Role of fungal endophytes and epiphytes in endangered species conservation

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Phyllostegia endophytes and pathogen resistance

Highlights:

- Greenhouse populations of Phyllostegia are dependant on regular fungicide treatments, possibly making them prone to infection when outplanted
- A filtered slurry from the leaves of wild relatives was effective at reducing powdery mildew infections
- It is probable that the antagonistic yeast, *Pseudozyma aphidis*, is conferring some resistance to fungal diseases
- Future goals are to isolate and grow *P. aphidis* for further tests and to incorporate outplanting of treated plants

Phyllostegia mollis and *Phyllostegia kaalaensis* are federally listed endangered plant species endemic to Oahu, HI. There are currently no known wild populations of *P. kaalaensis* and the few wild populations of *P. mollis* are failing to demonstrate long-term survival. Greenhouse populations of these plants are maintained by the Army Natural Resources division, but they show marked susceptibility to fungal pathogens, particularly the powdery mildew, *Neoerysiphe galeopsidis*. Greenhouse populations are, therefore, dependent on regular fungicide treatments which are impossible to maintain once individuals have been out-planted to habitats within their native ranges.

Current scientific consensus is that the fungi which coexist within plant tissues form an integral part of plant fitness. These beneficial endophytic and mycorrhizal fungi are not present in plants that have received regular fungicidal treatments, so they are not present in out-planted populations of *P. mollis* or *P. kaalaensis*. One of the major benefits that host plants receive from mutualistic fungi is increased resistance to disease, as mutualistic fungi can outcompete pathogens for habitable living space or even actively repel invasive fungi through excreting chemical compounds. The essentially sterile plants are presumed, therefore, to be highly susceptible to attack by pathogenic fungi in the environment.

We have completed two rounds of testing on the efficacy of transplanting fungal endophytes from healthy wild populations of *P. mollis* and *P. hirsuta* into greenhouse-raised *P. mollis* and *P. kaalaensis* individuals. Two experimental transplantation methods were tested: 1) Isolating individual fungal strains from wild hosts, culturing them in the lab, and spraying them onto the leaves of greenhouse-raised individuals; 2) Preparing a low-tech slurry from leaves of wild individuals, filtering out large particles, and spraying this onto the new host plants. The first method has the benefit that we know exactly what we are

applying to the new host leaves, the second method has the benefit of potentially passing on beneficial fungi that are not amenable to laboratory culture.

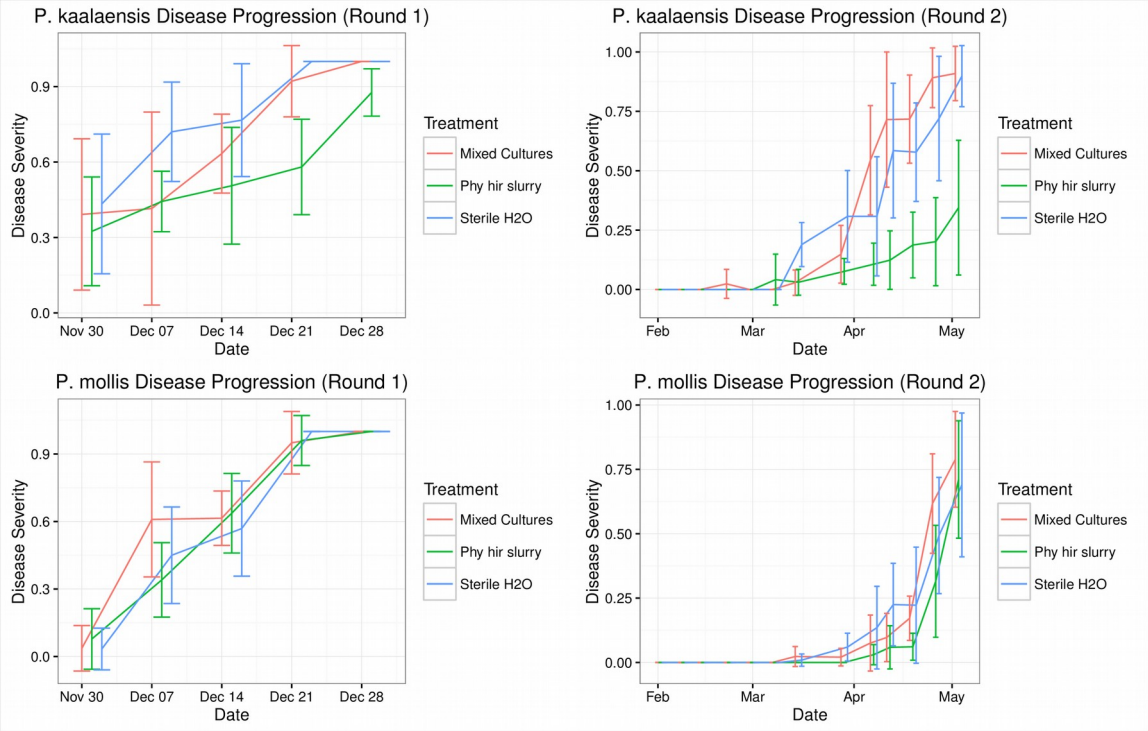


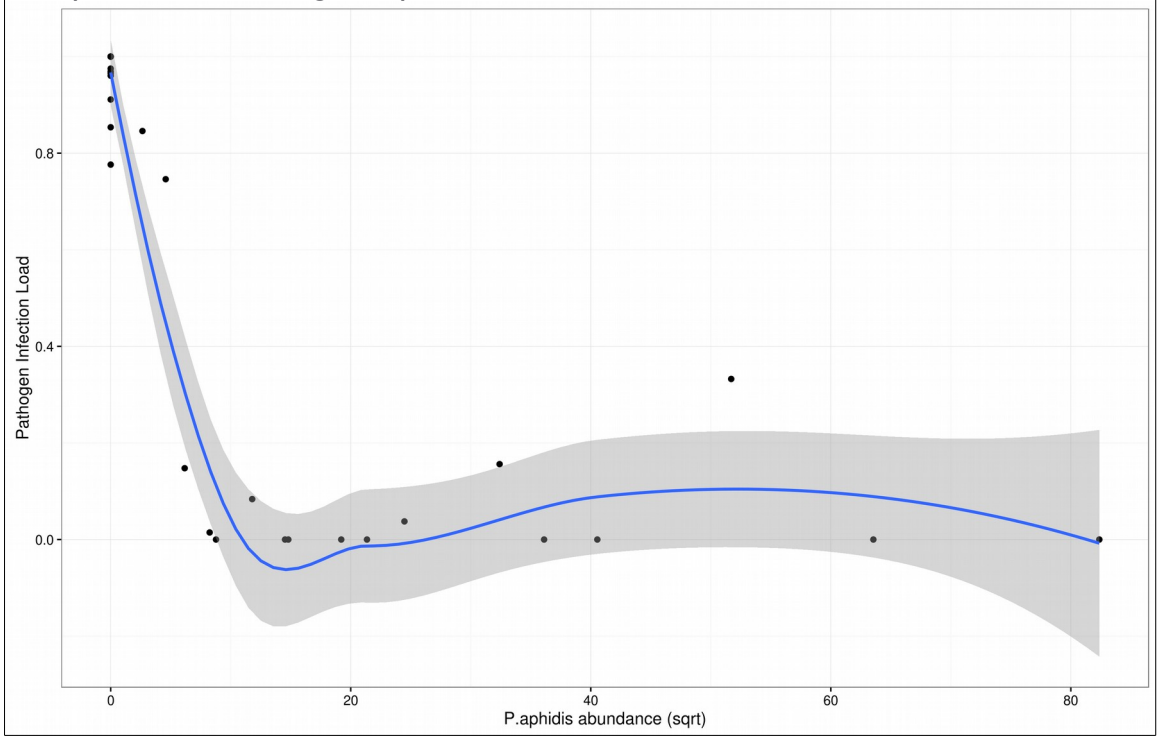
Figure 0: P. kaalaensis and P. mollis disease progression showing both rounds of tests.

Preliminary results are promising. The cultured fungal isolates did not appear to confer any advantage over the control group with respect to disease severity in either trial, but the group receiving the slurry of wild leaves showed delayed mortality and decreased disease severity in both cases. Figure 1 shows results for both plant species during both rounds of testing.

DNA from the inoculae and the plant endophyte loads was sequenced and the results are surprising. Roughly 90% of the fungal reads from the leaf slurry treatment, which showed such effectiveness against disease, come from *N. galeopsidis,* the same pathogen that appears to be killing the plants. However, there is a strong correlation between reduced disease and the presence of the basidiomycete yeast, *Pseudozyma aphidis* (Figure 2). It is likely that this yeast, which is a mycoparasite, is antagonistic against *N. galeopsidis* and could be an effective way of limiting pathogen damage without relying on fungicides.

Three *P. kaalaensis* plants treated with the leaf slurry have been outplanted in the Ekahanui snail enclosure and have been reported to be healthy after more than 3 months. Next steps will include isolating and testing *P. aphidis* as an inoculum on its own, along with scaling the inoculation method for army use and outplanting efforts.

Figure 0: Pathogen load on P. kaalaensis as a function of Pseudozyma aphidis abundance. As P. aphidis abundance increases, there is a sharp decline in N. galeopsidis infections.



Arabidopsis as a tractable laboratory system for feeding *Achatinella mustellina* snails

Highlights:

- *Achatinella* snails raised in the laboratory depend on regular time-consuming collections of leaves to supplement their diet of foliar epiphytes
- The ability to grow epiphytic fungi found in wild snail habitats in a tractable plant system would greatly ease conservation efforts for this species
- We are testing the ability to transplant wild epiphytes onto *Arabidopsis* leaves under varying environmental conditions
- Growth has completed, DNA has been extracted and is being prepared for sequencing to determine our success

Captive (laboratory) snail (*Achatinella mustelina)* populations are dependent on microbes from wild leaves to supplement their diet. These leaves are obtained by regular field forays which are costly and time consuming. The ability to grow diverse microbial communities on laboratory-amenable plants would be a major convenience and cost-savings for maintaining healthy laboratory snail populations.

We are investigating the efficacy of such a system using the model plant *Arabidopsis thaliana* (Figure 3). An study is under way to examine the factors that determine the composition of a newly-forming microbial community, such as would be seeded onto the plants in order to grow “snail food.” This plant is very fast growing and there are thousands of curated ecotypes that display a wide

range of phenotypic traits, so it potentially offers a highly customizable “delivery system” for supplementing snail captive diets without constant trips into the field.

Plants were grown in sterile conditions and were inoculated with fully-factorial combinations of four epiphytic fungi. Additional treatments include whole microbiome slurries from two field sites: The current snail location, Skeet Pass, and the proposed enclosure site for this population, Kaala Bog. The experiment was replicated under normal and drought-stressed conditions to concurrently investigate the role of epiphytic microbes in plant health. The design allows us not only to determine the feasibility of transplanting snail-associated food organisms into a laboratory setting, but the role of phylogenetic relatedness in determining fungal colonization outcomes, and whether this has any functional role in plant health. Growth trials have been completed and DNA libraries are being generated from each plant surface for sequencing.

Figure 0: Arabidopsis thalliana in growth chamber. This ecotype has nice "snail-friendly" leaves and can be grown from seed in 4 weeks.