Testing the effects of inoculation with arbuscular mycorrhizal fungi and the foliar endophytic mycoparasitic yeast *Moeziomyces aphidis* on the disease severity from *Neoerysiphe galeopsidis* in infected of *Phyllostegia kaalaensis* plants

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Introduction

We measured the percent infection of *Neoerysiphe galeopsidis* (powdery mildew) on the leaves of *Phyllostegia kaalaensis*, a critically endangered plant endemic to Hawaii. To combat the powdery mildew, we treated plants with an endophytic mycoparasitic yeast, *Moeziomyces aphidis* (END), arbuscular mycorrhizal fungi (AMF), as well as a combination of both AMF and END (ANE). We treated the plants before infecting them with the powdery mildew and measured disease severity after 11 weeks of exposure.

Methods

We collected soil from two different sites: Kapuna Gulch (KP) is a site where *P. kaalaensis* was located historically and Kaluaa Gulch (HK) is a site where there is a current outplanted population of the congeneric species, *Phyllostegia mollis*. From these sites we cultured and extracted the AMF from the soil to create our AMF inoculum. We extracted the spores to ensure we only added AMF to the plants rather than various pathogens or bacteria that could have possibly been in the soil. We cultured *M. aphidis* from isolates prepared previously by Dr. Geoff Zahn.

We treated the plants with AMF by pipetting ~150 spores from our spore inoculum which we extracted from the soils. The END was added by mixing the cultured *M. aphidis* with 0.1% agar and using a spray bottle to spray the contents onto the leaves. There was also a control treatment (CON) which added filtered END treatment through a 10 um filter and no added AMF. The leaves were sprayed until they were saturated. We sprayed the leaves once every four days for 3 weeks.

To infect the plants we received leaves of *P. kaalaensis* that were infected by powdery mildew from the greenhouse at the Oahu Army Natural Resources Program. We used those infected leaves to rub the infected areas on our healthy leaves. We did this everyday until there were signs of infections on our plants. The plants showed signs of infection after 5 days of exposure.

After 78 days we measured disease severity of the pathogen by image processing. We took the third-youngest leaf that showed signs of infection from the plant. We then took the image of the leaf by scanning it to the computer. Using the imaging software ImageJ, we estimated the total area of the leaf and what percentage of the leaf area was infected.

Data in Figure 1 are presented as mean percent disease severity and standard error of the mean. All data were analyzed using R 3.5.0. Comparisons between means were based on a test of analysis of variance (ANOVA) at an α=0.05.

Effect of Endophyte Treatment

The impact of the END treatment was significant in the defense against the pathogen powdery mildew (P=0.002). Our results suggest that the effect of END was 4.6-fold that of CON (Figure 1). This confirms the hypothesis of Dr. Geoff Zahn, who proposed in his study using whole leaf endophyte communities (Zahn & Amend 2017), that this mycoparasitic fungus may be responsible for defending *P. kaalaensis* against powdery mildew.

The ANE treatment had significant implications in the defense of powdery mildew relative to the control as well (P=0.001). The addition of both guilds of fungi, however did not perform significantly better than the addition of just the endophyte (P=0.97). The endophyte alone actually performed 1.3-fold on average better than the addition of both AMF and the endophyte. Thus there was intermediate effect of the performance of the ANE treatment relative to the END or AMF alone (see below and Figure 1).

Effect of AMF Treatment

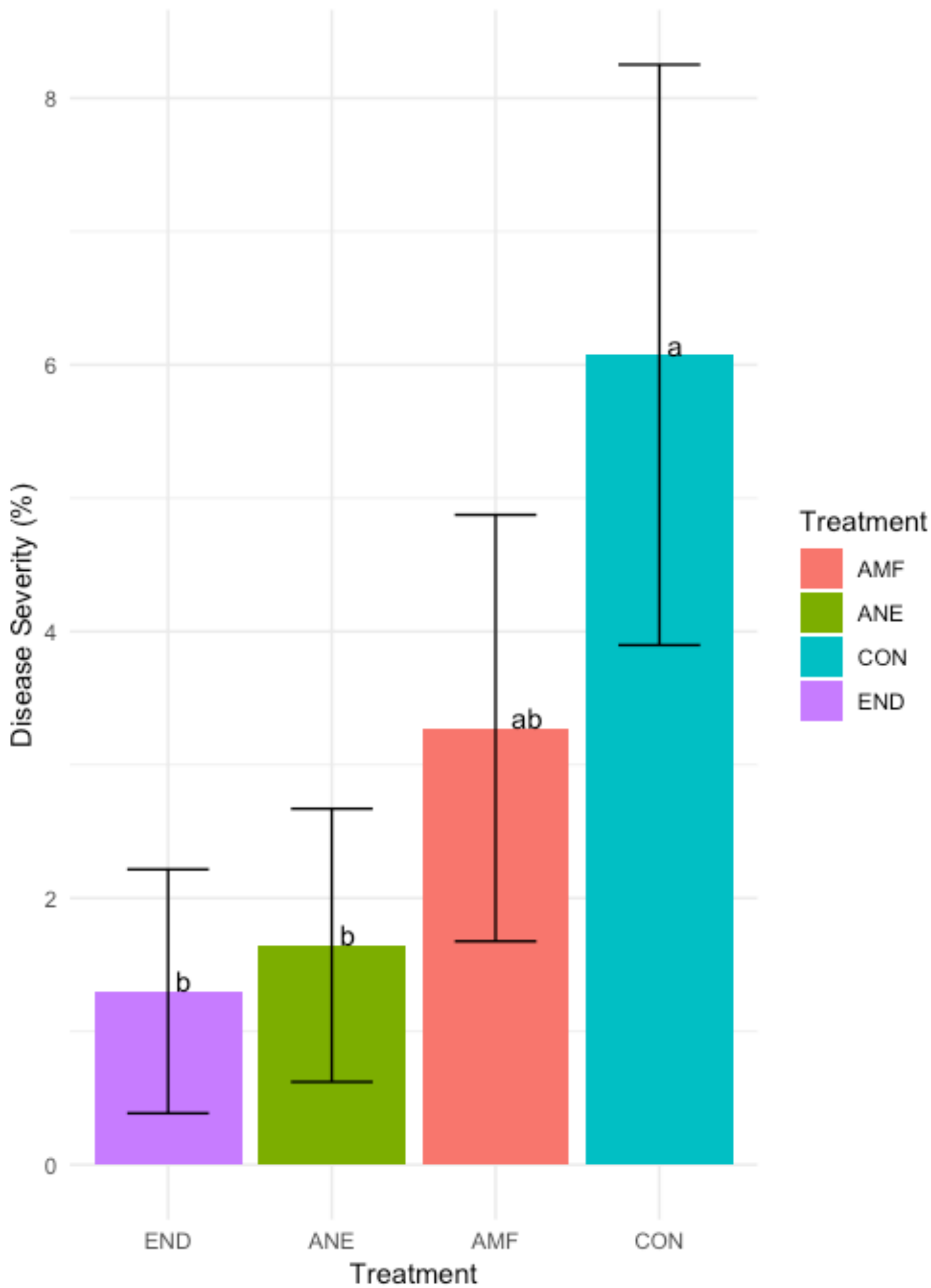
While average disease severity was lower, our AMF treatment was not significant in the prevention of powdery mildew compared to the CON (P=0.12). It was also not significantly different than the END and ANE treatments as well (P=0.19 and 0.28, respectively). However, The AMF treatment performed 1.9-fold on average better than the CON, indicating that AMF alone do confer some defense relative to untreated controls (Figure 1).

Discussion

With respect to the management of powdery mildew, the results suggest it would be best to spray the leaves of *P. kaalaensis* with *M. aphidis* before, or while, growing them in the greenhouse. Because we only observed the plants before they were outplanted, it's hard to say whether it is necessary to spray the plants prior to outplanting them in the wild or if it would be very effective once they are outplanted.

The addition of AMF alone did not significantly increase the defense of *P. kaalaensis* against powdery mildew. While focusing on different species of powdery mildew, another study also found that AMF does not have any significant effect on defending against powdery mildew (Liu et al. 2018). However, other studies have found that AMF had a significant effect in defense against powdery mildew (Yousefi et al. 2011, Mustafa et al. 2016). It could be that the particular pairing of AMF and P. kaalaensis doesn't confer increased defense under short-term greenhouse conditions relative to *Moeziomyces aphidis* alone or in tandem with AMF, but AMF alone may be important in field settings where plants are exposed to other pests and this deserves additional attention.

Figure 1 The effect of each treatment on Disease Severity (percentage of leaf area covered by powdery mildew). The treatments are the addition of *M. aphidis* (END), addition of arbuscular mycorrhizal fungi and *M. aphidis* (ANE), addition of arbuscular mycorrhizal fungi (AMF), and a control treatment with no addition of AMF and the addition of END after being filtered through a 10 um filter (CON). Different lowercase letters represent statistically significant differences (P< 0.05, Tukey's HSD). The error bars represent 1 standard error of the mean.



References

Liu Y, Feng X, Gao P, Li Y, Christensen MJ, Duan T. Arbuscular mycorrhiza fungi increased the susceptibility of Astragalus adsurgens to powdery mildew caused by Erysiphe pisi . Mycology. 2018;9(3):223-232. doi:10.1080/21501203.2018.1477849.

Mustafa, G., Randoux, B., Tisserant, B. et al. (2016). Phosphorus supply, arbuscular mycorrhizal fungal species, and plant genotype impact on the protective efficacy of mycorrhizal inoculation against wheat powdery mildew. Mycorrhiza (2016) 26: 685. https://doi.org/10.1007/s00572-016-0698-z

Yousefi, Zohreh & Riahi, Hossein & Khabbaz-Jolfaei, Hossein & Zanganeh, Sima. (2011). Effects of arbuscular mycorrhizal fungi against apple Powdery Mildew disease. Life Science Journal. 8. 108-112.

Zahn G, Amend AS. (2017) Foliar microbiome transplants confer disease resistance in a critically-endangered plant. PeerJ 5:e4020 https://doi.org/10.7717/peerj.4020