Summary of progress on Testing the effects of inoculation with beneficial symbiotic fungi on the survivorship of *Phyllostegia kaalaensis*

PI: Prof. Nicole Hynson

Summary
The goal of this project is to test the efficacy of pretreatment with mycorrhizal and endophytic fungal inoculum on increasing the survivorship of the endemic and endangered plant species *Phyllostegia kaalaensis* found only on the island of Oahu, HI. This speicies is currently extirpated from the wild due to the negative impacts of a pathogenic powdery mildew fungus (*Neoerysiphe galeopsidis*).

Progress to date
We have chosen two study sites, one where *P. kaalaensis* was found historically, but is now extirpated and an attempt at reintroduction failed, and a second where the congeneric *P. mollis* has been outplanted by OANRP, and is doing well. The former is located in the Pahole Natural Area Reserve (Kapuna), and the latter is in the Honouliuli Forest Reserve (Kaluaa). We have secured permits from Hawaii Department of Forestry and Wildlife as well as Hawaii Department of Land and Natural Resources to collect soil from these locations that will be used to cultivate the local mycorrhizal fungi. Soil collections were made in November and December 2016 from both sites. These soils were used for a greenhouse trap culture experiment at the University of Hawaii Manoa. Mycorrhizal generalist host plants (*Paspalum notatum*-bahia grass and *Sorghum* × *drummondii*-sudan grass) were grown in replicate in the two field soils to "trap" their arbuscular mycorrhizal fungi. In Summer 2017 we harvested the trap cultures, and after a resting period we extracted the arbuscular mycorrhizal spores in August.

These spore extracts were used to inoculate four different genotypes of *P. kaalaensis* that were propagated axenically by the rare plant lab at the Lyon Arboretum (Figure 1). Replicates of each genotype were inoculated with one of the following treatments: arbuscular mycorrhizal spores (from either Kaluaa or Kapuna), foliar endophytic fungi, arbuscular mycorrhizal spores and foliar endophytic fungi, or no treatment (control; Figure 2). For this experiment we isolated a specific foliar endophytic fungus, *Pseudozyma aphidis*, which is a mycoparasite that occurs naturally in Hawaii and has been shown to combat *N. galeopsidis*. The plants are currently being grown under controlled conditions while they become colonized with these fungal treatments. In two-three months we will introduce *N. galeopsidis*, which kills *P. kaalaensis* in the wild and measure disease severity under our various treatments. We anticipate that plants inoculated with both their above and belowground symbionts will be the most robust, and that these inoculations will lead to increased growth and survivorship of plants in the controlled environment, as well as when any survivors are introduced into the wild sometime in late 2017.

Summary
There are numerous threatened and endangered plant species in Hawaii and elsewhere that in their natural environments rely on beneficial symbiotic microbes for success.

However, current *ex situ* conservation practices rarely incorporate these microbes into their propagation or reintroduction methods. It is our hope that using *P. kaalaensis* as a model species, we can provide evidence of the benefit plant microbiomes to plant health as well as provide recommendations to conservationists and land managers on how to incorporate these microbes into current plant conservation practices.

Figure 1: *Phyllostegia kaalaensis* clones grown axenically from tissue culture.

Figure 2: *Phyllostegia kaalaensis* clones in controlled environment growth chambers post treatment. Treatments include: addition of arbuscular mycorrhizal spores, addition of mycoparasitic endophytic fungus, addition of both spores and endophyte and controls.