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

**Introduction and Background.**

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 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 2011) However, what factors influence this diversity and to what extent is still poorly understood. In this study, I 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 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 1999). 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 2013). The differences between endophytic communities in plants in their native environments *Cephalotaxus harringtona* versus overseas has also been studied to a certain degree, such as with in France and Japan (Landfield 2013). However, the full impact of urban environmental factors on endophytic communities has yet to be completely understood. 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 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 about 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 16 Internal transcribed Spacer (ITS) region using (??kit name). Fungi will be vouchered in tubes of distilled water, in order to preserve them (???)

**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 studies 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 2016). Even in large cities, plant life has been shown to impact temperature, air quality, and other aspects of human health (Willis 2017). For example, a study in China has shown that healthy plant life can reduce the urban heat island effect (Kong 2014). 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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