with 31 dead ferns and 3 with severe decline. The estimated area of decline is approximately 11,722 square feet (or 0.27 ac). This site is also located on a west-facing slope within a forested ravine. It is important to note, that comprehensive restoration began on this site in June 2015 to remove a blanket of ground ivy, Himalayan blackberry, and other invasive weeds. The site has had two years of follow-up maintenance. The decline could be the result of decade's long cover with English ivy, but staff will continue to monitor to determine if the die-off continues to spread.
Project Status:	Ongoing
Resources & External Links:	NA

Project Title:	Regional inventory using iNaturalist
Date:	April 2018 - Current
Principal Investigator(s):	Tim Billo, University of Washington; timbillo@uw.edu
Project Contributor(s):	Lisa Ciecko, Green Seattle Partnership; Lisa.Ciecko@seattle.gov Paul Shannon, Friends of Seward Park; pshannon@systemsbiology.org Public
Research Question(s) & Objective(s):	To identify potential sites of sword fern decline regionally using citizen science. Create an inventory of potential sites in which sword fern decline can “verified” by trained volunteers (Appendix 3.5).
Methods:	Project Terms and Rules sections of the Project Description provides the background of the project and implies the need to add entries of sword fern decline locations.
Results:	The project currently has 12 observations and 8 observers. There is a need to advertise the project more widely.
Project Status:	Ongoing
Resources & External Links:	https://www.inaturalist.org/projects/sword-fern-decline-in-washington-state

ii. Pathology and Edaphic Factors

Project Title:	Investigation into the plant pathology of affected western sword fern (<i>Polystichum munitum</i>) populations at Seward Park
Date:	June 8-23, 2015
Principal Investigator(s):	Olaf Ribeiro, Ribeiro Consultants; fungispore@comcast.net
Project Contributor(s):	NA
Research Questions & Objectives:	Understand the pathology of sword fern through tissue and root analysis. Determine whether root rot (<i>Rhizoctonia</i>) was the major cause of the sword fern population decline in Seward Park.
Methods:	Above and below-ground tissues were sampled and subject to a visual inspection followed by pathogen isolation and identification through laboratory culture. Ribeiro Consultants did not disclose specific methodology. Standard soil nutrient tests were also conducted on soils collected from plant sampling sites at Seward Park “Ground Zero”.
Results:	<p>The observed dieback of the terminal feeder roots was likely caused by desiccation rather than pathogen infection. <i>Rhizoctonia</i> was occasionally isolated but not in quantities that would explain the extent of the root dieback. The bases of the stems were extensively rotten, which is more typical of <i>Rhizoctonia</i> pathogen infection. <i>Rhizoctonia</i> was isolated from the stems and may have been the main cause in stem dieback. Extensive presence of <i>Phoma</i> (a common coelomycetous soil fungi) was also noted on the stem tissues. Fern fronds were primarily affected by the <i>Glomerella</i> fungus blight but it was not likely the cause for complete dieback. No foliar nematodes were isolated from fronds tested.</p> <p>The soil nutrient analyses (Appendix 3.6) indicated that phosphorus, magnesium, zinc, manganese, copper and iron were found to be excessive. Potassium, calcium and boron were high. Excess phosphorus inhibits the uptake of copper, iron, zinc and boron. Fertilization recommendations to facilitate nutrient availability were made (Appendix 3.6).</p>
Project Status:	Project completed, results are summarized (Appendix 3.6). Follow-up work needed to investigate plant pathology.
Resources & External Links:	NA

Project Title:	Phase I: Investigation of the role of soilborne plant pathogens in the genus <i>Phytophthora</i> as causal agents of western sword fern (<i>Polystichum munitum</i>) decline in Seward Park
Date:	November 2015-December 2016
Principal Investigator(s):	Marianne Elliott, Puyallup Research and Extension Center, Washington State University; melliott2@wsu.edu
Project Contributor(s):	Katie Coats, Puyallup Research and Extension Center; kpcoats@wsu.edu
Research Questions & Objectives:	To conduct a comparative evaluation of the presence of <i>Phytophthora spp.</i> in declining and healthy stands of western sword fern at Seward Park.
Methods:	Samples of fine roots and adjacent soil were collected from around healthy and affected ferns (at a distance of 12 and 24 inches away from plant center). DNA was extracted from six samples, with one being a pseudo-replicate (split from affected soil). The DNA samples were run in duplicate through the general <i>Phytophthora</i> (5.8S) quantitative polymerase chain reaction (qPCR) assay along with a positive control (DNA from a pure culture of <i>Phytophthora</i>) and a negative control (water). All samples were multiplexed with an amplification control that checks for evidence of PCR inhibition (Appendix 3.7).
Results:	<p>Counter to study hypothesis, that soil adjacent to and the tissue of affected plants would have higher levels of <i>Phytophthora spp.</i>, results indicate that healthy soil and plant generally had more <i>Phytophthora spp.</i> DNA (lower Ct values) compared to the affected soil and fine root tissue.</p> <p>Baseline <i>Phytophthora</i> levels across similar forest sites were not known; thus, it was difficult to contextualize these findings beyond the fact that <i>Phytophthora</i> is unlikely to be the primary the cause of the sword fern decline. The conducted analysis was genus-specific, and thus combined all possible <i>Phytophthora</i> species together in one value. It is possible that a causal <i>Phytophthora</i> was present at higher concentration in the affected plant; irrelevant of total amount of <i>Phytophthora spp.</i> DNA found and thus warrants further investigation.</p>
Project Status:	Phase I was completed and considered inconclusive (Appendix 3.7), thus Phase II of the project was initiated.
Resources & External Links:	http://sewardparkswordferndieoff.blogspot.com/2016/12/katie-coats-and-marianne-elliott-of-wsu.html

Project Title:	Phase II: Examination of the role and potential entry pathways of the soilborne plant pathogens in the genus <i>Phytophthora</i> as causal agents of western sword fern (<i>Polystichum munitum</i>) decline in Seward Park
Date:	April 2017 – July 2017
Principal Investigator(s):	Marianne Elliott, Puyallup Research and Extension Center, Washington State University (WSU); melliott2@wsu.edu
Project Contributor(s):	Katie Coats, WSU Puyallup Research and Extension Center; 2kpc coats@wsu.edu Jenny Glass, WSU Puyallup Research and Extension Center; jennyglass@wsu.edu
Research Questions & Objectives:	To improve our understanding of role human-assisted movement of plant pathogens (<i>Phytophthora</i> spp.) from the collection, propagation, or outplanting.
Methods:	<p>WSU surveyed one remnant old-growth forest stand in Seward Park that has been undergoing restoration for several years. Twenty-eight bait samples were collected from six species of potted plants designated for planting at the site (Appendix 3.7). These plant species were Douglas-fir (<i>Pseudotsuga menziesii</i>), Indian plum (<i>Oemleria cerasiformis</i>), Oregon grape (<i>Mahonia nervosa</i>), salmonberry (<i>Rubus spectabilis</i>), trailing blackberry (<i>R. ursinus</i>), and thimbleberry (<i>R. parviflorus</i>).</p> <p>In spring 2017, four areas at the park were sampled. These sites had undergone supplemental restoration planting in 2016 and included healthy and unhealthy ferns. Plant species that were installed and sampled at the restoration sites were the same six from the potted plants mentioned above, plus beaked hazelnut (<i>Corylus cornuta</i>).</p> <p>Soil samples (500 g) collected from beneath (1) “healthy”, (2) “declining”, and (3) “restored” sword ferns. Soil samples were evaluated for the presences of <i>Phytophthora</i> and other soil borne plant pathogens via laboratory culture using (a USDA-APHIS protocol, Appendix 3.7). Researchers used a comparative approach to determine whether differences in fungal and oomycete pathogens could explain differences between soil on sites with healthy and unhealthy sword fern populations.</p>
Results:	In the samples from the four restored areas at the remnant old growth forest site evidence of <i>Phytophthora cambivora</i> , <i>P. europaea</i> , and <i>P. chlamydospora</i> were found. These species were found in soil beneath the recently installed plants. Only <i>P. europaea</i> was detected in soil beneath the healthy sword fern at one of the sites. No <i>Phytophthora</i> species were detected in the two sword fern decline sites.
Project Status:	Complete
Resources & External Links:	http://sewardparkswordferndieoff.blogspot.com/2016/12/katie-coats-and-marianne-elliott-of-wsu.html http://sewardparkswordferndieoff.blogspot.com/

Project Title:	Comparative analysis of the soil invertebrate community and soil quality to address the decline of western sword fern (<i>Polystichum munitum</i>) in Seward Park
Date:	2016-2017
Principal Investigator(s):	Patrick Tobin, School of Environmental and Forest Science, University of Washington; pctobin@uw.edu Grace Masaoka; g.masaoka@gmail.com
Project Contributor(s):	NA
Research Questions & Objectives:	To explore the potential cause(s) of the western sword fern decline, by comparing below-ground differences in both the invertebrate community and soil quality among healthy and affected sites.
Methods:	<p>Three sites representing a gradient of decline were selected. These included a (1) “healthy” site, with no apparent signs of decline; (2) an “intermediate” site, containing both healthy and declining ferns; and (3) the “ground zero” site, which has experienced complete die-off with no signs of regeneration. All three sites were located adjacent to the Squebeqed Trail, which runs through the center of Seward Park.</p> <p>Three randomly selected fern plants (or fern remnants) were extracted at each site by carefully digging out the entire plants. Root biomass was collected and subject to microscopic examination to measure the presence of root herbivory from invertebrates (arthropods and nematodes).</p> <p>Soil samples (5 per site) were collected from the base of each fern (ca. 20 cm from the center of the fern and extend to a depth of 20 cm) and were used to assess the soil invertebrate community (n=13) and conduct soil analyses (n=2). Soil analysis included soil classification, pH, and bulk density (Appendix 3.8).</p>
Results:	Although not statistically different, soil invertebrate abundance and root mass differed between healthy and affected sites. The differences in soil bulk density between sites were statistically significant, but none of the bulk densities were high enough to be considered detrimental to root growth. Thrips were found on samples collected at the “intermediate” site and may have prevented the post-drought recovery of hydraulic conductance by damaging plant conductive tissue.
Project Status:	Complete
Resources & External Links:	NA

Project Title:	Soil analysis across a gradient of western sword fern (<i>Polystichum munitum</i>) decline within Seward Park
Date:	June 28, 2017
Principal Investigator(s):	Nelson Salisbury, EarthCorps; nelson@earthcorps.org Paul Shannon, Friends of Seward Park; pshannon@systemsbiology.org
Project Contributor(s):	UMass Soil Nutrient Laboratory
Research Questions & Objectives:	To assess differences between soil nutrient compositions across the gradient of severity in sword fern decline.
Methods:	<p>Investigators selected three sites across a gradient of sword fern decline severity at Seward Park (Appendix 3.9). These included: Site 1--complete die-off and no surviving ferns (Ground Zero); Site 2--active decline site (western location, across the Squebeqed trail); Site 3--an un-affected site with an intact fern population (north end of Seward Park).</p> <p>At each site, investigators collected ten to twelve soil samples from a 100 m² quadrats and combined to represent one sample per site. Sampling followed a standard protocol provided by the UMass Extension Service. A routine soil analysis and a test of the total sorbed metals were conducted on samples from each of the three sites.</p>
Results:	Relative to the un-affected site (Site 3), phosphorus, potassium, and calcium were above the optimal range at sites affected by the decline (Sites 1 and 2); with the site of active decline exhibiting the highest nutrient levels (Appendix 3.9). This may reflect the decomposition of the dead plant material following the initial sword fern mortality, which is followed by additional nutrient bioavailability in the soil. A more comprehensive regional assessment is needed to investigate these dynamics.
Project Status:	Complete
Research Questions & Objectives:	http://sewardparkswordferndieoff.blogspot.com/2017_10_29_archive.html

Project Title:	Sword fern soilborne pathogen transmission: Sandy Hook planter experiment
Date:	March 2018 - Current
Principal Investigator:	John O'Leary, Department of Natural Resources, Suquamish Tribe; joleary@suquamish.nsn.us
Project Contributor(s):	David Perasso, Friends of Martha Washington; perasso@cruzio.com Paul Shannon, Friends of Seward Park; pshannon@systemsbiology.org Tim Billo, University of Washington; timbillo@uw.edu Marianne Elliott, Puyallup Research and Extension Center, Washington State University; mellott2@wsu.edu
Research Questions & Objectives:	To determine whether soil from a die-off area will contaminate sword ferns that have previously been isolated from die-off.
Methods:	Planters were placed approximately 175 ft west of a saltwater shoreline at an elevation of approximately 55 ft NAVD88, and located within immediate geographic area of confirmed sword fern (<i>Polystichum munitum</i>) die-off/decline (Appendix 3.10). Bagged potting soil was wrapped in a tarp and stored on site from 3/2/18 until used on 3/13/18. The planters were partially assembled off site, prior to final assembly on site 3/12/18, the day prior to planting. Plants were brought to the site on 3/13/18. The two planters were constructed with ¾" AC plywood and 2 x 4 lumber pieces. Both planters had a calculated capacity of 10 ft ³ and were each filled with 9 ft ³ of bagged potting soil. Approximately 2 gal of local soil was collected from several sites within the die-off area. Collection sites included: adjacent to highly stressed ferns within the die-off zone and near the apparent edge, from root ball of dead fern, from duff layer, from sandy soil horizon about 6" deep. Local soil was mixed into the top few inches of "Red" contaminated planter. Subsequent soil additions were planned to occur several times until the end of the study. An equivalent amount of clean potting soil would be added to the "white" uncontaminated control planter. Visual inspections were conducted periodically. If decline of planted ferns is observed, frond counts will be measured.
Results:	No differences between plants were noted to date.
Project Status:	The experiment is ongoing and "contaminated" soil additions are planned in the spring of 2019.
Resources & External Links:	NA

Project Title:	Waterborne pathogen transmission pilot study
Date:	October – November 2018
Principal Investigator(s):	Paul Shannon, Friends of Seward Park; pshannon@systemsbiology.org
Project Contributor(s):	NA
Research Questions & Objectives:	Can foliar symptoms of die-off be transferred between affected and unaffected fronds through water?
Methods:	<p>Four pairs of sword fern fronds were collected from healthy and affected mature plants in the old-growth forest at Seattle's Seward Park, Seattle, WA. Each frond was cut from plants a few inches above the ground. Each pair was placed into a carefully washed bottle half-filled with tap water.</p> <p>Three bottles held two fronds each, one "affected" (showing symptoms of decline) and one "unaffected" (appearing healthy at the time of collection). The affected fronds (all three) were taken from separate plants at the edge of the (still expanding) active die-off zone at Seward Park. The unaffected fronds were collected from apparently healthy plants, located at least 10 m from any obviously affected plants. Each of the affected fronds showed a different amount and type of die-off phenotype: (A) "crinkly" phenotype: all pinnae curved, thought to be an early symptom of the decline, (B) 50% pinnae necrotic, or (C) all pinnae necrotic (brown, apparently dead; Appendix 3.11). A control pair of two "unaffected" fronds collected from healthy plants were placed in the fourth bottle. Healthy fronds were collected one week later than those that were affected. The four pairs were observed and photographed weekly for three weeks.</p>
Results:	At the end of the pilot study, all three of the healthy fronds exposed to affected fronds exhibited degradation compared to the control. The frond paired with the crinkly phenotype (A) showed the most dramatic decline. Conversely, for the frond paired with the 50% necrotic frond was the least affected (Appendix 3.11).
Project Status:	Complete. Appropriate levels of replication and tests of causality would need to be included in future work.
Resources & External Links:	NA

iii. Vertebrate Drivers

Project Title:	The potential role of mountain beaver (<i>Aplodontia rufa</i>) in western sword fern (<i>Polystichum munitum</i>) decline at Seward Park
Date:	September 2015 – September 2016
Principal Investigator(s):	Tim Billo, University of Washington; timbillo@uw.edu
Project Contributor(s):	Justin Beach, University of Washington; na Kramer Canup, EarthCorps; kramer@earthcorps.org Paul Shannon, Friends of Seward Park; pshannon@systemsbiology.org Tristan O'Mara, University of Washington; na
Research Questions & Objectives:	To assess the possible correlation between the presence of mountain beaver populations and the distribution of sword fern decline within Seward Park.
Methods:	Established monitoring plots (Appendix 3.1, Figure 1) were proposed as a framework for mountain beaver monitoring. As such, beaver dens within a 10-m radius from the center of each plot were to be identified. Two camera traps were to be installed in each plot and left for 1 week, then moved to subsequent plots within the established plot system. Additionally, exclosure shelters would be installed to conduct comparative tests between areas of decline subject to beavers.
Results:	The project was never carried out in full due to damage to exclosures, limited budget, and general SFDWG's consensus that it is unlikely that beaver activity was a major contributor to the decline. Results of a small pilot of the study suggest that there was no clear relationship between exclosure and fern survival (e.g. in a few cases, ferns inside exclosures were thriving, while ferns outside were not, and in other cases, all ferns in and outside exclosures had died). If initiated, a follow up study should include a larger sample size.
Project Status:	Incomplete
Research Questions & Objectives:	https://sewardparkswordferndieoff.blogspot.com/search?q=mountain+beaver https://sewardparkswordferndieoff.blogspot.com/2016/02/paul-talberts-skepticism-regarding.html

iv. Ecophysiology, Morphology, and Experimental Restoration

Project Title:	Ecophysiological assessment of western sword fern (<i>Polystichum munitum</i>) across a temporo-spatial gradient
Date:	April 2017 – Current
Principal Investigator(s):	Natalie Schwartz, University of Washington; nms35@uw.edu
Project Contributor(s):	Kramer Canup, EarthCorps; kramer@earthcorps.org Matthew Aghai, Verdant Consulting Group; verdantcg.llc@gmail.com
Research Questions & Objectives:	To establish a baseline understanding of sword fern ecophysiological dynamics. To understand whether physiological mechanism(s) are driving the decline across planted and established sword ferns at Seward Park. Can differences in ecophysiological metrics explain the mechanisms of the decline within plants (individual) and across sites (population-level)?
Methods:	Sword fern seedlings were planted at three locations within Seward Park that had experienced decline (including Ground Zero) (Appendix 3.12). Each location was considered to be a “planting block” and contained ca. 60 seedlings each. In addition to planted plots, existing plots from the 2015 fixed plot assessment (See Figure 3.2) were included to monitor plant physiological responses to active decline, and three plots were also established in un-affected stands. Measurements were conducted monthly from April 2018 through October 2018. Measurements included: plant heights, frond counts, canopy spread, aboveground tissue chlorophyll fluorescence and content (SPAD), water potential, gas exchange, soil moisture, photosynthetically active radiation (PAR), temperature and relative humidity. Additional components of the investigation will include a one-time destructive sample of healthy and diseased tissue and one-time sampling of study site soils.
Results:	Pending completion of sampling and data analysis.
Project Status:	Ongoing through December 2019; following an October 2018 measurement period, initial results will be summarized.
Research Questions & Objectives:	NA

Project Title:	Experimental planting of western sword fern (<i>Polystichum munitum</i>) in Seward Park
Date:	February 2018 – Ongoing
Principal Investigator(s):	Paul Shannon, Friends of Seward Park; pshannon@systemsbiology.org
Project Contributor(s):	Suzanne Bouchard, Seward Park Volunteer; na
Research Questions & Objectives:	To test the capacity for survival of sword fern seedling planted at and adjacent to the primary die-off site at Seward Park (Ground Zero). Will the spread of the die-off zone result in mortality of planted ferns?
Methods:	Two lines of 12 (n=24) sword fern seedlings were planted at the primary die-off site (Ground Zero) and one line of seedlings (n=12) were planted ca. 1 meter beyond what was, at the time of planting, a northern boundary of the affected die-off area (AD) (Appendix 3.13, Figure 1). Every other fern (in each line) was inoculated with mycorrhizal spores. Seedling survival and status were monitored by weekly photographs. Seedlings of western sword fern (<i>P. munitum</i>) were provided by Seattle Parks and Recreation as part of the Green Seattle Partnership. Every plant received two liters of water at intervals of one to two weeks, from June through mid-September to minimize the confounding effects of transplant stress and drought on survival.
Results:	Following 8 months of monitoring, 100% of ferns in the Ground Zero plots remain healthy (Appendix 3.13). Within the AD plots, one fern died in May 2018 another has shown signs of decline by September 2018. Both affected ferns were located closest to the original boundary of the affected die-off area. The remaining 10 AD ferns are uniformly healthy (Appendix 3.13). These findings may have important consequences for future restoration planning and vegetation management.
Project status:	Ongoing (Expected completion in 2023)
Research Questions & Objectives:	https://sewardparkswordferndieoff.blogspot.com/2018/03/experimental-planting-at-seward-park.html

3.2.2. Restoration Measures

To address the need to re-establish native vegetation at the “Ground Zero” site (Seward Park), which is currently void of understory vegetation, Seattle Parks and Recreation is moving forward with installing 400 plants in 2019. Plants will be installed at “Ground Zero” adjacent to the fish hatchery trail.

Latin Name	Common Name	Type	Quantity
<i>Abies grandis</i>	grand fir	Trees	5
<i>Acer circinatum</i>	vine maple	Shrubs	20
<i>Alnus rubra</i>	red alder	Trees	10
<i>Berberis nervosa</i>	dwarf Oregon grape	Shrubs	50
<i>Cornus nuttallii</i>	Pacific dogwood	Trees	5
<i>Corylus cornuta var californica</i>	beaked hazelnut	Shrubs	20
<i>Gaultheria shallon</i>	salal	Shrubs	50
<i>Holodiscus discolor</i>	ocean spray	Shrubs	10
<i>Oemleria cerasiformis</i>	Indian plum	Shrubs	50
<i>Rosa gymnocarpa</i>	baldhip rose	Shrubs	20
<i>Salix scouleriana</i>	Scouler's willow	Trees	5
<i>Symphoricarpos albus var laevigatus</i>	common snowberry	Shrubs	40
<i>Taxus brevifolia</i>	Pacific yew	Trees	5
<i>Tiarella trifoliata var trifoliata</i>	Three-leaf foamflower	Forbs and Ferns	50
<i>Tsuga heterophylla</i>	western hemlock	Trees	10
<i>Vaccinium ovatum</i>	evergreen huckleberry	Shrubs	50
TOTAL			400

3.3. Aims, Goals, and Strategic Objectives

This Strategic Plan aims to provide a roadmap for understanding and addressing the decline of western sword fern at Seward Park and the greater Puget Sound Region.

In it, we identify **two key goals**:

1. To determine the cause and mechanism of spread of the decline
2. To identify effective restoration strategies to maintain forest ecosystem health

To achieve these goals, we propose **four strategic objectives** as a means of project prioritization. These objectives were largely synthesized from meetings and discussion among SFDWG members, municipal program managers, and consultants during 2018.

1. Monitoring and mapping
2. Multi-disciplinary research
3. Active management and restoration
4. Prevention and mitigation

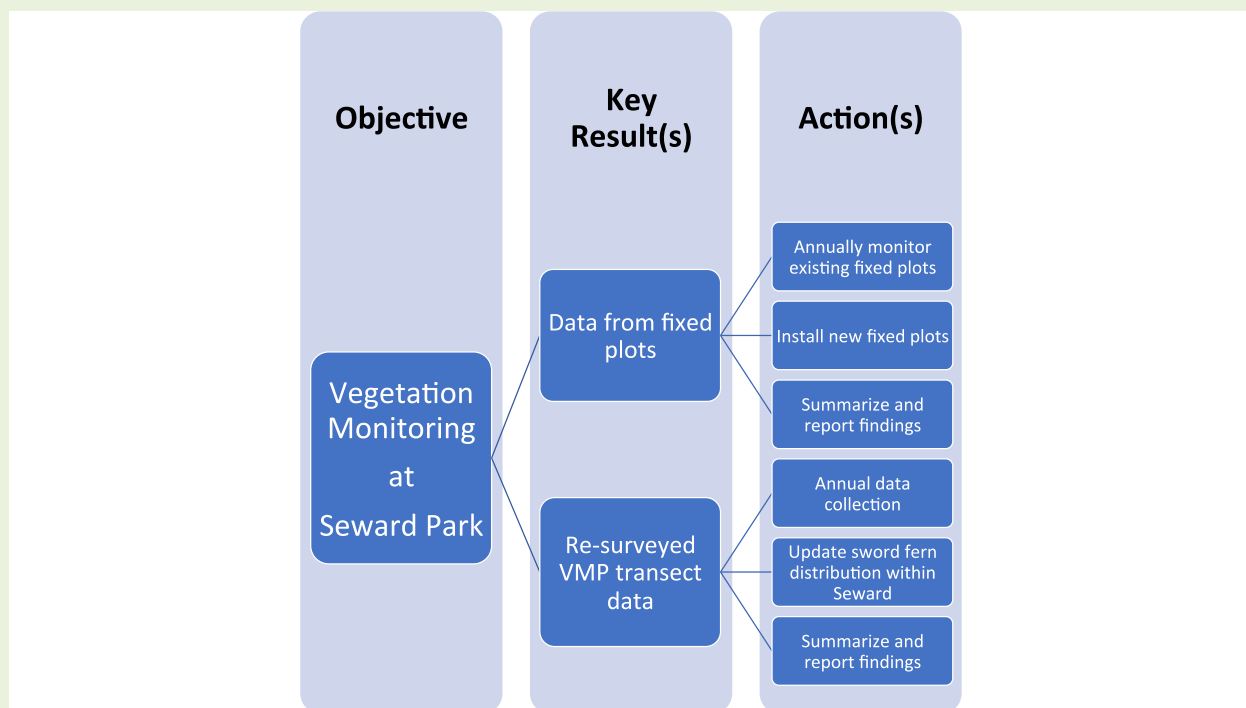
Given that western sword fern is a prolific and widespread component of the Pacific Northwest vegetative communities, it may serve as a *de facto* sign of the general status of the region's forest. The scope of the issue is large and complex enough that it must be addressed systematically both locally (e.g. Seward) and across the region. The use of a comprehensive strategy to collect information, specifically landscape-level data on plant community, edaphic, environmental, and biotic factors across affected areas would allow scientists to identify common themes that are involved in the decline phenomenon. Combining this approach with ongoing site-specific efforts already underway at Seward Park can help address the question from multiple directions. The continuation of existing efforts would also allow the park to continue to meet its management goals of protecting native vegetation and promoting public outreach and education.

3.3.1. OBJECTIVE 1: MONITORING AND MAPPING

i. Vegetation Monitoring

Permanent forest monitoring plots and transect-based surveys create a basis of reference that allows for the documentation of changes in forest vegetation composition through time. This approach can ease decision-making and facilitate a rapid response to alterations in plant community health. Finally, regional mapping may be crucial for securing funding from a broader cross-section of funding sources.

Ongoing vegetation monitoring endeavors by the SFDWG should continue with the support from Seattle Parks and Recreation. Specifically, a network of fixed plots (established in 2015 and expanded in 2018) should be monitored annually to quantify the spread and extent of the decline in Seward Park (see Section 3.2). Additionally, the existing vegetation inventory included in the Seward Park Vegetation Management Plan (VMP) (Hanson and Davidson, 2005) should be used as a baseline for continued transect-based vegetation monitoring as it pertains to sword fern populations. Specific actions proposed and tested by the SFDWG should be implemented fully in 2019 (see Section 3.2). Annual biometric sampling (i.e. vegetation presence and absence, qualitative health status), analysis, and summary of findings with SFDWG and GSP are critical to tracking and managing sword fern decline within Seward Park.



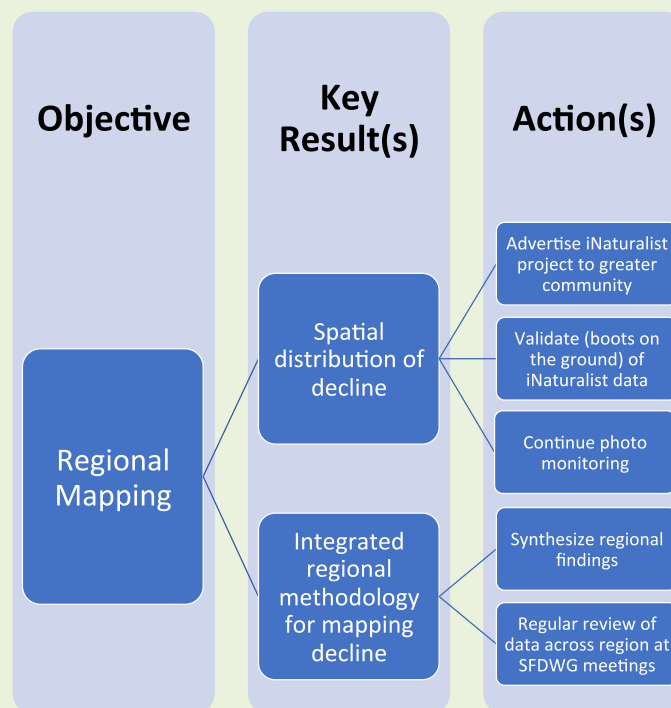
ii. Regional Mapping

Reports of sword fern decline from across the region (Appendix 3.12) highlight the need to validate and describe the breadth and severity of the issue. The process of identifying sites exhibiting signs of sword fern decline at this scale would require significant efforts and exceed the current capacity and jurisdiction of SFDWG and Seattle Parks and Recreation. The limited capacity for data collection is a common challenge, which has been met by crowd-sourcing data through citizen science efforts (Dickinson et al., 2012; Theobald et al., 2015). With more than 150,000 observers, iNaturalist is one of the most popular crowd-sourcing platforms in natural sciences (iNaturalist, 2017). Existing examples of projects such as Nature’s Notebook (USA-NPN, 2018) or MeadoWatch (MeadoWatch, 2018) have relied on hundreds of citizen scientists to amass significant quantities of field data that can help advance the speed and scale of scientific research in many domains.

Recognizing the value of the crowd-sourcing approach, in 2014 SFDWG used iNaturalist to establish the “Western Sword Fern Decline in Washington State” Project to help identify potential sites of sword fern decline (Shannon, 2014). As with all iNaturalist projects, this project is self-maintaining, with all data available to the public. In this context, iNaturalist would function as the source for potential sites exhibiting sword fern decline. However, the quality of the data collected can significantly shape its long-term value (Dickinson et al., 2012). Thus, we recommend that a specific set of guidelines are included in the Observation Rules (NHM-LA, 2015; p.5) to help guide users to submit accurate contributions (Appendix 3.5). This data, which contains georeferenced site photos, observation dates, and other relevant details can be used to guide a formal systematic verification of the distribution of the decline across the region. Site verification can be accomplished by trained volunteers or as part of a student project. To date, the project has not been widely advertised. Soliciting public engagement through outreach at local and regional events such as conferences, volunteer events, and recreation hubs (e.g. the Mountaineers, REI, Park Visitor Centers) can facilitate greater buy-in from interested citizen scientists.

Starting in 2017, several semi-permanent photo points were established in three parks in Seattle: Seward, Lakeridge, and Cheasty Greenspace at Mountain View. Initial photographs were taken from June to August of 2017. In addition to the three designated parks, Al Smith has also begun documenting signs of the decline regionally. To date, more than 90 points in more than 30 parks or natural areas have been recorded. This work should continue in 2019, with a schedule for follow up visits to established sites, expansion into other areas, and validations of sites reported through iNaturalist.

To date, several techniques for monitoring the decline have been used by members of SFDWG at other regional parks. In this process, members have accumulated an understanding of which approaches are most likely to be useful in quantifying the mosaic of decline. While these efforts have been valuable, to systematically assess the distribution of the decline through the Puget Sound, future work should focus on developing an integrated approach for mapping. This would allow the group to leverage more funding and scientific interest needed to address this issue region-wide.



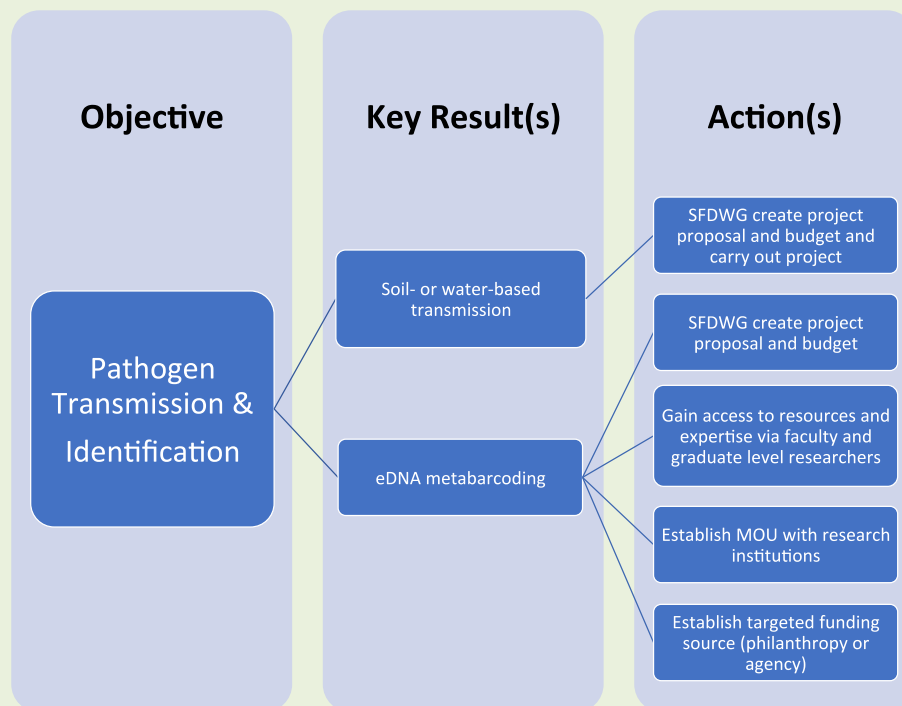
3.3.2. OBJECTIVE 2: MULTI-DISCIPLINARY RESEARCH

i. Pathogen Transmission and Identification

Confirming the involvement of a biotic agent and describing the symptoms of the decline is critical step necessary to directly exclude other potential causes of the sword fern decline. Experimental laboratory techniques such as investigations of soil and waterborne pathogen transmission may be effective low-cost approaches to achieving this goal (See Appendix 3.10-3.11). This approach side steps culture-based techniques, which can limit discovery of pathogens only to those organisms that are able to grow under laboratory conditions (Mendes et al., 2013; Rondon et al., 2000) thus restricting the scope of investigation.

If a biotic pathogenic agent (or agents) is deemed responsible, molecular techniques, such as metabarcoding, can be a robust tool to enable the identification of microbes *in situ* (Ravin et al., 2015). Metabarcoding is a

rapid method of specimen identification that combines DNA-based identification with high-throughput DNA sequencing (Abdelfattah et al., 2018; Fahner et al., 2016). In this process, environmental DNA (eDNA) collected from soil and/or plant tissue is extracted to undergo high throughput sequencing. The resulting sequences are then compared with reference libraries to identify potential pathogens. This approach has been used to identify a number of previously unknown pathogens and soil microbes (Cerri et al., 2017; Fahner et al., 2016). There are caveats to using eDNA barcoding techniques. Given the high risk of contamination and the possibility of confounding results due to hybrid and novel species, high sample replication is necessary. Additionally, results can vary significantly throughout the growing season, thus data collection should occur over time. The need for high replication presents a particular challenge because this technique is costly and necessitates the use of an external database (fee-based service) to classify and sequence eDNA. However, regional centers such as the University of Washington (UW), Department of Biological Sciences have active laboratories (e.g. SEFS Shared Genetics Laboratory) that can facilitate this analysis at a reduced cost. For this reason, agreements such as a Memorandum of Understanding (MOU) between Seattle Parks and Recreation and research institutions such as UW may be particularly worthwhile. To move towards utilizing metabarcoding and eDNA or other high-throughput analytical studies of pathogens at Seward Park, the SFDWG research committee should create a project outline and associated budget. To access resources and additional expertise (e.g. faculty or graduate level researchers), SFDWG should work with Seattle Parks and Recreation to formulate a request to establish an MOU with the regional research institutions (e.g. UW, WSU). Once the extent of the work is fully understood, the SFDWG should seek out additional funding from philanthropic organizations or government agencies (e.g. professional plant conservation societies, private donors, state and federal institutions).

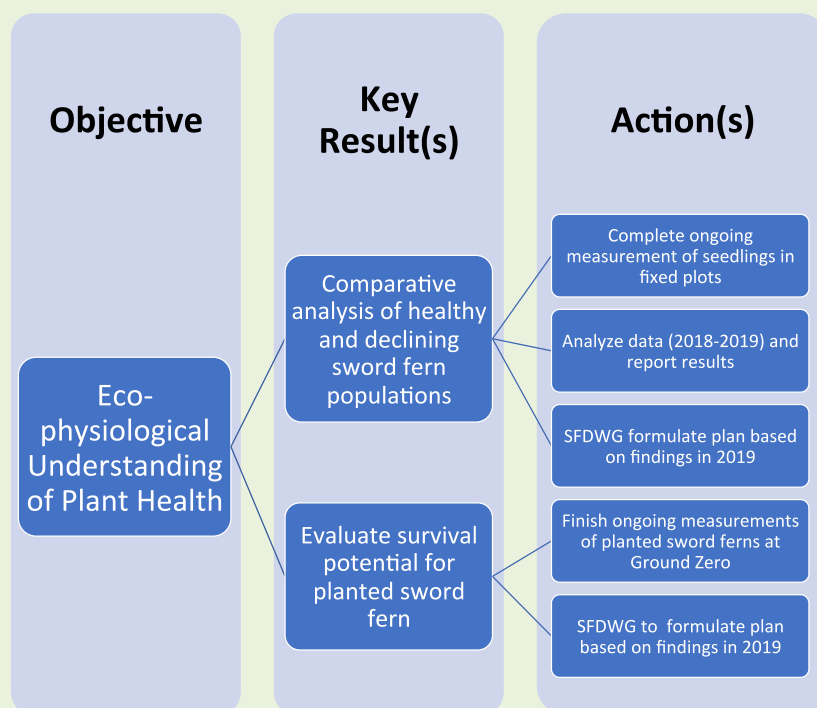


ii. Ecophysiological Assessment of Plant Health

Abiotic and biotic changes in the environment – specifically, changes to plant community composition, microclimate (aboveground), soil structure, and soil nutrients – can impact plant health (Kimball et al., 2016). The status of a plant can be determined by evaluating a suite of physiological factors in tandem with key environmental conditions (e.g. temperature, soil moisture, light). This information can be used to create the basis for an ecophysiological understanding of individual plants, plant communities, and ecosystem-level dynamics affecting plant health. Measurements related to plant photosynthetic capacity (e.g. gas exchange, chlorophyll content, chlorophyll fluorescence, and light response curves) and those that pertain to water status in the plant (e.g. water use efficiency, mid-day and pre-dawn water potential) explain the plant’s ability to capture resources needed for growth, survival, and reproduction.

Comparative ecophysiological assessment of healthy and declining sword fern populations can help shed light on the drivers of the decline. In 2018, a UW undergraduate researcher (Natalie Schwartz) working in association with SFDWG initiated a study to examine the ecophysiological drivers of sword fern decline in Seward Park. Because many of these measurements collected required the use of specialized instruments and analytical techniques, the student established an informal partnership with a UW laboratory to allow for equipment access.

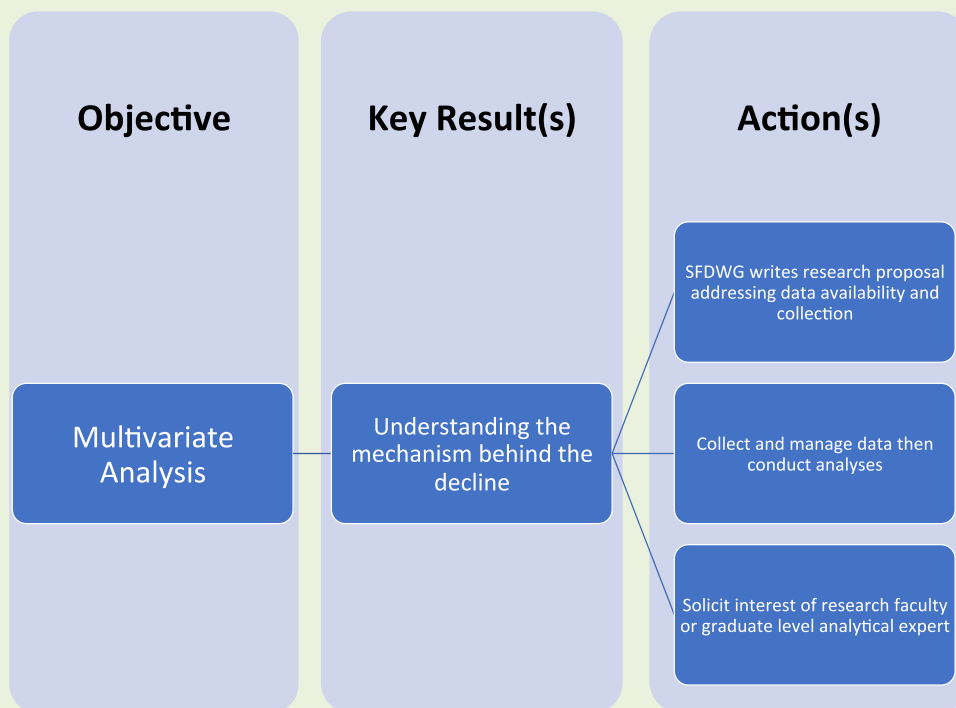
Data collection in fixed plots and monitoring of planted ferns at “Ground Zero” should continue in 2019, led by Natalie Schwartz. The final data collection period will take place no later than May 31, 2019. The project has been funded through grants from (1) the Society for Ecological Restoration, and (2) the Washington Native Plant Society. This funding will continue to support the associated field and lab work. To meet project objectives and granting agency requirements, Natalie will also carry out the analysis of the collected data, synthesize findings, and communicate research outcomes in the form of a peer-reviewed publication and/or public presentation by December 2019. The data can serve as a performance baseline for healthy established populations, as well as planted populations of sword fern, building knowledge for both restoration planning and species-specific ecology. In 2020, the SFDWG research committee should re-evaluate the need for project continuity or expansion based on findings at the end of the planned research period.



iii. Multivariate Analysis of Biotic and Abiotic Factors

A comprehensive regional study looking at a variety of factors (soil type, nutrient profile, soil moisture, slope, aspect), site conditions (light levels, plant community composition), and plant-level metrics (speed of decline, plant morphological and physiological responses) across a gradient of “healthy”, “declining”, and “affected” ferns may help identify significant factors or combination of factors that enable the decline phenomenon. Standard linear statistical approaches are limited in their capacity to compare multiple correlated variables. Principal components analysis (PCA) can be used to analyze a large number of disparate variables within one analysis (Summerville, 2008). This approach can help identify relationships between several domains to determine the factors that correlate with sword fern decline.

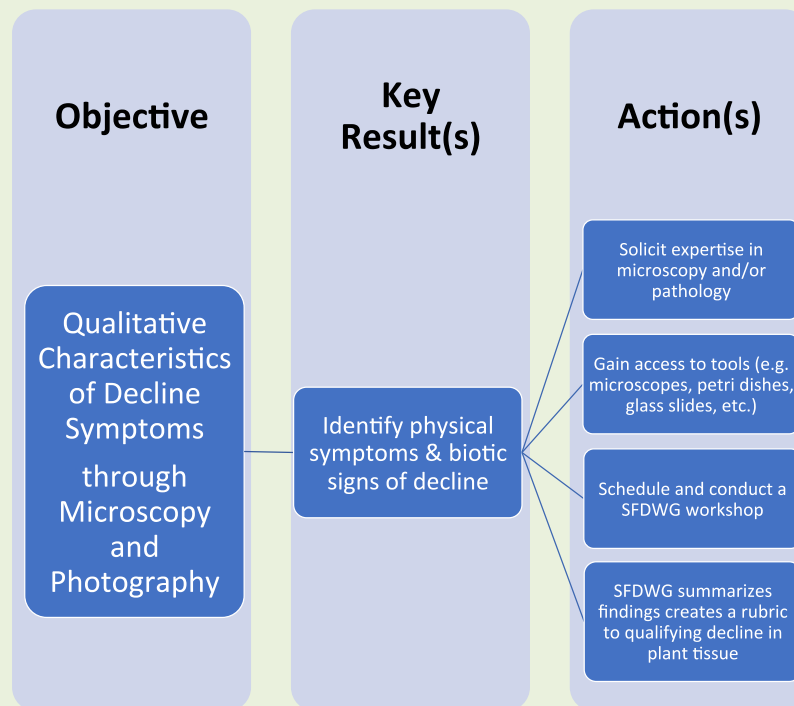
A research proposal that outlines the study design and scope is needed. This proposal should identify the potential variables that can contribute to the decline (an example of potential factors can be found in Appendix 3.13.), data attributes to capture across a range of regional sites, the associated costs, and a project timeline. The SFDWG research committee may be well suited to carry out the initial planning steps. However, given the scale of the project, data collection, processing, analysis, and interpretation of findings, this project will likely require the involvement of an academic partner (within or outside current SFDWG membership) in the role of a primary investigator to ensure project completion. Utilizing graduate student or faculty interest may be most advantageous.



iv. Qualitative Characteristics of Decline through Microscopy and Photography

The current characterization the affected sword fern tissue is vague, and only late-stage decline symptoms are a reliable sign of the decline phenomenon. Thus, there is a need to identify decline symptoms earlier in its progression. To better characterize the physical symptoms and biotic drivers of the decline, microscopy and photography techniques can be used to conduct a close examination of plant tissue of healthy and affected ferns. These techniques can also identify the presence of invertebrates on the plant tissues and quantify any differences in the composition (and presence/absence) of these organisms at different stages of fern decline. While volunteers can carry these assessments, specialized equipment and guidance of subject experts will be needed.

A research proposal that outlines the project needs, objectives, equipment, and personnel needs, as well as associated costs and planned outcomes should be created by the SFDWG (see Appendix 5.3). The quality of the examination will depend on an unbiased sampling of plant tissue, which should include appropriate replication and randomization (i.e. equal number of replicates from healthy and declining ferns, tissues collected from the same crown position, equal size of samples). The resulting images can be used as a foundation for a rubric to qualify decline, especially during early stages of development. If determined to be valuable, a database for image collection, storage, and use can be established by SFDWG. This database should follow a clear organizational structure and be accessible by a SFDWG member or committee representative charged with managing the system.



v. Developing Strategies for Ecological Recovery

Given the extent of the decline and the lack of natural regeneration on affected sites (e.g. Ground Zero), research into the best methods for restoration, followed by or in tandem with active restoration is needed. Specifically, investigations should address whether sword fern re-planted following decline are able to survive. In addition, it is important to identify other native plant species that may serve as effective alternatives for the restoration of affected sites. Some of these efforts have already begun and are planned to continue in subsequent years (December 2019 and 2023, respectively).

3.3.3. OBJECTIVE 3: ACTIVE MANAGEMENT AND RESTORATION

Prioritizing management objectives for Seward Park, a large multi-use park, presents major challenges. Actions to reduce sword fern decline should be carried out within the context of the existing management framework. The Seward Park Vegetation Management Plan (Hanson and Davidson, 2005) outlines the key management agenda as follows:

“to conserve Seward Park’s increasingly rare native plant communities by arresting past and latent habitat loss and enhancing survival of the park’s indigenous flora and fauna”, while also strengthening “park stewardship by an ever-widening array of users, to encourage engagement in this unparalleled natural environment.” (Hanson and Davidson, 2005, p.7)

In line with the plan’s agenda, management actions should facilitate the protection of intact, non-impacted sword fern stands and restore affected sites with native understory vegetation. Given the currently ambiguous nature of the decline, restoration work should occur in tandem with and draw upon the results of experimental planting (See Section 3.3.2) to identify the suite of species best-suited for restoration on sites left bare following sword fern mortality. Restoration planning should also consider the high risk of further pathogen introduction and spread to actively mitigate these risks (see Section 3.3.4).

3.3.4. OBJECTIVE 4: PREVENTION AND MITIGATION

Recent evidence of pathogen-driven plant population declines has been widespread throughout the world (Littell et al., 2010; McGregor et al., 2016; Rippey, 2018; Tobin, 2016), with both native and exotic pathogens causing plant mortality. For example, five native fungal pathogens are reported to be responsible for the majority of conifer losses in forestry programs through Oregon and Washington (Hadfield et al., 1986). These include: laminated root rot (*Phellinus weirii*), Armillaria root disease (*Armillaria obscura*), annosus root disease (*Fomes annosus*), black stain root disease (*Ceratocystis wageneri*), and Port Orford-cedar root disease (*Phytophthora lateralis*). Other, less common root pathogens have typically been reported to cause only minor losses (e.g. Schweinitzii root rot [*Phaeolus schweinitzii*], tomentosus root rot [*Inonotus tomentosus*], and yellow or Stringy butt root rot [*Perenniporia subacida*]), but may be increasing plant susceptibility to insect damage and mortality under climate change.

In addition to native pathogens already present across the majority of the Pacific Northwest forests, exotic pathogens can also influence plant health. These pathogens can be spread by two means: (1) through restoration efforts, or through (2) public use of parks, especially in sensitive areas of active plant decline. Early Detection Rapid Response (EDRR) approaches advocate for preemptive planning to prevent the introduction of potentially destructive pathogens (Rabaglia et al., 2008; White et al., 2010). Although a pathogen has not been identified as the cause of the sword fern decline, a cautious approach can help reduce the risks associated with the introduction and spread of exotic plant pathogens that have the potential to negatively impact all native vegetation within the park.

Established permanent trail networks are important for promoting park use, ensuring accessibility, and maintaining the integrity of sensitive areas by restricting foot traffic. However, visitor- or wildlife-created informal trails, often referred to as social trails, do not fall within a park's formal trail system (Leung et al., 2002) and are abundant in Seward Park. These trails can also promote pathogens and invasive species spread into sensitive areas (Holmquist, 2004; Knight, 2000; Lindenmayer and Fischer, 2013). Maintenance of an electronic inventory of these trail networks is advantageous for park planners and restoration experts as they reinforce stable approved trails and decommission and rehabilitate social trails back to native habitat.

i. Nursery Stock Tracking

Unknowingly including pathogen-infected plant material in restoration projects can lead to the introduction of the pathogen into forests or natural sites (Frankel et al., 2016; Grünwald et al., 2012). Creating a process for tracking externally produced plant nursery stock used in restoration and mapping areas where planting has occurred, can help systematically monitor the correlation between these activities and the spread of the decline. Nursery stock should be ordered and tracked from source to site by planting program managers. Maintaining regular dialogue with the source nursery and conducting site visits to inspect growing stock prior to transport and planting operations is key.

ii. Decline Severity Risk Map

An updated map of decline locations can serve as a decline severity risk map. This map can be generated from findings of the mapping and monitoring efforts currently underway (see Section 3.3.1) and used to (1) guide restoration planning among project personnel and (2) to minimize potential cross contamination and transfer of materials from areas of decline to those that are healthy.

iii. Risk Mitigation Protocol

In addition to plant material, equipment (e.g. shovels, worker's boots, gloves, and field vehicles) is a high-risk vector for dispersing pathogens (Working Group for Phytophthoras in Native Habitats, 2016). The risk is

increased when crews operate during the rainy season. Awareness of hazards and the use of equipment hygiene methods can the reduce risk of infection.

To identify critical areas of hazard, a preliminary risk management analysis should be carried out to identify primary risk factors for pathogen transport and susceptibility in Seward Park (Tables 1.1 and 1.2). Once critical areas of risk are identified, SFDWG in partnership with GSP and Seattle Parks and Recreation should discuss the feasibility of creating a Seward Park phytosanitation plan. A detailed list of strategies for reducing the risk of pathogen spread has been outlined by the WSU Sudden Oak Death Program (WSU Puyallup Research and Extension Center, 2013) and the Phytophthoras in Native Plant Habitats Working Group (Working Group for Phytophthoras in Native Habitats, 2016), which could serve as useful references.

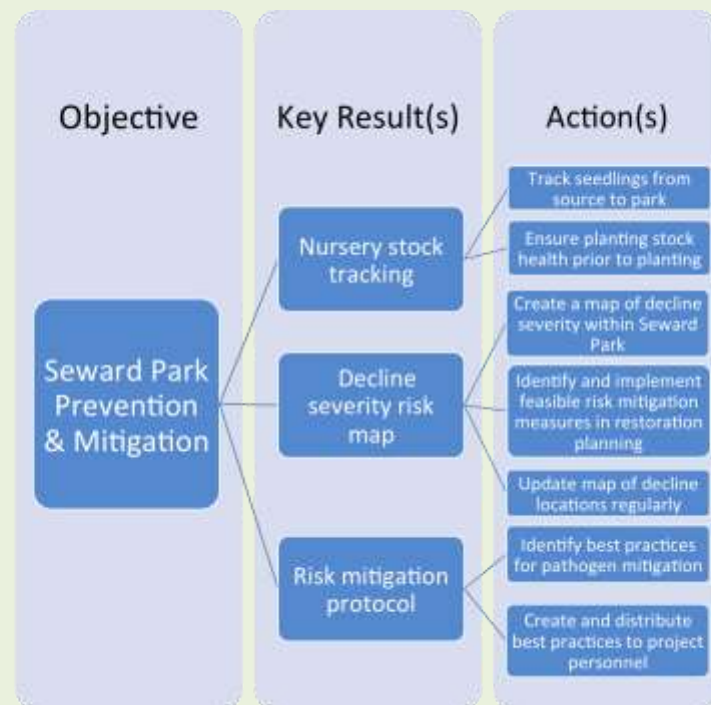


Table 1.1. Key for ranking critical risk factors (Table 1.2)

LEVEL OF RISK					
Likelihood	Impact				
	Insignificant (1)	Minor (2)	Moderate (3)	Major (4)	Catastrophic (5)
Almost certain (5)	<i>Medium</i>	<i>Medium</i>	<i>High</i>	<i>Extreme</i>	<i>Extreme</i>
Likely (4)	<i>Low</i>	<i>Medium</i>	<i>High</i>	<i>High</i>	<i>Extreme</i>
Possible (3)	<i>Low</i>	<i>Medium</i>	<i>Medium</i>	<i>High</i>	<i>High</i>
Unlikely (2)	<i>Low</i>	<i>Low</i>	<i>Medium</i>	<i>Medium</i>	<i>Medium</i>
Rare (1)	<i>Low</i>	<i>Low</i>	<i>Low</i>	<i>Low</i>	<i>Medium</i>

Table 1.2. Key risk factors associated with park management activities.

Key risk factor(s)	Associated activities	Likelihood (1 to 5)	+ Impact (1 to 5)	= Level of risk (Extreme, Med, Low)	Ability to Control (Y/N)
Movement of infected soil	Direct or inadvertent soil movement through equipment used for landscape management				
Movement of infected plant materials	Direct or inadvertent planting of infected plants in restoration efforts				
Movement of infected water or raw materials	Direct or inadvertent materials movement through landscape management at high risk regional sites (e.g. mulching, irrigation)				
Public traffic on trails and roads	Inadvertent pathogen spread from contact with eroding or unsanctioned trails				
Public traffic off trails and roads	Inadvertent pathogen spread from contact with plants and soil off trail				
Stress from drought, flood, or climate change	Increased frequency and intensity of pronounced drought periods, increased flood risk in winter, higher/lower temperature thresholds				
Invasive flora	Increased stress from resource competition and encroachment				
Invasive fauna	Outbreaks associated with increased herbivory and animal-spread pathogens				

SECTION 4. OUTREACH AND PUBLIC ENGAGEMENT

The sword fern decline phenomenon is an important opportunity to engage further with park users, Green Seattle Partnership stakeholders, the scientific community, and regional land managers. The following section summarizes past outreach endeavors and formulates a plan for outreach in 2019 and beyond.

To date, Sword Fern Decline Working Group (SFDWG) has developed substantial and effective *ad hoc* strategies for sharing information about the status of sword fern decline with the general public and as a means of soliciting informal assistance. However, given the complexity of the drivers of the decline, a framework is needed to solicit (a) municipal and external support, (b) institutional partnerships, and (c) public engagement.

4.1. Summary of Past and Ongoing Outreach

4.1.1. Media Coverage

Working Group members engaged with the media. This has resulted in the following press:

- KUOW – Acres of Dead Forest Plants Puzzle Scientists
- Sierra Club Magazine – Mystery of the Missing Ferns
- King 5 – Massive Mystery Fern Die-Off at Seattle Park
- Kitsap Sun – Ferns are dying in Kitsap Forests, and nobody knows why

4.1.2. Web Presence

A public website has been created by the Green Seattle Partnership to outline the known information regarding the severity, extent, and associated work surrounding the sword fern decline. The Sword Fern Decline at Seward Park webpage serves as an avenue for updates, information sharing, and outreach. SFDWG member Paul Shannon continues to curate the Seward Park Sword Fern Die-off Blog, which captures his communications with fern experts, reports from around the region, hypothesis, and some research results.

4.1.3. Signage

Temporary signage was installed in 2017 at the Ground Zero site in Seward Park, as well as at park kiosks and in the Seward Park Audubon Center. Updated signage, produced pro bono by Johnson+Southerland, will replace the temporary signage at Seward during winter 2018.

4.1.4. Presentations

Working Group members have had the opportunity to present on the sword fern decline. The following is a list of presentations:

- Tim Billo, Washington Native Plant Society Meeting, November 13, 2016
- Paul Shannon, Green Seattle Partnership Field Committee Meeting, June 2, 2016
- Lisa Ciecko, Society for Ecological Restoration Northwest Conference, October 15, 2018
- Tim Billo, Washington Native Plant Society Meeting, February, 2019

4.2. Future Priority Efforts

4.2.1. Communications Planning

To further identify priority outreach efforts, the SFDWG outreach committee will work with Seattle Parks and Recreation to develop a communications plan and additional materials. This can include a messaging platform, communications best practices, and a media kit with images and talking points. In addition, the plan will outline next steps for website updates, signage, and presentations, and photo sharing.

4.2.2. Individual Outreach

On occasion, SFDWG members have directly sought out and contacted experts in the region to solicit their professional advice or to form collaborations. These are beneficial relationships that merit documentation, discussion among the larger SFDWG members, and tracking for continuity of project objectives or individual objectives. These relationships, like those with larger entities and agencies would be best served through the project tracker (Appendix 5.2). Initial contact, additional correspondence and relevant milestones can be logged as with any ongoing project.

4.2.3. Municipal and External Support

Relationships with industry, environmental groups, other municipalities and agencies can provide pathways for information flow beneficial to both management and mitigation of sword fern decline at Seward Park. Additionally, formal partnerships with researchers and institutions with analytical resources can enhance the quality of inference. The critical component to these collaborations is offering a functional framework for maintaining relationships and facilitating research. This Strategic Plan, the SFDWG Charter, and formation of best practices surrounding procedure and implementation of projects at Seward Park will demonstrate capacity and build trust among external entities. Trust in the SFDWG and its members can facilitate access to shared resources, funding, personnel, and information, all of which can contribute to greater project efficacy.

4.4.4. Institutional Partnership(s)

There are several regional institutions including the University of Washington (UW), Washington State University (WSU), and Evergreen State College that have both research faculty and academic research trajectories aligned with natural resource management and human dimensions of resource management. Already in place at Seward are pro-bono relationships with researchers from UW and WSU that have provided access to intellectual and laboratory support. Further, there is opportunity to procure a student lead work force on a semi-regular basis to assist with field and analytical ventures and with access to appropriate support facilities to promote sound scientific inquiry on Seward Park.

Partnerships with research institutions in hopes of securing funding for individual research efforts can be complicated by overhead costs. When the Principal Investigator (PI) is a primary affiliate of an institution, upwards of 40% of awarded funding can be dispersed to the institution. An alternative approach utilizes one or a series of agreement types with institutions in order to procure the services or support of individual faculty, laboratories, analytical services, student services, or myriad other exchanges where monetary considerations can be constricting.

Agreement types typically considered by top tier public institutions like UW and WSU include:

- A Memorandum of Understanding (MOU), where an entity can “memorialize an expression of intent, or expressing goals and aspirations or activity without committing to a legally binding agreement implicating legal remedies.” An MOU with a regional institution would allow for blanket collaborative agreements to be negotiated among individuals or representatives of each entity towards a larger collaboration without monetary exchange.

- A Data Use Agreement (DUA), for the “transfer of data collected or developed, as opposed to other types of information. The data being transferred is subject to restriction for regulatory purposes and not typically for proprietary interests.” A DUA would serve as an understanding for sharing relevant project data among faculty and SFDWG members towards agreed upon project goals.
- Unfunded Research Agreement (URA), for “where monetary consideration is not received by and institution, but an external entity may provide other types of support (e.g. samples, data, materials, equipment).”
- Affiliation Agreements (AA), for “Collaboration between employees of the University and the cooperating agency without specific assignment of facilities to the cooperative effort.”

To move towards these agreements, a project level or greater collaborative narrative needs to be established with individual researchers or administrators. Subsequent planning will involve negotiating an agreement, funding strategy, and timelines. This can be accomplished through SFDWG committee work, where a committee formulates an outreach letter to experts or agencies of interest. This Strategic Plan can serve as a reinforcing document for outreach, by demonstrating group efforts, structure, and function.

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SECTION 5. THE WORKING GROUP CHARTER

5.1. Purpose

The working group's purpose is to:

- Determine the cause of the sword fern decline
- Document the distribution of the decline through systematic monitoring
- Understand how to prevent the spread of the decline
- Identify effective restoration efforts
- Implement outreach strategies to extend collaboration regionally and build capacity
- Advise Seattle Parks and Recreation on sword fern decline management at Seward Park

5.2. Structure

The working group was formed in 2015 as interested individuals initiated projects to address the issue of sword fern decline in Seward Park. Seattle Parks and Recreation, as the land manager of Seward Park, has taken on a coordination role, including overseeing partner organizations EarthCorps and Verdant Consulting Group, LLC to facilitate working group efforts starting in 2017.

The SFDWG is made up of self-identified members; people who have come to the group willing to contribute their time and expertise to addressing the sword fern decline phenomenon. There are currently 19 active members and a total of 29 interested participants (Working Group Roster; Appendix 5.1). Together the members of the group share a diverse skillset, which includes: computation, mapping, grant writing, data collection, experimental design, research planning, plant science/horticulture, botany, plant pathology, student/volunteer supervision & mentorship, public outreach, and knowledge of Seward Park.

Members of the group agree to the coordination elements listed in Section 5.3 below, as well as the Seattle Parks and Recreation Behavioral Expectations. Although the following expectations may seem obvious, they are included to reflect without doubt what is required of working group members. As an advisory group to Seattle Parks and Recreation, members are ultimately responsible to the Superintendent of Seattle Parks and Recreation (Seattle Parks & Recreation, 2018).

Behavioral Expectations:

- 1) As a volunteer, you represent Seattle Parks and Recreation. Like all Parks employees, you must treat the public, park users, and other volunteers and Parks employees with respect.
- 2) Threatening or harassing behavior will result in removal from the volunteer event and could result in expulsion from the park.
- 3) Individuals must not be under the influence of alcohol or controlled substances while participating in volunteer activities.
- 4) Safety is of utmost importance at all times. Take safety seriously and consider the use of safe work practices for yourself and others as a personal responsibility.
- 5) Recognize parks as public places. All users have rights to the parks. Volunteer events are open to the public unless Parks staff has approved a closure or exclusive event.

5.3 Coordination

5.3.1. Project Reporting

To meet requirements for research and restoration activities carried out on Seattle Parks and Recreation managed lands, all projects are required to provide a written report documenting project methods, analysis, and results at the culmination of a project.

To ensure continued coordination, accountability, adaptive management, and recognition, all research projects should be recorded in the tracking platform (see Appendix 5.2). This system provides a structure for project leaders to share with the group, allows municipal staff and managers to be apprised of project status, and simplifies reporting and documentation. At each quarterly meeting, the meeting facilitator should follow up on the status of each project (see Appendix 5.2. for project status codes). Between meetings, regular reporting among the working group should occur by email.

5.3.2. Project Proposal Submission

To facilitate a strategic approach to continued research and management of the sword fern decline efforts in Seward Park, the SFDWG will continue to use the project proposal review process (Appendix 5.3). This process allows Seattle Parks and Recreation to provide approval for research conducted on its land and to provide a broader review of research efforts by SFDWG peers.

5.3.3. Data Management

Document and data storage will be maintained on a DropBox system until another option becomes available. Standards for document storage, maintenance, project reporting, and data sharing should be developed and adopted by the working group in 2019 to support continued collaboration.

5.3.4. Meeting Schedule

The working group will meet in full twice annually to share accomplishments and work on planning efforts. Working group committees will meet more regularly, at least quarterly, by phone or in person. The individual(s) charged with the coordination role for a given year will set the schedule. Seattle Parks and Recreation will continue to track working group members and other interested parties.

5.3.5. Communication Expectations

Because members of the working group come from all backgrounds and often have limited capacity to contribute to the sword fern effort, communication expectations should be reasonable. Working group members agree to limit emails to the full group to issues of meeting coordination. The working group will continue to consider alternatives for effective communication.

5.3.6. Volunteer Tracking

Work at Seward by individuals who are not affiliated with an institution should be logged, as volunteer hours and a liability form should be signed. Seattle Parks and Recreation staff will coordinate volunteer tracking.

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SECTION 7. APPENDICES

Appendix 2.1.

Sword Fern (*Polystichum munitum*) Decline Symptoms



Figure 1

Early Stage of Decline

- Isolated fronds show signs of stress, while the majority of the fern still appears healthy (Figure 1)
- Affected fronds can display a variety of symptoms:
 - Brown rotting color
 - Pale color similar to bleaching, yellow to white
 - Loss of vigor and lose their upright form

Middle Stage of Decline

- Fronds and leaflets begin to dry out and/or wilt
- 25- 75% of fronds display characteristics of stress related to the decline (Figure 2)
- Some fronds have completely wilted and died (Figure 2)



Figure 2

Late Stage of Decline



Figure 3

- Most fronds is wilting and brown (Figure 3)
- Eventually there is a complete loss of fronds, leaving a crown stump that may remain intact for several years without regenerating (Figure 4)



Figure 4



Appendix 3.1

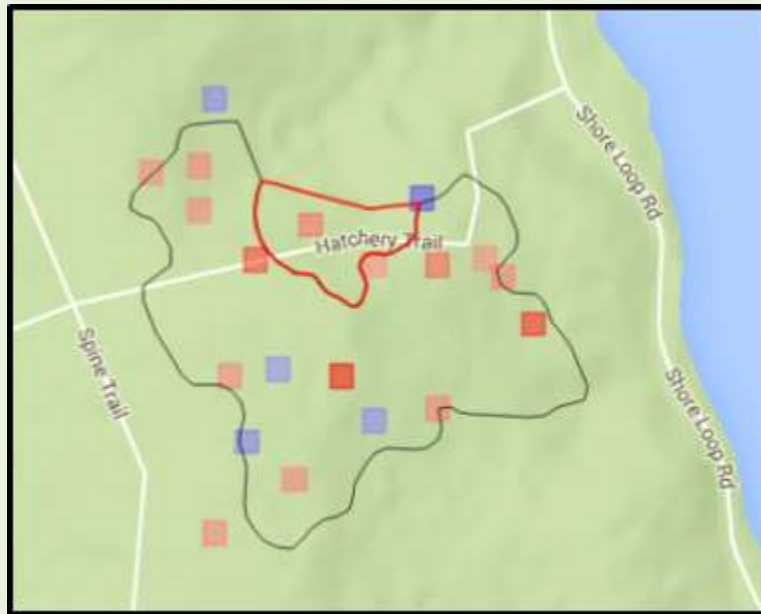


Figure 1. Plot layout around Ground Zero and initial decline area.



Figure 2. Healthy (left) and declining (right) fronds of western sword fern.



Figure 3. Polygons outlining the decline of the sword fern.

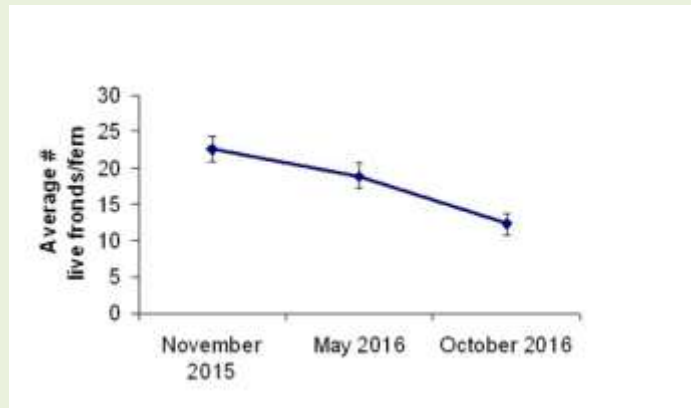


Figure 4. Average quantity of sword fern live fronds from sampling in November 2015 through May and October 2016. Note a decline through 3 distinct sampling periods at sample area in Seward Park.

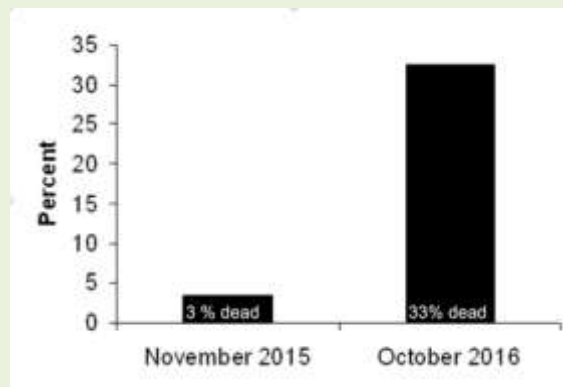


Figure 5. Percent mortality of entire sword fern plants from sampled population evaluated in November 2015 and then October 2016. Note a 30% increase in mortality across the sampled area in Seward Park.

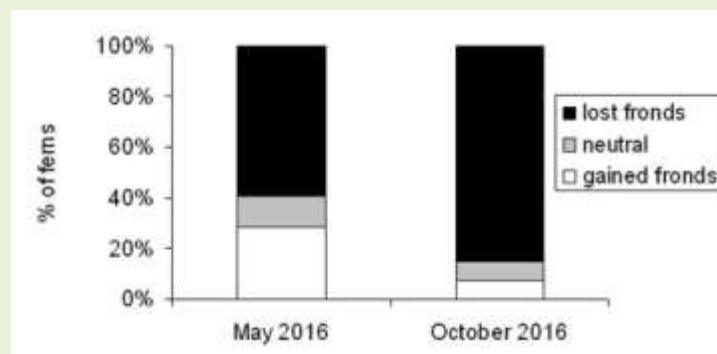


Figure 6. The average percent variation in frond quantity among a sampled sword fern individuals sampled at Seward Park. Plants frond dynamics were sampled at the individual level for this data set. Note increase in percentage of individuals that lost fronds from May 2016 through October 2016.

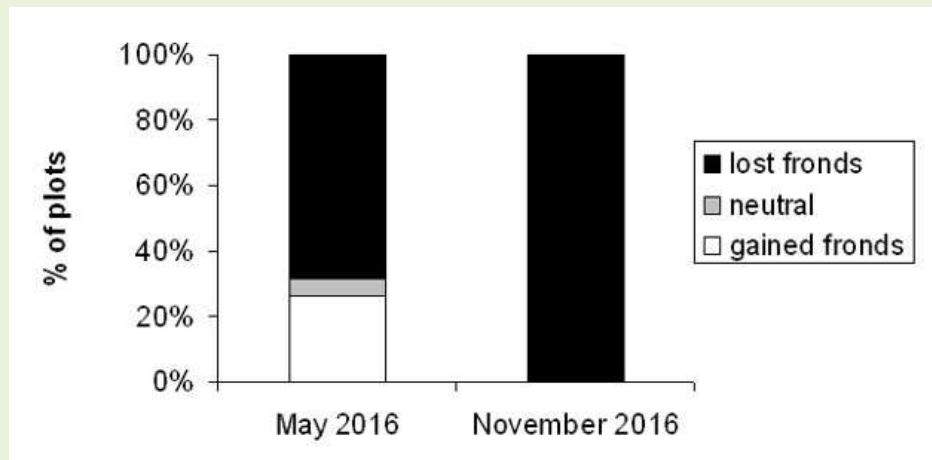
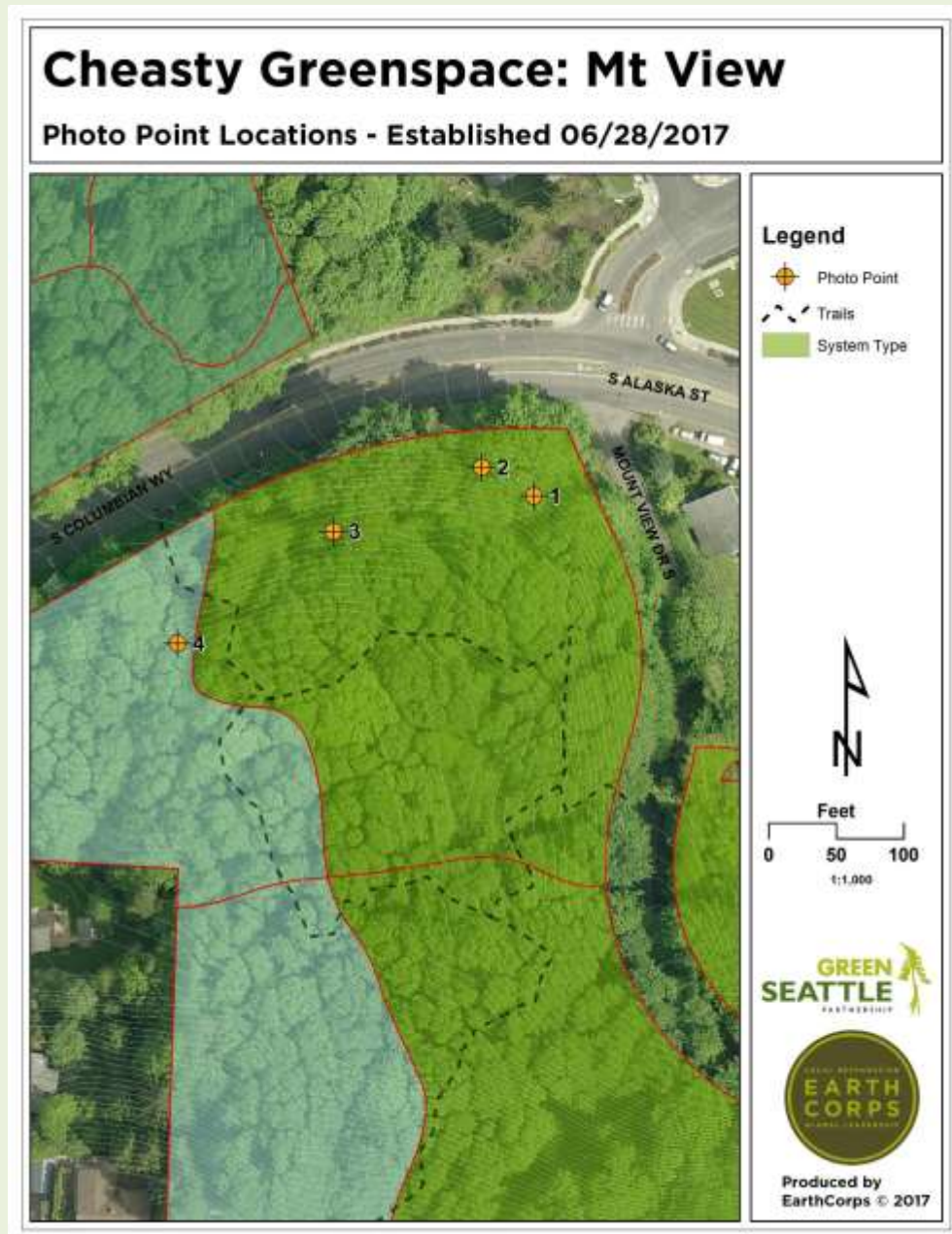


Figure 7. The average percent variation in frond quantity among a sword fern monitoring plots sampled at Seward Park. Plants frond dynamics were assessed at the plot level for this data set. Note increase to 100 percent of plots having lost fronds by October 2016.

Appendix 3.2.



Cheasty Greenspace: Mt View Photo Monitoring: 2017

PP1 (1 of 1): 06-28-2017_ENE



Cheasty Greenspace: Mt View Photo Monitoring: 2017

PP2 (2 of 2): 06-28-2017_WSW



Cheasty Greenspace: Mt View Photo Monitoring: 2017

PP3 (1 of 1): 06-28-2017_SSE



Cheasty Greenspace: Mt View Photo Monitoring: 2017

PP4 (1 of 1): 06-28-2017_WSW



Cheasty Greenspace: Mt View Photo Monitoring: 2017

PP2 (1 of 2): 06-28-2017_SSW



Seward Park Photo Monitoring: 2017

PP1 (1 of 1): 06-28-2017_NNW



Seward Park Photo Monitoring: 2017

PP2 (1 of 1): 06-28-2017_ESE






Dead Horse Canyon

Photo Point Locations - Established 08/25/2017



Legend

-  Photo Point
-  Trails
-  System Type



Feet
0 280 560
1:5,209

**GREEN
SEATTLE**
PARTNERSHIP



Produced by
EarthCorps © 2017

Dead Horse Canyon Photo Monitoring: 2017

PP1 (1 of 1): 08-25-2017_N



Dead Horse Canyon Photo Monitoring: 2017

PP2: 08-25-2017_Stake Location



Dead Horse Canyon Photo Monitoring: 2017

PP2 (1 of 1): 08-25-2017_N



Dead Horse Canyon Photo Monitoring: 2017

PP1: 08-25-2017_Stake Location



Dead Horse Canyon Photo Monitoring: 2017

PP3 (2 of 3): 08-25-2017_S



Dead Horse Canyon Photo Monitoring: 2017

PP3 (1 of 3): 08-25-2017_SE



Dead Horse Canyon Photo Monitoring: 2017

PP3 (3 of 3): 08-25-2017_SW



Dead Horse Canyon Photo Monitoring: 2017

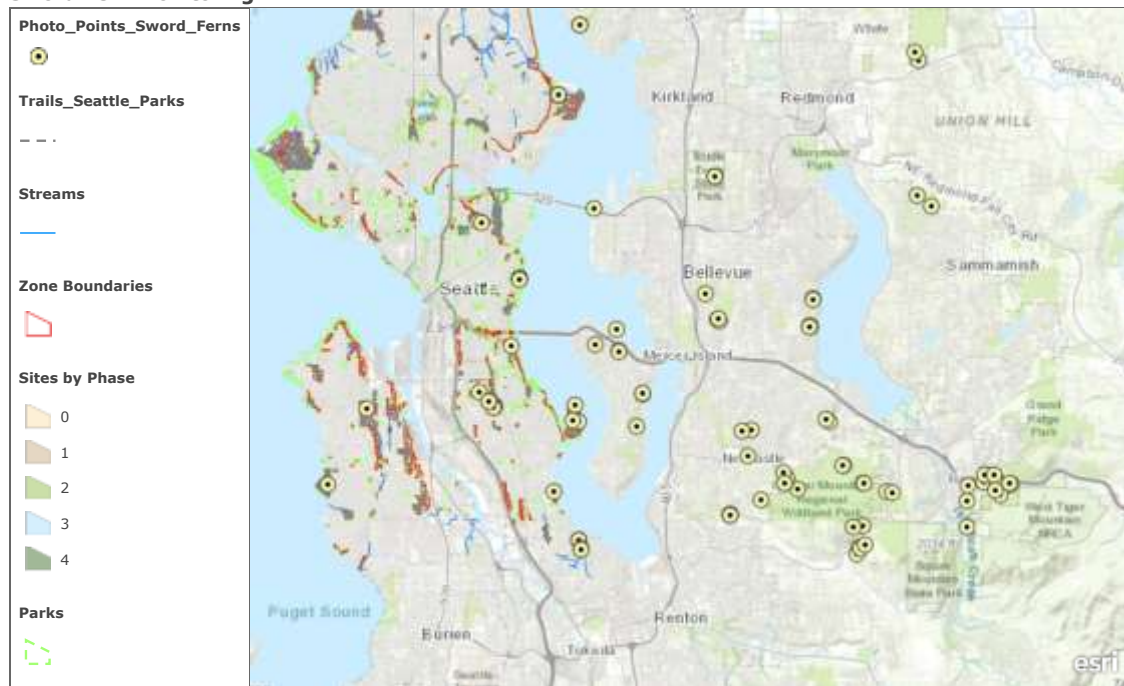
PP3: 08-25-2017_Stake Location



Dead Horse Canyon Monitoring: 2017



Sword Fern Monitoring



App for collecting photo monitoring data at known and potential locations of sword fern die-off.

City of Bellevue, WA, Bureau of Land Management, Esri Canada, Esri, HERE, Garmin, USGS, NGA, EPA, USDA, NPS

Rapid Sword Fern Photo Monitoring Protocols

Repeat photography can be an effective and economical method to track changes to a landscape over time. The following protocols have been streamlined to allow them to be applicable to a wide range of potential users across varied locations using different cameras/devices. These protocols were designed to capture and track changes to the vegetative conditions at potential sites where sword fern die-off is suspected. More comprehensive methodologies may be warranted at known locations where die-off has been documented and more detailed tracking may be deemed necessary.

In order to establish monitoring locations, the following information is recommended:

1. Semi-permanently mark the precise location where the photo was taken.
 - a. Recommended method of monumentation is to drive a wooden stake ($\frac{3}{4}$ " x $\frac{1}{2}$ ") into the ground with a piece of flagging nailed to the top surface. Write on the flagging indicating the photo location ID number.
 - b. If the photo point is along a trail, the stake should be inserted flush with the ground to avoid tripping hazards. If the photo point is off-trail, use your discretion to allow for relocation and safety.
 - c. DO NOT place stakes unless you have express permission from the landowner/manager!
2. Clearly record the following information for each photo taken at each location:
 - a. The locality of the photo (City, Park/location, Address)
 - b. The unique point ID (recommended format: SEWARD_PP1, SEWARD_PP2, etc.)
 - c. The date the photo was taken
 - d. The general direction you were facing (N, NNE, NE, ENE, E, etc.)
 - e. The number of photos taken at each location (if applicable)
 - f. Any comments or observations that may be pertinent.
3. Create a map (GPS aided where possible) to visually indicate the location of each photo point in relation to known objects or features (such as trails or roads etc.).
 - a. King County [iMap](#) or google maps may be useful resources.

Apps like TimeShots or Before and After Cam may also be useful, although they currently do not seem to be fully developed or reliable at this time (at least for Android).

Another option could be an open-accessible web-map where users would be able to take or upload photographs and associate them with user-defined point locations (using ArcGIS online). This would provide a central database where all interested parties could update, add to, and access the photo monitoring locations and images.

Appendix 3.3.

Seward Park's Sword Fern Decline Line Point Intercept and Health Monitoring Protocols

Line Point Intercept

1. 50 meter transect
2. Random pin drop every $\frac{1}{2}$ meter
 - a. 100 points per 50 meter transect
3. Always read the left side of the line beginning at meter 0.
4. Record every hit.
 - a. If the pin hits any part of an alive sword fern then it is a hit.
 - b. If the pin does not hit any part of an alive sword fern then it is a miss.
 - i. Do not record misses.
5. What is considered a hit and a miss?
 - a. Hit:
 - i. Pin drop touches a live piece of sword fern that is still connected to the central base of the plant.
 - ii. Fern and or frond must have green foliage and must be attached to the central base of the plant.
 - b. Miss:
 - i. Pin drop does not touch any part of a sword fern
 - ii. Pin drop touches a fern frond that is still connected to the central base but the frond is entirely brown and not alive
 - iii. Pin drop touches the central base of a sword fern plant but there are no fronds growing out of the central base and no new little green fronds are emerging. The fern is entirely dead and only the central base remains.

Photo Monitoring

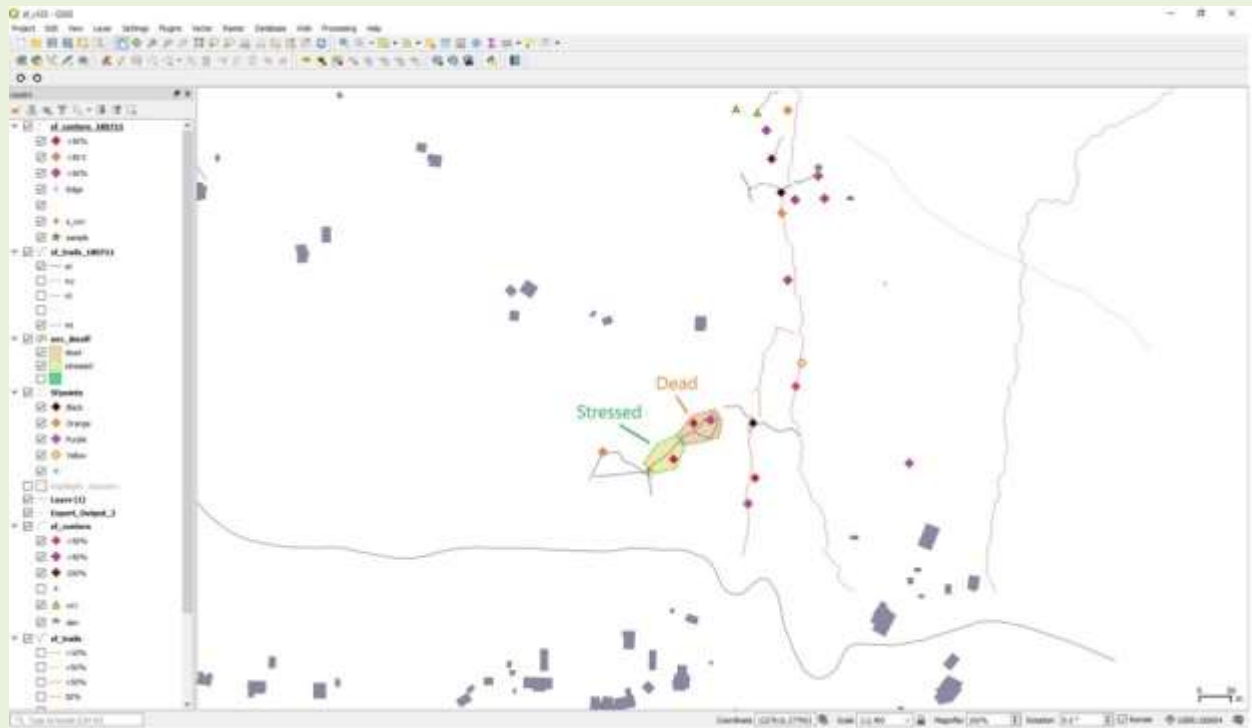
1. At least 2 photos per transect. One photo at meter 0 and another photo at meter 25.
 - a. Stand 3 meters back from the photo point so that the beginning of the transect is included in the photo.
 - b. If there is vegetation blocking the view of the photo at any point along the line, then take more photos so that the entire transect can be seen in the photos.
 - c. Use photo board that shows:
 - i. the plot name
 - ii. date
 - iii. transect azimuth
 - iv. at what meter along the transect the photo was taken at.

Health Assessment

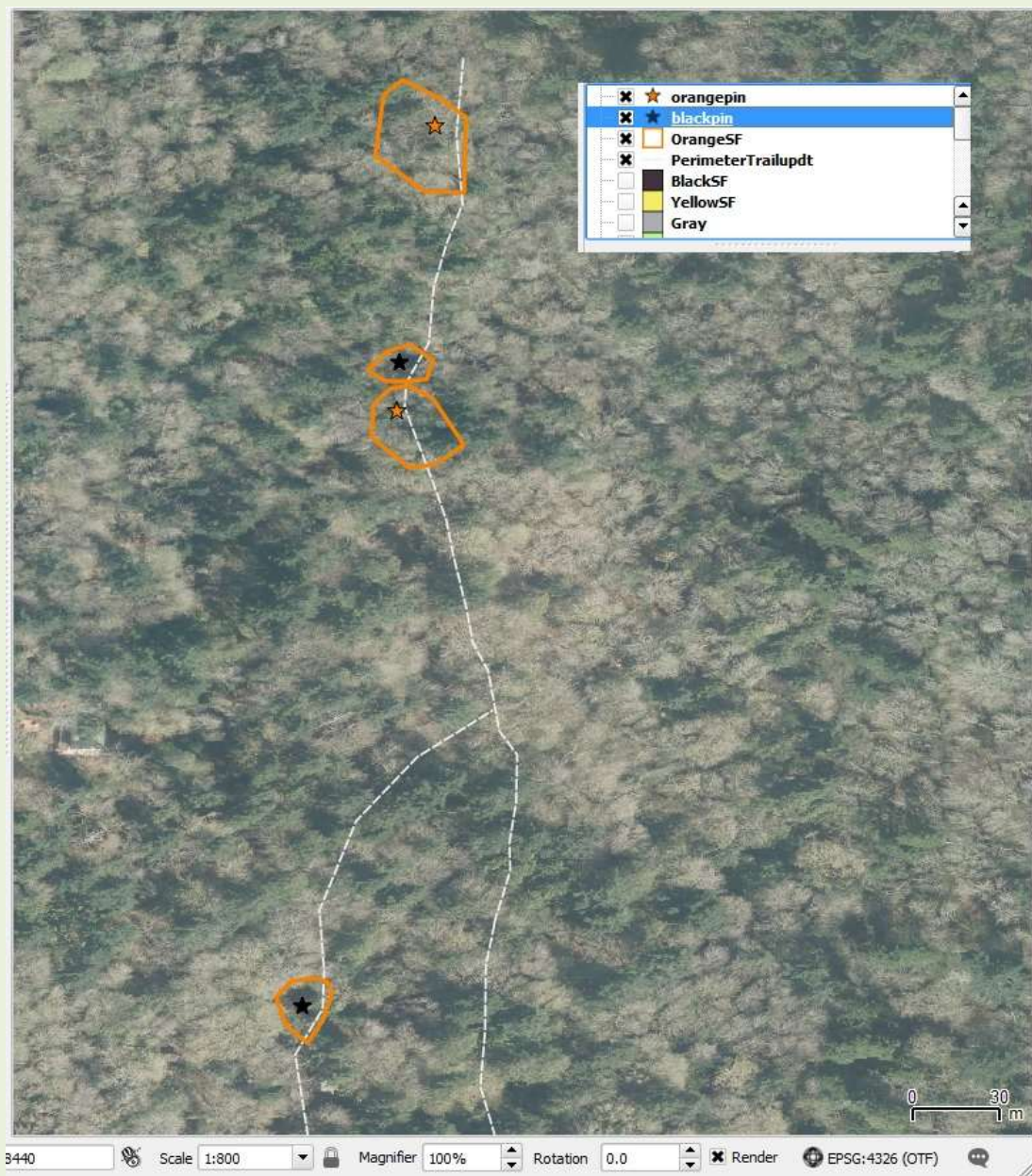
1. Use the same 50 meter as for the Line Point Intercept
2. Begin at meter 0 and identify every sword fern that is alive or dead within 1 meter of the left side of the transect.
 - a. The central base of the plant must be within the 1-meter width on the left side of the transect or touching the edge of the 1-meter width.

- b. If the central base does not fall within the 1-meter width or touch the edge of the 1-meter width, then the fern is not included in the plot.
 - c. If the frond is within the 1-meter wide plot but the central stem base is not, then the fern is not included in the plot.
- 3. Identifying individual ferns can be very difficult. To help alleviate this confusion, we will use a standard method to identify ferns whose central stem base are in very close proximity to each other.
 - a. The central stem base of each individual fern must be at least 4 inches from the central stem base of any other sword fern.
 - i. 4 inches is equivalent to about the width of a human fist.
 - ii. If you can not fit your fist between two central stem bases, then those two central stem bases are considered to be the same fern because their roots are significantly overlapped to where it can be certain that they are two individual ferns.
- 4. Identify every fern based on these four categories:
 - a. 1: Alive and thriving
 - i. Fern has <20% brown foliage and the fern in general appears to be healthy and not showing any signs of stress.
 - b. 2: Stressed
 - i. Fern has >20% brown foliage, does not appear to be thriving, and is not entirely dead.
 - c. 3: Dead
 - i. Fern does not have any green foliage and does not have any new growth coming out from the central root base.
 - ii. Fronds may still be attached to the central stem base but there is no green foliage on the leaflets or stems of these fronds.
 - d. 4: Recovering
 - i. Some ferns that appear to have been dead for many months or even years, begin growing small new fronds that can be smaller than a human finger.
 - ii. These ferns are considered alive, but they do not seem to have the health capacity at this time to grow into mature ferns. Thus, they are being put into their own category because they still appear to be alive but need to be separated from categories 1 and 2.
 - e. 5: Seedling Juvenile Fern- fronds shorter than your index finger.
 - i. Some plots have very small ferns that are likely less than a year or two old. We need to track this natural regeneration but also need to keep a record of the juvenile ferns separate from categorizing them as a 1, 2 or 3.

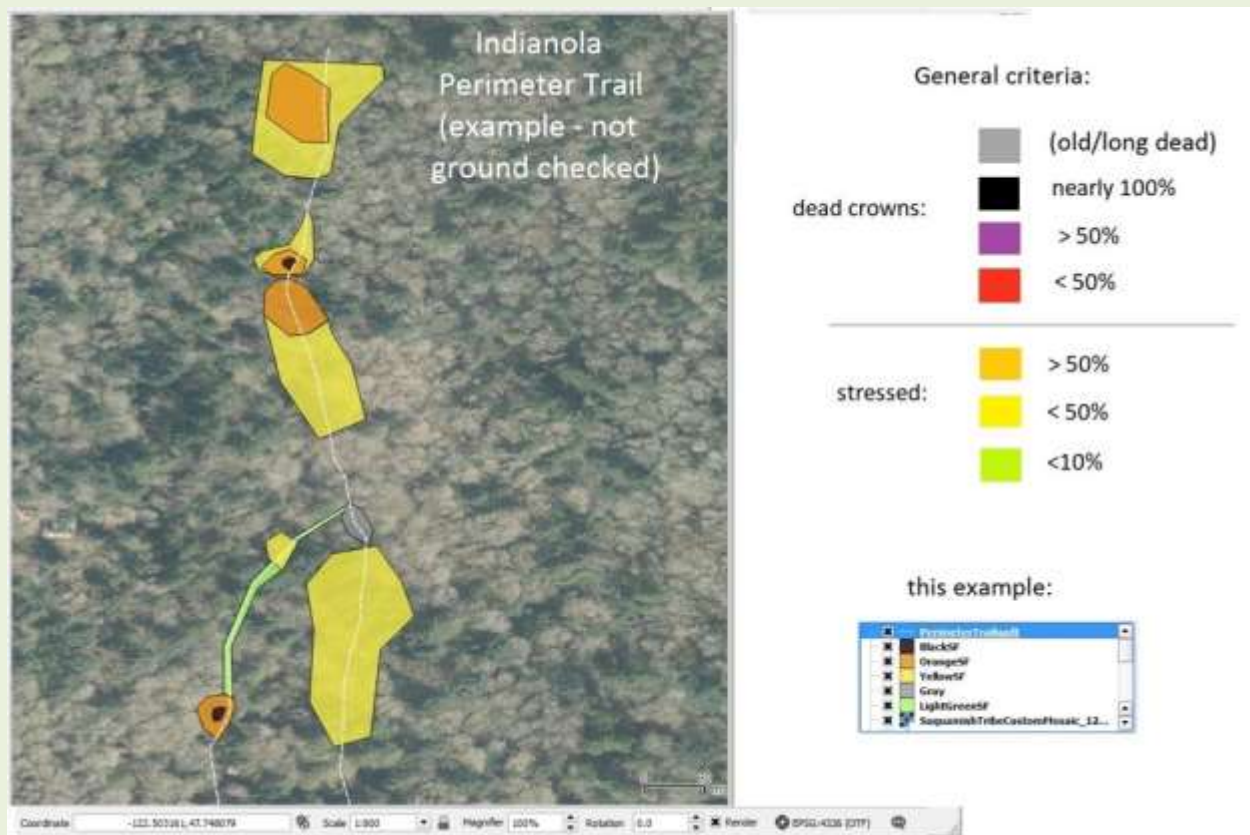
Appendix 3.4.



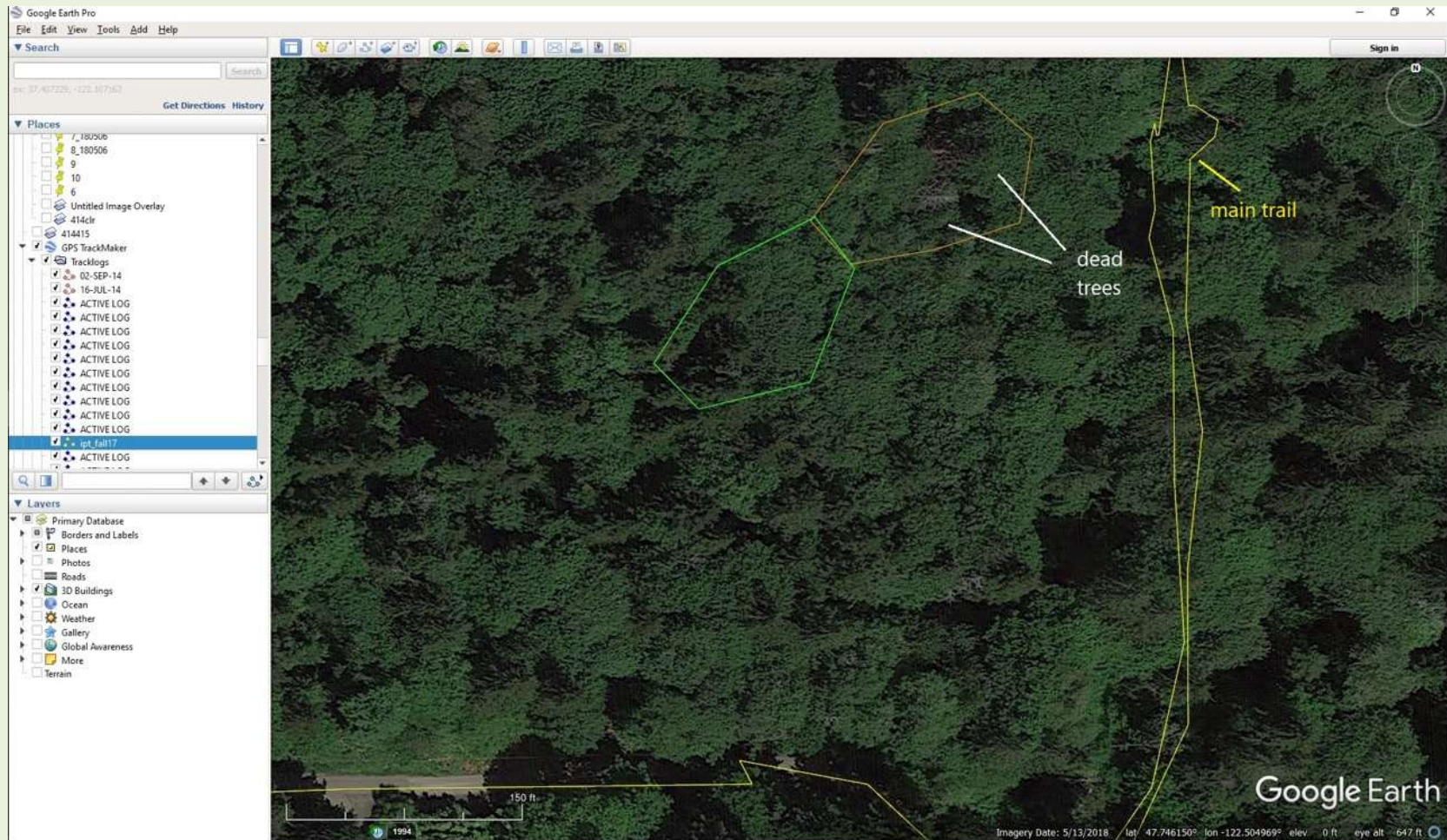
IPT_180711.jpg



sf_points.jpg



sf_polygons.jpg









Appendix 3.5.

iNaturalist guidelines

1. Observations should be collected on sites where: at least 4 dead ferns in one area are present (near complete browning and wilting of fronds, complete loss of fronds with only a crown stump remaining, or crown stump failing to regenerate new fronds in spring/summer)
2. Observations must include geographic coordinates (please be sure that the “Geoprivacy” category in the “Add observation” menu is set to “Open”)
3. Please include an estimate of the size of the impacted area in the notes.
4. Repeated observations of the same area in spring and fall (to note regeneration failure) are encouraged.

Appendix 3.6.



Ribeiro Consultants

10744 NE Manitou Beach Drive, Bainbridge Island, WA 98110
Phone & Fax: 206-842-1157. e-mail: fungispore@comcast.net. website: www.ribeirotreeconsultants.com
Consultants to the Arboricultural & Horticultural Industries – Specializing in Plant Disease Diagnosis

PLANT PATHOLOGY: LABORATORY REPORT

Report #: 15041A
Date: June 23, 2015
Client: Lisa Ciecko: Plant Ecologist
Seattle Parks and Recreation, Natural Resources Unit
Jefferson Horticulture Facility. 1600 South Dakota St | Seattle | 98108 -1546
Your Ref: **Seward Park Fern dieback**
Plant spp.: *Polystichum munitum* (western sword fern)
Material analyzed: Soil ☒ Roots ☒ Tissue ☒ Other:

Sample #	Your Reference	Tissues/Roots
15041A	Dying Sword Ferns	Roots: <i>Rhizoctonia</i> . Tissues: <i>Glomerella</i> . Stems: <i>Phoma</i> (extensive); Base of stems: <i>Rhizoctonia</i> .

Seward Park was visited on June 8, 2015 in the company of Paul Shannon, Paul Talbert, Barbara DeCaro, Lisa Ciecko, Joseph Manson, and Flip Reilly to evaluate the extensive dieback of sword ferns in the park.

The dieback observed appeared to follow a pattern more consistent with a root rot problem (see pictures below). However, there were other symptoms that indicated that the dieback observed was being caused (or initiated) by several mitigating circumstances.

Conditions observed:

1. Extensive browning and total collapse of ferns.
2. Ferns showing initial signs of stress (browning of the margins of the fronds).
3. Soil very dry.
4. Canopy in the area of fern dieback more open than other areas where ferns appear healthy.
5. Majority of trees in this area are western red cedars.
6. There are no Douglas Firs in the north-facing "ground zero" slope.
7. Big leaf Maple trees presently provide some canopy. However, this canopy is lost after leaf fall in the autumn.



Figs.1& 2: Fern dieback (arrows)

Pathology results:

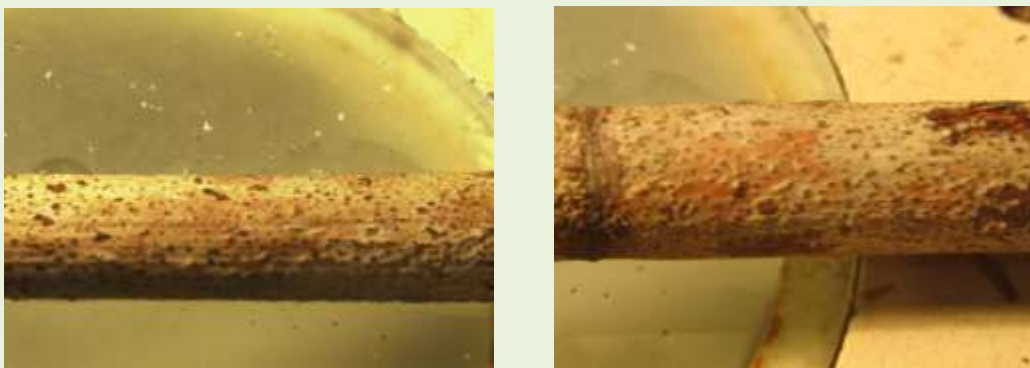
Roots examined exhibited dieback of the feeder root terminally. This appeared to be a condition caused by desiccation rather than pathogen infection. The root rotting fungus pathogen *Rhizoctonia* was occasionally isolated but was not considered the cause of the root dieback observed. The bases of the stems were extensively rotted (see Figs. 3, 4 & 5 below). This is more typically of pathogen infections. *Rhizoctonia* was isolated from the diseased stems and is believed to be the main cause of stem dieback.



Figs. 3, 4 & 5: Base of fern stems showing extensive rot (arrows) caused by *Rhizoctonia*

Rhizoctonia is a difficult pathogen to control. **Composted** bark chips used as mulch have been reported to reduce the levels of this pathogen in the soil. Uncomposted amendments that is high in cellulose (e.g. straw) increase disease. Deficiency of potassium or calcium also increases *Rhizoctonia*. Infections increase with excess nitrogen applications. Several fungicides are available for control of *Rhizoctonia*. These will require to be applied by qualified pesticide applicator. Actinovate is one biological fungicide with efficacy against *Rhizoctonia*.

Stems were also extensively infected with the stem blight pathogen – *Phoma* (see Fig. 6 & 7 below). This pathogen is known to cause stem dieback in a number of plants such as asparagus and oil rape seed.



Figs. 6 & 7: Extensive *Phoma* infections (black dots) on Fern Stems

Fixed copper sprays, such as Kocide DF or Champ, control *Phoma*. Due to the levels of pathogen present on these stems, more than one fungicide application will be required. Rake up and destroy as many of the infected stems as is feasible. This will help reduce inoculum. Avoid overhead irrigation. If overhead irrigation is being used, irrigate in the early morning hours so leaves have time to dry during the day.

The fronds are infected with *Glomerella* fungus blight though not in sufficient amounts to cause complete dieback of the frond. No foliar nematodes (a problem reported on ferns), were isolated from fronds tested.

It is difficult to control *Glomerella* infections. Champ Formula 2, Kocide DF, Protect T/O, Heritage, and Spectro 90 WDG are some fungicides that may help reduce infections caused by this pathogen. Rake up and destroy as many of the fallen leaves as is feasible. Prune only in the dormant season.

Soil Nutrient Analyses: The nutrient analyses (see attached) indicated that Phosphorus, Magnesium, Zinc, Manganese, Copper and Iron are excessive. Potassium, Calcium and Boron are high. Excess Phosphorus inhibits the uptake of copper, iron, zinc and boron; excess potassium inhibits the uptake of nitrogen, calcium and magnesium; excess iron inhibits the uptake of manganese and phosphorus and excess zinc inhibits the uptake of manganese and iron.

See attached report on amount of fertilizer to apply to correct some of these problems.

Summary:

Polystichum munitum (western sword fern) is an evergreen fern native to western North America, where it is one of the most abundant ferns. According to the literature I have read Sword ferns perform best in moist shady conditions. However, as long as there is good drainage, the sword fern can easily adapt to a number of soil conditions. They can even thrive in sun when given plenty of moisture.

“The favored habitat of this fern is the understory of moist coniferous forests at low elevations. It grows best in a well-drained acidic soil of rich humus and small stones. Sword ferns are very tough and can survive occasional dry periods, but do well only with consistent moisture, light sunlight, and prefer cool weather to overly warm. In cultivation, they also respond well to regular, light applications of fertilizer” Paul Alaback *et al. Plants of the Pacific Northwest Coast* (Revised ed.). Vancouver, British Columbia: p. 53.

Recommendations:

1. It would not be cost effective to treat for the pathogens isolated. Better to remove all diseased plants and rake up and destroy any remaining plant debris.
2. Before replanting, add a layer of composted mulch to increase biological activity.
3. Monitor during periods of little rainfall in summer months. Irrigate at periods when soil becomes excessively dry.
4. Always move from healthy to diseased areas to prevent spreading inoculum. **Do not enter healthy fern areas after walking in diseased fern areas.**
5. Consider planting more conifers in these areas to increase canopy cover in the future.

Please call or e-mail should you have any further questions.

Olaf K. Ribeiro, Ph.D. CPAg
ARCPACS Certified Plant Pathologist/ Certified Arborist PN#6390A

Member: American Phytopathological Society ~ International Society of Plant Pathologists ~ International Society of Arboriculture ~ American Registry of Certified Professionals in Agronomy, Crops & Soils

Analytical Results		Sample: 'Ferns - 15056'				Simply Soil Testing	
Customer:		Ribeiro Consultants 10744 Manitou Beach Dr. Bainbridge Island, WA 98110		Date received: 06/10/15 Report date: 06/16/15 Sample reference #: 00819 Plant code: 99 - Ferns Tests requested: Basic, OrgM, B, S, Zn, Mn, Cu, Fe			
RESULTS		INTERPRETATION					
Nutrient	Level	Low	Medium	High	V High	Result	
Phosphorus (P)	128 ppm	<div></div>				Very High	
Potassium (K)	150 ppm	<div></div>				High	
Calcium (Ca)	1121 ppm	<div></div>				High	
Magnesium (Mg)	189 ppm	<div></div>				Very High	
Boron (B)	0.9 ppm	<div></div>				High	
Sulfur (S as SO4)	3.4 ppm	<div></div>				Medium	
Organic Matter	9.6 %	<div></div>				High	
Soluble Salts	0.32 mS/cm	<div></div>				Satisfactory	
Micronutrients		Critical Range		Result			
Zinc	4.7 ppm	0.4 - 0.8 ppm		Very High			
Manganese	11.8 ppm	0.5 - 1.0 ppm		Very High			
Copper	2.2 ppm	0.2 - 0.5 ppm		Very High			
Iron	50.9 ppm	1.9 - 3.8 ppm		Very High			
pH and Lime Requirements							
Current pH = 5.27 (within optimal range)				Lime Recommendation			
<div><div>Acidic</div><div></div><div>Alkaline</div></div> <div>3456789</div> <div>Optimal pH range: 4.5 - 6.5</div>				None required.			
Fertilizer Recommendations							
Nutrient	Weight / 1000 sq ft	Nutrient	Weight / 1000 sq ft				
Nitrogen	1.4 lbs (60 lbs/acre)	Boron (B)	none required				
P (as phosphate)	none required	Sulfur (S)	8 oz (2.5 lbs as gypsum)				
K (as potash)	none required	Iron (Fe)	none required				
Calcium	none required	Manganese (Mn)	none required				
Magnesium	none required	Copper (Cu)	none required				
		Zinc (Zn)	none required				
N-P-K Fertilizer Options							
N, P and K are recommended in the proportions 20:0:0. Phosphorus and potassium are already present at high levels. Only a standard annual application of nitrogen is recommended. Below are some nitrogen-containing fertilizer options, along with the quantity needed.							
Ammonium sulfate - 7 lbs/1000sf							
Milorganite - 23 lbs/1000sf							
Schultz Super 21-0-0 (Home Depot) - 7 lbs/1000sf							
Urea - 3.0 lbs/1000sf							
Scott's Organic Choice - 13 lbs/1000sf							

Simply Soil Testing, 20312 Lafayette Rd, Burlington, WA 98233 Email: service@simplysoiltesting.com

Appendix 3.7.

The following is a direct transcript from communication with Paul Shannon.

Katie Coats and Marianne Elliott of the WSU Plant Diagnostics Lab in Puyallup: a report after PCR assay of *Phytophthora* at the genus level (received 18 November 2016)

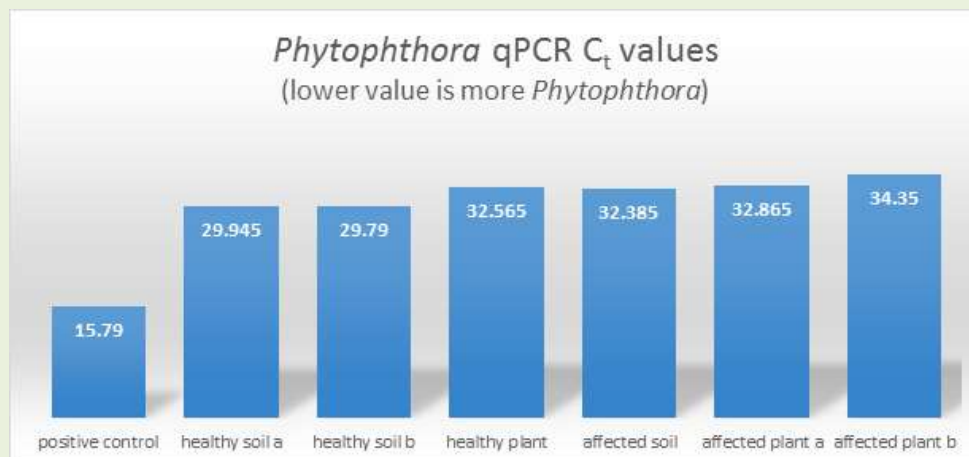
I have qPCR results on the sword fern samples but they're not conclusive.

I extracted DNA from the following samples:

1. "healthy soil a" = 4 grams of soil from 12" away from healthy plant
2. "healthy soil b" = 4 grams of soil from 24" away from healthy plant
3. "healthy plant" = roots, surface sterilized in 10% bleach for 1 minute then rinsed twice in sterile water to assure qPCR is detecting infection inside the roots, photo attached
4. "affected soil" = 4 grams of soil from near affected plant
5. "affected plant a" = roots, surface sterilized in 10% bleach for 1 minute then rinsed twice in sterile water, photo attached
6. "affected plant b" = split from sample 5, run as a replicate

The DNA samples were run in duplicate through the general *Phytophthora* (5.8S) qPCR assay along with a positive control (DNA from a pure culture of *Phytophthora*) and a negative control (water). All samples were multiplexed with an amplification control that checks for evidence of PCR inhibition, something that's common in root and soil samples.

Results are shown in the bar graph below... it's crude... I don't usually use the Ct values, but I think it's a quick way to get the results across to you. The lower the Ct value, the more target DNA is present... so it makes sense that the pure DNA sample has a Ct around 16. The qPCR runs up to 42 cycles, but data above 40 cycles is dubious. If no *phytophthora* is present, then there will be no Ct value at all, which was the case with the water control. Theoretically the DNA doubles in each cycle, so a Ct value one more than another represents twice as much target DNA.



The bottom line is that the healthy soil and plant generally had lower Ct values (more Phytophthora DNA) than the affected soil and plant. I don't have a soil sample from another location outside the park to give us an idea of how normal these levels of phytophthora are, but they're counter what we expected... One thing to keep in mind is that this test lumps all phytophthoras together, so it remains possible that a causal phytophthora is in higher concentration in the affected plant even though the total amount of phytophthora is lower.

Therefore, we will proceed with another strategy referred to as baiting. We'll add some rhododendron leaves as baits to the individual plant and soil samples along with water... and then plate in culture medium to see if/how much/which phytophthoras may be present. That will take a couple weeks from start to finish... so more wait time.

I'll send you another update once we know the timeline of those results; ballpark of early December
I'm including Marianne in case she has other comments... and let me know if you have any questions.



WSU Puyallup Diagnostic Laboratory Report

Plant Clinic Number: 0572

Date: 12/04/2015

Client: Paul Shannon
Seward Park

Submitter:

Host or Habitat: sword fern

County: King

Agent:

Sample Quality: Excellent

Information Quality: Excellent

Payment: Extension

Enclosures:

Diagnosis by: Jenny Glass, Plant & Insect Diagnostician

Diagnosis and Recommendations:

Jenny Rebecca Glass

The visit to the dying sword fern patch at Seward Park was very interesting. Soil, root tissue, crown tissue, and foliar symptomology were collected to bring back to the labs for a *Phytophthora*-baiting test as well as microscopic observation of the tissue.

At the WSU Puyallup Plant & Insect Diagnostic Lab, root and crown tissue from the damaged areas was surface disinfested and plated onto sterile laboratory agar to check again for the presence of pathogens. Tissue was also incubated in moist chamber. Given the dead and dry nature of the sample material, numerous secondary saprophytic fungal species were recovered by both methods. Some *Pythium* was present but this is common in soils/dead material so I don't believe its presence is associated with the origin of the problem. Our findings of no recovered *Phytophthora* correspond with the work done by Dr. Marianne Elliott and her team via baiting, as well as with previous work completed for this problem. I was checking specifically for *Rhizoctonia*, as this pathogen group is a known blight-causing species on fern types, but the work did not reveal further evidence of this pathogen group. When the plates from the various sample areas were compared, nothing stood out as a strong indication of a single organism connecting the damage.

I will be curious to hear the results of the student's survey work.

2606 W. Pioneer, Puyallup, WA 98371-4998
253-445-4500 • Fax: 253-445-4571

Cooperating agencies: Washington State University, U.S. Department of Agriculture, and Washington counties. Extension programs and employment are available to all without discrimination. Evidence of noncompliance may be reported through your local Extension office.

Soil and Container Mix Protocol

Protocol for Detecting *Phytophthora ramorum* in Soil and Container mix Revised November 2010

See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for latest approved protocol.

Soil or container mix infested with *Phytophthora ramorum* appears to the unaided eye exactly the same as non-infested soil or container mix. Therefore, all soil and container mix samples must be handled carefully. All tools used to collect soil or container mix samples must be disinfested with a solution of 10% bleach, quaternary ammonium at the labeled rate, or full-strength Lysol™ spray, then rinsed thoroughly with distilled water and allowed to dry. Alternatively, tools may be flame-sterilized with a propane torch between sample collections. All soil and organic material should be removed from the tools prior to being disinfested. Care should be taken to not transfer soil or container mix from one sample location to another on shoes, gloves, or clothing. All sampling equipment should be cleaned and disinfested prior to entering a new nursery sampling location or block. Care must be taken to ensure that soil or container mix, within a sampling location, are not cross-contaminated in any manner. To reduce the risk of contamination, samples should be collected starting with the least infested area and moving toward the most infested area.

Definitions

Aliquot – a volume of substrate from a composite sample that is placed into a container and assayed; usually three aliquots (approx. 50-150 ml or 2-5 oz) from each composite sample are baited.

Block of plants – A contiguous group of host and associated plants with less than a 2 m (6.5 ft) break of non-host plants or empty space.

Composite sample – A mixture of subsamples that are physically combined to form a single representative sample from a designated area.

Container mix – Soilless substrates (also referred to as growing media) placed in pots and used to grow plants; usually consists of bark and peat but also may contain slow-release fertilizer, sand, vermiculite, perlite, etc.

Lot – Set of plants that can be identified or grouped by shipment, cultivar, or production unit.

Soil – Substrates in the field on which potted plants are located; often this consists of peat and bark fines washed from pots, plant debris, gravel, or any combination of these.

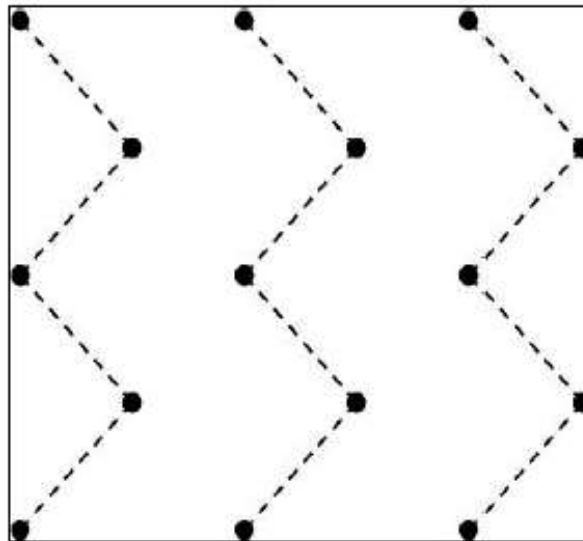
Subsamples – Small amounts of soil or container mix that are combined to form a single, composite sample; collecting subsamples increases the chances of finding *P. ramorum* if it is present.

Sampling Soils and Container Mixes

Sample Collection

1. Record the physical location (address) of the nursery site along with GPS reference coordinates. (*Tip: If a GPS unit is not available, many cellular phones have GPS capabilities. Also Google Earth can be used to obtain coordinates using an address.*)
2. Prepare or secure from the facility manager/owner, a diagram of the nursery or sampling area, which includes row or block numbers and plant species/cultivars. If possible, collect reference GPS coordinates for each block of plants.
3. Each 1 L (1 qt) composite sample should consist of a *minimum* of 15 subsamples collected from soil or container mix within the targeted area. Subsamples should be collected in zigzag transects according to the pattern in the diagram below (Figure 1).

Figure 1. Recommended pattern for collecting subsamples of soil or container mix to produce a representative composite sample



4. Referencing Table 1, collect composite samples from both soil and container mix for each block of plants. An exception to this would be if all plants (including container mix and pots) were destroyed or the plants are not on a soil substrate (e.g., concrete or asphalt). Each sample should contain approximately 1 L/1 qt (volume) of soil or container mix and be placed in a 4 L (1 gallon) size zip-to-close plastic bag. The number of composite samples collected will depend on the size of the block of plants being sampled (Table 1).

Table 1. Number of composite samples to collect per block

Survey Area Size		Composite Samples of Soil	Composite Samples of Container Mix
(m ²)	(ac)		
<1,000	<0.25	5	5
1,000-2,000	0.25-0.5	10	10
2,000-4,000	0.5-1	20	20
>4,000	>1	30	30

Note: *If the soil surface is covered with gravel having a large amount of plant debris on top, collect as much debris as practical. If the gravel has little plant debris on top, collect subsamples from the soil beneath the gravel. If water permeable weed block (landscape cloth) is present, either covered with gravel or under gravel, small slits should be made in the cloth to allow for sample collection.*

Soil

- a. Collect a representative composite sample (approximately 1 L (1 qt)) from the surveyed area (e.g., a block of plants, a nursery bed, a shade house, etc.) with a trowel to a depth of approximately 5-10 cm (2-4 in). (*Tip: If soil is loosely packed, a plastic spoon can be used to collect sample. The spoon can then be sealed in the corresponding sample's plastic bag for easy disposal in the laboratory. This method is NOT recommended for sampling container mix because substrate at the bottom of pots cannot be sampled.*)
- b. Samples should be collected from around and under pots containing plants suspected of being infested or infected with *P. ramorum* or from areas where diseased plants were previously located. This may require scraping soil from on or under nursery cloth or anything else on which pots are or were located.
- c. Place each composite sample into an individual plastic bag; if the soil is wet or saturated from rain or excessive irrigation, double bag the slurry to avoid leaks.

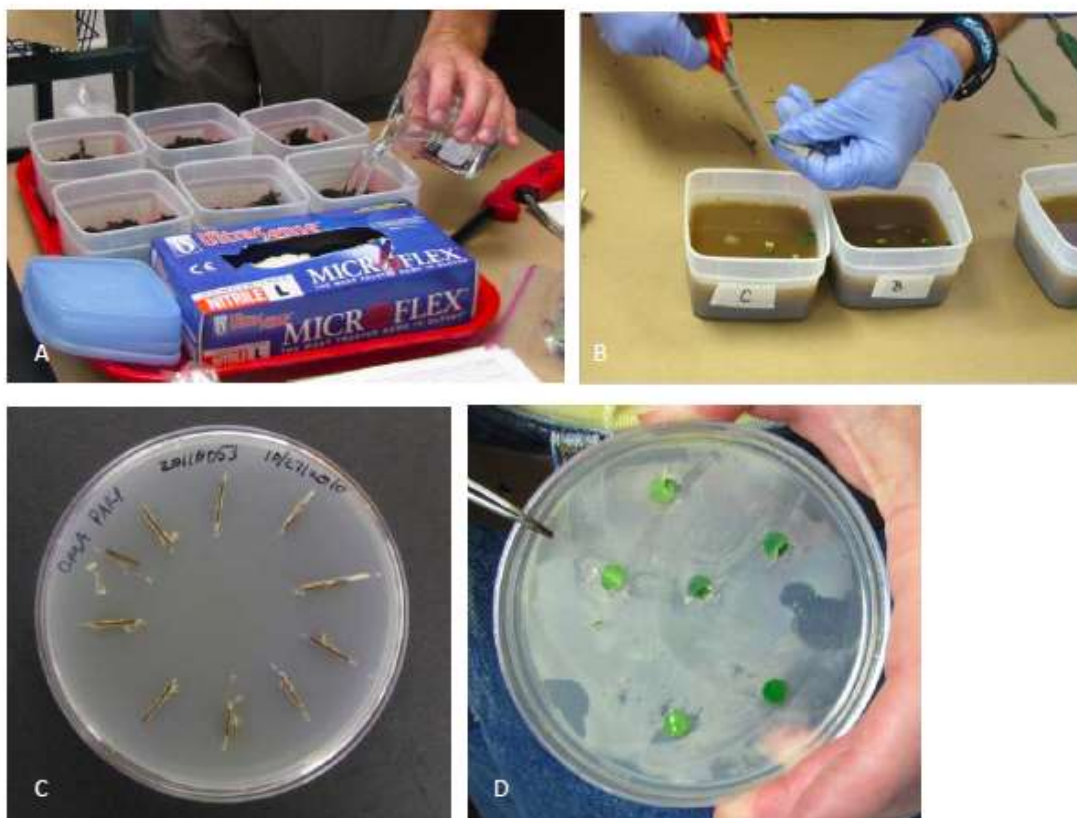
Container mix

- d. Collect a representative composite sample from each block of plants using a wide bore soil tube (highly recommended); one core from each or every other pot in the block of plants is sufficient depending on the number of pots present.
5. For each composite sample, break up clods and root masses, then thoroughly mix the sample in the bag; this can be done in the field or laboratory.
6. Moisten the sample with distilled water if it appears dry, as desiccation will severely affect the ability to recover *P. ramorum* from a soil sample.
7. Disinfest sampling tools and soles of shoes (e.g., 10% bleach, quaternary ammonium at the labeled rate, or full-strength LysoI™ spray) between samples to prevent potential dissemination of the pathogen. Next, thoroughly rinse tools with distilled

3. Thoroughly mix the 1 L (1 qt) sample within the bag, breaking up any clods. Divide the sample equally into two 500 ml (17 oz) aliquots, placing one sample into a new sealed zip-to-close bag or container then placing it in cold storage (4-10°C/39-50°F) for a minimum of 30 days.
4. Prepare and label three containers (e.g., small 0.5 L (1 pt) plastic containers, resealable 1 L (1 qt) bags, etc.) for each composite sample to be baited.
5. Thoroughly mix the remaining sample, then place an aliquot approx. 1 to 2 cm (0.5 to 1 in) deep into each of the three containers; soil deeper than this may inhibit zoospores from swimming to the surface.
6. Add distilled water to a depth of 2.5 cm (1 in) above the soil surface; stir the mixture and allow it to settle. *Note: organic debris may continue floating.*
7. Bait leaves should be free of blemishes, damage, disease, and pesticides. *Rhododendron catawbiense* and/or *Camellia japonica* are recommended bait types (if both bait types are available it is recommended to use both). Using a standard hole-punch or scissors, prepare enough leaf pieces (~10/container) to bait all containers (Figure 2). Leaf pieces cut with scissors should be approximately 5 mm (< 0.25 in) across. (*Tip: Use different-shaped leaf pieces to differentiate between bait types if two types of bait are being used.*)
8. Using sterile forceps, add 8-10 leaf pieces of each bait type (or 15-20 leaf pieces if only using one bait type) to each container. Baits should float on the water surface. If some of the baits sink, do not remove them, instead add additional baits. Cover containers to avoid evaporation and desiccation.
9. Store containers at 18-22°C/64-72°F for three days (an incubator maintained at 20°C/68°F or a closed cabinet works best).
10. For each container, remove six baits of each host type (or 12 baits of one host type) with sterile forceps and blot dry on a clean paper towel. Dispose of paper towels after each sample.
11. Place the six bait pieces of each host type from one container on a separate plate of PARPH-V8¹ medium (e.g., one plate with six rhododendron leaf pieces and one plate with six camellia leaf pieces, or alternatively, two plates of same host tissue baits) so they are embedded completely in the agar. Leaf pieces placed on the agar surface will dry-out and curl up. There should be six plates and 36 baits from each composite sample: 3 containers × 2 plates/container × 6 baits/plate (Figure 2). (*Tip: Baits can be inserted into the medium vertically, which prevents shadowing during microscopic examination. To prevent media tearing, a scalpel can be used to make small incisions where baits are going to be placed. Vertical placement may require slightly thicker agar or smaller bait pieces, however the plates no longer have to be read on both sides.*)

¹ See Appendix A for recipe

Figure 2. Baiting soil samples and plating baits (images courtesy of Grace O'Keefe, PPQ and Jennifer Falacy, Washington State Dept. of Ag)



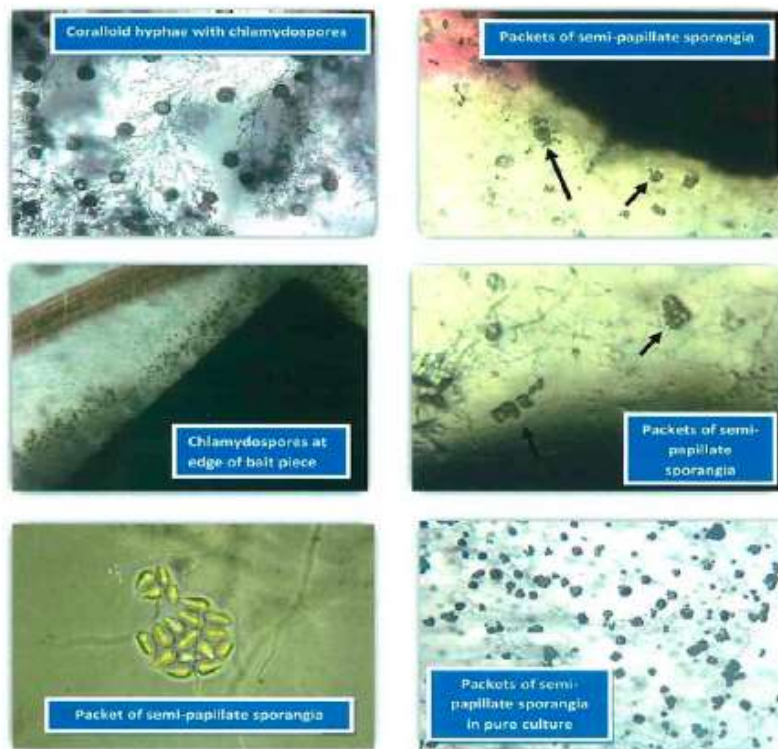
A) Preparing soil samples for baiting. B) Baiting soil samples using *Rhododendron* and *Camellia* leaves. C) Plate with baits inserted vertically. D) Plate with baits inserted horizontally.

12. Place plates upside-down in a plastic box or zip-to-close bag to prevent desiccation; incubate plates at 15-20°C/59-68°F in the dark for up to 28 days; a designated incubator works best but a closed cabinet in an air-conditioned room can also be used.
13. Using a dissecting or inverted microscope, examine plates frequently (starting 2 days after baits have been plated) for colonies that resemble *P. ramorum* – i.e., those with typical coraloid hyphae, large golden chlamydospores, and packets of semi-papillate sporangia on the surface (Figure 3); mark these with a permanent marker or grease pencil.
14. Once colonies form on plates, follow the morphological identification protocol found at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/cultur_eprotocol6-07.pdf
15. *P. ramorum* hyphae often are visible 2-5 days after baits have been plated. However, patience, persistence, and good observational skills often are the keys to finding *P.*

ramorum on the isolation plates; *P. ramorum* may be recovered from only one of the 36 bait pieces and may not be recognizable until several weeks after baits are plated.

16. Subculture isolates to fresh PARPH-V8 and then to PAR-V8². *Note: P. ramorum grows and sporulates better in the absence of hymexazol (i.e., on PAR-V8). It is best to subculture from suspect colonies early, before these colonies become over grown by faster-growing organisms.*

Figure 3. Characteristic structures of *Phytophthora ramorum* (images courtesy of S. N. Jeffers, Clemson University)



Second Baiting of Soil and/or Container Mix Samples

17. Remove composite samples from cold storage and hold at room temperature (22-24°C/72-75°F for 3 days to acclimate before baiting begins.
18. Bait samples again as described above in Steps 1-14.
19. After samples have been baited a second time, destroy or sterilize any remaining soil and/or container mix using an appropriate method (e.g., autoclaving).

² See Appendix B for recipe

Materials and Supplies

- Disposable gloves
- 4 L (1gallon) zip-to-close bags (at least 4 mm in thickness), avoid the bags with a “zipper” mechanism
- Trowel or other soil-sampling tool
- Wide bore soil tube (2.5 cm/1 in or larger)
- Permanent marker
- Insulated ice chest (with ice in bags or blue ice if external temperatures are above 21° C/70°F)
- Disinfesting solution (10% bleach, quarternary ammonium at the labeled rate or full-strength Lysol™ spray)
- Plastic or glass containers with lids; square, wide bottom containers work best (e.g., 0.5 L (1 pt) freezer boxes)(see Figure 2)
- Distilled water
- Single hole punchers, scissors or razor blades
- Forceps and scalpel
- 70% alcohol and flame for sterilizing laboratory utensils
- Paper towels
- Baits –Use rhododendron and/or camellia leaves that have been on the plant for at least one year; leaf pieces should be 5×5 mm squares (< 0.25 in) or 5 mm (< 0.25 in) dia. disks
- PARPH-V8 selective medium; 2 plates per baited container
- PAR-V8 selective medium (as needed for subcultures)

Growing Media Formulae

PARPH-V8 Selective Medium: For *Phytophthora* species (Adapted from Jeffers and Martin, 1986; Ferguson and Jeffers, 1999)

Ingredient	Amount Per:	
	1.0 Liter	0.5 Liter
<u>Basal Medium</u>		
Clarified V8 Concentrate*	50 ml	25 ml
Distilled Water	950 ml	475 ml
Difco Bacto Agar	15 g	7.5g
<u>Amendments</u>		
Delvocid [50% pimaricin]	10 mg	5 mg
Sodium Ampicillin	250 mg	125 mg
Rifamycin-SV [sodium salt]	10 mg	5 mg
Terraclor [75% PCNB]	66.7 mg	33.4 mg
Hymexazol	50 mg	25 mg

* Clarified V8 Concentrate is made from buffered V8 Juice (1.0 g CaCO₃/100 ml V8 Juice) clarified in one of three ways:

- centrifugation at 4000 RPM for 20 min followed by filtration using two layers of Whatman No. 1 filter paper under vacuum
- centrifugation at 7000 rpm for 10 min then filtration is not necessary
- vacuum filtration alone through a 1-2 cm deep layer of Celite

Clarified V8 should be frozen at -20°C in 50-ml aliquots (e.g., in disposable 50-ml centrifuge tubes).

PCNB and hymexazol are optional and can be omitted [e.g., to make PAR, PARP, & PARH]

• PCNB is useful to inhibit soilborne fungi on soil dilution plates

• Hymexazol inhibits most species of *Pythium* while allowing most species of *Phytophthora* to grow, although they may grow more slowly

Directions

1. Add ingredients for basal medium to a 2-L flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask
2. Autoclave for 20 min at 121°C and 15 psi; turn waterbath on to ~50°C
3. Add each amendment to a sterile water blank [5 ml distilled water in a 16-mm test tube]; vortex to mix
4. Cool medium in waterbath
5. Slowly stir medium with a magnetic stirrer in laminar flow hood
6. Vortex each amendment thoroughly and add to mixing basal medium
7. Use one additional sterile water blank to sequentially rinse all amendment tubes and then add rinse water to the medium; continue mixing medium
8. Pour plates relatively thin (i.e., about 15 ml/plate = 60 plates/liter); pour molten medium so it does not quite cover the entire plate; therefore, plates will need to be swirled gently to evenly distribute medium before it hardens
9. Cool plates at room temperature
10. Store plates inverted in plastic bags in the dark in a refrigerator
11. Plates should be used within 30 days

PAR-V8 Selective Medium: For *Phytophthora* species (Adapted from Ferguson and Jeffers, 1999)

Ingredient	Amount Per:	
	1.0 Liter	0.5 Liter
Basal Medium		
Clarified V8 Concentrate*	50 ml	25 ml
Distilled Water	950 ml	475 ml
Difco Bacto Agar	15 g	7.5g
Amendments		
Delvocid [50% pimaricin]	10 mg	5 mg
Sodium Ampicillin	250 mg	125 mg
Rifamycin-SV [sodium salt]	10 mg	5 mg

* Clarified V8 Concentrate is made from buffered V8 Juice (1.0 g CaCO₃/100 ml V8 Juice) clarified in one of three ways:

- centrifugation at 4000 RPM for 20 min followed by filtration using two layers of Whatman No. 1 filter paper under vacuum
- centrifugation at 7000 rpm for 10 min then filtration is not necessary
- vacuum filtration alone through a 1-2 cm deep layer of Celite

Clarified V8 should be frozen at -20°C in 50-ml aliquots (e.g., in disposable 50-ml centrifuge tubes)

Directions

1. Add ingredients for basal medium to a 2-L flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask
2. Autoclave for 20 min at 121°C and 15 psi; turn waterbath on to ~50°C
3. Add each amendment to a sterile water blank [5 ml distilled water in a 16-mm test tube]; vortex to mix
4. Cool medium in waterbath
5. Slowly stir medium with a magnetic stirrer in laminar flow hood
6. Vortex each amendment thoroughly and add to mixing basal medium
7. Use one additional sterile water blank to sequentially rinse all amendment tubes and then add rinse water to the medium; continue mixing medium
8. Pour plates relatively thin (i.e., about 15 ml/plate = 60 plates/liter); pour molten medium so it does not quite cover the entire plate; therefore, plates will need to be swirled gently to evenly distribute medium before it hardens
9. Cool plates at room temperature
10. Store plates inverted in plastic bags in the dark in a refrigerator
11. Plates should be used within 30 days

References

Jeffers, S.N., and Martin, S.B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70: 1038-1043.

Ferguson, A.J., and Jeffers, S.N. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83: 1129-1136.

Baiting protocol for soils

Label 1L bottles with sample and rep numbers. Use rhododendron leaves and healthy sword fern leaves. Place one intact rhody leaf and one sword fern frond in each of 3 bottles containing 100 g soil and 500 ml water. Cap tightly and incubate bottles on their sides for 48-72h.

After incubation, remove baits from bottles and rinse with water, then blot dry. If asymptomatic incubate an additional 3-7 days in ziplock bags containing moist paper towels. Plate symptomatic areas of foliage (5 per plate) on PARPHV8. Use two plates for each bait type per bottle.

Check for Phytophthora colonies on all plates after 2-5 days and isolate onto small PARP plates for identification.

Please see table on following page.

Sample #	Description	Treatment
1	5 x 5 plot, healthy, symptomatic fern foliage	Surface sterilize, plate 5 segments on PARPHV8 (3 plates)
2	5 x 5 plot, healthy, crown from healthy fern plant	Surface sterilize 10 necrotic root segments per plate (PARPHV8), dissect crown and look for brown staining symptoms (3 plates). If present, surface sterilize and plate 5 segments on PARPHV8 (1 plate).
3	5 x 5 plot, healthy, soil from base of 3 healthy ferns, upper 5 cm depth + litter & roots	Bait in 1 L bottles. Use rhododendron leaves and healthy sword fern leaves. Place one intact rhody leaf and one sword fern frond in each of 3 bottles containing 100 g soil and 500 ml water.
4	Ground zero, foliage and fronds from dying ferns	Surface sterilize, plate 5 segments on PARPHV8 (3 plates)
5	Ground zero, crown and roots from dead ferns	Surface sterilize 10 necrotic root segments per plate (PARPHV8), dissect crown and look for brown staining symptoms (3 plates). If present, surface sterilize and plate 5 segments on PARPHV8 (1 plate).
6	Ground zero, soil from base of 3 dead ferns, upper 5 cm depth + litter & roots	Bait in 1 L bottles. Use rhododendron leaves and healthy sword fern leaves. Place one intact rhody leaf and one sword fern frond in each of 3 bottles containing 100 g soil and 500 ml water.
7	Ground zero, soil from base of 3 dead, planted cedars	Bait in 1 L bottles. Use rhododendron leaves and healthy sword fern leaves. Place one intact rhody leaf and one sword fern frond in each of 3 bottles containing 100 g soil and 500 ml water.
8	Symptomatic foliage from other hosts along trail by Ground zero and road	Surface sterilize, plate 5 segments on PARPHV8 (3 plates).

**Investigation of the Western Sword Fern Disappearance in Seward
Park**

By:
Grace Masaoka

A Senior Thesis Submitted in Partial Fulfillment of the Requirements of the
Degree of
Bachelor of Science

School of Environmental and Forest Science
University of Washington
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Seattle, Washington 98105

Approved by Dr. Patrick Tobin
Faculty Thesis Supervisor

March 2017

Abstract

Seward Park is a forested area that spans about 300 acres and is home to many native plants including the western sword fern (*Polystichum munitum*), which is among the most dominant ferns in the Pacific Northwest, providing a multitude of ecosystem services. For several years, a mysterious dieback and continued absence of sword ferns in one particular area has been observed in the park. A drought during the summer of 2015 was originally thought to be the cause but has been ruled out, as other drought affected areas have since recovered. This study tested the hypotheses that there are significant differences between the three study sites in terms of soil invertebrate abundance and root weight. It also assessed bulk density to determine if the soil in any of the three sites may have inhibited root growth. To analyze the differences between sites, I collected soil samples three ferns in each site and used in lab techniques to extract quantitative data. The data was then graphed in R Studio and I did an Analysis of Variance (ANOVA) test for each dependent variable (soil invertebrates and root weight). This analysis led me to conclude that though there tended to be differences in soil invertebrate abundance and root weight between sites, it was not significantly different. The differences in bulk density between sites were statistically significant, but none of the bulk densities were high enough to be considered detrimental to root growth. Performing this study again with a much larger sample size would be necessary in order to determine if the small sample size was the only reason the differences were not statistically significant.

Introduction

Western sword ferns are one of the most dominant shrubs in the Pacific Northwest, playing a large role in ecosystem services as a food and habitat source for many animals (Zouhar 2015). In recent years Seward Park has experienced a decline in western sword ferns and continued

root mass, and stored in a Ziploc back for later examination. For invertebrate and bulk density analysis, I collected samples in Ziploc bags consisting of soil from each fern in all three sites. I collected one sample per fern for invertebrate analysis, and two samples per fern for bulk density analysis. In between sites, I used the phytosanitary procedure in order to minimize the movement of plant pathogens. To do this, I dipped my shoes in a tray with 10% bleach solution, then rinsed them in a separate tray with clean water. This procedure was also used with the shovels I was using to collect my soil samples.

Analysis

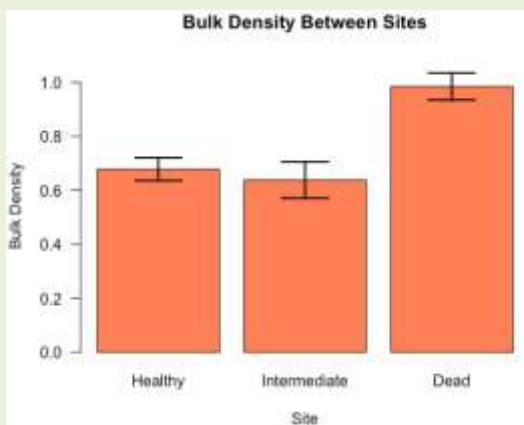
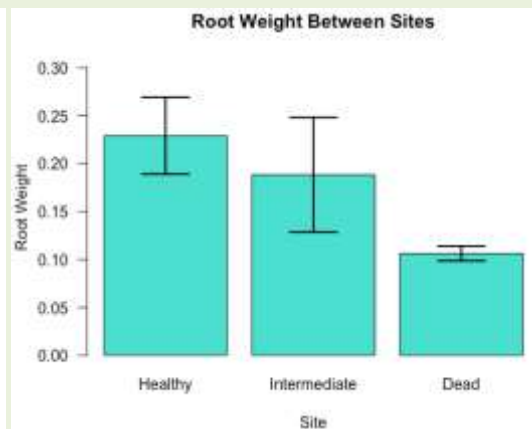
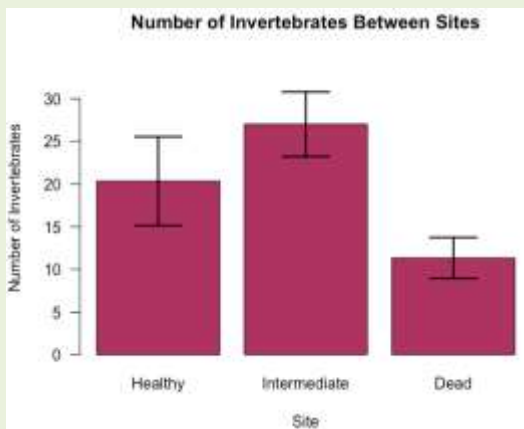
My root and invertebrate analysis was done in the Tobin Disturbance Ecology Lab (106 Winkenwerder Forestry Sciences), and were processed on the same day as collection. I used 9 self-built Tullgren funnels to extract the soil community over the course of about a week. After extraction, I examined the invertebrates under a microscope to get an estimate of relative abundance in each site. For root assessment, I washed and cut 10 individual roots (6 cm long each) from each root mass in all three sites. The root segments were dried on paper towels, and then weighed. I also looked at individual roots from each site under the microscope to see if there was any noticeable root herbivory that may have inhibited nutrient uptake. My bulk density analysis was done in the soil lab (210 Bloedel). I began by air drying my soil samples for one week. After that I weighed each sample, put a subsample of each one in a ceramic crucible, recorded that weight, and dried all samples ($n=18$) in the oven for 48 hours at 105 degrees C. After taking them out of the oven I weighed them again and then used a conversion (which accounts for crucible weight) to calculate bulk density of each sample (g/cm^3). After processing all of my data, I used R Studio for the remainder of my analyses.

Results

The three factors I looked at in each site were 1) invertebrate abundance, 2) root weight, and 3) bulk density. For all three factors I wanted to see if there were any significant differences between the sites, so I ran a simple ANOVA (Analysis of Variance) test for each factor based on this hypothesis:

H_A: there is at least one difference between means

H_O: there is no difference between means



ANOVA results

Response	p-value(*=significant)
Number of invertebrates	0.08123
Root weight	0.1874
Bulk density	<0.001*

key suspects in question. Based on this information, the presence of thrips in the intermediate site of Seward Park warrants further investigation. I would also recommend doing a chemical analysis on the soil and testing to see if any pathogens are present in future studies.

Acknowledgements

Many thanks to **Patrick Tobin** for helping me collect data, perform in-lab techniques, and being all around the best advisor I could ask for, plant ecologist **Lisa Ciecko** for allowing me to collect data at Seward Park, and graduate student **Cole Gross** for helping me process and analyze my bulk density samples.

References

- Abe, Hiroshi, Jun Ohnishi, Mari Narusaka, Shigemi Seo, Yoshihiro Narusaka, Shinya Tsuda, and Masatomo Kobayashi. "Function of Jasmonate in Response and Tolerance of Arabidopsis to Thrip Feeding." *Plant and Cell Physiology*. Oxford University Press, 01 Jan. 2008. Web. 10 Mar. 2017.
- Lavelle, Patrick, T. Decaens, M. Aubert, S. Barot, M. Blouin, F. Bureau, P. Margerie, P. Mora, and J. P. Rossi. "Soil Invertebrates And ecosystem Services." *Soil Invertebrates and ecosystem Services*. Elsevier Masson SAS, n.d. Web. 10 Mar. 2017.
- Zouhar, Kris. 2015. *Polystichum munitum*, western sword fern. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: <http://www.fs.fed.us/database/feis/plants/fern/polmun/all.html> [2017, March 10]

Appendix 3.9.

Seward Park Sword Fern Study – 2017 Soil Sampling

Soil samples were collected on June 28, 2017 and sent to the [University of Massachusetts Soil and Plant Nutrient Testing Laboratory](#). A routine soil analysis and total sorbed metals tests were performed on samples from each of three areas (see map). Sampling procedures were followed as per the [listed instructions](#). At each location, 10-12 subsamples were collected within an approximate 10-meter by 10-meter square area and combined for each sample.



Figure 1. Comparison between select macronutrients across sites. Phosphorus (P), potassium (K), and to a lesser extent calcium (Ca) are higher at the affected sites.

Site 1: complete die off and no surviving ferns (Ground Zero)

Soil Test Interpretation

Nutrient	Very Low	Low	Optimum	Above Optimum
Phosphorus (P):	██████████	██████		
Potassium (K):	██████████	██████████	██████████	
Calcium (Ca):	██████████	██████████	██████████	
Magnesium (Mg):	██████████	██████████	██████████	██████

Site 2: active die-off site (western location, across the Squebeqed trail)

Soil Test Interpretation

Nutrient	Very Low	Low	Optimum	Above Optimum
Phosphorus (P):	██████████	██████████		
Potassium (K):	██████████	██████████	██████████	██████████
Calcium (Ca):	██████████	██████████	██████████	
Magnesium (Mg):	██████████	██████████	██████████	██████████

Site 3: an un-affected site with an intact fern population (north end of Seward Park)

Soil Test Interpretation

Nutrient	Very Low	Low	Optimum	Above Optimum
Phosphorus (P):	████			
Potassium (K):	██████████	██████		
Calcium (Ca):	██████████	██████████	██████	
Magnesium (Mg):	██████████	██████████	██████████	██████████



Soil and Plant Nutrient Testing Laboratory

203 Paige Laboratory
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website: soiltest.umass.edu

Soil Test Report

Sample Information:

Sample ID: SP #1

Order Number: 31638
Lab Number: S170711-131
Area Sampled: 400 sq ft
Received: 7/11/2017
Reported: 7/19/2017

Results

Analysis	Value Found	Optimum Range	Analysis	Value Found	Optimum Range
Soil pH (1:1, H ₂ O)	5.9		Cation Exch. Capacity, meq/100g	16.7	
Modified Morgan extractable, ppm			Exch. Acidity, meq/100g	8.0	
Macronutrients			Base Saturation, %		
Phosphorus (P)	2.7	4-14	Calcium Base Saturation	42	50-80
Potassium (K)	136	100-160	Magnesium Base Saturation	8	10-30
Calcium (Ca)	1406	1000-1500	Potassium Base Saturation	2	2.0-7.0
Magnesium (Mg)	156	50-120	Scoop Density, g/cc	1.01	
Sulfur (S)	11.7	>10	Optional tests		
Micronutrients *			Soil Organic Matter (LOI), %	7.0	
Boron (B)	0.2	0.1-0.5	Soluble Salts (1:2), dS/m	0.05	<0.6
Manganese (Mn)	8.8	1.1-6.3	Nitrate-N (NO ₃ -N), ppm	13	
Zinc (Zn)	1.9	1.0-7.6			
Copper (Cu)	0.0	0.3-0.6			
Iron (Fe)	7.8	2.7-9.4			
Aluminum (Al)	77	<75			
Lead (Pb)	2.6	<22			

* Micronutrient deficiencies rarely occur in New England soils; therefore, an Optimum Range has never been defined. Values provided represent the normal range found in soils and are for reference only.

Soil Test Interpretation

Nutrient	Very Low	Low	Optimum	Above Optimum
Phosphorus (P):				
Potassium (K):				
Calcium (Ca):				
Magnesium (Mg):				



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Recommendations for Deciduous Trees, Shrubs & Vines-Maintenance

Limestone (Target pH of 6.0)	Nitrogen, N	Phosphorus, P ₂ O ₅	Potassium, K ₂ O
0	.1 - .2	0.25	0.1

Comments:

-For instructions on converting nutrient recommendations to fertilizer applications in home gardens, lawns and landscapes, see Reference "Step-by-Step Fertilizer Guide for Home Grounds and Gardening" (listed below).

References:

Home Lawn and Garden Information

<http://ag.umass.edu/resources/home-lawn-garden>

Step-by-Step Fertilizer Guide for Home Grounds and Gardening

<https://soiltest.umass.edu/fact-sheets/step-step-fertilizer-guide-home-grounds-and-gardening>

Recommendations for Needleleaf Trees & Shrubs-Maintenance

Limestone (Target pH of 6.0)	Nitrogen, N	Phosphorus, P ₂ O ₅	Potassium, K ₂ O
0	.1 - .2	0.1	0.05

Comments:

-For instructions on converting nutrient recommendations to fertilizer applications in home gardens, lawns and landscapes, see Reference "Step-by-Step Fertilizer Guide for Home Grounds and Gardening" (listed below).

References:

Home Lawn and Garden Information

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Step-by-Step Fertilizer Guide for Home Grounds and Gardening

<https://soiltest.umass.edu/fact-sheets/step-step-fertilizer-guide-home-grounds-and-gardening>

General References:

Interpreting Your Soil Test Results

<http://soiltest.umass.edu/fact-sheets/interpreting-your-soil-test-results>

For current information and order forms, please visit

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UMass Extension Nutrient Management

<http://ag.umass.edu/agriculture-resources/nutrient-management>

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Total Sorbed Metals**Sample Information:**

Sample ID: SP #1

Order Number: 31736

Lab Number: M170719-107

Received: 7/19/2017

Reported: 7/25/2017

Results

<i>Analysis</i>	<i>Value Found</i>	<i>USEPA Heavy Metals Thresholds *</i>
Lead (Pb)	41.2 mg/Kg	400 mg/kg
Nickel (Ni)	31.0 mg/Kg	1600 mg/kg
Cadmium (Cd)	0.4 mg/Kg	39 mg/kg
Chromium (Cr)	24.5 mg/Kg	100 mg/kg
Zinc (Zn)	72.4 mg/Kg	23,600 mg/kg
Copper (Cu)	13.8 mg/Kg	Not Available
Arsenic (As)	13.6 mg/Kg	Varies by state
Selenium (Se)	9.3 mg/Kg	390 mg/kg
Molybdenum (Mo)	4.3 mg/Kg	Not Available

** This information is for general guidance. The threshold values are based on toxicity characteristic leach potential (TCLP). It should be noted that the degree of environmental risk could be influenced by soil characteristics. Contact your local Environmental Protection Authority for specific recommendations.*

General References:

Soil Lead Testing Interpretation <http://soiltest.umass.edu/fact-sheets/soil-lead-testing-interpretation-recommendations-0>

Learn About Lead <http://www2.epa.gov/lead/learn-about-lead>

Arsenic Cleanup Criteria for Soils in the US and Abroad <https://soiltest.umass.edu/fact-sheets/arsenic-cleanup-criteria-soils-us-and-abroad>

Selenium http://rais.ornl.gov/documents/eco-ssl_selenium.pdf

Ecological Soil Screening Levels <http://rais.ornl.gov/documents/ecossl.pdf>

Plant Remediation (Berkeley) http://www.berkeley.edu/news/media/releases/2005/02/01_plantremediation.shtml

For specific heavy metals regulations, refer to your state's Department of Environmental Protection.



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Soil Test Report

Sample Information:

Sample ID: SP #2

Order Number: 31638
Lab Number: S170711-132
Area Sampled: 400 sq ft
Received: 7/11/2017
Reported: 7/19/2017

Results

Analysis	Value Found	Optimum Range	Analysis	Value Found	Optimum Range
Soil pH (1:1, H ₂ O)	5.6		Cation Exch. Capacity, meq/100g	20.1	
Modified Morgan extractable, ppm			Exch. Acidity, meq/100g	11.1	
Macronutrients			Base Saturation, %		
Phosphorus (P)	4.7	4-14	Calcium Base Saturation	34	50-80
Potassium (K)	213	100-160	Magnesium Base Saturation	8	10-30
Calcium (Ca)	1375	1000-1500	Potassium Base Saturation	3	2.0-7.0
Magnesium (Mg)	190	50-120	Scoop Density, g/cc	0.87	
Sulfur (S)	12.4	>10	Optional tests		
Micronutrients *			Soil Organic Matter (LOI), %	10.2	
Boron (B)	0.4	0.1-0.5	Soluble Salts (1:2), dS/m	0.05	<0.6
Manganese (Mn)	15.5	1.1-6.3	Nitrate-N (NO ₃ -N), ppm	8	
Zinc (Zn)	4.2	1.0-7.6			
Copper (Cu)	0.1	0.3-0.6			
Iron (Fe)	13.6	2.7-9.4			
Aluminum (Al)	134	<75			
Lead (Pb)	3.2	<22			

* Micronutrient deficiencies rarely occur in New England soils; therefore, an Optimum Range has never been defined. Values provided represent the normal range found in soils and are for reference only.

Soil Test Interpretation

Nutrient	Very Low	Low	Optimum	Above Optimum
Phosphorus (P):				
Potassium (K):				
Calcium (Ca):				
Magnesium (Mg):				



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Recommendations for Deciduous Trees, Shrubs & Vines-Maintenance

Limestone (Target pH of 6.0)	Nitrogen, N	Phosphorus, P ₂ O ₅	Potassium, K ₂ O
17.5	.1 - .2	0.1	0

Comments:

- For instructions on converting nutrient recommendations to fertilizer applications in home gardens, lawns and landscapes, see Reference "Step-by-Step Fertilizer Guide for Home Grounds and Gardening" (listed below).
- Do not topdress with more than 5 lb limestone per 100 sq ft at one time. Split the above application between early spring and mid-autumn.
- Avoid overfertilization. In addition to threatening water quality, excessive nutrient applications can compromise plant health and contribute to insect and disease problems. For details, see Reference "Corrective Measures and Management of Over-Fertilized Soils" (listed below).

References:

Home Lawn and Garden Information	http://ag.umass.edu/resources/home-lawn-garden
Step-by-Step Fertilizer Guide for Home Grounds and Gardening	https://soiltest.umass.edu/fact-sheets/step-step-fertilizer-guide-home-grounds-and-gardening
Corrective Measures and Management of Over-Fertilized Soils	https://ag.umass.edu/soil-plant-tissue-testing-lab/fact-sheets/corrective-measures-management-of-over-fertilized-soils

Recommendations for Needleleaf Trees & Shrubs-Maintenance

Limestone (Target pH of 6.0)	Nitrogen, N	Phosphorus, P ₂ O ₅	Potassium, K ₂ O
17.5	.1 - .2	0.05	0

Comments:

- For instructions on converting nutrient recommendations to fertilizer applications in home gardens, lawns and landscapes, see Reference "Step-by-Step Fertilizer Guide for Home Grounds and Gardening" (listed below).
- Do not topdress with more than 5 lb limestone per 100 sq ft at one time. Split the above application between early spring and mid-autumn.
- Avoid overfertilization. In addition to threatening water quality, excessive nutrient applications can compromise plant health and contribute to insect and disease problems. For details, see Reference "Corrective Measures and Management of Over-Fertilized Soils" (listed below).

References:

Home Lawn and Garden Information	http://ag.umass.edu/resources/home-lawn-garden
Step-by-Step Fertilizer Guide for Home Grounds and Gardening	https://soiltest.umass.edu/fact-sheets/step-step-fertilizer-guide-home-grounds-and-gardening
Corrective Measures and Management of Over-Fertilized Soils	https://ag.umass.edu/soil-plant-tissue-testing-lab/fact-sheets/corrective-measures-management-of-over-fertilized-soils



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Recommendations for Deciduous Trees, Shrubs & Vines-Maintenance

Limestone (Target pH of 6.0)	Nitrogen, N	Phosphorus, P ₂ O ₅	Potassium, K ₂ O
17.5	.1 - .2	0.1	0

Comments:

- For instructions on converting nutrient recommendations to fertilizer applications in home gardens, lawns and landscapes, see Reference "Step-by-Step Fertilizer Guide for Home Grounds and Gardening" (listed below).
- Do not topdress with more than 5 lb limestone per 100 sq ft at one time. Split the above application between early spring and mid-autumn.
- Avoid overfertilization. In addition to threatening water quality, excessive nutrient applications can compromise plant health and contribute to insect and disease problems. For details, see Reference "Corrective Measures and Management of Over-Fertilized Soils" (listed below).

References:

Home Lawn and Garden Information	http://ag.umass.edu/resources/home-lawn-garden
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Corrective Measures and Management of Over-Fertilized Soils	https://ag.umass.edu/soil-plant-tissue-testing-lab/fact-sheets/corrective-measures-management-of-over-fertilized-soils

Recommendations for Needleleaf Trees & Shrubs-Maintenance

Limestone (Target pH of 6.0)	Nitrogen, N	Phosphorus, P ₂ O ₅	Potassium, K ₂ O
17.5	.1 - .2	0.05	0

Comments:

- For instructions on converting nutrient recommendations to fertilizer applications in home gardens, lawns and landscapes, see Reference "Step-by-Step Fertilizer Guide for Home Grounds and Gardening" (listed below).
- Do not topdress with more than 5 lb limestone per 100 sq ft at one time. Split the above application between early spring and mid-autumn.
- Avoid overfertilization. In addition to threatening water quality, excessive nutrient applications can compromise plant health and contribute to insect and disease problems. For details, see Reference "Corrective Measures and Management of Over-Fertilized Soils" (listed below).

References:

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Corrective Measures and Management of Over-Fertilized Soils	https://ag.umass.edu/soil-plant-tissue-testing-lab/fact-sheets/corrective-measures-management-of-over-fertilized-soils



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Soil Test Report

Sample Information:

Sample ID: SP #3

Order Number: 31638
 Lab Number: S170711-133
 Area Sampled: 400 sq ft
 Received: 7/11/2017
 Reported: 7/19/2017

Results

Analysis	Value Found	Optimum Range	Analysis	Value Found	Optimum Range
Soil pH (1:1, H ₂ O)	5.5		Cation Exch. Capacity, meq/100g	18.4	
Modified Morgan extractable, ppm			Exch. Acidity, meq/100g	10.2	
Macronutrients			Base Saturation, %		
Phosphorus (P)	0.5	4-14	Calcium Base Saturation	31	50-80
Potassium (K)	70	100-160	Magnesium Base Saturation	13	10-30
Calcium (Ca)	1122	1000-1500	Potassium Base Saturation	1	2.0-7.0
Magnesium (Mg)	291	50-120	Scoop Density, g/cc	0.99	
Sulfur (S)	10.0	>10	Optional tests		
Micronutrients *			Soil Organic Matter (LOI), %	5.3	
Boron (B)	0.1	0.1-0.5	Soluble Salts (1:2), dS/m	0.04	<0.6
Manganese (Mn)	11.6	1.1-6.3	Nitrate-N (NO ₃ -N), ppm	1	
Zinc (Zn)	2.0	1.0-7.6			
Copper (Cu)	0.1	0.3-0.6			
Iron (Fe)	40.4	2.7-9.4			
Aluminum (Al)	93	<75			
Lead (Pb)	5.9	<22			

* Micronutrient deficiencies rarely occur in New England soils; therefore, an Optimum Range has never been defined. Values provided represent the normal range found in soils and are for reference only.

Soil Test Interpretation

Nutrient	Very Low	Low	Optimum	Above Optimum
Phosphorus (P):	<div></div>			
Potassium (K):	<div></div>			
Calcium (Ca):	<div></div>			
Magnesium (Mg):	<div></div>			

**Soil and Plant Nutrient Testing Laboratory**

203 Paige Laboratory
161 Holdsworth Way
University of Massachusetts
Amherst, MA 01003
Phone: (413) 545-2311
e-mail: soiltest@umass.edu
website: soiltest.umass.edu

Recommendations for Deciduous Trees, Shrubs & Vines-Maintenance

Limestone (Target pH of 6.0)	Nitrogen, N	Phosphorus, P ₂ O ₅	Potassium, K ₂ O
		lbs / 100 sq ft	
15	.1 - .2	0.25	0.25

Comments:

-For instructions on converting nutrient recommendations to fertilizer applications in home gardens, lawns and landscapes, see Reference "Step-by-Step Fertilizer Guide for Home Grounds and Gardening" (listed below).
-Do not topdress with more than 5 lb limestone per 100 sq ft at one time. Split the above application between early spring and mid-autumn.

References:

Home Lawn and Garden Information

<http://ag.umass.edu/resources/home-lawn-garden>

Step-by-Step Fertilizer Guide for Home Grounds and Gardening

<https://soiltest.umass.edu/fact-sheets/step-step-fertilizer-guide-home-grounds-and-gardening>

Recommendations for Needleleaf Trees & Shrubs-Maintenance

Limestone (Target pH of 6.0)	Nitrogen, N	Phosphorus, P ₂ O ₅	Potassium, K ₂ O
		lbs / 100 sq ft	
15	.1 - .2	0.1	0.1

Comments:

-For instructions on converting nutrient recommendations to fertilizer applications in home gardens, lawns and landscapes, see Reference "Step-by-Step Fertilizer Guide for Home Grounds and Gardening" (listed below).
-Do not topdress with more than 5 lb limestone per 100 sq ft at one time. Split the above application between early spring and mid-autumn.

References:

Home Lawn and Garden Information

<http://ag.umass.edu/resources/home-lawn-garden>

Step-by-Step Fertilizer Guide for Home Grounds and Gardening

<https://soiltest.umass.edu/fact-sheets/step-step-fertilizer-guide-home-grounds-and-gardening>

General References:

Interpreting Your Soil Test Results

<http://soiltest.umass.edu/fact-sheets/interpreting-your-soil-test-results>

For current information and order forms, please visit

<http://soiltest.umass.edu/>

UMass Extension Nutrient Management

<http://ag.umass.edu/agriculture-resources/nutrient-management>

**Soil and Plant Nutrient Testing Laboratory**

203 Paige Laboratory
161 Holdsworth Way
University of Massachusetts
Amherst, MA 01003
Phone: (413) 545-2311
e-mail: soiltest@umass.edu
website: soiltest.umass.edu

Total Sorbed Metals**Sample Information:**

Sample ID: SP #3

Order Number: 31736

Lab Number: M170719-109

Received: 7/19/2017

Reported: 7/25/2017

Results

<i>Analysis</i>	<i>Value Found</i>	<i>USEPA Heavy Metals Thresholds *</i>
Lead (Pb)	52.2 mg/Kg	400 mg/kg
Nickel (Ni)	42.8 mg/Kg	1600 mg/kg
Cadmium (Cd)	0.2 mg/Kg	39 mg/kg
Chromium (Cr)	39.0 mg/Kg	100 mg/kg
Zinc (Zn)	57.3 mg/Kg	23,600 mg/kg
Copper (Cu)	19.1 mg/Kg	Not Available
Arsenic (As)	10.7 mg/Kg	Varies by state
Selenium (Se)	10.2 mg/Kg	390 mg/kg
Molybdenum (Mo)	4.3 mg/Kg	Not Available

** This information is for general guidance. The threshold values are based on toxicity characteristic leach potential (TCLP). It should be noted that the degree of environmental risk could be influenced by soil characteristics. Contact your local Environmental Protection Authority for specific recommendations.*

General References:

Soil Lead Testing Interpretation <http://soiltest.umass.edu/fact-sheets/soil-lead-testing-interpretation-recommendations-0>

Learn About Lead <http://www2.epa.gov/lead/learn-about-lead>

Arsenic Cleanup Criteria for Soils in the US and Abroad <https://soiltest.umass.edu/fact-sheets/arsenic-cleanup-criteria-soils-us-and-abroad>

Selenium http://rais.ornl.gov/documents/eco-ssl_selenium.pdf

Ecological Soil Screening Levels <http://rais.ornl.gov/documents/ecossl.pdf>

Plant Remediation (Berkeley) http://www.berkeley.edu/news/media/releases/2005/02/01_plantremediation.shtml

For specific heavy metals regulations, refer to your state's Department of Environmental Protection.

Appendix 3.10.

(please do not publish without permission)

Sandy Hook Sword Fern Die-off Planter Experiment Preliminary Information

John O'Leary, Suquamish Tribe Water Resources Program Manager
3/23/18

Objective: To determine whether soil from a die-off area will contaminate sword ferns that have previously been isolated from die-off. A clear difference regarding evidence of die-off in the contaminated planter versus the uncontaminated planter would indicate a soil organism or organism with a soil dependent life stage is very likely the culprit.

Location: Within immediate geographic area of confirmed sword fern (*Polystichum munitum*) die-off. Die-off was first noted in this location in 2010. Planters are sited approximately 175 ft west of a saltwater shoreline at an elevation of approximately 55ft NAVD88. A big leaf maple, *Acer macrophyllum*, dominates the overstory directly above the planters, Western Red-cedar, *Thuja plicata* is dominant over most of the immediately adjacent die-off zone, with some Douglas-Fir, *Pseudotsuga menziesii*, interspersed. [these sections consist mostly of memory-based descriptions for general context, to be confirmed on subsequent site visits]

Several dead crowns were observed within 20 feet of planters. North and west (upslope/denser canopy) of the planters is the area with the most complete die-off, the local ground zero, with several dozen dead crowns marking an area that was once a sword fern dominated understory. On 3/13/18, small indian plum *Oemleria cerasiformis* appeared to be leafing out in some portions of the die-off area. Small, dead Western Red-cedar were also noticed in several spots, apparently planted about 2 winters ago.

Materials and Methods: In general, the entire area was regarded as contaminated, so basic precautions were used to prevent contamination of the soil and plants prior to installation. Bagged potting soil was wrapped in a tarp and stored on site from 3/2/18 until used on 3/13/18. The planters were partially assembled off site, prior to final assembly on site 3/12/18, the day prior to planting. Plants were brought to the site on 3/13/18.

The two planters were constructed with ¾" AC plywood and 2x4s. Beds were levelled and level within an inch of each other. Both planters had a calculated capacity of 10 ft³ and were each filled with 9 ft³ of bagged potting soil. The bags most likely to have been exposed during on site storage (placed on the outside and bottom) were used to fill the Red (contaminated) planter. Once both planters were filled, approximately one gallon of potting soil was transferred from Red to White planter (1 ft³ = 7.5 gal).

Plants were paired with nearest size match of the same provenance and split between planters. The 13 plants were placed on each bed generally from largest to smallest/south to north without regard to pair number. Some space was left at the north end of each planter in case more ferns are added. David Perasso planted the ferns in the uncontaminated planter.

Between one and two gallons of local soil was collected from several sites within the die-off area. Collection sites included: adjacent to highly stressed ferns within the die-off zone and near the apparent edge, from root ball of dead fern, from duff layer, from sandy soil horizon about 6" deep. Local soil was mixed, then mixed into the top few inches of contaminated planter. We intend to repeat collection and addition of local soil three to four more times until the end of the growing season, for a total of approximately one cubic foot (about 10% of total). An equivalent amount of clean potting soil will be added to the uncontaminated bed.

David Perasso planted the ferns in the contaminated planter.

Froned counts will be conducted periodically using methodology similar to Tim Billo's protocol. We will also try to establish a reliable method focusing on comparing new springtime growth. Close up photos will be taken of plants when counted.

(please do not publish without permission)

Discussion: There were and are several plausible scenarios where the uncontaminated planter may have been or may soon be contaminated. We are being cautious regarding the possibility that we could contaminate the planter meant to remain uncontaminated.

If the plants in the uncontaminated planter show early signs of die-off similar to those in the contaminated planter, we may surmise that the die-off causing agent moves very easily and is very aggressive. If the plants in the uncontaminated planter show delayed signs of die-off compared to the contaminated planter, the agent would still need to move very easily, however it may need time to grow in the soil before it reaches the point where the ferns are noticeably afflicted.

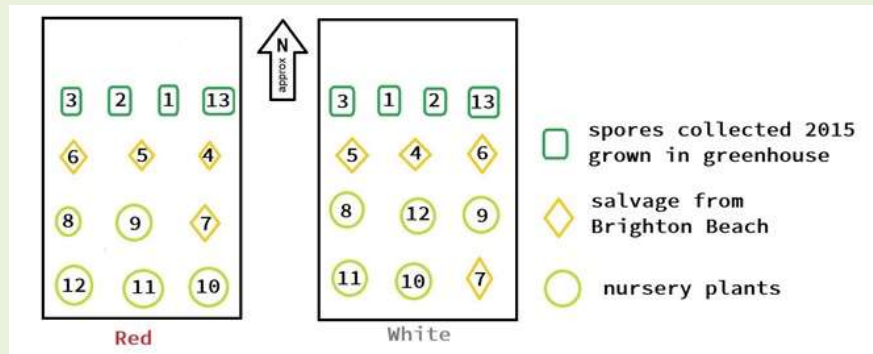
If neither planter shows signs of die-off, it could indicate that soil dependent organisms are not responsible – however it could also mean that an insufficient quantity of contamination was introduced into the planter; or that the timing and/or process of the transfers missed or interfered with the organism's ability to cause die-off; etc...



Red pin marks die off ground zero. Orange/yellow polygon extended 1/3 of a mile to the WNW broadly shows area where I encountered patches of apparent die off during autumn 2017.

(please do not publish without permission)

Sandy Hook Planters			
	Red (contaminated)	White (uncontaminated)	Date
Top of Bed	3'x4'	3'x4'	
Bottom of Bed	2'x4'	2'x4'	
Depth	1'	1'	
Calc volume	10 ft ³	10 ft ³	
Potting soil added	9 ft ³ – 1 gal	9 ft ³ + 1 gal	3/13/18
Local soil added	1 to 2 gal	–	3/13/18
Percent local soil	2%	–	
Pairs 1-13 planted			3/13/18



Appendix 3.11.

The following is a direct copy of materials provided by Paul Shannon.

Some Tentative Results Regarding the Transmission of the Agent/s of the Puget Lowlands Sword Fern Die-Off

Paul Shannon
October 6th 2018

P. munitum die-off has been observed since 2010 in the Puget Lowlands of Washington state, from the Kitsap Peninsula to Seattle. The die-off, apparently unprecedented, threatens the present and future health of the Pacific Northwest forests - in which the sword fern is an understory dominant. Individual plants live for more than 100 (and perhaps 1000) years, often in large clusters. They rarely reproduce under a closed forest canopy.

Seattle Parks staff and consultants, UW ecologist Tim Billo, and WSU plant pathologist Marianne Elliott, their students, and citizen scientists have all contributed valuable lab and field work, but have as yet been unable to establish the cause or causes of the die-off. A few plausible hypotheses have been eliminated - or identified as playing at most a minor role in the spread of the die-off. Phytophthora, mountain beaver, drought, and human and canine overuse: none of these appear to be the primary cause of the die-off

The loosely constituted sword-fern working group agrees that progress towards understanding the die-off depends upon our ability to reproduce the phenomenon on demand in a laboratory setting. This would permit the use of new assays, perhaps including metagenomics. We cannot understand the agent or agents of the die-off if we cannot first isolate and reproduce the phenomenon.

I here report some possible progress towards that goal from an experiment conducted over three weeks at my home, with only modest rigor and producing somewhat ambiguous results. Readers of this report will be - and should be - skeptical. At best, these results may motivate a rigorous experiment conducted by actual laboratory scientists.

Method

I collected four pairs of sword fern fronds from healthy and affected mature plants in the old-growth forest at Seattle's Seward Park. Each frond was cut from the plant a few inches above the ground. Each pair was placed into a carefully washed bottle half-filled with tap water. The four pairs were observed and photographed for three weeks.

Three bottles held fronds in which one frond was affected and one was unaffected at the time of collection. The unaffected fronds were collected from the same mature and apparently healthy plant, located at least ten meters from any obviously affected plants. The affected fronds (all three) were taken from separate plants at the edge of the (still expanding) active die-off zone at Seward Park. As the photos show, each of the affected fronds showed a different amount and type of die-off phenotype:

1. The "crinkly" phenotype: all pinnae curved, thought to be an early sign of the die-off's effect in some plants. The "A" fern.
2. 50% pinnae necrotic: B
3. All pinnae necrotic (brown, apparently dead): C

The pair of fronds in the fourth bottle were both apparently healthy and unaffected at the time of collection. This pair was the (admittedly imperfect) control for this admittedly unrigorous experiment. They were cut from a mature plant near the plant from which the other three healthy fronds were collected.

Due to lack of foresight, I collected the healthy fronds for the fourth control bottle one week after I collected the first three pairs. Observations of that pair therefore were not completed until an extra week had passed.

Tentative Conclusions

After three weeks, all three of the healthy fronds exposed to affected fronds (in shared bottles filled with tap water) show noticeably more degradation than do the two control ferns. The **A** frond - paired with the crinkly phenotype affected frond - shows the strongest effect. Our Kitsap collaborators (who came up with “crinkly” to describe curled pinnae) suggest that this can be the first visible sign of the die-off phenomenon. Thus we may conjecture, *with huge uncertainty and only anecdotal evidence* - that the presumed agent/s of the die-off may be at heightened virulence, and/or capable of increased mobility at this early stage.

The **B** frond, paired with the 50% necrotic frond, shows the least affect. In fact, its degradation is not so different from that of the more degraded control frond.

Photographs (below)

1. The contrasting final state of 3 experimental and 2 control fronds after 3 weeks paired in bottles
2. Bottle A at the beginning of the experiment
3. Bottle B at the beginning
4. Bottle C at the beginning
5. The two healthy control fronds the day they were collected
6. Time course pair A
7. Time course pair B
8. Time course pair C
9. Time course Control pair

1. Results: the healthy frond from bottles A,B,C
and the 2 Control Ferns at end of 3 weeks



2. Bottle A: crinkly frond & healthy frond, day zero



3. Bottle B: 50% necrotic front & healthy frond, day zero



4. Bottle C: 100% necrotic frond & healthy frond, day zero



5. Control: two healthy fronds, day zero



6. Bottle A, weeks 0-3

initially health fern on right, left, right, left in photos below



7. Bottle B, weeks 0-3

initially health fern on right, left, right, left in photos below



8. Bottle C, weeks 0-3

initially health fern on left, right, left, left in photos below



9. Control Bottle, weeks 0-3
(disturbed pinnae at the bottom are only the result of inserting and removing the fronds into the bottle)





Appendix 3.13.

The following is a direct transcript from communication with Paul Shannon.

Experimental planting at Seward Park, in two distinct zones: 24 ferns at Ground Zero, 12 ferns in a currently active die-off zone.

February 2018 – Ongoing (Expected finish date is 2023)

Principal Investigators: Paul Shannon and Suzanne Bouchard

Research Hypothesis

Ferns planted into Ground Zero (GZ) will exhibit a significantly better survival rate than those planted into a presumed active die-off zone (AD) ferns, measured by a simple t-test, where survival is defined as loss of all green fronds, and which is foreshadowed by loss of fiddlehead productivity measured in April of each year. This prediction is based on the presumption (based on preliminary data) that the presumed pathogen causing the die-off is now absent from, or less virulent in, Ground Zero, but that it is fully present and active in the presumed AD zone.

Methods

Two lines of 12 ferns (n=24) were planted at the primary die-off site (ground zero) and a third line (n=12) were planted a meter beyond what was, at the time of planting, a northern boundary of the affected die-off area. Note that with die-off now covering most of Seward's forest, this northern boundary is relative to the radially expanding original Ground Zero. See map below. Every other fern (in each line) was inoculated with mycorrhizal spores. Fern survival and status was monitored by approximately weekly photography. Ferns were donated by the Seattle Parks District and the Green Seattle Partnership. Every plant received two liters of water at intervals of one to two weeks, from June through mid-September, to minimize the confounding effects upon survival of transplant stress and drought.

Results

After 8 months, all 24 GZ ferns are healthy. The northernmost AD fern died in May. The second-most northern AD fern was, by early September, in steep decline. The remaining 10 AD ferns are uniformly healthy. All 24 GZ ferns are healthy. See photos below.

Discussion

This experiment was inspired by preliminary data obtained from two nursery ferns (in 1-gallon pots) planted in December 2014 at Ground Zero, watered somewhat irregularly through the summer months of 2015 and 2016. One of these ferns is flourishing today; the other died in 2017. This 50% survival is consistent with – in fact, better than – average success rates with sword fern restoration in unaffected areas at Seward Park since 2010, perhaps due to two seasons of watering. 50% survival, we observe, may indicate that GZ no longer harbors the presumed agent/s of the die-off. The good health (see photos) of all 24 2018 GZ ferns

after eight months is consistent with this hypothesis. Confirmation or contradiction will emerge in the remaining 4+ years in which this study will run.

The unknown agent/s of the die-off, however, are apparently active at the expanding edge of the die-off. There is a particularly clear boundary to the north of Ground Zero (see region of AD planting on the map below) beyond which, in February 2018, and within 20 meters in a fern-rich area, no affected ferns could be found. Immediately south of this boundary, many affected ferns, and dead fern crowns, are easily found.

The 12 AD ferns were planted in a north-south line just beyond this boundary, into the then unaffected area. However, by late summer 2018, several large (5' high) ferns just north of the AD planting (hence “NADZ”, or New Active Die-off Zone) began to show the distinctive signs of die-off: “crinkly” fronds and some pinna necrosis. Though a formal protocol with which to distinguish die-off effects from normal frond decline has not been defined, these formerly healthy ferns (see photo “NADZ” and map) appear to be in the early stages of die-off. The crinkly phenotype and/or necrosis appearing in yearling frond pinnae, rather than in second year fronds closer to the ground, are among the informal criteria at present used to indicate the early stages of die-off.

If these large ferns are indeed affected (to be confirmed or contradicted by observing their health in 2019) then we may suppose that the agent/s of die-off are currently active – perhaps especially active - in exactly this spot. And that activity, if real, may explain the death and decline of, so far, only the two nursery ferns planted closest to NADZ.



Figure 1. Map of experimentally planted western sword fern seedlings across the region of decline. GZ – “Ground Zero” original location of decline that has resulted in complete die-off; AD – “Active Die-off” at the time of planting in February 2018.

**NADZ: new die-off, mature fern, 16 June 2018
(healthy in February)**



Two affected ferns from AD, the Active Die-off Zone, counting from the north, at three dates:

- AD1: dead by June
- AD2: nearly dead in September

6 May 2018



2 June 2018



23 Sep 2018



AD 1



AD 2

- AD6, AD11: representative of 3-12, healthy throughout

6 May 2018



2 June 2018



AD 6

23 Sep 2018



AD 11



AD dozen 23 September 2018



Ground Zero Ferns, north dozen, 23 Sep 2018



Appendix 3.14.

List of reported potential location of sword fern decline.

Location	Report
Fort Worden State Park, Jefferson County, WA	http://sewardparkswordferndieoff.blogspot.com/2017/10/extensive-die-off-at-fort-warden-state.html
Port Ludlow, Jefferson County, WA	https://sewardparkswordferndieoff.blogspot.com/2018/07/port-ludlow-die-off-history-2008-present.html
Lord Hill Regional Park, Snohomish County, WA	https://sewardparkswordferndieoff.blogspot.com/2018/01/report-of-die-off-in-snohomish-county.html
Suquamish (private property), Kitsap County, WA	https://sewardparkswordferndieoff.blogspot.com/search?q=indianola
Blakely Harbor, Kitsap County, WA	https://sewardparkswordferndieoff.blogspot.com/2017/12/bainbridge-island-blakely-harbor-die.html
Sandy Hook (private property), Kitsap County, WA	https://sewardparkswordferndieoff.blogspot.com/2017/04/two-informally-validated-die-off-sites.html
Cheasty Mt. View, King County, WA	https://sewardparkswordferndieoff.blogspot.com/search?q=indianola
Upper Luther Burbank Park, King County, WA	https://sewardparkswordferndieoff.blogspot.com/search?q=indianola
Baring (private property), King County, WA	https://sewardparkswordferndieoff.blogspot.com/search?q=indianola

Appendix 3.15.

Potential variables to be included in the multivariate analysis of biotic and abiotic factors.

Domain	Variables
Topographic	Elevation, slope, aspect, distance from road.
Edaphic	Soil composition, pH, water holding capacity, salinity, conductivity, organic matter content, soil nutrient analysis.
Vegetative (community-level)	Dominant forest community, species associations, stand structure and age, pathogen presence (e.g. decline of tree species).
Ecophysiological (individual)	Gas exchange, chlorophyll content, chlorophyll fluorescence, light saturation, water use efficiency, mid-day and pre-dawn water potential.
Environmental	Temperature (e.g. max/min, maximum diurnal, degree deviation from normal), precipitation (e.g. daily and monthly average, mean number of precipitation-free days), canopy cover, photon flux density.

Appendix 5.1.

Roster of Sword Fern Working Group members. Names in plain text indicate core members while italicized text indicated other interested parties and associates with relevant expertise. An active copy of the roster can be found [here](#).

Name	Association	Title	Email
Lisa Ciecko	Seattle Parks and Recreation	Plant Ecologist	lisa.ciecko@seattle.gov
Tim Billo	University of Washington - Program on the Environment	Lecturer	timbillo@uw.edu
Kim Frappier	City of Mercer Island	Natural Resources Specialist	kim.frappier@mercergov.org
Joey Manson	Seward Park Audubon Center	Director	jmanson@audubon.org
Paul Shannon	Friends of Seward Park	Forest Steward	pshannon@systemsbiology.org
Marianne Elliot	Washington State University (WSU) Puyallup Research and Extension Center	Research Associate	melliott2@wsu.edu
Nelson Salisbury	EarthCorps	Ecologist	nelson@earthcorps.org
John O'Leary	Suquamish Tribe	Water Resources Program Manager	joleary@suquamish.nsn.us
David Perasso	Friends of Martha Washington,	Forest Steward	perasso@cruzio.com
Al Smith	Friends of Seward Park	Volunteer	alsticta@yahoo.com
Kramer Canup	EarthCorps	Research Assistant	kramer@earthcorps.org
Olga Kildisheva	Verdant Consulting Group, LLC	Scientist	Verdantcg.llc@gmail.com
Matthew Aghai	Verdant Consulting Group, LLC	Scientist	Verdantcg.llc@gmail.com
Bri Castilleja	NA	NA	casti.bri@gmail.com
Jeanne Schollmeyer		Extension Horticulturalist	Jeanne.schollmeyer@seattle.gov
Patrick Tobin	University of Washington - School of Environment and Forest Sciences	Assistant Professor of Disturbance Biology	pctobin@uw.edu
Ed Dominguez	Lead Naturalist	Seward Park Audubon Center	edominguez@audubon.org
Paul Talbert	Friends of Seward Park	Volunteer	ptalbert@fredhutch.org

Name	Association	Title	Email
<i>Patti Bakker</i>	<i>Seattle Parks and Recreation</i>	<i>GSP Planning and Development Supervisor</i>	<i>Patricia.Bakker@seattle.gov</i>
<i>Barb DeCaro</i>	<i>Seattle Parks and Recreation</i>	<i>Resource Conservation Coordinator</i>	<i>barbara.decaro@seattle.gov</i>
<i>Alaine Sommargren</i>	<i>City of Mercer Island</i>	<i>Director of Natural Resources</i>	<i>Alaine.Sommargren@mercergov.org</i>
<i>Kathryn Coats</i>	<i>WSU Puyallup Research and Extension Center</i>	<i>Scientific Assistant</i>	<i>kpc coats@wsu.edu</i>
<i>Emilie Bess</i>	<i>NA</i>	<i>Entomologist</i>	<i>hello.em@gmail.com</i>
<i>Darin Gemmer</i>	<i>Camp Indianola</i>	<i>Director</i>	<i>darin@campindianola.org</i>
<i>Darren Streng</i>	<i>Bloedel Reserve</i>		<i>ddstreng@gmail.com</i>
<i>Olaf Ribiero</i>	<i>Ribeiro Plant Lab, Inc</i>	<i>Pathologist</i>	<i>fungispore@comcast.net</i>
<i>Dan Hinkley</i>	<i>NA</i>	<i>Plantsman</i>	<i>djhinkley@me.com</i>
<i>Jake Betzen</i>	<i>University of Washington</i>	<i>Graduate Student</i>	<i>jakebetzen@gmail.com</i>
<i>Dominic Maze</i>	<i>City of Portland Environmental Services</i>	<i>Biologist/ Invasive Species Coordinator</i>	<i>dominic.maze@portlandoregon.gov</i>
<i>Jenny Glass</i>	<i>Washington State University Puyallup Research and Extension Center</i>	<i>Plant Diagnostician</i>	<i>jennyglass@wsu.edu</i>
<i>Soo Hyung Kim</i>	<i>University of Washington, Center for Urban Horticulture</i>	<i>Associate Professor of Plant Science</i>	<i>soobkim@uw.edu</i>

Appendix 5.2.

Project progress tracker

A		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD
Calendar Legend		Code																												
Project outline developed (goals, methods, timeline)		PO																												
Project started		PS																												
Data collection		DC																												
Field visit		FV																												
Data analysis		DA																												
Project reporting (goals, methods, outcomes, discussion, next steps)		PR																												
Project complete		X																												
Project underway		→																												
New related project planned		+																												
Experiment/Project		Experiment #	Participants	Complete	Jan-15	Feb-15	Mar-15	Apr-15	May-15	Jun-15	Jul-15	Aug-15	Sep-15	Oct-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16	Apr-16	May-16	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Nov-16	Dec-16	Jan-17	Feb-17
Plant pathological investigation of affected western sword fern (<i>Polystichum munitum</i>) populations at Seward Park		SWRD 15 - 01	Ribeiro Consultants	X																										
Mapping sword fern decline at Seward Park		SWRD 15 - 02	Nelson Salisbury, Paul Shannon	X										PO	PS	DC	→	→	→	→	→	→	→	→	→	→	→	→	→	
genus <i>Phytophthora</i> as causal agents of western sword fern (<i>Polystichum munitum</i>) die-off in Seward Park		SWRD 15 - 03	Marianne Elliott, Katie Coats	X																										
Assessment and monitoring of western sword fern (<i>Polystichum munitum</i>) and a description of the phenomenon decline with fixed plots		SWRD 15 - 04	Tim Billo, Kramer Kanup, Paul Shannon, Nelson Salisbury, Tristin O'Mara													PS	→	DC	→	→	→	→	→	→	→	→	→	→	→	
Phase II: Examination of the role of and the pathways of entry for soil borne plant pathogens in the genus <i>Phytophthora</i> as causal agents of western sword fern (<i>Polystichum munitum</i>) die-off in Seward Park		SWRD 15 - 05	Marianne Elliott, Katie Coats, Jenny Glass	X												PS	→	→	→	→	→	→	→	→	→	→	→	→	→	
The potential role of mountain beaver (<i>Aplodontia rufa</i>) in western sword fern (<i>Polystichum munitum</i>) decline at Seward Park		SWRD 15 - 06	Tim Billo, Kramer Kanup, Paul Shannon, Nelson Salisbury, Tristin O'Mara													PS	→	→	→	→	→	→	→	→	→	→	→	→	→	
Comparative analysis of the soil invertebrate community and soil quality to address the decline of western sword fern (<i>Polystichum munitum</i>) in Seward Park		SWRD 16 - 01	Patrick Tobin, Grace Masoka	X												PS	→	→	→	→	→	→	→	→	→	→	→	→	→	
Marianne, is this the same as the bating experiment?		SWRD 17 - 01	Nelson Salisbury, Al Smith																											
Soil analysis across a gradient of western sword fern (<i>Polystichum munitum</i>) decline within Seward Park		SWRD 17 - 02	Nelson Salisbury, Paul Shannon																											
Ecophysiological assessment of western sword fern (<i>Polystichum munitum</i>) across a temporo-spatial gradient		SWRD 18-01	Natalie Schwartz, Matthew Aghai, Olga Kildisheva, Kramer																											
Vegetation Management Plan (VMP) transect resurvey pilot study		SWRD 18 - 02	Nelson Salisbury, Kramer Kanup																											
Mapping sword fern decline on the Kitsap Peninsula		KTSP 18 - 01	John O'Leary																											
Mapping sword fern decline in Mercer Island Natural Area Parks		MRCR 18 - 01	Kim Frappier																											
Regional inventory using iNaturalist		SWRD 18 - 03	Paul Shannon, Tim Billo																											
Experimental planting of western sword fern (<i>Polystichum munitum</i>) in Seward Park		SWRD 18 - 04	Paul Shannon, Suzanne Bouchard																											

Project Tracker Legend	Code
Project outline developed (goals, methods, timeline)	PO
Project started	PS
Data collection	DC
Field visit	FV
Data analysis	DA
Project reporting (goals, methods, outcomes, discussion, next steps)	PR
Project complete	X
Project underway	→
New related project planned	+

Appendix 5.3.

Template and criteria used in the review of projects relating to sword fern decline in Seward Park.

Project Title:

Investigators: (Name, affiliation, email)

Project Summary (500 words max):

Sampling Methods (500 words max):

Data Analysis (300 words max):

Data Management (100 words max):

Project Timeline (100 words max):

Project Participants:

Are any of the investigators volunteers?

Yes _____ No _____

If Yes, please contact Lisa.Ciecko@seattle.gov to complete a Volunteer registration form

Budget:

Expense	Details	Type	Cost
Total:			\$

Deliverables (100 words max):

Literature Cited (optional):

Proposal Evaluation Criteria

1. Clarity of the Proposal:
 1. Clarity of project goals and objectives
 2. Completion
2. Scientific/Practical significance of the proposal objectives:
 1. Quality and feasibility of the proposed plans
 2. Research focus and themes aligned to the mission of the Sword Fern Working Group
 3. Feasibility of the proposed approach and research methods
3. Data Management and Analysis:
 1. Presence and reliability of a data management strategy
 2. Outline of a data analysis approach
4. Project timeline:
 1. Realistic and achievable timeline
5. Budget estimation:
 1. Reasonable budget for the proposed goals and activities and proper budget breakdown
6. Deliverables:
 1. Indication of deliverables
 2. Value of deliverables

Research Proposal Scoring

Evaluation comments	Score
All relevant aspects of the criterion are successfully addressed.	5
The criterion is well addressed, although certain improvements are possible.	4
The criterion is broadly addressed, yet significant weaknesses need to be corrected.	3
There are serious weaknesses in relation to the criterion	2
The criterion is addressed in an unsatisfactory manner.	1