**Project Title: A bioassay to determine whether the sword fern dieoff agent can be transmitted in sap of symptomatic plants**

**Investigators: (Name, affiliation, email)**

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Paul Shannon

Paul Talbert

others?

**Project Summary (500 words max):**

Earlier disease transmission experiments involving soil have not resulted in symptomatic sword ferns. A trial was done using a frond from a symptomatic fern and a frond from a healthy fern placed together in water. The healthy frond paired with a frond showing the "crinkly" symptom characteristic of early stages of the dieoff became symptomatic. Based on these preliminary results that show the potential for a sap-borne disease organism, we propose to repeat the experiment with more replication under controlled conditions.

Diseases that move in the xylem tend to be caused by organisms like bacteria, phytoplasmas, or viruses and are often vectored by insects or nematodes (Yadeta and Thomma 2013, Valverde and Sabanadzovic 2009, Sandeno 1962). Perhaps the vector wasn’t alive in the soil in the previous soil experiments or the conditions weren’t right. The vector could also be feeding on the foliage and not the roots. The outward spread can be explained by it being a slow moving (crawling) vector as opposed to something that can fly.

In this project sword fern fronds of the same age class will be collected for all experiments from 3-5 different host plants to allow for genetic variation. The trials will be conducted in the greenhouse at WSU Puyallup. Additional trials can be done by volunteers since the methods are simple and do not require specialized equipment.

**Sampling Methods (500 words max):**

Collect 3 fronds with 'crinkly' symptom from 5 plants at Seward Park active dieoff zone n=15

Collect 8 fronds from 3 healthy sword ferns at WSU Puyallup (An area where there is no dieoff nearby) n=24

Keep fronds from each plant separate and number the plants in case there is genetic variability among plants. Sterilize cutting tool between samples using an alcohol or clorox wipe. Place fronds in separate gallon ziplock bags in cooler and take to lab the same day if possible.

One frond from each plant at the dieoff site will be paired with one frond from each of the control ferns (3 x 5). The control ferns will also be paired with each other (3 x 3). Each pair of fronds will be given a number between 1-18.

|  |  |  |
| --- | --- | --- |
| Plant # | Location | number of fronds |
| 1 | Seward Pk, dieoff zone | 3 |
| 2 | Seward Pk, dieoff zone | 3 |
| 3 | Seward Pk, dieoff zone | 3 |
| 4 | Seward Pk, dieoff zone | 3 |
| 5 | Seward Pk, dieoff zone | 3 |
| 6 | WSUP | 8 |
| 7 | WSUP | 8 |
| 8 | WSUP | 8 |

In the lab, cut off 1 cm of the stem of each fern and place immediately into a sterile 50 ml Falcon tube or beaker containing sterile water. Flame-sterilize the cutting tool between samples and do all the control samples first. Seal around the stems with parafilm. Place into rack in greenhouse and observe for symptoms.

Take photos at beginning and at 1 week intervals for 3 weeks. Also rate each frond using the scale:

0 = healthy

1 = "crinkly" symptom, all pinnae curved with "crinkly" edges, possible early stage of infection

2 = 25-50% of pinnae necrotic

3 = >50% of pinnae necrotic, advanced infection or dead

If symptoms develop using this method, the experiment can be repeated with fronds from other dieoff sites.

**Data Analysis (300 words max):**

Differences in frond ratings between control and symptomatic treatments will be analyzed using the Mann-Whitney U test.

**Data Management (100 words max):**

The data collected and analyzed in this project will be presented in the project report. It will be used to inform future experiments.

**Project Timeline (100 words max):**

Sample collection and experiment setup – Winter 2019

Experiment conducted – Winter/spring 2019

Data analysis and report writing - Spring/summer 2019

Two trials will be done to confirm results.

**Project Participants:**

David Perasso

John OLeary

Are any of the investigators volunteers?

Yes\_\_\_X\_\_\_\_ No\_\_\_\_\_\_\_

*If Yes, please contact* [*Lisa.Ciecko@seattle.gov*](mailto:Lisa.Ciecko@seattle.gov) *to complete a Volunteer registration form*

**Budget:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Expense** | **Details** | **Type** | **Cost** |
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| **Total:** |  |  | **$** |

**Deliverables (100 words max):**

We expect to determine whether the disease agent is transmitted in the xylem of the host plant and be able to rule it in or out as a potential mechanism for the dieoff. The results of the experiments will be presented in a written report and at a meeting of the sword fern working group.

**Literature Cited (optional):**

Sandeno, J. 1962. Diseases of western sword fern *Polystichum munitum* (Kaulf.) Presl. M.S. thesis, Oregon State University.

Valverde, R.A. and S. Sabanadzovic. 2009. A novel plant virus with unique properties infecting Japanese holly fern. Journal of General Virology, 90, 2542–2549. DOI 10.1099/vir.0.012674-0.

Yadeta, K. A. and Thomma, B.P.H.J. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Frontiers in Plant Science. doi: 10.3389/fpls.2013.00097