

**Comments and Suggestions in Response to  
Marianne Elliot's December 2018 Proposal: *A  
bioassay to determine whether the sword fern  
dieoff agent can be transmitted in sap of  
symptomatic plants***

Combined and lightly edited by Paul Shannon  
January 6th 2019

**From David Perraso, email (29 dec 2018)**

1. "Earlier disease transmission experiments involving soil have not resulted in symptomatic sword ferns."

Though this statement is obviously true, I would delete any reference to the soil experiments as it's too easy for someone who casually follows the work to jump to conclusions. As is stated later in the proposal, there are possible reasons why soil transmission experiments have not yet produced no results among them: the age of the ferns, the soil used, the lack of "in the ground" stresses, the possibility that the samples weren't taken properly, storage of the samples before use. IMHO we have only scratched the surface in testing for soil borne transmission. I think it is best to justify this experiment on its own, without reference to any other experiments. It's simply a test for Xylem transmission, a repeat of what Paul did earlier, and that's enough. If you don't want to delete the above statement, at least include the word "yet" at the end of the sentence.

2. We need a clear definition of a crinkly frond.

About 2 months ago, Paul provided me with 2 "crinkly" fronds. These were blended in water and added to soil in which some small sword ferns were planted. So far, no sign of disease transmission. (I'll go into detail on what I did when I report results in a couple more months, but obviously there were controls). I was puzzled. I expected that if the disease could be transmitted thru the water in a bottle, it should be transmittable thru the roots. After all, the the mountain beavers aren't going around putting fronds in beer bottles. Any transmission would have to be thru the roots,

wouldn't it? I don't know the answer to that, but I took a look at the photos of the fronds used in Paul's experiment and compared them to the photos of the fronds in my experiment and guess what, they look completely different (at least to me). Take a look at the photos in Paul's report titled "bottleExperimentSummary-smallerImages.pdf." In addition to the fronds being wavy or curled, the tips of the pinnae are curled back and/or shriveled and look desiccated. Actually I can't be sure from the photos if those fronds are crispate or simply shriveled up. On the other hand, the fronds that Paul provided for my experiment looked perfectly healthy, fully hydrated with clearly crispate distal pinnae edges. I later checked with some fern experts and Richie Steffans (one of the best fern people in the PNW) sent me pictures of perfectly healthy *Polystichum munitum* with crispate pinnae and said there were several populations in WA where crispate pinnae are common. So, I'm wondering. Were the fronds I used actually infected or not? I assume that Paul took the fronds from an active die off area and probably from ferns that showed other signs of die off. And maybe the crispate edges at Seward are really only happening on infected ferns. I don't know. My point is that we need a clear, repeatable definition of what a "crispy" frond is.

3. The fronds should be cut again right before placing them in the water. 15 seconds is a good time limit before the frond begins to seal itself.

#### 4. Water source

It would be good to use distilled water (so there's no chloramine in the water) and buffer the pH. Multiple pH values could be used, e.g. pH=6, pH=7.5. Or the pH of the juices of a healthy sword fern could be measured and the pH of the water set to that value (near it, anyway). At the very least I think it's important to avoid municipal tap water which is buffered to be basic so it won't dissolve the lead in old pipes.

4. Please remove my name from the participant list. I'm not doing this experiment.

## **From John O'Leary, email (30 dec 2018)**

1. I definitely like David's comment "We need a clear definition of a crinkly frond." Definitely worth some effort getting that done.

2. In Marianne's proposal, having an intermediate step in between her first two rating steps might be worthwhile, perhaps enough to show they are very likely symptomatic without being 100% crispate, like this:

0 = healthy

1 = >25%

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

3. Looking through this and your other material, it occurred to me that fronds physically touching one another may allow transmission. I'm not sure if her experiment would be the place to test this, as it would probably call for more samples. One thing I've noticed along animal trails is that the fronds hanging into the trail itself are often the first to show contamination, that is, animals would brush them while walking by, so some agent physically moving from frond to frond may be a common way for the disease to travel.

----- more from David (30 dec 2018)

Good points, John, especially the point about touching. I'd have to go back thru the proposal to see if that is in there, but the experiment should make a clear choice between fronds touching (i.e. the part out of the water touches), not touching or doubling the sample size and having some touch and some not touch. It's a variable that should be controlled.

And while I'm at it, the fronds should all be cleaned before being used to make sure that no surface beasts or contaminants are unknowingly part of the experiment.

My apologies to Marianne who is putting in a lot of effort. It's so much easier to be strict when someone else is doing the experiment. I only wish I were as careful in my own experiments.

**-- From Tim Billo (1 jan 2019)**

This looks reasonable to me. Sort of a more professional reproduction of your experiment. I would only say that one frond should be taken per fern. You have a pseudo replication problem taking multiple fronds from one individual. Seems like the impact on Seward is minimal. I would try to assure Lisa about the purpose and what you hope to conclude, and that the results will be reported on Verdant's system. Hearing that directly from Marianne will probably reassure Lisa.

**From Paul Talbert (2 jan 2019)**

In the paragraph about taking photos, would it be helpful to explicitly mention that you are taking photos of both controls and experimentals? It might be a good idea to take photos of the plants that you take the fronds from, in addition to the progress of the fronds in the experiment. For the budget, you should at least include the cost of 18 Falcon tubes (Marianne should know, or I can see what they cost in my lab), unless you think Parks will object to spending a few bucks. Even with digital photos, there are probably a few costs of preparing a report, unless everything is digital and done by volunteers. Is Marianne volunteering her time, or should you ask for some compensation for her? Or are students volunteering their time?

Even if all labor is donated, it might be good to give an estimate of the time/value. This might improve credibility and might be good for Lisa to be able to show to her bosses.

## **From Nelson Salisbury (2 jan 2019)**

I agree with all of the questions/comments raised by Paul [Talbert] below – especially with regards to cost/time estimates. I also wondered if there was any utility in tracking the “donor” plants (repeat photographs or even more thorough monitoring of each plant – you could adapt Tim’s plot monitoring protocols?). I’m not certain this would necessarily be warranted, although it could be useful to compare the greenhouse results with speed and/or severity of disease symptoms of donor plants.

I would also recommend more clarity in the Sampling Methods section:

1. How will you control for possible airborne disease organisms?
2. How exactly will the fronds be suspended in the tubes? Are you going to rely on parafilm only or could you use some kind of septa cap?
3. Will the affected and control frond stalks be physically touching in each tube? Will the frond pinnae of each pair be physically touching each other above the tube? Will this be consistent for all pairs?
4. How will the 18 paired samples be positioned in the greenhouse? Will all pairs be in the same rack or will treatments be physically separated? Will controls be interspersed with treatment pairs?

## **From Paul Shannon (6 jan 2019)**

To follow up on David and John's concerns about "crinkly fronds". They are right to point out that this phenotype is ill-defined.

David tells us of reports from PNW fern expert Richie Steffans of healthy wild sword fern populations with common crispate pinnae.

We do not yet know if this healthy phenotype exists at Seward Park. My very casual observation is that "crinkly" preceeds necrosis, but we definitely should not bank of this. It -may- be that the die-off begins with disrupted sap flow in the pinnae, and that - for pinnae which previously lay flat - curling ensues. Then further disruption leads to necrosis, first at the tip, sometimes covering an entire single side of a frond, with the other side mostly healthy.

Some preliminary (or concurrent) study of the stages of dying of ferns at Seward Park, or elsewhere, would be useful. Is there a single trajectory? Several? How often do we observe the crinkly phenotype closely preceeding necrosis?

We have offers of assistance from two new volunteers coming out of Tim's talk at the WNPS on January 3rd. They may be willing to do some detailed field tracking of the die-off trajectories.