Urban biogeography of fungal endophyte communities in *Metrosideros excelsa* throughout San Francisco

Introduction and Background.

As of 2014, more than 40% of people in the US live in urban locations (OECD 2014). In recent years, ecologists have begun studying the urban environment just as they would a natural environment, in order to understand the novel environmental conditions this setting presents to the organisms that live there. For example, recent studies have shown that plant life in large cities can impact temperature, air quality, and other aspects of human health (Willis and Petrofsky, 2017). One study across the United States showed that plant life can take up significant amounts of carbon dioxide from the local air (Nowak *et al.* 2014). Another study in China has shown that healthy plant life can reduce the urban heat island effect (Kong *et al.* 2014). Therefore, understanding the impact of urban environments on plant health could help to allow those plants to thrive, benefitting the human inhabitants of the city as well as the environment as a whole.

One potentially major factor of plant health that has yet to be studied in an urban environment in great detail is the endophytic microbiome. Endophytes are microbial organisms, generally bacteria and fungi, that live symbiotically inside the leaves of plants. Inoculation experiments have shown that specific species of endophytes have an impact on their host's overall health, including factors such as resistance and susceptibility to disease (Busby *et al.* 2016). In the wild, endophytic communities display species diversity comparable to that of any macroscopic community, even among individual trees from the same species (Gazis *et al.* 2011). However, what factors influence this diversity and to what extent is still poorly understood. The urban setting is unique because factors such as rainfall and elevation will be less apparent in a smaller geographic area, but new factors such as proximity to roads and tall buildings may have present differences of their own. Studies of suburban forests in Japan have indicated that an urban setting has a notable impact on endophytic diversity (Matsumura and Fukuda, 2013). However, the full impact of urban environmental factors on endophytic communities has yet to be completely understood.

In this study, we plan to use culturing and barcode sequencing to identify the species makeup of endophytic communities *Metrosideros excelsa* individuals across San Francisco to relate environmental factors with species of endophytes. A previous study on fungal endophytes in a related species, *Metrosideros polymorpha*, in Hawaii showed that the species makeup of fungal endophyte communities can vary greatly with environmental factors such as elevation and rainfall (Zimmerman and Vitousek, 2012). Other studies have been conducted on *Metrosideros excelsa* itself in its native home of New Zealand, but there have been few studies about the endophytes in this species outside of its native environment or in an urban setting (McKenzie *et al.*1999). When completed, the results of this study will demonstrate which species of endophytes are associated with specific environmental factors, as well as which environmental factors are related to an increase or decrease of endophytic species diversity.

Methods.

We will select sampling sites based on average traffic, elevation, and temperature data from around San Francisco, aiming to represent a variety of urban environmental factors. Variables such as tree size and proximity to the ocean and other trees will also be taken into consideration. We will use the Urban Forest Map, which pinpoints the location of every *M.* excelsa individual in the city, to locate the trees that we will sample from. We will sample from at least 7 different sites, and culture from at least 4 trees from each site. Leaves will be collected by clipping three branches from each tree. Each piece of branch will come from a different part of the tree, although all will come from the outer layer of leaves. After the branches are collected, we will select 6-8 asympromatic leaves to culture from for each tree.

Then, we will surface- sterilize the leaves, cut them into 2mm squares, and put each square into a slant tube of Malt Extract Agar (MEA) media. We will surface sterilize the leaves by rinsing them in 95% ethanol for 10 seconds, then 10% bleach for 2 minutes, and then 70% ethanol for 2 minutes. We will make 100 slant tubes from each tree. After a week, we will subculture the fungi from the tubes that grew onto 35mm petri dishes with MEA media. We will continue to check the slant tubes on a weekly basis to check for new growth, and subculture any additional growth as well.

The extra fungal tissue from the petri dishes will be used for both barcode sequencing and vouchering. We will sequence the Nuclear Ribosomal Internal Transcribed Spacer (ITS) region using the Sigma Extract-N-Amp kit. Barcode sequencing data will be used to identify the species using Genbank and UNITE database sequences, and will also be analyzed using Geneious. Fungi will also be vouchered in tubes of distilled water, in order to preserve them for future research.

Expected Results and Significance.

Earlier this year, we conducted some preliminary research using the methods above on three testing sites. This research showed a significantly (Kruskal-Wallis $p < 0.5$) lower isolation frequency in the downtown site than in the other two. Based on this research, we expect to see a lower isolation frequency and less species variation for fungal endophytes cultured from downtown, more urban sites. We will focus on these sites in order to determine if the variance in our preliminary data is indicative of a pattern or simply an anomaly. We will also try to sample from sites that differ from the downtown sites in only one variable, such as sites with similar traffic levels but a lower temperature. We expect that the best predictor of endophytic diversity will be either traffic levels or proximity to other trees, because high traffic likely lowers the local air quality, and neighboring plants could potentially expose the studied trees to new species of endophytic fungi. As discussed above, endophytes can have a significant impact on overall plant health, which can in turn effect the local environment (Busby *et al.* 2016). Understanding how the endophytic microbiome is affected by urban environmental factors could provide valuable insight into how trees adapt and thrive in such locations.

Literature Cited.

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