Sword ferns from the Seward Park die-off site can induce wilting and obstruct water uptake in healthy ferns

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Abstract

The regional decline of sword ferns (*Polystichum munitum*) in the Puget Sound lowlands remains unexplained. However, anecdotal observations and some preliminary evidence from a pilot study suggest that a transmissible pathogen may be driving the phenomenon. We used sword fern fronds as a bioassay in a paired-sample greenhouse experiment to determine if symptoms could be transmitted from symptomatic fronds to healthy fronds with water as a shared medium. After 5 days, fronds in the infected treatment group were significantly more desiccated and had significantly lower foliar moisture content than fronds in the control group which were grown in water with apparently healthy fronds. We were not able to identify whether the causal agent was transmitted through physical contact, an aboveground vector or through the water. Nonetheless, our results suggest that a vascular pathogen is present in the foliage of sword ferns located at the edge of an active die-off site in Seward Park.

Background

Since 2013, over 20 acres of sword ferns (***Polystichum munitum***) have died in Seward Park in an outwardly spreading pattern while leaving other native plant species alive, indicative of a potentially host-specific pathogen (Doughton, 2019). Other sword fern die-off areas have been identified throughout the Puget Sound region but the cause for the phenomenon remains unknown (Sooter, 2018). Diseases that move through the xylem of ferns tend to be caused by organisms like bacteria, phytoplasmas, or viruses and can be vectored by insects or nematodes (Sandeno, 1962; Valverde and Sabanadzovic, 2009; Yadeta and Thomma, 2013). In a small pilot trial, in which a frond from a symptomatic fern and a frond from an apparently healthy fern were placed together in a vessel of water, the healthy frond eventually developed necrotic pinnae characteristic of the die-off area, providing some preliminary evidence of disease

transmission. We repeated this study in a greenhouse experiment with greater replication, randomization and control over potentially confounding factors. Under the assumption that the die-off phenomenon is largely driven by a pathogen, we hypothesized that desiccation and necrosis of foliar tissue would be induced in healthy fronds when grown in water shared with symptomatic fronds from the Seward Park die-off area. Furthermore, we predicted that the water media would induce symptoms in a second set of healthy fronds, after the initial fronds were removed.

Methods

For Trial 1, we harvested 50 "receiver" fronds in pairs from 25 individual ferns in Schmitz Preserve Park while 25 symptomatic "donor" fronds were simultaneously harvested at the edge of an active die-off site in Seward Park. Symptomatic fronds were identified based on the following criteria: i) multiple dead sword fern crowns within 3 meters, ii) relatively low proportion of fronds from the current year's cohort compared with previous frond cohorts, iii) presence of crispate and/or discolored pinnae. An additional 25 healthy donor fronds were obtained from Schmitz Preserve Park to serve as a control.

Figure 1: Example of a pair of genetically identical receiver fronds. Bar represents 15 cm.

Fronds were cut near the base of the rachis and placed in water-filled florist tubes for transportation to the greenhouse. Cutting tools were sterilized with 70% isopropanol between samples. At the Center for

Urban Horticulture, the ends of the fronds were then recut underwater and placed in 500 mL glass jars filled with distilled water. For each sample pair, one receiver frond was placed in a jar along with a healthy donor frond (control group), while the other was placed with a symptomatic donor frond (infected group). Sample pairs were arranged in a randomized block design along the primary environmental gradient of the greenhouse to control for potential differences in rates of evapotranspiration. Water was refilled as necessary through the duration of the experiment.

A semi-quantitative visual assessment scale (0 to 4) was periodically used to assess relative differences in desiccation: 0) no signs of drought stress, 1) <50% of pinnae crispate or curling, 2) >50% of pinnae crispate or curling, 3) <50% of pinnae dry and brittle to touch, 4) >50% of pinnae dry, brittle or necrotic. Foliar moisture content (FMC) was determined by drying foliar tissue in an oven at 80°C until a constant weight. Subsamples of water, stem sections and pinnae were preserved for potential microscopy and molecular analysis to be performed later.

In a follow-up experiment (Trial 2), we selected a subset of the jars to determine if symptoms could be induced in a new set of healthy fronds, in the absence of the original donor and receiver fronds. Healthy fronds were sourced from a forested section of private property adjacent to Schmitz Preserve Park and were collected and prepared as describe for Trial 1. Six days following the removal of the original fronds, the new set of receiver fronds were placed in the jars used for the control and infected treatment groups (n = 7). Distilled water was used in a third treatment group as an additional control. Desiccation and foliar moisture content were determined as described for Trial 1.

Data were analyzed using ANOVA and paired-sample t-tests.

Results

In Trial 1, signs of drought stress rapidly developed among receiver fronds in the infected group which were paired with symptomatic fronds from the Seward Park die-off site (Figure 2 – 4), with significant differences observed after 5 days ($t_{24} = -7.91$, $p < 0.001$). The mean desiccation value was 2.5 ± 0.1 SE for the infected group, meaning that on a typical frond, the majority of the pinnae had crispate margins and/or were curled along the proximodistal axis, and a minority of the pinnae were dry and brittle. In contrast, the mean desiccation value of the control group was 0.8 ± 0.2 SE, meaning that a minority of the pinnae on a typical frond had crispate margins and/or were curled along the proximodistal axis.

Similarly, foliar moisture content (FMC) was significantly lower in the infected group compared to the control group (t_{24} = 4.579, p < 0.001). The mean FMC was 0.52 $g \cdot g^{-1}$ ± 0.05 SE in the infected group and 0.73 $g \cdot g^{-1}$ ± 0.04 SE in the control group (Figure 5).

Several noteworthy anecdotal observations were also made. By the conclusion of Trial 1, the cut ends the rachises had developed a discoloration (Figure 6) which tended to be more pronounced and extensive in the infected group. We also noted that fronds in the control group absorbed water more rapidly than the infected group, requiring us to refill the jars with distilled water more frequently.

Although similar patterns in water uptake were initially observed in Trial 2, the desiccation values of the fronds were not significantly different by day 5 ($F_{2,18}$ = 1.258, p = 0.308). Likewise, FMC was similar between the treatment groups of Trial 2 ($F_{2,18}$ = 0.126, p = 0.882).

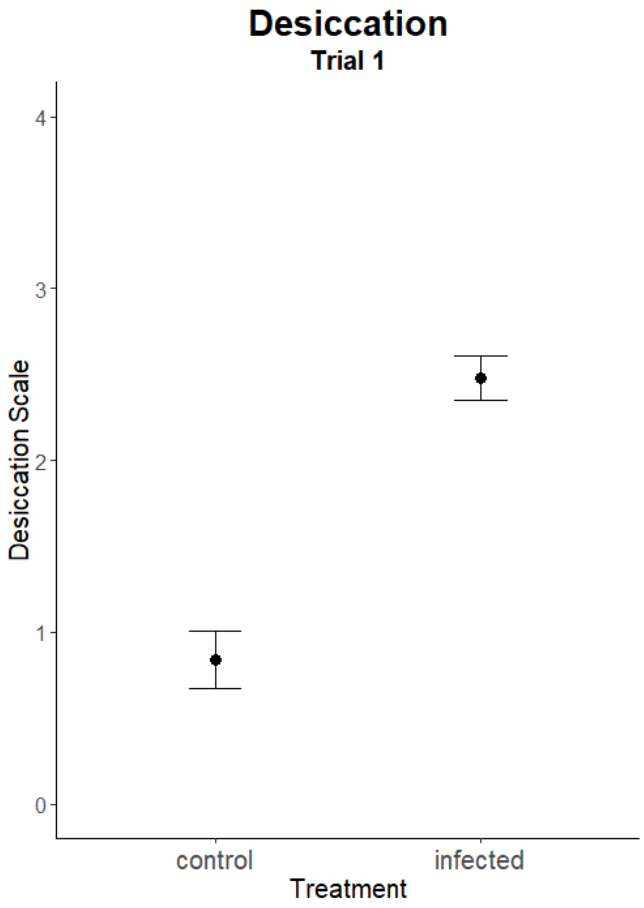

Figure 2: Desiccation values of the control and infected groups (n = 25) on day 5 of Trial 1 using a semi-quantitative visual assessment scale. Values are means (points) ± standard error (lines).

Figure 3: Sample pair #3 on day 9 of Trial 1, showing control frond (left) and infected frond (right). Bar represents 15 cm.

Figure 4: Sample pair #16 on day 9 of Trial 1, with control frond (left) and infected frond (right). Bar represents 15 cm.

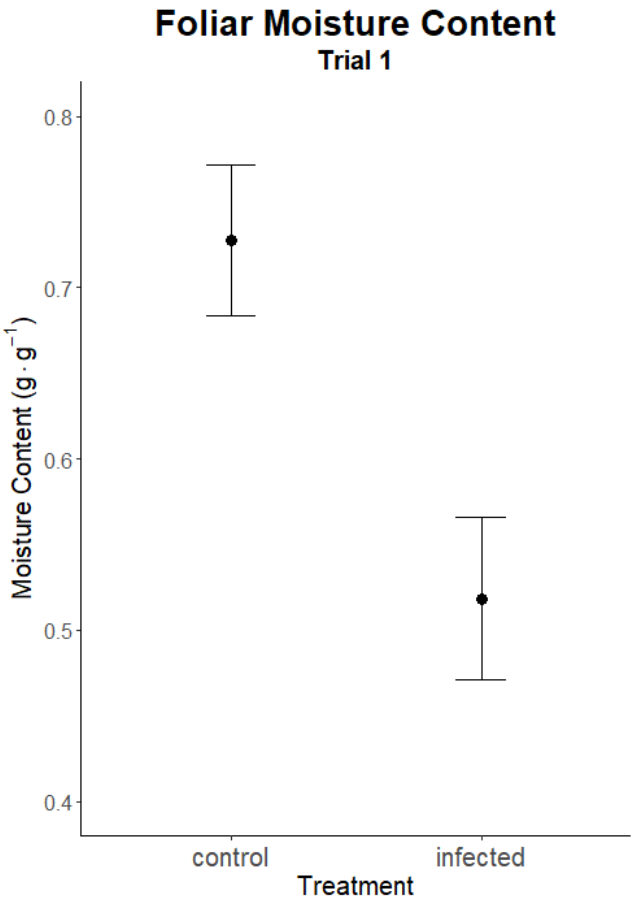

Figure 5: Foliar Moisture Content (g water per g dry mass) of the control and infected groups (n = 25) on day 12 of Trial 1. Values are means (points) ± standard error (lines).

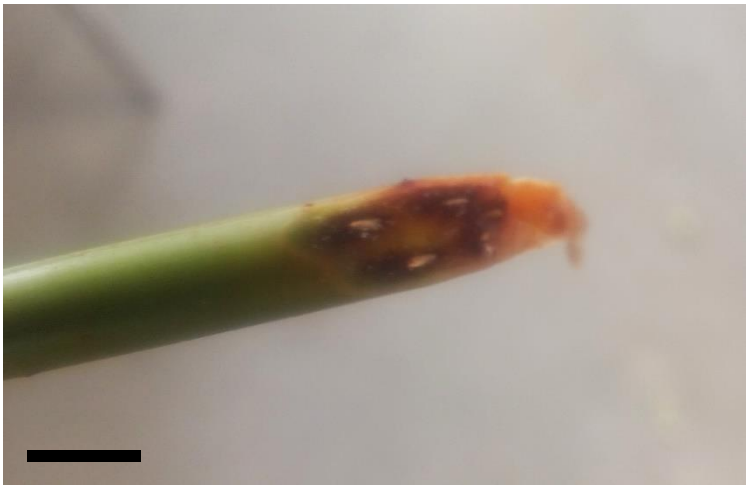

Figure 6: Example of discoloration that developed at the cut ends of most rachises in Trial 1. Bar represents 5 mm.

Discussion

The results from Trial 1 support our hypothesis that similar symptoms can be transmitted from fronds associated with a die-off site to fronds lacking any apparent signs of stress or symptoms of disease. Because our experimental design rigorously controlled for nuisance variables such as temperature and moisture gradients in the greenhouse (Cox and Cochran, 1946) or genetic differences between individual plants (Lawrence, 1955), these results are best explained by an abiotic or biotic agent being transmitted from the donor fronds to the receiver fronds and subsequently facilitating the symptoms observed. Although we have yet to isolate and identify the agent, the low water uptake and rapid desiccation of the fronds in the infected treatment group are consistent with the expected behavior of a water-borne pathogen obstructing the vascular tissue of its host (Yadeta and Thomma, 2013). Microscopy and molecular analysis of the stem and foliar samples may confirm whether or not a pathogen is indeed responsible for these symptoms.

As a criticism of our study, we note that the symptomatic donor fronds sourced from the die-off zone of Seward Park were from a previous-year age-cohort, whereas the healthy donor fronds sourced from Schmitz Preserve Park were all from the current-year age-cohort. The fungal microbiome of sword fern fronds is known to undergo succession over time (Younginger and Ballhorn, 2017), thus it is plausible that the age of the donor fronds was a confounding factor influencing the composition of microorganisms transferred between donor and receiver fronds. As this experiment was conducted in October, the microbiome of the current-year fronds from Schmitz Preserve Park had likely reached a stable state dominated by a single newly discovered fungal species (Younginger, pers. comm., 2019). While there is no evidence to suggest that the fungal microbiome of sword fern fronds changes significantly following the first year of their emergence, future research should nonetheless account for the age of sword fern fronds as a potentially confounding factor.

Results from Trial 2 conflicted with our hypothesis that the water used as media for the infected group would continue to induce symptoms in a new set of healthy fronds after the original fronds were removed. Under the assumption that a biological agent was responsible for the transmission of symptoms observed in Trial 1, we offer two potential explanations for the null results of the follow-up trial: i) the pathogen is transmitted primarily through physical contact and/or an aboveground vector, or ii) the pathogen is unable to survive in water for 6 days in the absence of its host.

Conclusion

Our study has demonstrated that symptoms similar to those observed in a sword fern die-off area can be induced in otherwise health-looking fronds in a controlled environment. Although we have yet to isolate the agent involved in the transmission, the wilting and desiccation of the fronds suggest that a water-borne vascular pathogen may be responsible. These results justify further research to identify the agent and understand its biology.

We intend to repeat our experiment to control for temporal differences in the microbiome of sword ferns by using donor fronds belonging to the same age-cohort. Under the continued assumption that a pathogen is responsible for the die-off phenomenon, we will also seek to identify whether it was transmitted through physical contact, an aboveground vector, or through shared growing media.

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References

Cox, G.M., Cochran, W.G., 1946. Designs of greenhouse experiments for statistical analysis. Soil Science 62, 87.

Doughton, S., 2019. The Case of the Vanishing Ferns: Citizen sleuths can't figure out what's killing Seattle's sword ferns [WWW Document]. The Seattle Times. URL https://www.seattletimes.com/pacific-nw-magazine/whats-killing-our-sword-ferns/ (accessed 9.9.19).

Lawrence, W.J.C., 1955. Techniques for experiments with pot plants. Plant Soil 6, 332–346. https://doi.org/10.1007/BF01343643

Sandeno, J.L., 1962. Diseases of Western Sword-Fern, Polystichum munitum (Kaulf.) Presl. Oregon State University, Corvallis, Oregon.

Sooter, T., 2018. Ferns are dying in Kitsap forests, and nobody knows why [WWW Document]. Kitsap Sun. URL https://www.kitsapsun.com/story/news/2018/09/15/kitsap-forests-ferns-dying-why/1290844002/ (accessed 9.9.19).

Valverde, R.A., Sabanadzovic, S., 2009. A novel plant virus with unique properties infecting Japanese holly fern. J. Gen. Virol. 90, 2542–2549. https://doi.org/10.1099/vir.0.012674-0

Yadeta, K., Thomma, B., 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Front. Plant Sci. 4. https://doi.org/10.3389/fpls.2013.00097

Younginger, B.S., Ballhorn, D.J., 2017. Fungal endophyte communities in the temperate fern *Polystichum munitum* show early colonization and extensive temporal turnover. Am. J. Bot. 104, 1188–1194. https://doi.org/10.3732/ajb.1700149