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

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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. Phythophthora, 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 affect 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

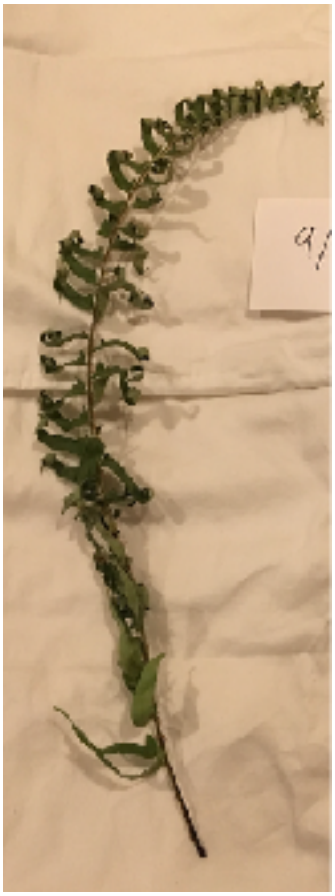
After three weeks, all three of the healthy fronds exposed to affected fronds (in shared bottles filled with tap water) show noticeably more degradation than do the two control ferns. The **A** frond - paired with the crinkly phenotype affected frond - shows the strongest effect. Our Kitsap collaborators (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 fonds 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)